Freshness and Shelf Life
of Foods

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Preface

Freshness is a particularly appreciated attribute of many fresh foods, (e.g., vegetables, fruits, fruit juices, milk, and fish), but also of processed foods such as bread, fine bakery products, and coffee. Shelf life is often closely associated with freshness, but shelf life is also increasingly important for products not normally associated with the attribute *fresh*. The latter category includes a variety of foods such as dairy products, beverages, confectionery products, frozen products, as well as meat and meat products. Industry and consumers both benefit from ways to preserve the fresh character of foods or to increase their shelf life. Improving the fresh character and the shelf life of foods starts with understanding what causes a product to be perceived as fresh as well as understanding what limits the shelf life of products.

The underlying functionalities of freshness can be described in terms of sensory and nutritional properties. That is, a product is perceived as fresh if the sensory and nutritional properties are the same as when the product was freshly harvested or prepared. The shelf life of a product is traditionally determined primarily by whether a product can safely be consumed; however, the sensory and nutritional properties are becoming increasingly important. Improving shelf life can lead to prolonged freshness, and that is the link between shelf life and freshness.

The symposium *Freshness and Shelf Life of Foods* was held at the American Chemical Society (ACS) National Meeting in Chicago, Illinois, from August 26–31. The symposium focused on the flavor and texture aspects of the freshness and shelf life of foods. This book contains the proceedings of this symposium, and includes contributions from flavor chemists, food physicists, sensory scientists, and food technologists from universities, research institutes, and companies. It is divided into three sections: (1) general aspects of freshness and shelf life, (2) texture aspects of freshness and shelf life, and (3) flavor aspects of freshness and shelf life. The first section starts with a study, on the perception of freshness by consumers. This section includes a general

overview of shelf-life issues and two chapters on packaging. The second section includes studies not only on which physical chemical parameters are most critical for preserving freshness but also on methods of how to improve freshness and shelf life. The third section focuses on the characterization of aroma changes during storage and the underlying mechanisms of these changes. Products that are covered in this book include tomatoes, citrus fruits, milk, cereals, bread, peanuts, beer, meat, and vegetable oils.

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Chapter 1

The Shelf Life of Foods: An Overview

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This chapter presents an overview on the factors affecting deterioration of foods and approaches used in shelf life testing. It further outlines the need for characterization of the various mechanisms of bio-/chemical and microbial deterioration of each type or group of foods, so that improved methods of testing may be developed, and longer shelf lives can be achieved.

Introduction

The fact that foods are diverse, complex and active systems, in which microbiological, enzymatic and physicochemical reactions are simultaneously taking place, makes their study an arduous task. Food preservation is dependent on the understanding of these reactions and their respective mechanisms and the successful limitation of the ones most responsible for spoilage or loss of desirable characteristics and sometimes the channeling of other reactions towards beneficial changes. Essentially, the shelf life of a food can be defined as the period it will retain an acceptable level of eating quality, from a safety and sensory point of view.

The drive by the food and beverage industry to achieve higher quality and extended shelf life food products accelerated in the 1990s. Some of the

contributing factors in the search for improved shelf life include increased consumer demand for fresh, convenient, safe and superior quality foods available year-round, and the continued globalization of food distribution systems. This has placed pressure on the food industry to ensure shelf stability and storage times as products travel further and further from their point of origin. But even as innovative packaging, new technologies and improved testing method have spurred achievements for some food manufacturers in successfully extending the shelf life of some products, most notably, fresh-cut salads, other emerging pressures indicate that the need for improved shelf life testing and assessment procedures is significant. One such trend is worth noting: the continued introduction of legal drivers for shelf life testing. Although there is no federally mandated, uniform open dating system, many U.S. government organizations have ruled that certain foods must have some type of open date (1,2). The European Union also has such legislation in place for all food products.

For the food industry, meeting these ostensibly contrary objectives of consumer demand for longer shelf life (but minimally processed foods) better: and at the same time minimizing processing requires the implementation of enhanced preservation parameters, improvement in testing and analytical procedures, a better understanding of food quality factors as related to their sensory characteristics, and continued education of scientists in food quality modeling and accelerated shelf life testing procedures. This chapter will briefly review the current state of shelf life determination testing methods, tools and technologies employed to ensure that consumers receive high quality food products with the added convenience of extended shelf life.

Essential Factors of Shelf Life

The study of food preservation is not likely to produce clear-cut results for across-the-board application, primarily because foods are very complex, active systems. To attain knowledge of a food's expected shelf life, one must understand the microbiological, enzymatic and physicochemical reactions that simultaneously take place in any given food, identify the mechanisms responsible for spoilage or loss of desirable characteristics such as texture, flavor, odor or nutrients, and implement scientific models for estimating the period it will retain an acceptable level of eating quality from a safety and sensory perspective. The four critical factors in this endeavor are:

- formulation,
- processing,
- packaging, and
- storage conditions.

All four factors are critical but their relative importance depends on food. In general, most perishable foods that are properly stored have under 14 days of shelf life, which is limited in most cases by biochemical (enzymatic/senescence) or microbial decay. With new aseptic technology, irradiation or high pressure processing, as well as controlled atmosphere/modified atmosphere packaging (CAP/MAP), such foods may last up to 90 days. Properly stored semi-perishable foods, such as some cheeses and frozen desserts, have a shelf life of up to 6 months, while shelf-stable foods, such as most canned goods, last more than six months and as long as three years under proper storage conditions (3).

An understanding of the interplay between these factors is key to shelf life estimation and testing. For example, a change in a single processing parameter may lead to undesirable chemical or physical changes in a product, or it may require reformulation or a change in packaging in order to attain the required shelf life. Similarly, the very act of processing may subject the formulated materials and ingredients to conditions that are unfavorable or inhibitory to undesirable deteriorative reactions and promote desirable physical and chemical changes thus giving the food product its final form and characteristics. And, once the food leaves the processing stage, its keeping properties and the extent to which it will retain its intended attributes is a function of its microenvironment. The important parameters are gas composition (oxygen, carbon dioxide, inert gases, ethylene, and so on), the relative humidity (RH), pressure or mechanical stresses, light and temperature. These parameters are dependent on two of the other critical factors: packaging and storage conditions. Appropriate shelf life testing is normally required to take into account the different scenarios brought about by this interplay.

Of course, the real-world pressure on the product development scientist is to provide a good estimate of product shelf life in the face of very real time constraints placed on him by marketing and research and development (R&D) managers. These food scientists gather information on the specific processing method to be employed, the types of raw materials and functional ingredients used, prior experience with similar formulations, packaging, and so on, and perform confined experiments under abuse conditions to extrapolate limited data to the projected shelf life in order to answer the basic questions, "What is the shelf life of the food?" and "Will it reach the consumer with an acceptable quality?"

Approaches to Shelf Life Estimation of Food Products

There are several established approaches to gathering this information, including estimating shelf life based on published data, utilizing known distribution times for similar products on the market, or using consumer

complaints as the basis for determining if a problem is occurring. These methods have their downsides, however, including the fact that most shelf life data on specific engineered foods is proprietary, similar products to benchmark against do not exist, or there is no information on actual consumer home storage times. If one is confident in a product's shelf life or it is already in the marketplace, one can use a distribution test method. Product is collected at supermarket sites and stored in the lab under home-use conditions. Only one such study has been reported in the literature, although this method has been used by others, especially in cases when states or countries instituted new open dating legislation (4). This method results in the product shelf life based on both distribution and home storage conditions.

The most frequently used methodology is accelerated shelf life testing (ASLT), where the objective is to store the finished product/package combination under some abuse condition, examine the product periodically until the end of shelf life occurs, and then use this data to project shelf life under true distribution conditions (5). Of course, ASLT has garnered much attention in the last 20 years or more, since it offers a way to estimate shelf life without having to wait from one to two years for the answer.

Modeling for Shelf-Life Estimation and ASLT

Selecting an appropriate, reliable approach to modeling quality loss of a food product is an important first step in estimating shelf life, and allows for the efficient design of appropriate shelf life tests. Shelf life predictions are based on fundamental principles of food quality loss modeling, primarily kinetic modeling of different deterioration mechanisms that occur in food systems, which have been detailed extensively in the literature (6-15). A general equation describing quality loss in a food system may be expressed as:

$$rQ = \phi(Ci, Ej)$$

which states that the rate of quality degradation (rQ) is a function of a number of composition factors (Ci), such as concentration of reactive species, microorganism levels, catalysts, reaction inhibitors, pH and water activity, as well as environmental factors (Ej) such as temperature, relative humidity, light, mechanical stress and total pressure (3). According to Troller and Christian (16) water activity, temperature and pH are the most important factors that control rates of deteriorative changes and microbial growth in foods. These parameters were also referred to as "hurdles" by Leistner (17).

Following modeling, desirable and undesirable quality factors then can be measured using chemical, physical, microbiological or sensory parameters.

Environmental factors can significantly affect the rates of the reactions and need to be defined and closely monitored during kinetic experiments. A kinetic model for quality loss is not only particular to the studied food system, but also to the set of environmental conditions of the experiment, including the permeability of the packaging material. It would be desirable to generalize the models so that they include, as parameters, the environmental factors that more strongly affect the quality loss rates and which are susceptible to variation during the life of the food. Some of these important factors in food preservation and quality are detailed.

Temperature

The important effect of temperature on reaction rates has long been recognized. Raising the storage temperature will accelerate many ageing processes and this is the basis of many of the accelerated methods. The most prevalent and widely used model is the Arrhenius relation (6), derived from thermodynamic laws as well as statistical mechanics principles. The Arrhenius relation, developed theoretically for reversible molecular chemical reactions, has been experimentally shown to hold empirically for a number of more complex chemical and physical phenomena (e.g., viscosity, diffusion, and sorption). Food quality loss reactions described by the kinetic models have also been shown to follow an Arrhenius behavior with temperature.

An alternative way of expressing temperature dependence which has been extensively used by the food industry is the Q_{10} approach. Q_{10} is defined as the ratio of the reaction rate constants at temperatures differing by 10°C. This model can be used to describe how much faster a reaction will go if the product is held at some other temperature, including high abuse temperatures. If the temperature-accelerating factor is given, then extrapolation to lower temperatures, such as those found during distribution, may be used to predict expected product shelf life. This is the principle behind ASLT. As described previously, ASLT involves the use of higher testing temperatures in food quality loss and shelf life experiments and extrapolation of the results to regular storage conditions through the use of the Arrhenius equation, which cuts down testing time substantially. A reaction of an average activation energy (Ea) of 20 kcal/mol may be accelerated by 9 to 13 times with a 20°C increase in the testing temperature, depending on the temperature zone (3). This principle and the methodology in conducting effective ASLTs are described in the literature (7, 18, 19). However, caution should be exercised when interpreting results and extrapolating data to other conditions. For example, when the product/package system is tested, the package also controls shelf life so that the true shelf life of the food itself is unknown; thus, if a new package with different permeabilities to

oxygen, water, or carbon dioxide is chosen, the prior results may not be applicable. Package geometry and shaking rate must also be closely controlled as these parameters significantly affect product movement and shearing.

If the ASLT conditions are chosen properly and the appropriate algorithms for extrapolation are used, then shelf life under any "known" distribution conditions should be predictable.

A few other practical problems that may arise in the use of ASLT conditions include, but are not limited to:

- Error may occur in analytical or sensory evaluation. Generally, any analytical measurement should have a variability of less than ±10% to minimize prediction errors.

- Generally, as temperature rises, phase changes (such as fat changing from a solid to liquid) may occur which can accelerate certain reactions. Thus, the actual shelf life at the lower temperature may be longer than predicted. Similarly, it has been known since 1990 that for dry foods with a given moisture content put at a higher temperature (above the glass transition temperature), the projection of shelf life using a shelf life plot to room temperature storage could be wrong, resulting in either a prediction of greater or shorter time than actual shelf life. This has opened the door for a whole set of new laboratory testing procedures such as measuring the glass transition using differential scanning calorimetry (DSC), or by some thermal rheological method like dynamic mechanical analysis (DMA) or dynamic mechanical thermal analysis (DMTA).

- Upon freezing, such as used for storage of control samples, reactants are concentrated in the unfrozen liquid, creating a higher rate of quality loss at certain temperatures, which is unaccounted for by the Q_{10} value, and will cause prediction errors.

- Storage defects in frozen products can be accelerated by storage at higher than normal temperatures. For example, more rapid changes will occur at -18 °C compared with normal long term storage temperature of below -25 °C, still faster changes will occur at -10 °C. Certain forms of deteriorations, such as ice crystal growth and freezer burn (i.e., sublimation of ice as water vapor from the surface of the frozen food) will also be accelerated if the temperature is made to fluctuate while food still remains frozen (20).

- Cycling the product between 0°C and room temperature will accelerate watery separation in starch-thickened foods. Absence of any separation after 30 cycles over 2 months normally suggests that product will be stable for 2 years at ambient temperature (20).

- If high enough temperatures are used, proteins may become denatured. This can result in both increases and decreases in the reaction rate of

certain amino acid side-chains and thus cause either under- or over-prediction of true shelf life.

- The solubility of gases, especially oxygen, in fat or water decreases by almost 25% for each 10 °C rise in temperature. An oxidative reaction (loss of Vitamin E, A, C or linoleic acid) can decrease in rate if oxygen availability is the limiting factor. Thus, at the higher temperature, the rate will be lower than the theoretical rate, resulting in under-prediction of true shelf life at normal storage temperature.

The potential problems and possible errors that can arise in the use of accelerated techniques have been described in greater detail by Robertson (21).

Water activity

Water activity (a_w) is the ratio of the vapor pressure in a solution or a food material, p_w, and that of pure water at the same temperature, p^o_w : $a_w = p_w / p^o_w$. Therefore, the equilibrium or steady state a_w is related to equilibrium relative humidity (ERH) of the surrounding atmosphere and a_w can be considered to be a temperature dependent property of water which is used to characterize the equilibrium or steady state of water within a food material.

$$ERH = a_w \times 100\%$$

The relative humidity of the immediate environment which directly affects the moisture content and water activity (a_w) of a food is the second most important environmental factor that affects the rate of food deterioration reactions (22). Water activity describes a thermodynamic energy property of water in the food, and in part, water acts as a solvent and participates in chemical reactions (23). Rates of deteriorative changes and microbial growth under normal food storage conditions often depend on water content and a_w (Figure 1). Food deterioration due to microbial growth is not likely to occur at $a_w < 0.6$ (24). However, chemical reactions and enzymatic changes may occur at considerably lower a_w values (Figure 1). Although a higher a_w does not necessarily mean a faster reaction rate, critical levels of a_w can be established above which undesirable factors that lead to the deterioration of food occur, such as microbial growth or textural changes. Controlling the a_w is the basis for preservation of dry and intermediate moisture foods (25). Besides the specific critical a_w limits, water activity has a pronounced effect on chemical reactions in these foods. Generally, the ability of water to act as a solvent, reaction medium and as a reactant itself increases with increasing a_w up to a point, where other factors decrease reaction rates. As a result, many deteriorative reactions increase exponentially in rate with increasing a_w above the value corresponding to the monolayer moisture, the value at which

most reactions have a minimum rate. For example, if one has a wet food and tries to dehydrate it to different water activities from the wet state, in fact, the reaction rate will increase, reach a maximum, and then decrease (Figure 1). This is a key concept from the standpoint of intermediate moisture foods, for example, especially with regard to semi-soft food bars, which are generally in a water activity range where rates of deterioration are very high. The rate of lipid oxidation increases again as the a_w decreases below the monolayer, and for most aqueous phase reactions, one rate decreases again above a certain a_w in the 0.6 to 0.8 range.

Mathematical models that incorporate the effect of a_w as an additional parameter can be used for shelf life predictions of moisture-sensitive foods. Also, ASLT methods have been used to predict shelf life at normal conditions based on data collected at high temperature and high humidity conditions (27).
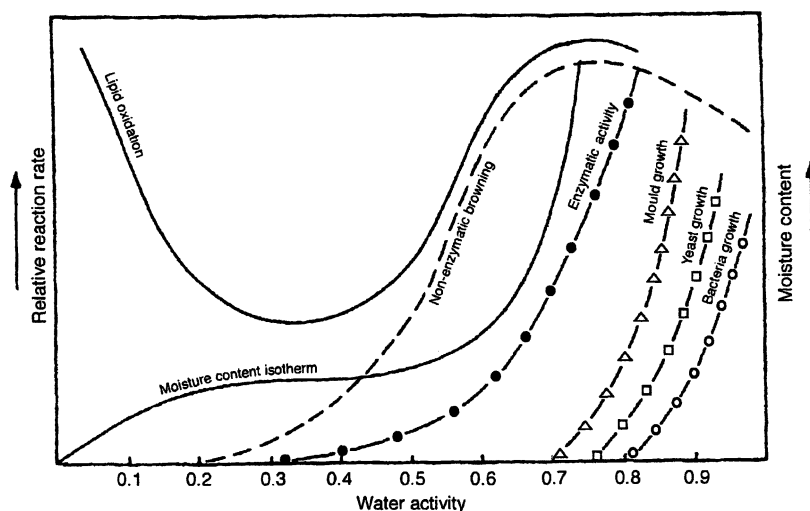

Figure 1. Relationship between biochemical changes and water activity (reproduced from reference 26. Copyright 1972 Institute of Food Technologists).

Other Environmental Factors

Understanding *gas composition*, which is also a factor that may play a significant role in some quality loss reactions, is important but not clearly understood or researched. Oxygen availability is very important for oxidative

reactions and can affect both the rate and reaction apparent order, depending upon whether it is limiting or in excess (13). It also affects the respiration rates and senescence of plant materials and microbial growth depending on the redox potential. Vacuum packaging and nitrogen flushing is based on slowing down undesirable reactions by limiting the availability of O_2. Controlled and modified atmosphere packaging are based on these principles. Further, the presence and relative amount of other gases, especially carbon dioxide, strongly affects biological and microbial reactions in fresh meat, fruit and vegetables. The mode of action of CO_2 has not been completely elucidated, but is partly connected to surface acidification (3). Different commodities have different optimum O_2-CO_2-N_2 gas composition requirements for maximum shelf life. Excess CO2 in many cases is detrimental. Spencer and Humphreys (in the present book) describe a novel method of packaging of different food products using inert gas argon, which is more efficient in excluding O_2 due to its physico-chemical properties than N_2 based packaging. This system of packaging with argon was shown to inhibit oxidation and microbial growth leading to significant extension of shelf life and improvement in quality parameters such as flavor, aroma, color and overall consumer acceptability. Other important gases are ethylene and propylene oxides (epoxides) and ozone (21). Ideally by selecting a packaging material with the desirable permeation properties, the concentration of gases and the RH inside the package can be kept within predictable limits determined by the conditions set at processing.

One key problem in gas composition analysis is that analysts will flush with a certain gas, but because films are permeable to gases and some gases may react with a food (e.g., CO_2 may dissolve in the food or oxygen may be used up in oxidation reactions), it is very possible for the gas composition to change over time. Therefore, it is important to know what those changes are.

Quality Indices Used in Shelf Life Testing

Obtaining a reliable approach to modeling quality loss of a food product is based on defining an appropriate index that measures, or directly corresponds to, food quality. Again, shelf life can be defined as the time until a product becomes unacceptable to consumers under a given storage condition. These indices include sensory evaluation, as well as chemical, microbiological and physical testing through instrumental or classical methods. The quality indices used most commonly today in shelf life and storage studies are detailed below.

Physical Changes

Physical changes are caused by mishandling of foods during harvesting, processing and distribution; these changes lead to reduced shelf life of foods. Some of the common physical changes are as follows:

- bruising of fruits and vegetables during harvesting and post-harvest handling which lead to development of rot,
- wilting or loss of moisture from fruits and vegetables during storage in low humidity environment,
- absorption of moisture by a dry food leading to sogginess, e.g., bakery, dried milk powders, infant formulas, dry pet foods, etc,
- growth of ice crystal due to temperature fluctuation in frozen stored food products, e.g., ice cream, frozen fruits and vegetables,
- freezer burn,
- textural changes due to thawing/refreezing,
- melting and solidifying of fat, e.g., confectionary /bakery products,
- viscosity changes eg. mayonnaise viscosity changes in salad,
- phase separation, e.g., separation of whey or syneresis in yoghurt.

Many of the above mentioned physical changes can be prevented by careful handling, proper packaging and stricter control of storage temperature.

Chemical changes

During the processing and storage of foods, several chemical changes occur that involve the internal food constituents and the external environmental factors. These changes may cause food deterioration and reduce the shelf life. The most important chemical changes are associated with the enzymatic reactions, oxidative reactions, particularly lipid oxidation, and non-enzymatic browning.

The least stable macro-constituents in foods are the lipids. Depending on the degree of unsaturation, lipids are highly susceptible to oxidation resulting in the development of oxidative rancidity. When this occurs, the food becomes unacceptable and is rejected by the consumers. While the development of off-flavor is markedly noticeable in rancid foods, the generation of free-radicals during autocatalytic process leads to other undesirable reactions, e.g., loss of vitamins, alteration in color, and degradation of proteins (6). In addition to the development of off-flavors, many of the oxidized products of rancidity are now considered to be unhealthy (28).

The rates of lipid oxidation are influenced by several factors. Presence of oxygen in the vicinity of food and temperature play critical roles in influencing the rate of reaction. Similarly, water plays an important role; lipid oxidation

occurs at high rates at very low water activities (Figure 1). In determining the shelf life of foods that contain lipid, especially higher concentrations of unsaturated fatty acids, the important reaction mechanisms and their rates of reactions must be known (6). A number techniques have been used to study oxidation of lipid, namely, peroxide value, 2-thiobarbituric acid method and gas chromatographic methods to monitor volatiles (29). In recent times, static or dynamic headspace or solid-phase microextraction sampling followed by GC separation of volatiles generated during lipid oxidation has been the preferred method due to relatively simple sample preparation, sensitivity and fast analysis time. In this method, the primary lipid oxidation marker compounds can be separated and quantified, e.g. aldehydes- pentanal, hexanal, heptanal, octanal, nonanal, and decadienals; ketones 2,3-octanedione, 2-heptanone, and hydrocarbons-pentane (29). Kim and Morr (30) reported major volatile compounds recovered and identified from 12 hr light exposed milk (200 foot candle fluorescent light, 0-5°C; simulating commercial milk storage conditions), resulting from photosensitization and decomposition of riboflavin and oxidation of lipids and sulfur compouns, including: hexanal, pentanal, dimethyl disulfide, 2-butanone and 2-propanol. Hexanal has been used as a marker oxidation product in various studies (29, 31, 32, 33, Lee *et al.*, present book).

In addition to lipid oxidation, there are other chemical reactions that are induced by light such as loss of vitamins, and browning of meat (6). Light sensitivity of riboflavin in milk has been previously reported (30).

Specific knowledge on the loss of flavor ingredients during storage is slowly building up. Thiols contribute to the flavor of most foods, but tend to be somewhat unstable especially in aqueous solutions, and in the presence of oxygen. Instability can be due to oxidation (34-36), but the instability of 2-methyl-3-furanthiol, an important flavor impact component for meat, appears to be due to electrophilic mechanisms (37). Cysteine was found to increase the stability of thiols, but to decrease the stability of some other important flavor components such as 4-hydroxy-2.5-dimethyl-3-furanone and sotolone.

Non-enzymatic browning is a major cause of quality change and degradation of nutritional content in many foods. This type of browning occurs due to the interaction between reducing sugars and amino acids. These reactions result in the loss of protein solubility, darkening of lightly colored dried products and the development of bitter flavors. Environmental factors such as temperature, water activity and pH have an influence on non-enzymatic browning.

At favorable temperatures such as room temperature, many enzymatic reactions proceed at rapid rates altering the quality attributes of the foods. For example, fruits upon cutting tend to brown rapidly at room temperature due to the reaction of phenolase with the cell constituents released due to the cutting of the tissue in the presence of oxygen. Enzymes such as lipoxygenase, if not denatured during the blanching process, can influence the food quality even at

sub-freezing temperatures (38). In addition to the temperature, other factors such as oxygen, water and pH induce deleterious changes in foods that are catalyzed by enzymes.

Microbiological Changes

Spoilage of food and beverages is the result of microbial activity of a variety of microorganisms. The micro flora that colonize a particular food or beverage depends on the characteristics of the product (e.g., composition, pH etc.) and the way it is processed and stored. Some of the consequences of microbial growth in food products are changes in pH, formation of toxic compounds, gas production, slime formation and off-flavor production.

Classical microbial evaluation of perishable foods is of limited value for predictive prognoses since these foods are sold or eaten before the microbiological results become available. As a result of these problems, many workers in recent years increasingly investigating techniques that measure chemical changes produced by bacteria rather than measuring the total numbers of bacteria. A popular technique is electrical impedance, which has been applied in case of shelf life evaluation of milk and milk products (39). A widely used rapid technique for determining the number of bacteria present in milk is the measurement of adenosine triphosphate (ATP), using luciferase and cofactor to produce light (40). However, this test correlate better with the total bacterial counts than they do with actual product shelf life because they do not necessarily measure the direct cause of off-flavor formation (e.g., malodorous bacterial metabolites) and the end of shelf life. During extended refrigerated storage various psychrotrophic bacteria can grow and produce heat stable proteinases (*Pseudomonas, Aeromonas, Serratia* and *Bacillus* species) (41) and lipases (*Pseudomonas, Flavobacteria* and *Alcaligenes* species). Once present these heat stable enzymes could continue to cause defects even in UHT-treated milks (42).

New highly sensitive and specific microbiological methods based upon immunological and molecular techniques have already been developed for the detection of pathogenic microorganisms (43). These techniques could also be applied for the early detection of specific spoilage organisms. However, before such techniques could be used for the detection of specific organisms, those microorganisms must be identified for each type of product and their effect on spoilage characteristics must be determined (44, 45).

An alternative way is to pin point the specific microbial metabolite, formed during the metabolism/growth of specific microorganism, causing spoilage or off-flavour in the food product (46). Formation of such metabolite could be followed upon isolation by sensitive instrumentation, such as High Performance Liquid Chromatography/Mass Spectrometry or Gas Chromatography

Olfactometry/Mass Spectrometry. One promising non-microbiological technique that has been shown to be a better predictor of shelf life than microbial plate counts is the determination of volatiles in milk by dynamic headspace capillary gas chromatography (DH-GC) followed by multivariate interpretation of GC peaks (47, 48). Solid-phase microextraction-GC/MS with multivariate analysis (MVA) has also been successfully used to classify abused milk samples as to the cause of off-flavors-i.e. oxidation (light- or copper-induced) and microbial spoilage (31, 32).

But the major problem is to find the relation between microbial composition and presence of microbial metabolites, with the presence and possible prediction of microbial spoilage. Table I summarizes a number of such metabolites, which could be used as quality indices (49-55). This kind of unifying description of the interaction between the micro flora developing in the product and the chemical changes in the same product yield important information, which can be used for developing newer methodologies for shelf life evaluation of foods. Such an integrated understanding of each type/group of food product would indeed be beneficial for the overall understanding and help in increasing interest in developing newer processing techniques to make foods safer and with longer shelf lives.

Sensory Evaluation

When the spoilage is due to changes in texture or the development of off-flavor, caused by physicochemical and biochemical or microbial reactions, the underlying mechanism might be difficult to identify. Therefore, the evaluation of spoilage will always, directly or indirectly, be related to a sensory assessment. Sensory evaluation by a trained panel usually gives a good estimate of the overall quality state of a food. One approach in sensory testing is to try to determine, at a certain level of probability, whether a product has changed (difference tests). Hence, this approach gives "endpoint" information and does not allow for modeling quality loss with time (3). Hedonic testing is a somewhat different approach that attempts to model the progressive loss of overall quality characteristics, using a graded hedonic scale (3). The requirements on the sensory panel for uniformity, experience and size for hedonic testing are stricter than the difference tests and often these requirements are not met, resulting in unreliable data. Another problem with this approach is the considerable difficulty in establishing a meaningful scale for each food product- an expert panel is not necessarily representative of consumers, let alone different consumer segments (56).

A usual approach to sensory testing is to assign the zero time value as 100% and the end of shelf life value as 0% quality, and thus the times in between

correspond proportionally to different levels of quality. This is based on the assumption that the sensory response is linear with time, which is often not true (3). Typically, however, industry does not test to determine end of shelf life. With the exception of rapid decay foods - refrigerated products like milk - the consumer is not able to detect a sensory difference from day-to-day in shelf-stable items such as cereal or canned soup. Hence, typical sensory testing using hedonics or difference testing is not going to give you a consistently accurate view of shelf life for a given product (3). The different statistical and graphical approaches for using sensory data in shelf life testing were evaluated by Labuza and Schmidl (57) and Kilcast (58). The maximum likelihood graphical procedure (Weibull method) that is widely used in the electrical and machinery industries but has been scarcely used for food, was described as a good systematic approach to sensory testing (4). The Weibull method is simple in that it asks only "Is the product acceptable?". The intensity of testing is increased near the end of shelf life so that a true shelf life is determined. This method was successfully applied to determine the end of shelf life of pasteurized milk (59) and roasted and ground coffee (60).

Besides the practical problems with regard to using sensory data in shelf life modeling further factors are the high cost that is involved with large testing panels and the problems connected to tasting spoiled or potentially hazardous samples. In some cases microbial growth or nutrient degradation could reach unacceptable levels while the food is still judged organoleptically acceptable. Sensory data are not "objective" enough for regulatory purposes and in cases of legal action or dispute. Sometimes consumers can be "trained" to accept lower standard products by being exposed to products of gradually slipping quality. That makes the need of alternative ways of assessing quality apparent (3).

Use of Time Temperature Indicators as Shelf Life Monitors

Generally a Time-Temperature Indicator (TTI) can be defined as a simple, inexpensive device that can show an easily measurable, time-temperature dependent change that reflects the full or partial temperature history of a (food) product to which it is attached. TTI operation is based on mechanical, chemical, enzymatic or microbiological systems that change irreversibly from the time of their activation. The rate of change is temperature dependent, increasing at higher temperatures in a manner similar to most physicochemical reactions. The change is usually expressed as a visible response, in the form of a mechanical deformation, color development or color movement. The visible reading thus obtained gives some information on the storage conditions that have preceded it.

Table I. Some examples of marker chemicals, produced in food by microbial activity, which can be used as quality indices (compiled from *references 49-55*)

Food product	Chemical	Spoilage problem (or off flavor note)
Miscellaneous chemicals		
Prawn, Lobster	bis(methylthio)-methane, trimethylarsine	garlic
Prawn	dimethyltrisulfide, indole	rotten onion-like
Mushroom (canned)	2-methyl isoborneol, geosmin	earthy, muddy
French fries	p-cresol, skatole, indole	pigsty-like
Cocoa beans (packaged)	chlorinated anisole	muddy
Meat (vacuum packed)	indole, H_2S	off-flavor
Fish	trimethylamine	off-flavor loss of freshness
Meat, fish and cheese	histamine, tyramine, cadavarine, and putrescine	past microbial activity off-flavor, and health issues
Volatile fatty acids		
Canned products Vegetables, fruit, meat, fish, and dairy products	n-butyric acid	swelling of low acid canned foods
Canned Meat	n-valeric acid, n-butyric acid	off-flavor
Paperboard*	3-methyl butyric 3-methyl propionic and valeric acids	transfer to food
Metabolites of sorbate		
Cheese, wine, and non-carbonated drinks	trans-1,3-pentadiene	hydrocarbon-, paint- and solvent-like
Wine	2-ethoxyhexa-3,5-diene	geranium defect
Metabolites of ferulic acid		
Lagers, Beers and Stouts	4-vinylguaiacol	phenolic taste
Presence of D-alanine		
Fruit juices	D-Alanine (>1ppm)	quality indicator

TTIs may be classified as either critical temperature indicators, partial history indicators or full history indicators (6).

- *Critical temperature indicators.* Such indicators give a response only when the temperature either goes above or below a certain pre-determined value. They do not provide temperature history effect.
- *Partial history indicators.* This type of indicators provide response only when the temperature goes above a certain pre-determined value. This type of indicators are based on melting of a chosen chemical and its diffusion along a porous matrix, which in turn yields a time-temperature integrated response.
- *Full history indicators.* This kind of indicators provide continuos response based on the changes in the temperature. The response of these indicators is at a rapid rate at higher temperature and at slow rate at lower temperature. These indicators could be based on an enzymatic reaction, such as lipolysis, or polymerization reactions.

TTIs can be used to monitor the temperature exposure of individual food packages, cartons or pallet loads during distribution up to the time they are displayed at the supermarket. By being attached to individual cases or pallets they can give a measure of the preceding temperature conditions at each receiving point. These points would serve as information gathering and decision making centers. The information gathered from all stations could be used for overall monitoring of the distribution system, thus allowing for recognition and possible correction of the more problematic links.

Another application involves their use as quality monitors. With quality loss being a function of temperature history and with TTIs giving a measure of that history, their response can presumably be correlated to the quality level of the food. If that can be achieved, TTIs can be used in either (or both) of two ways. The first would be as an inventory management and stock rotation tool at the retail level. Secondly, TTIs attached to individual packaged products, can serve as dynamic or active shelf life labeling instead of (or in conjunction with) open date labeling. The TTI would assure the consumers that the products were properly handled and would indicate remaining shelf life.

A variety of TTIs based on different physicochemical principles have been described by Byrne (61), Taoukis et al.(62) and Singh (6). Statistical correlations of TTI performance and product quality characteristics have been reported for a variety of perishable and frozen foods (3, 6).

Shelf Life and Open Dating Regulations

Another driving force for shelf life testing is a legal one. Many government organizations have stipulated that certain foods must have some type

of open date. This has been reviewed by the U.S. Congress Office of Technology Assessment (1) and summarized as an IFT status summary (2). In Europe, under new European Union regulations, all foods must have some type of shelf life date. These dates may include the following (63):

- *Production Date or Pack Date*: This can be the actual date the product was processed or harvested and packaged and lets the consumer know how old the product is so they can make a selection judgment. Used on pre-packed fresh fruits and vegetables, where the shelf life depends on the freshness of the product when harvested.

- *Sell by Date*: In some U.S. states and in some European countries many perishable processed foods require a sell by date. Helps in stock rotation to get the product out, so that the consumer can purchase the product at a point which will still give them adequate time for home storage before the end of shelf life. Printed dates are usually very good guesses based on prior experience or industry practice.

- *Best-if-Used-by-Date*. The estimated point where the product quality loss reaches a level still generally acceptable but after which it fails to meet the high quality standard. Ambiguous date as to when the product should be taken off the supermarket shelves, but confusing for the stock rotators.

- *Use by Date*. The sell by date with a warning to consume within __ days or an actual "use by date" are other approaches that can be used. The date determined by manufacturers as the end of the useful quality life of the product.

- *Combination Date*. Best if used within __ days of (date). The "__ days of" part makes this phrase a best-if-used-by date, while the date given means a sell by date.

- *Freeze-by-Date*. Often used on meat and poultry in conjunction with another date, such as a use-by-date. Helpful to the consumer and helps the store in terms of product movement.

- *Closed or Coded Date*. Numbers used by the industry that indicates production lots. May represent a packing date, but not written for the consumer to understand. Important numbers in the case of product recalls.

Conclusions

It is obvious from the above discussion that a wide range of methodologies have been employed for the purpose of shelf life evaluation of foods. It is still not possible to describe any magic solutions towards solving the puzzle of shelf life testing. But we think that an integrated approach might work better, this will

involve careful consideration of the product composition, processing parameters, packaging, environmental factors, bio-/ chemical reactions and type of microorganisms present. This will help in working out the food deterioration mechanisms. From this information one can chose proper chemical/microbial indicators and identify the right tools/techniques to put in use for analysis of the product. With the rapid progress in molecular technique or analytical instrumentation technology it is possible to detect and quantify spoilage using chemical (and also microbial) indicators in minute quantities, indicating the onset of spoilage.

Better understanding of the spoilage mechanisms may help in developing new technology, especially in the area of ingredients, processing and packaging, which will ensure the supply of safe, wholesome and quality food products.

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The Concept of Food *Freshness:* Uncovering Its Meaning and Importance to Consumers

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A series of conjoint analytic studies were conducted with civilian and military consumers in order to assess the factors that contribute to the concept of food freshness and the relative importance of freshness to other product variables. Respondents rated the freshness of 33 different food product concepts that varied in terms of food type, method of processing / preservation, retail source, etc. Respondents also rated their purchase interest for 28 food product concepts that varied in taste, price, healthfulness, safety, freshness, convenience, retail source, and brand type. Results showed the concept of freshness to be determined primarily by time from harvest/production and food type. Foods that were described as "minimally processed", e.g. by high pressure or pulsed electric fields, or "frozen and thawed" were rated as less fresh than refrigerated or frozen foods. The taste of the food was found to be the most important driver of purchase interest, with freshness having equal importance to such factors as convenience, healthfulness, and retail source.

Introduction

The Meaning of Food Freshness

The term "freshness" is one that is commonly used in association with foods. Scientists, food industry professionals, and the public would all agree that

"freshness" is a critical variable that affects the overall quality and acceptability of a food and that preserving freshness should be a primary objective throughout the food production and distribution chain. But what is 'freshness" and how is it measured? Examination of the other chapters in this volume will quickly reveal that it is defined and measured in a wide variety of ways by different professionals working in the area. A chemist might say that freshness is related to the oxidative changes occurring in the food, while a rheologist might say it is related to the integrity of the physical structure of the food. A nutritionist would look to index freshness by the retention of nutrients in the product, while a microbiologist might say it is reflected in the activity of spoilage bacteria. At the two extremes of the production and distribution chain, farmers and foodservice managers might tell you that freshness refers to the time from harvest or slaughter. In a sense, all of these statements are true, but do any of them really capture the essence of food freshness? Breene (1) asked the question this way:

> "Is fresh synonymous with raw? Are apples, potatoes and other raw products that have been stored for up to a year before being marketed as table stock or being processed considered to be fresh? Are semi-processed or minimally processed products such as precut salad and soup mixes and freshly squeezed juices fresh or processed? The term "fresh-like" has been introduced to describe some of these. Does this mean they are fresh or that they are processed foods resembling fresh foods? Are sprouts fresh or is sprouting a process? Can strawberries or other produce that have been mildly irradiated to control microorganisms and insects or to delay ripening to be called fresh or is irradiation a process? Is ethylene induced ripening of fruits a process?"

Clearly, the definition and meaning of freshness is a complex and challenging one. However, it may well be argued that the only meaning of freshness that matters is the one used by consumers. In a very real sense, the consumer is the ultimate arbiter of what constitutes freshness in a food product, because it is only his/her interpretation of freshness that determines purchase behavior, not any intrinsic element of the food itself. This primacy of consumer opinion is reflected in the fact that all physicochemical measures of freshness rely for their validity on the fact that they correlate with some change in appearance, odor, taste, or texture that consumers associate with "loss of freshness". To some degree, even microbiological and nutritional criteria of freshness rely for their importance on consumer beliefs and opinions, because the absence of microbiological or nutritional degradation does not ensure freshness in the mind of the consumer. This point is aptly reflected in the attitudes of consumers toward irradiated foods. Although these foods are both

microbiological safe and nutritionally sound, consumers do not consider them to be "fresh". The importance of consumers' opinions regarding the meaning of the word "fresh" is also recognized in federal regulations governing the use of the "fresh" label on foods. The Food and Drug Administration weighs heavily the opinions and perceptions of consumers about the freshness of products that have been in any way treated, processed, stored, or packaged, and their rulings often focus on the fact that labeling must be based on consistency with consumer beliefs and perceptions, not simply on the scientific arguments supporting the petition. Past controversies over the use of the term "fresh" or "fresh-like" with regard to juice products and the ongoing controversy over the use of these terms for "minimally processed" foods are but two salient examples of cases where the FDA has sought the opinions of consumers in formulating their decision in order to avoid rulings that conflict with consumer opinions and perceptions.

Given the importance of the consumer's perspective on what constitutes freshness in a food, what do we really know about how consumers conceive of freshness? Unfortunately, while an examination of the literature confirms the general importance of freshness to the consumer, there is not much data to help us understand the factors that contribute to the concept of freshness from the consumer's perspective. For example, in a survey of American and Irish consumers, George (2) found that freshness and brand name are the two attributes most frequently mentioned when consumers are asked what they look for in a "high quality product". This study also found that "offering fresh produce" was an important factor in consumer judgments of the quality of supermarket foodservices. In a 16 country survey of consumers from the European Union, Lappalainen, et al. (3) found quality/freshness to be a critical factor influencing food choice. Freshness has also been shown to be a primary reason why consumers shop at roadside and farmers' markets (4, 5). In fact, in the study by Rhodus, et al. (4), 94% of shoppers who were queried cited freshness as a very important factor in buying produce, while 98% cited freshness as the basis upon which they decide where to purchase produce. In a recent study aimed at addressing the meaning of freshness to consumers, Fillion and Kilcast (6) found that both sensory factors, such as the product's appearance, flavor and texture, and time from harvest were important elements in consumers' perceptions of the freshness of fruits and vegetables. Although much of the consumer literature on freshness in foods focuses on fruits and vegetables, a large number of studies have been conducted to assess freshness and/or related sensory quality attributes for other natural or unprocessed commodities, such as meat (7-11), fish (12-15), and dairy products (16, 17), as well as baked goods (18, 19).

Clearly, freshness is an attribute that is most commonly associated with raw or natural products. The concept loses much of its meaning to consumers when it comes to processed foods, especially those that are thermally processed, or those that have been frozen for even short periods of time. In keeping with this

fact, the FDA in 1993 published regulations allowing the use of the term "fresh" only for "unprocessed foods". However, while this regulation precludes the use of the term "fresh" for thermally processed and frozen foods, it does not preclude its use with foods that have undergone certain benign or "common practice" treatments, e.g. treatment with approved waxes and coatings, post-harvest application of approved pesticides, the use of mild chlorine or acid washes, and the use of ionizing radiation not to exceed 1 kG on raw foods. Other uses of the term "fresh" allowed by FDA include those cases where the term is applied to a processed food, but where the word is not used to imply that it is unprocessed or to otherwise confuse the consumer, e.g. the use of the term "fresh" to describe pasteurized milk (20).

Freshness and the Role of Emerging Technologies

The regulatory approval of the use of the term fresh to describe certain foods that have been treated by pasteurization, irradiation, and certain "minimal" processes and treatments raises the important question of just what kind of processes may be considered "mild" enough to still warrant the use of the term fresh on the final product. Recently, this question has come to the fore because of the rapid progress in the development of new methods of food preservation that involve only "minimal processing" of the food. Such methods as high pressure, pulsed electric fields, ultrasound, and ultraviolet light processing can all be considered to involve only minimal processing of the food. But does that constitute a basis for labeling them as fresh? In July 2000, in response to the growing controversy over whether foods processed by a variety of "emerging" technologies should be called fresh, the FDA scheduled public hearings on the topic and invited comments on several questions related to the petition. Among these questions, several pertained directly to the issue of how consumers define and perceive freshness in a food product. These included:
1)   Do consumers associate the term "fresh" with organoleptic (sensory) characteristics, nutritional characteristics, or some other characteristic?
2)   Do consumers want a way to identify foods that taste and look fresh but have been processed to control pathogens?
3)   Is the term "fresh", when applied to foods processed with the new technologies, misleading to consumers?
4)   Is there a term other than "fresh" that can be used for foods processed with the new technologies?
5)   Would consumers understand a new term?

The nature of the consumer-oriented questions asked by FDA in this petition reflects the concern of regulators with whether or not foods that are minimally processed by these emerging technologies and that result in "fresh-

like" food are, in fact, perceived as "fresh" by consumers. In their response to these and the other questions asked by FDA, the Institute of Food Technologists replied with comments that associate the concept of food freshness even more closely with consumer perception. In their reply, IFT (21) stated:

"The ultimate measurement of "fresh", however, is likely to be based on an individual consumer's own sensory perception. Consumers are likely to compare products that are labeled "fresh" to similar products that they have become accustomed to purchasing with that label. The consumer's ultimate satisfaction with the product involves a comparison of the expected sensory quality (based on previous "fresh" label product experiences) with the actual product characteristics."

As the above questions and responses imply, in the minds of both food regulators and industry scientific groups, the ultimate meaning of "freshness" is likely to be found with the consumer and his/her perceptions of it. In addition, consumer acceptance of products labeled as fresh are likely to be based on a comparison of expected sensory freshness attributes with actual product characteristics (see 22-24 for a discussion of the role of consumer expectations in consumer food quality perception). Unfortunately, as noted previously, our understanding of how consumers use the word fresh, what they think it means, and what variables contribute to it, are very limited.

Background to the Present Research

As a leader in the development of nonthermal food processing techniques for use in military rations, the U.S. Army Natick Soldier Center has been concerned with consumer perceptions of food freshness and with the relative importance of food freshness to other food characteristics, e.g. nutrition, taste, convenience, etc. In order to provide data useful to FDA and to industry groups charged with overseeing the development of these emerging technologies, a series of focus groups and market research studies were conducted with both civilian and military consumers in order to uncover some of the meaning behind the concept of food "freshness". In particular, these studies were aimed at determining the relative importance of various product factors (e.g. type of food, method of processing / preservation, time from harvest) to consumer conceptions of freshness and to assess the importance of freshness vs. other product variables (e.g. taste, price) to purchase interest. In addition, since the U.S. military constitute a group of consumers who regularly consume shelf stable foods that are months or years away from their original pack date, these

consumers were compared to civilian consumers to determine what, if any, differences exist in their concept of freshness and the factors that determine it.

Methods

In order to uncover the factors contributing to consumers' conceptions of freshness and the role of freshness in purchase interest, a conjoint analytic approach was used. Conjoint analysis (25, 26) is a consumer research technique that seeks to uncover the factors controlling consumer choice behavior using multiattribute choice alternatives in conjunction with a specified experimental design. Consumers are presented with a large set of multiattribute choice alternatives and choose or rate them on the basis of the perceptual dimension or behavior of interest. The "attributes" in this case constitute the factors that are believed to underlie the perception or behavior of interest. By varying the factors or their levels in each choice alternative using a statistically-determined experimental design, conjoint analysis enables the researcher to "work backwards" from the choices/ratings made by the consumer to uncover the relative importance of each factor without the need to ever directly ask the consumer his opinion of the importance of the factor to his/her choices or ratings.

In the present research, a series of conjoint analytic studies were conducted with civilian and military consumers (Table I) using a main effects fractional factorial design. A subset of respondents completed questionnaires in which

Table I. Demographics and Sample Sizes of Consumer Groups

	Military	*Civilian*
Gender	95% male, 5% female	70% male, 30% female
Age (median)	25	40
Location of Testing	Fort Polk, LA	Natick, MA
n for Purchase Conjoint	84	107
n for Freshness Conjoint	65	90

they rated the freshness of 33 different food product concepts, each comprised of four factors (food type, food source, method of processing/preservation, and time from arrival at store/market). For each factor, one of several different factor levels were used to construct the concepts (see Table II for factors, factor levels, and rating scale). A different subset of respondents completed questionnaires in which they rated their purchase intent for 28 food product concepts, each comprised of variable levels from among eight factors (see Table

III). Figure 1 shows example concept rating pages from the "freshness" (top) and "purchase interest" (bottom) questionnaires.

Table II. Factors and Factor Levels Used in "Freshness" Study

Dependent Variable:
  Freshness (Not all Fresh – Extremely Fresh)
Factors:
  Food Type:
    Fruits & Vegetables
    Meats & Poultry
    Milk & Dairy Products
    Fish & Seafood
    Bread & Bakery Products
    Orange/Fruit Juices
  Food Obtained from (purchase source):
    Supermarket
    Warehouse Store
    Specialty Market (Butcher Shop, Fish Market, Farmer's Market)
  Methods of Processing/Preservation:
    Refrigerated/Frozen
    Frozen Then Thawed
    Irradiated
    Minimally Processed (treated by high pressure or pulsed electric fields)
  Time from Product's Arrival at Store/Market:
    1-2 days
    3-5 days
    6-14 days
    >14days

Military consumers completed the questionnaires during a rest period that was part of a scheduled field training exercise. Questionnaires were administered in small groups while the subjects were seated in an open training building. Civilian consumers completed the questionnaires upon being called to attend a scheduled survey session. Questionnaires were administered to the civilian consumers in small groups while they were seated in a traditional consumer testing facility. Both consumer groups were given written and oral instructions on how to complete the questionnaires, and both groups were allowed to ask questions for further clarification. All consumers were given as much time as they needed to complete the questionnaire. Questionnaires were

**How fresh would you consider
a product to be that had
the following characteristics?**

- An Orange Or Fruit Juice
- From A Warehouse Store
- Irradiated
- 3-5 Days From The Product's Arrival At Store/Market

**Using the scale below, indicate
how fresh you would consider
this product to be.**
(Please circle only one answer.)

**1. Not At All Fresh
2. Slightly Fresh
3. Moderately Fresh
4. Very Fresh
5. Extremely Fresh**

**How interested would you be
in purchasing a product with
the following characteristics?**

- Slightly Pleasant Taste
- Low Priced
- Not Fresh
- Very Convenient To Prepare
- From A Supermarket/Commissary
- National Brand
- Very Healthful
- Definitely Safe To Eat

**Using the scale below, indicate
how interested you would be
in purchasing this product.**
(Please circle only one answer.)

**1. Not At All Interested In Purchasing
2. Slightly Interested In Purchasing
3. Moderately Interested In Purchasing
4. Very Interested In Purchasing
5. Extremely Interested In Purchasing**

Figure 1. Example concept rating pages from the freshness (top) and the
purchase interest (bottom) conjoint questionnaires.

completed by pencil, with subjects indicating their responses by circling one of the multiple choice rating categories for the dependent variable of interest (see Tables II and III). No significant problems were encountered in the administration of the questionnaires.

Results

The data from the "freshness" survey were analyzed using SPSS Conjoint 11.0 software (SPSS Inc., Chicago, Il). This software uses a general linear model analysis to calculate the part-worths or "utility values" for each level of each factor. These utility values indicate the influence of each factor level on the respondent's ratings. An "averaged importance" value is also calculated for each factor that reflects the relative range of utility values for the levels within each factor. The calculated utility values can be summed to produce a predicted value for the dependent measure, which can then be correlated with the actual ratings on the dependent measure in order to determine the degree of fit of the model to the data. In the present case, the data were well fit by the statistical model for both consumer groups (civilians: r = .987, military: r = .993). Figure 2a shows the averaged importance values for the four factors utilized in the freshness conjoint study (data for civilians are in gray, data for military are in black). As can be seen, time (in days) from the product's arrival in the store/market was the primary determinant of freshness judgments for both subject groups. This was followed, in order, by the type of food, the nature of the processing / preservation method, and the source of the food for both groups of subjects.

Figures 2b-e show the utility values for the different levels of each factor shown in Figure 2a. The utility values reflect the contribution of that element to the consumers' rating of the concept. A positive value reflects a positive contribution to the rating, while a negative value reflects a negative contribution. Figure 2b shows that the shorter the time from the product's arrival at store/market, the greater the positive contribution to freshness scores. Figure 2c, which shows the utility values for the different food types, also shows a much greater range of utility values for civilians than for military consumers. For civilians, "orange juice" had a high positive utility value, while "bread/bakery products" had a large negative utility value. In Figure 2d it can again be seen that civilians had a much greater range of utility values for the different methods of food processing / preservation. "Frozen then thawed" foods had a large negative utility value, whereas "irradiated" foods had a large positive utility value among civilians. "Minimally processed" foods produced neither a large negative nor large positive utility value. Lastly, in Figure 2e it can be seen that "warehouse store" contributed negatively to ratings of freshness in both subject groups, but that "specialty market" produced a positive utility value only for civilian consumers.
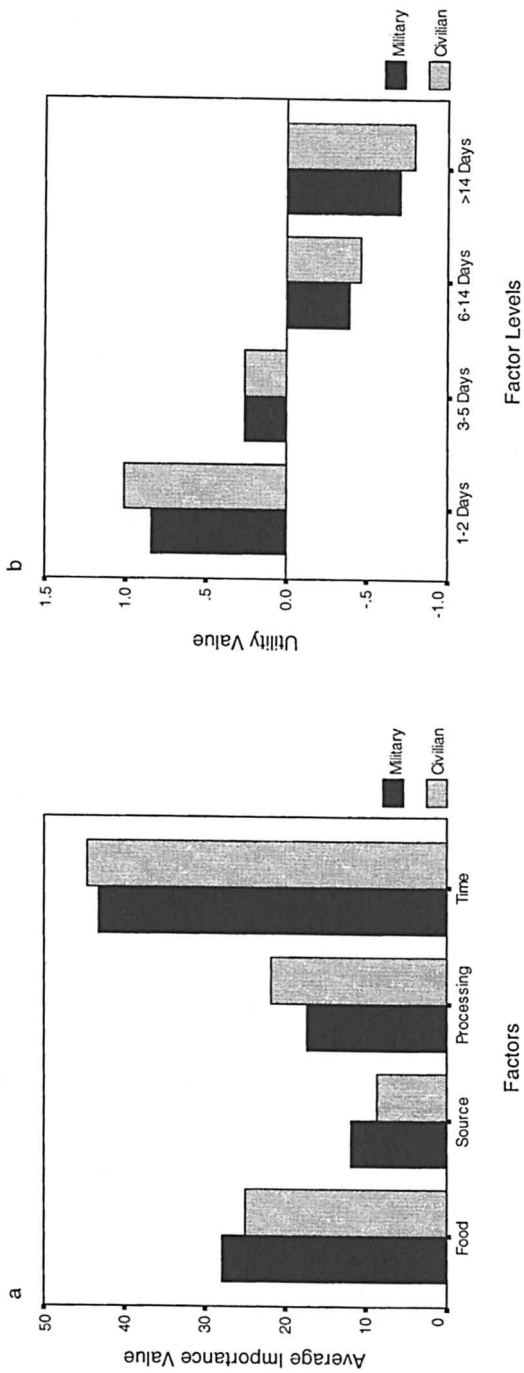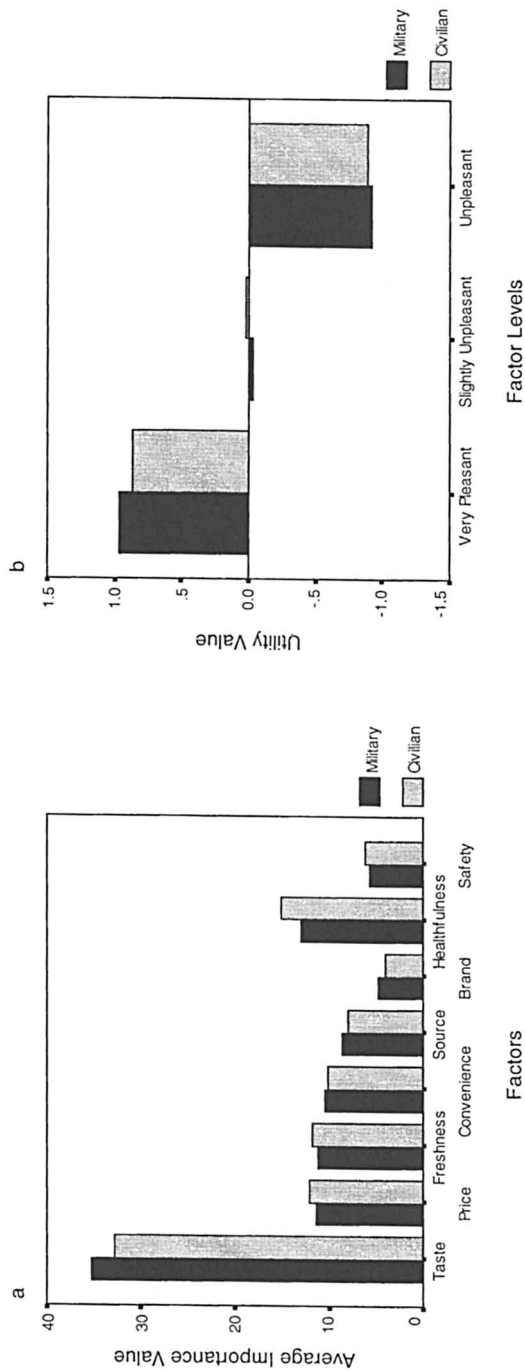
Figure 2. Average importance values (panel a) for the factors contributing to consumers' judgments of freshness and the associated utility values obtained for each level of each factor (panels b-e).
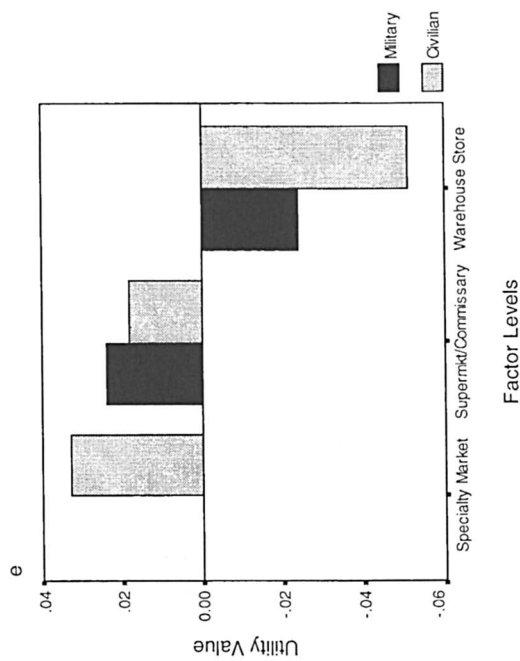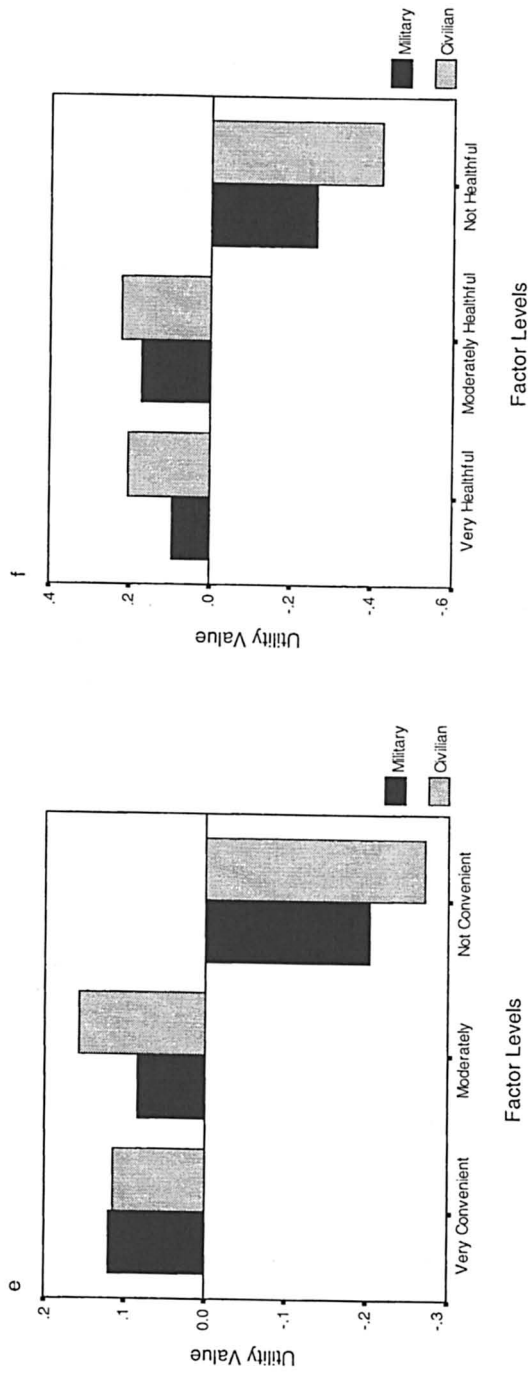
Figure 2. *Continued.*

**Table III. Factors and Factor Levels Used in "Purchase Interest"
Study**

Dependent Variable:	
Interest in Purchasing (Not at all interested – Extremely interested)	
Factors:	
Taste:	*Price:*
Very Pleasant Taste	High Priced
Slightly Pleasant Taste	Moderately Priced
Unpleasant Taste	Low Priced
Freshness:	*Convenience of Preparation:*
Very Fresh	Very Convenient
Moderately Fresh	Moderately Convenient
Not Fresh	Not Convenient
Food Obtained from (source):	*Healthfulness:*
Supermarket	Very Healthful
Warehouse Store	Moderately Healthful
Farmer's Market	Not Healthful
Brand Type:	*Safety:*
National	Definitely Safe to Eat
Store/Generic	Probably Safe to Eat

The data from the "purchase interest" survey was also well fit by the statistical model for both consumer groups (civilians: r = .988, military: r = .993). Figure 3a shows the averaged importance values for the eight factors used in the purchase interest study. As can be seen, taste had the greatest importance, by far, for both subject groups, followed by healthfulness at a much lower level of importance. Interestingly, brand type had the least importance for both subject groups. Figures 3b-f show the utility values for the different levels of the factors of taste, price, freshness, healthfulness, and convenience (data for source, brand type, and safety are not shown because the utility values were all extremely low). Taste, price and freshness (Figures 3b,c,d) all show a monotonic relationship between increasing factor level and utility value. For taste and freshness, this relationship is positive, while for price it is negative. For convenience (Figure 3e), the "very convenient" and "moderately convenient" descriptor variables produced similar positive utility values, while "not convenient" produced a large negative utility value among both groups of consumers. A similar pattern of utility values can be seen for "healthfulness" (Figure 3f), where the descriptor variable "not healthful" produced a large negative utility value, as contrasted with "very healthful" and "moderately

Figure 3. Average importance values (panel a) for the factors contributing to consumers' judgments of purchase interest and the associated utility values obtained for each level of each factor (panels b-f).

Continued on next page.
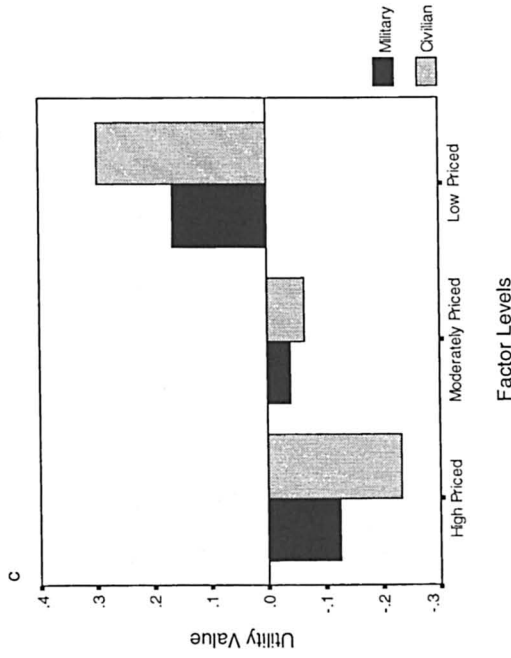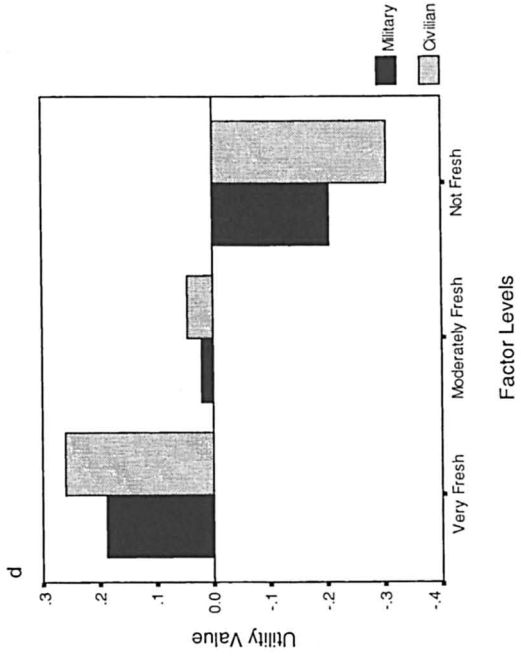
Figure 3. *Continued.*

healthful", which both had similar positive utility values among both consumer groups.

Discussion

In spite of the relatively large differences in the subject groups (military vs. civilian), the differences in the factors that contribute to the freshness of food and how freshness compares to other factors in determining purchase, interest were small. In terms of the concept of freshness, time from arrival of the product at the store/market was the most important variable contributing to freshness ratings. These data are consistent with the findings of Fillion and Kilcast (6), who found that time from harvest was one of the most important contributors to the perception of freshness, at least for fruits and vegetables. The type of food also clearly affected ratings of freshness, with juices producing higher ratings of freshness and bread/bakery products lower ratings, especially among civilians. These data most likely reflect the long-standing association of the term "fresh" with juice products and the fact that staling is a common characteristic associated with baked products.

Although food type had a relatively high importance for freshness, processing method had a somewhat lower importance. Foods described as "frozen, then thawed" had a strong negative impact on ratings of freshness, especially among civilian consumers. The more positive attitude of military respondents toward both "frozen then thawed" and "refrigerated / frozen" foods is likely due to the frequent exposure of military consumers to shelf-stable entrees as part of their combat rations, which makes refrigerated and/or frozen foods more desirable by comparison. Also interesting is the fact that the term "irradiated" had a high positive influence on freshness ratings for civilians, but not for the military sample. In the present study, the civilian group likely had a higher level of education than the military sample, due simply to the fact that they were older. Previous research has shown that consumers with higher educational levels have generally more positive attitudes toward food irradiation (27). However, the larger percentage of females in the civilian sample my have had an interactive effect, because research has also shown that females have a less positive attitude toward food irradiation than males (27, 28).

As it relates to the issues of food regulations and labeling that were discussed in the Introduction, foods described as "minimally processed" were viewed as being fresher than "frozen then thawed" food and on a par with "refrigerated / frozen" food among civilian consumers. However, among military consumers, "minimally processed" fared poorest among the four methods of processing / preservation that were cited. This may be due to either the gender or age difference between the two groups, which may have led to a more literal interpretation of the word "minimally" among the military

consumers. A similar difference between subject groups was observed for the responses to the term "specialty market". Here, the military consumers rated foods purchased from "specialty markets, e.g. butcher shop, fish market, farmers' market" (which one would normally consider to have very fresh products) lower in freshness than foods sold in supermarkets or commissaries. Of course it is not known whether the quality of "specialty markets" that military consumers encounter in the vicinity of their posts are the same as those encountered in non-military locales.

Concerning the purchase interest results, "taste" was clearly the most important factor driving the purchase interest of both sets of consumers. The high relative importance of the taste of the food, as compared to other, extrinsic product factors, e.g. nutrition, packaging, etc., is a common finding in the consumer food and marketing literature (3, 29-32). The fact that healthfulness of the food was the second most important factor influencing purchase interest is also consistent with previous findings showing the high importance of health and nutrition to food choice behaviors (3, 33). Although freshness had a somewhat lower level of importance to purchase interest than did the former two factors, it had about the same level of contribution as did price and convenience. Overall, the differences between military and civilian consumer samples were quite small for the purchase interest data. Minor differences, such as in the range of utility values for price (Fig. 3c) are likely attributable to differences in the economics of food pricing in military commissaries versus other, public sector markets. In general, it can be concluded that similar factors drive the purchase interests of these two disparate consumer groups.

Conclusions

The above research leads to the following set of conclusions about the concept of food freshness, the factors that contribute to it, and its relative importance to other factors in food purchase:

1. Taste is clearly the most important factor contributing to consumer interest in purchasing a food item, followed at some distance by nutrition for both military and civilian consumers.
2. Freshness is fourth in order of importance to purchase interest for both military and civilians, and is more important than either brand type or safety.
3. Degree of freshness is primarily a function of time since the arrival of the food at the store for both military and civilian consumers.
4. There are major differences in consumer ratings of freshness, depending upon the type of food, its source of purchase, and the method by which it is processed.

5. Although there were some differences between military and civilian consumers in terms of their concept of freshness and how freshness contributes to purchase interest, overall the two subject groups were very similar in terms of the relative importance of the factors influencing their responses.

Although the similarity in the results for the two consumer groups supports the convergent validity of the data, it would be desirable to replicate this research with consumer groups from other geographic areas within the U.S. in order to assess the external validity of the findings.

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Chapter 3

Flavor Issues in Maintaining Freshness

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This paper discusses causes of flavor deterioration, i.e. a loss of freshness, in foods during storage. Freshness may be lost for several reasons including: a loss of specific aroma compounds that are 'fresh' in character; the loss of characterizing aroma compounds (e.g. bready, beany, etc); or the masking of a fresh flavor due to the development of off-flavors. The hypothesis that "fresh" aroma compounds are lost during storage may be true but is difficult to prove since there are few, if any, aroma compounds identified to date that are characterized as being "fresh". The validity of each of these hypotheses is discussed and evidence from the literature is presented in support of the discussion.

One of the first questions that needs to be addressed is a definition of the term "freshness". According to Webster's Collegiate Dictionary, a "fresh" food is a food that is "free from taint" or "having its original qualities unimpaired". We may want to be slightly more liberal in the "free from taint" definition and add free from "off-flavors" as well since we generally consider that a "taint" is an external contamination while an "off-flavor" occurs due to the deterioration of the food itself (e.g. lipid oxidation, enzymatic action, or non-enzymatic browning). The concept that a food is free from "taint or off-flavors" vs. having its original qualities unimpaired can be viewed as two different considerations. The first is that the food has not developed off notes that mask or overwhelm

the desirable flavor of the food. We assume that the desirable flavor of a food is present and unimpaired but not sensed due to the occurrence of off notes. The second consideration is that freshness is lost due to the loss of the desirable qualities (desirable aroma components). This is a very important distinction and will form the basis of much of the discussion that follows.

Definition of Freshness

I would like to start with a discussion of what is responsible for freshness in a food. Are there aroma components in a food that define "fresh" or does freshness result from specific characterizing aroma components? A look at the literature shows that there are very few aroma compounds characterized as being fresh. That is understandable since it is hard to imagine what fresh is out of context. It is easy to visualize a "bread-like" aroma or a "fishy" aroma but what is a fresh aroma?

If one goes to the Flavornet web site established by Terry Acree (1), only ethyl-3-hydroxyhexanoate, α-phellandrene, benzyl acetate, pentadecanal, and isopropyl hexanoate have been defined as fresh (or having fresh as one of the descriptors). This site lists the sensory properties of 313 aroma compounds. If one checks one of the well established references in the flavor creation field, ethyl-3-hydroxyhexanoate is described as being fruity, α-phellandrene as being citrus, fresh, pleasant, and minty, benzyl acetate as being jasmine-like, pentadecanal is not listed and isopropyl hexanoate as having a sweet, delicate, fruity odor of pineapple with a fresh, berry-like taste (2). When fresh is mentioned, it is not the single or a primary attribute but is a part of the overall aroma character.

A more comprehensive listing of odor descriptors (approximately 1500 aroma compounds are described) is provided on a web site organized by Mottram (3). In this extensive listing, only 3-hydroxy-2-butanone (acetoin) has fresh as a component of its description. Acetoin is described as having a butter, fresh, fruity, green, moldy, slightly rancid sensory character. Fresh is again a minor descriptor as opposed to being a primary note. Burdock (2) describes acetoin as having a "pleasant butter-like odor and flavor". There is no mention of fresh in this description.

A final source of information is a professional flavorist i.e. a person who deals daily with creating flavors and therefore, must be intimately familiar with the creation of specific flavor notes. Frank Fischetti (4) defined freshness as "a nondescript *lightness* added to a flavor. I say nondescript (never-the-less contributing) as opposed to a definite character because these notes add the *front*, initial part of a flavor, the first thing perceived, which is then followed by the character (those compounds that make the flavor taste like bread or meat, for

example) items. These notes must be pleasant, immediately perceived and then disappear. I think freshness is often confused with impact, which I define as the initial impression of a flavor, apperceived indication of the strength and character of a flavor." It is relevant and appropriate (in my opinion) that Fischetti uses the term "nondescript" in his definition.

When asked for examples of fresh aroma compounds, he said that the first thing he thinks of is esters, at least for all fruit flavors. He emphasized that especially the lower boiling esters contribute freshness e.g., ethylacetate, ethylbutyrate etc. Fischetti also mentioned acetaldehyde as a compound that imparts freshness to many flavors especially citrus flavors. A final example was cis-3 hexenol which he feels adds a fresh note to many fruit flavors, especially berry flavors. It is worthwhile to note that a flavorist considers "fresh" components to be "nondescript" and offers very volatile non-characterizing components as examples. Fischetti also notes that fresh notes are product dependent. One may consider that the contributors to freshness are different from food to food.

Research on "Fresh" Aroma Compounds

The limited number of compounds in the literature characterized as being "fresh" is understandable considering how these compounds are being identified today. Most of our work in identifying and characterizing the sensory properties of aroma compounds is done by aroma isolation, gas chromatography and then gas chromatography/olfactometry (GC/O, sniffing the GC effluent). If there are aroma compounds that impart freshness through sensory character, one would expect these components to be quite volatile. This would make isolation by many traditional methodologies problematic. Aroma compounds might be lost during distillation, or concentration, or not adequately retained on adsorbent traps and thus be present in the aroma isolate at extremely low concentrations. Furthermore, the selection of potent aroma compounds is done by a GC sniffing methodology (e.g. a dilution, detection or intensity method). The ability of a panelist to detect an aroma compound is enhanced by his/her recognition of it. Thus, compounds that are not recognizable (e.g. fresh) would tend to be missed or underestimated in a sniffing experiment. It is possible that there are a group of chemicals, albeit unique for different classes of food products, that impart freshness and have not been characterized to date. To find these compounds, an alternative methodology may have to be used.

An approach for finding fresh aroma compounds could involve combining instrumental and sensory methodologies. One could produce an aroma isolate that is judged to be "fresh" bread aroma, for example. The aroma isolate could be subjected to fractionation by GC and then by recombining fractions, one could determine if there is a fraction that imparts freshness to the aroma profile. If there is such a fraction, then through refined fractionation, one could focus on the aroma compounds that are fresh.

Off-Flavors vs. Loss of Desirable Characterizing Flavor

Two additional alternatives remain for explaining a loss of freshness: one is the loss of desirable characterizing aroma and the other the formation of off-flavors that will mask the desirable flavor. In one case, we are considering that a loss of freshness is due to the loss of characterizing aroma components (e.g. 2-acetyl-1-pyrroline for bread). This loss can occur due to chemical reactions (e.g. between aroma compounds or between aroma compounds and the food matrix), chemical binding to major food constituents (e.g. linear starch fractions binding volatiles in bread) or through volatilization (including scalping by the package). In this scenario, we are assuming that there are no aroma components that are fresh but that freshness comes from characterizing aroma compounds.

The remaining alternative is that a loss of freshness is due to the formation of off-flavors which will mask the desirable flavors. The intensity of the off-flavor may be too low to be recognizable but high enough to mask desirable notes. Lipid oxidation and the Maillard reaction come to mind as mechanisms for the production of off-flavors in most foods. There are few studies in the literature to resolve this issue. However, bread aroma has been the topic of considerable research and some of the data are helpful in considering this question.

Staling of Bread

While there is considerable data published on flavor changes in bread during storage, two articles from Prof. Grosch's laboratory are quite relevant *(5,6)*. Schieberle and Grosch *(5)* determined the characterizing (key) aroma compounds in freshly baked white bread and then monitored the FD-factors (potential contribution to odor) during storage. A tabulation of the changes in FD-factors of the key aroma contributors is presented in Table 1. These data show that the characterizing aroma

Table I. Changes in the FD-factors of Crust Odorants During Storage of White Bread

Odorant	FD-Factor[a] at 0-hr	FD-Factor at 96-hr
3-Methylbutanal	128	16
Diacetyl	64	16
2-Acetyl-1-pyrroline	64	8
2-Methylpropanal	32	4
Oct-1-en-3-one	32	16
2-Ethyl-3,5-dimethylpyrazine	32	16
(E)-Non-2-enal	32	32
Pentane-2,3-dione	16	2
Butanoic acid	16	16
Methional	8	<1

[a] Flavor dilution factor

SOURCE: Adapted from Reference 5. Copyright 1992.

compounds (e.g. the short chain aldehydes, diketones, and pyrroline (character impact compound of bread crust) decreased substantially during storage. The compounds reminiscent of oxidation, e.g. oct-1-en-3-one and (E)-non-2-enal, either decreased slightly or remained the same. Thus, one would expect these oxidized notes to make a greater (or more noticeable) contribution to bread odor in the absence of the characterizing bread aromas and thus may be responsible for the stale flavor.

Zehentbauer and Grosch (6) provided further insight into the staling of bread (baguettes). They reported on a more comprehensive study to determine the reasons that commercially made baguettes staled more quickly than those made via traditional processes. Their data support the conclusions above as shown in Table II. These data are quantitative which is a somewhat different view than Schieberle and Grosch (5) presented in Table I. Briefly, the desirable baked, browned aroma components decreased rapidly after baking and the components more characteristic of lipid oxidation (the aldehydes and the ketone) either remained about the same or decreased to a lesser extent. Thus, it appears that staleness in bread is due to the loss of desirable components as opposed to the formation of undesirable components (at least in the short run). Their study also suggested that the traditionally made bread retained its freshness longer because it had much higher initial levels of desirable components. That is, desirable components had to drop much further in concentration before fresh aroma was masked by the lipid oxidation products.

**Table II. The Concentration of Odorants in the Headspace of Baguettes
Made by Traditional and Commercial Processes During
Storage at Ambient Temperature**

	Commercial			Traditional		
	Headspace Concentration after Specified Time[a]					
Odorant	1 hr[b]	4 hr	25 hr	1hr	4 hr	25 hr
Methylpropanal	830	400	300	1560	1160	900
2 and 3-Methylbutanal	470	228	250	553	420	362
Methional	6.6	6.4	4.6	6.8	6.5	4.8
Diacetyl	980	670	730	535	314	343
2-Acetylpyrroline	3.7	3.7	1.5	1.1	1	0.4
2-Ethyl-3,5-dimethylpyrazine	0.9	0.8	0.4	0.6	0.4	<0.3
Hexanal	216	254	180	160	190	145
1-Octene-3-ol	6.7	4.8	5.9	3.7	4.0	2.2
(E)-2-nonenal	28	44	24	16	26	20
(E,E)-2,4-Decadienal	7.8	6.0	7.1	3.8	2.9	4.0

[a] ng/L of air
[b] Time since baking
SOURCE: Adapted from Reference 6. Copyright 1997.

Peanuts and Peanut Products

The loss of flavor in peanuts or peanut products during storage is a problem. It has been hypothesized that flavor "fade" is due to some combination of the occurrence of rancidity and potentially the polymerization of heterocyclic nitrogen-containing volatiles (7). It is generally accepted that the characteristic desirable aroma of peanuts is primarily due to pyrazines which are known to polymerize under certain conditions thereby losing the odor character and aroma contribution. Thus, it was the goal of this work (7) to determine which of these two mechanisms was most likely responsible for the loss of peanut flavor during storage.

The data in Figure 1 show that pyrazines are quite stable in this system during storage. The concentration of pyrazines (this plot is the sum of four pyrazines: 2-methyl-2,6-dimethyl, 2,3,5-trimethyl and 3-ethyl-2-methylpyrazine) was virtually constant with storage even at such a high storage temperature. It is evident that lipid oxidation increased very rapidly during storage. This plot shows the formation of hexanal and total aldehydes (pentanal,

hexanal, heptanal, octanal and nonanal). It is clear that hexanal formation dominated the aldehyde profile.

The analytical data are useful in helping to interpret the sensory data presented in Figure 2. As can be seen, sensory scores for "rancid" increased very rapidly while the roasted nutty note decreased until both attributes leveled off. The analytical data suggest that the decreases in sensory scores for "roasted, nutty" notes are due to masking as opposed to the loss of the desirable character. Therefore, unlike the situation for bread staling, it appears that lipid oxidation is masking the desirable character of the peanuts resulting a loss of freshness (i.e. become stale). The desirable, characteristic peanut flavor components are quite stable.
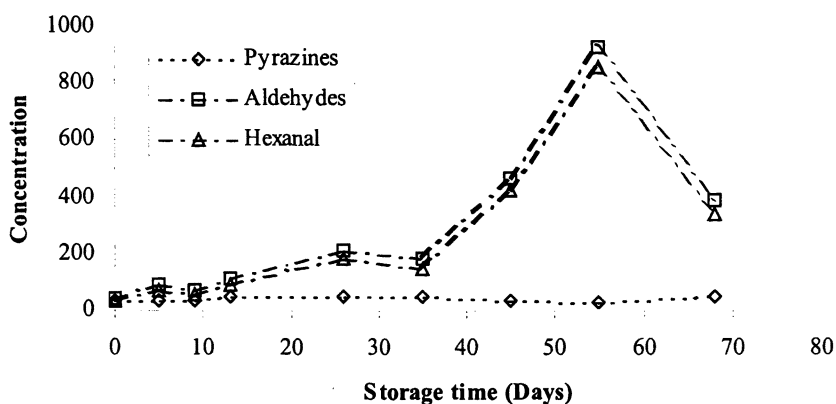
Figure 1. The influence of storage (65°C) on the amount of pyrazines, hexanal and total aldehydes (hexanal plus pentanal, heptanal, octanal and nonanal) present in ground peanuts. (Adapted from Reference 7. Copyright 1996 American Chemical Society.)

General Comments

The "bottom line" of this topic is that the loss of freshness is likely product dependent. Foods that depend upon very stable aroma compounds for character (e.g. pyrazines) most likely develop staleness from the masking of desirable

flavor by off-flavors e.g. lipid oxidation and/or nonenzymatic browning. These two degradation reactions are specifically mentioned because these reactions are common storage problems in foods and initially are difficult to recognize. As either of these reactions progresses to advanced stages, the consumer may recognize the aromas as being cardboard, metallic, painty or glue-like and no longer consider the food fresh (or stale) but off-flavored e.g. rancid. Thus, initially, the off-flavor is not recognizable but is judged as being stale.
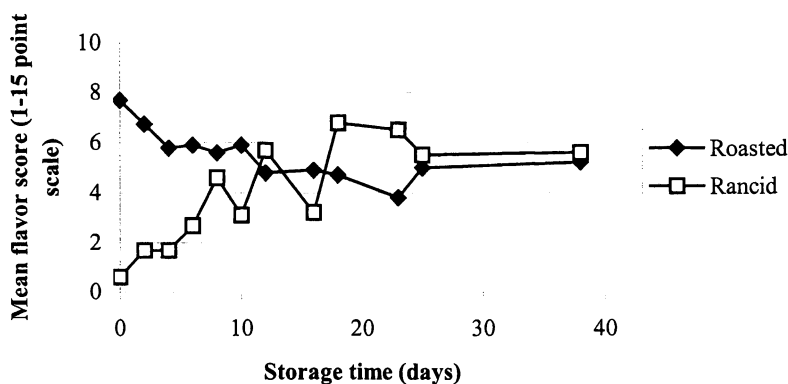
Figure 2. The influence of storage time on rancid off notes and the characteristic roasted nutty notes in stored ground roasted (Adapted from Reference Warner et al. Copyright 1996 American Chemical Society.)

Foods that depend upon unstable aroma compounds lose freshness, i.e. become stale, due to the loss of the characterizing flavor compounds or some combination of this loss and off-flavor development. "Unstable" compounds, as defined here, would include very volatile compounds since these compounds would be lost from the food system by evaporation. Hydrogen sulfide or acetaldehyde, for example, is very readily lost from foods during storage. Unstable compounds also would include very reactive compounds e.g. aldehydes *(8)*, sulfur compounds *(8-12)*, diketones (e.g. diacetyl) and others (such as 2-acetyl-1-pyrroline *(5,6)* or citral in acidic beverages, *(13)*). There is substantial information in the literature showing the loss of these types of aroma compounds during the storage of foods or model systems. These compounds very often include highly odor active or characterizing compounds.

The instability of sulfur compounds may be particularly problematic. Mottram's group has shown that disulfides will readily form covalent bonds with proteins, e.g. ovalbumin, thereby negating aroma contribution *(9,11,12)*. This reaction increases at higher pHs (8 or above) and/or when the protein is denatured. Interchange reactions between protein sulfhydryl groups and the disulfides were considered to be responsible for the loss of these disulfides. Mottram et al. *(9,11)* also found that bis(2-methyl-3-furyl) disulfide and bis(2-furfuryl) disulfide were readily decomposed to the corresponding thiols, (2-methyl-3-furanthiol and 2-furanmethanethiol, respectively) when heated in the presence of egg albumin. Under some conditions, the original disulfides were lost completely. The formation of some mixed disulfides suggested that the disulfides were cleaved and then new disulfides were formed. The fact that the loss of disulfides did not occur when heated in the presence of carbohydrates supported the hypothesis that disulfide interchanges had occurred. The loss of these disulfides would undoubtedly contribute to the loss of desirable flavor and thereby favor the occurrence of staleness.

Summary

The reason for a loss of fresh flavor in foods during storage is not well understood. There are at least three potential explanations for this loss and include: 1) the loss of a group of aroma compounds that are inherently fresh but contribute little to the basic character of the food; 2) the loss of desirable characterizing aroma compounds or 3) the formation of off-flavors that mask the desirable aroma compounds. We have little information in the literature to evaluate the potential validity of the first hypothesis i.e. there are a group of fresh aroma compounds in foods that are inherently unstable and are lost during storage. This hypothesis requires further study for validation or rejection.

The validity of hypotheses 2 and 3 are likely to be dependent upon the food product. Foods whose flavors are based on very stable aroma compounds, e.g. pyrazines, likely lose freshness due to the formation of off-flavors which eventually dominate or mask the desirable aroma character and thus, are ultimately judged as being stale. Alternatively, foods based on unstable aroma compounds (particularly sulfur compounds) are likely to lose freshness due to the loss of these characterizing compounds resulting in the food lacking in flavor and being judged as stale. Considering that staleness is a major consumer complaint and reason for product rejection, this area would benefit from substantially greater understanding.

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Chapter 4

Biochemical Changes Associated with Fresh-Cut Fruit Processing and Storage

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Fresh-cut fruit processing reduces product shelf life. Cut cantaloupe melon stored at 4 °C, unlike those kept at 20 °C, did not show significant changes in titratable acidity, pH, °Brix and organic acid content over a period of 14 days. Organic acids in the fresh fruit are oxalic, citric, malic and succinic acids. At 20 °C, lactic acid was by far the dominant acid present after two days. Gram-positive mesophilic bacteria also grew rapidly at this temperature unlike the slow growing Gram-negative rods that were observed at 4 °C. Lactic acid and Gram-positive microflora are potential quality markers of temperature abuse during fresh-cut fruit storage. Peroxidase (POD) enzymes in cantaloupe, honeydew, kiwifruit, pineapple and watermelon are anionic ascorbate POD apparently produced in response to wound induced oxidative stress. The POD isozymes are stable for 20 min. at 50 °C, hence mild heat treatments for short periods of time will not reduce activity significantly. In the muskmelons, ascorbic acid is suggested to inhibit oxidation by complexing with POD and metal ion cofactors.

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Fresh-cut produce is the fastest growing food category in the supermarket. Total sales have more than doubled from $5.2 billion in 1994 to an estimated $11 billion in 2000. Increased respiration rate, water and enzymatic activities that occur during fresh-cut fruit processing and storage cause the fruit tissue to senesce rapidly (1-4). Sugars, organic acids and amino acids significantly contribute to sweetness and aroma of fruits. Sweetness and aroma are the two most important quality indicators in melons (5,6). Organic acids are also important flavor precursors and respiratory energy sources in plant cells. Loss of fruit characteristic flavor is usually the first deteriorative change that occurs. Initial flavor changes are not indicated by appearance of the fruit. There is considerable interest in the factors and reactions that occur in the fruits to cause flavor changes. Degradative changes also include tissue softening/texture changes, nutrient losses, browning, and microbial growth. Cold storage generally retards many biochemical processes in foods, and fresh-cut fruits are normally stored under refrigeration. In the uncut fruit, enzymes are normally sequestered within the vacuole. Tissue disruption causes the mixture of the enzymes with other cytoplasmic and nucleic substrates.

Changes that occur in POD activity in wounded and fresh cut fruits significantly contribute to their product quality (7-9). In addition to the role played by POD in flavor of fresh fruits and vegetables POD isozymes are involved in many cell alterations (10). They appear to influence flesh firmness through catalysis of the cross-linking between tyrosine residues of the cell wall extensins and ferulic acid substituents of pectins (11), and the synthesis of lignin and suberin polymers (12).

The mechanism of POD peroxidative action essentially involves an oxidative action by way of an initial formation of a complex intermediate with a hydrogen acceptor. The transfer of hydrogen from a donor substrate results in a second complex intermediate before the regeneration of the POD enzyme and formation of a reaction product (10). Plant PODs are classified into two types (13). Ascorbate POD (APX; class I), which is from the plant chloroplast and cystol, is distinguished from classical secretory plant POD (guaiacol POD; class II) isozymes by significant differences in their primary structure. APX is an H_2O_2-scavenging POD that uses ascorbate as an electron donor in plants and algae (14,15). Most secretory plant POD are glycosylated (16). Unlike guaiacol POD that is characterized by broad specificity with respect to electron donors, and participates in many physiological processes such as the biosynthesis of lignin and ethylene, ascorbate POD exhibits high sensitivity for ascorbate as the electron donor (14,17), and is specific in its physiological role in scavenging potentially harmful H_2O_2 (18) and free radicals (19). Most of our ongoing research relates to the nature of POD enzymes and how they potentially affect the quality and shelf life of fresh-cut fruits. The possible roles of POD

enzymes in maintaining freshness, and other biochemical effects on fresh-cut fruit quality are discussed.

Materials and Methods

Determination of pH, Titratable Acidity and °Brix. Cantaloupe melons slices (~3mm thick) were prepared after skins and seeds were removed from the whole fruit. A set of fruit pieces placed in plastic containers with airtight lids and kept in an incubator at 4 °C while the rest were stored at 20 °C inside a laminar flow hood. Juice obtained after centrifugation (5000 x g) of pulverized fruit samples taken periodically were used for pH, soluble solids and titratable acidity determinations. Titratable acidity was determined as malic acid equivalents by titration with NaOH (0.1M).

Organic Acid Analysis. Fruit slices were frozen in liquid nitrogen and lyophilized. The dried fruit was mixed with liquid nitrogen and crushed into powder in a mortar. Organic acids were extracted from the powder in acetic acid solution (pH 2.5) and analyzed by HPLC as previously described (20,21).

Determination of Microbial Activities. Total microbial counts were carried out aerobically on Nutrient agar. Suspended homogenates (0.1 mL) of the fruit (10-20 g) in sterile water (190 mL) were overlaid on the surface of Nutrient agar plate after series dilutions. Bacterial colonies were counted after 48 h of incubation at 30 °C.

Effect of Ascorbic Acid on Cantaloupe Color During Storage. Cut fruit pieces (200 g) in polypropylene baskets and dipped in cold water (4 °C) and other solutions containing the following additives: ascorbic acid (1.25 mM), ascorbic acid (2.5 mM), ascorbic acid (2.2 mM) plus EDTA (10 mM) and ascorbic acid (2.5 mM) plus $MnCl_2$ (2.5 mM) in separate treatments for 1 min. After the fruits were dipped in the respective solutions, they were allowed to drain for about 1 min before being transferred into sealed plastic containers. A set of fruit pieces that were not dipped in water was used as control. Treated fruits were stored at 4 °C for 20 days. Color changes determined from Tristimulus L*, a*, b* measurements on a HunterLab DP-9000 (Reston, VA) colorimeter were used to determine changes in L* (ΔL), Chroma (ΔC).

Peroxidase Enzyme Assays. Acetone powders were prepared by homogenizing cut fruits (200 g) with cold acetone (500 mL; -20 °C) for 30 s and successive washings of filtrate with cold acetone. In the preparation of watermelon acetone powder, water (10% w/w) was added to the fruit prior to addition of cold acetone and homogenization. Enzyme extractions were carried out by homogenizing

acetone powders (1 g) in a mixture of phosphate buffer (0.05 M; pH 8.0, 15 mL) and Triton X (0.1%) and centrifugation of the homogenate.

Peroxidase assay with guaiacol as substrate was carried by monitoring changes in absorbance at 470 nm in mixtures consisting of 0.02 M Na_2HPO_4 and 0.8 M NaH_2PO_4, 20 mM guaiacol, 4 mM H_2O_2, enzyme extract (10 µL), pH 7, in a total volume of 3 mL (22). The effects of the following compounds: $MnCl_2$ (0.5 mM), EDTA (10 mM) $CaCl_2$ (0.5 mM), diethyldithiocarbamate (DETC; 0.5 mM), tiron (1 mM), superoxide dismutase (100 units/mL), ascorbic acid (AA; 0.5 mM), and β-mercaptoethanol (0.5 mM) on enzyme activity were determined. The compounds were added to the buffer at stated concentrations prior to preparation of reaction mixtures and enzyme activity assays. POD activity as a function of pH was determined by adjusting pH of the buffer using suitable amounts of 1.0 M NaOH or 1.0 M HCl containing 0.5 M NaCl . The effect of temperature on enzymatic activity was determined by incubating the reaction mixtures at different temperatures (30 – 70 °C) prior to addition of the enzyme, and measuring increase in absorbance at the corresponding temperatures. Thermal stability of cantaloupe melon peroxidase was determined by incubating enzyme extracts at 30 - 70 °C in a waterbath for 5 and 15 min at each temperature. Reaction mixtures were prepared at these time intervals and assayed for residual enzyme activities at each temperature.

Peroxidase assay with AA as substrate was carried out in a buffer with a reaction mixture containing potassium phosphate (50 mM; pH 7.0), ascorbate (0.5 mM) , H_2O_2 (0.1 mM) and EDTA (0.1 mM). Adding the enzyme extract started the reaction and the absorbance decrease was recorded 10 to 30 s after this addition (23). Enzymatic activities in the absence of H_2O_2 were generally very weak or absent. Thus, correction for the oxidation of ascorbate as a result of ascorbate oxidase was unnecessary.

Gel electrophoresis. Electrophoretic separations were carried out on Xcell II Mini-Cell on 8cm x 8cm x 1mm, 10-well polyacrylamide pre-cast gels. Novex Tris-glycine buffer system was native gel electrophoresis. Isoelectric fcosing (pH 3-10) buffers were used with IEF gels for isoelectric focusing. Protein separations were performed according to the manufacturer's instructions (Novel Experimental Technology, San Diego, California). Peroxidase assay with benzidine was done by immersing gels in acidic benzidine (100 mL; 0.5% w/v benzidine and 4.5% w/v glacial acetic acid) for 5 min after which H_2O_2 (3 mL of 30% v/v H_2O_2) was added. Gels were photographed immediately. Staining with o-dianisidine was carried out by immersing gels in a solution containing sodium phosphate (10 mM, pH 6.0), o-dianisidine (1 mM) and H_2O_2 (50 mM) for 2 h at room temperature.

Results and Discussion

Cold storage (4 °C) helps to maintain a number of cut fruit quality parameters. Titratable acidity, pH and soluble solid content did not change significantly over a period of two weeks in cut cantaloupe pieces stored at 4 °C (Table 1). Organic acid content and composition in the fruit were also stable over the same period of time (Table 2). Organic acids identified in fresh-cut and fruit stored at 4 °C are oxalic, citric, malic, and succinic acids.

Table I: Changes in pH, total acidity (g/100 mL) and soluble solids in minimally processed cantaloupe with storage at 20°C and 4°C.

Storage Days	pH	Acidity	°Brix
	4 °C		
0	6.47a*	0.21a	8.53a
1	6.42a	0.20a	8.60a
2	6.51a	0.18a	8.00a
3	6.51a	0.26a	7.87a
5	6.55a	0.22a	7.73a
14	6.30a	0.19a	7.37a
	20 °C		
0	6.58a	0.21e	9.03a
1	6.61a	0.25e	9.07a
2	5.99b	0.44d	6.60b
3	5.25b	0.83c	5.80b
5	4.60a	1.61b	4.74c

*Values in the same column for each temperature without the same letters are significantly different (P<0.1; n=3). Reproduced with permission from ref. 46. SOURCE: Reproduced with permission from reference 46. Copyright 1995.

Citric and malic acids were the dominant acids, occurring at concentrations of 3.8 and 6.3 μm/100g of fresh fruit while oxalic and succinic acids were 0.06 and 0.56 μm/100g of fruit respectively. At 20 °C, significant changes in fruit acidity, °Brix and pH occurred with storage. A 17% reduction in °Brix and 2-fold

increase in total acidity occurred after 2 days of storage. Malic acid content decreased whilelactic acid began to form. Lactic acid content increased, from being absent in the freshly cut fruit, to about 1.7 μm/100g after one day of storage at 20 °C. Lactic acid was by far the dominant acid present after two days of storage. The observed decrease in pH and increased acidity with storage obviously resulted from the production of lactic acid. The coincident loss of malic acid during the production of lactic acid is indicative of a synthetic pathway similar to that of the malo-lactic fermentation by lactic acid bacteria (24).

Table II. Changes in organic acid contents of cantaloupe (μmol/100g of fruit) with storage at 20°C and 4°C.

Storage Days	Oxalic	Citric	Malic	Succinic	Lactic	Total
			4 °C			
0	0.06a*	4.45a	6.05a	0.83a	0	11.4a
1	0.08a	3.75a	7.11a	0.80a	0	11.7a
2	0.06a	4.84a	5.86a	0.77a	0	11.6a
3	0.07a	3.02a	5.57a	0.77a	0	9.21a
5	0.07a	4.93a	5.35a	0.54a	0	11.2a
14	0.07a	3.79a	5.07a	0.50a	0	9.45a
			20 °C			
0	0.05c	3.22a	6.22a	0.37ba	0e	9.87e
1	0.03c	3.37a	3.46b	0.22b	1.65ed	8.75e
2	0.07bc	2.53ba	2.20cb	0.30ba	7.65d	12.8ed
3	0.09bc	3.11a	1.38cd	0.26b	19.4c	24.4c
5	0.13ba	2.70ba	0.47d	0.31ba	34.3b	38.7b

*Values in the same column for each temperature without the same letters are significantly different (P<0.1; n=3). Reproduced with permission from ref. 46.
SOURCE: Reproduced with permission from reference 46. Copyright 1995.

Microbial growth on Nutrient agar plates was predominantly bacterial at both temperatures (Figure 1). An increase in microbial growth from 1.05 to 132.3 CFU/g occurred within 24 hours of storage at 20 °C. At 4 °C, there was an induction period of about five days before a more rapid bacterial growth
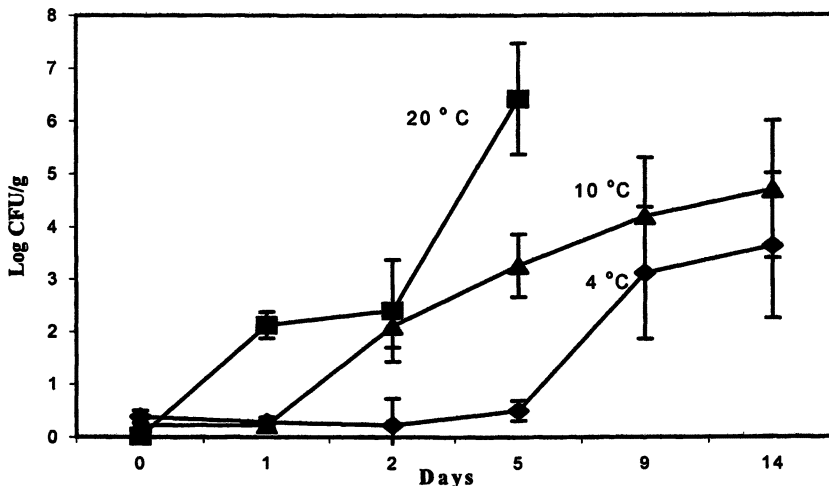
Figure 1. Microbial growth on fresh-ut cantaloupe. Reproduced with permission from reg. 46.
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occurred. Gram staining results indicated Gram-negative rods for cantaloupe stored at the 4 °C, while the flora was Gram-positive in fruits stored at 20 °C.
At 10 °C, the induction period for Gram-negative bacteria bacterial growth was reduced to one day. As part of the defense mechanism to reduce biological stress in cut cantaloupe, the fruit tissue produces terpenoid phytoalexins that are inhibitory to microbial growth (25). The ability of lactic acid bacteria to multiply on fresh-cut fruits and vegetables is dependent on temperature and the medium of growth (26). The potential use of the presence and amount of this mesophilic microflora as a determinant of temperature abuse in vegetable salads and for predicting shelf-life has been demonstrated (27,28). Thus, the presence of lactic acid, otherwise absent in cantaloupe stored under adequate refrigeration, has the potential to be used as a marker for determining temperature abuse in fresh-cut cantaloupe melon. Production of lactic acid and lactic acid bacteria is also expected to alter the flavor of fresh cut cantaloupe possibly by way of an increased lipase production (29,30).

A recent observation (31) concerning the apparent lactic acid bacteria mediated maceration of cut cantaloupe at 20 °C is inconsistent with the general belief that lactic acid bacteria do not directly attack plant cell wall polymers. At 4 °C fruit stored did not show significant degradation over 14 days. Gram-negative rods believed to be psychrotrophic pseudomonads were the dominant microflora with no lactic acid present. These pseudomonads are capable of producing pectic enzymes that would have been expected to degrade the fruit

tissue. The observation is an indication of the need for further research on the effects of microorganisms on fresh-cut fruit tissue surfaces. Preliminary results from our research also indicate the production of polygalacturonase enzyme during cold storage (4 °C) of cut cantaloupe melon, and that the rate of production of this microbial enzyme might be higher in fruits stored at 15 °C than those kept at 4 °C (Lamikanra et al., unpublished). The effects of this enzyme on fresh cut fruit texture and flavor are being determined.

A good indication that appearance alone cannot be used to predict the quality of fresh-cut products is the fact that significant changes in reflectance values (ΔH, ΔC, ΔL) do not occur in cut cantaloupe stored 4 °C for over 7 days. Visually observed discoloration at 20 °C is also consistent with absence of browning, and appears to be the result of disruption of tissue and cell wall structure. The absence of polyphenol oxidase induced darkening would normally result from the absence of these enzymes and/or oxidizable phenols in the fruit. We recently demonstrated that PPO activity in cantaloupe melon is very weak relative to those in apple and lettuce (32). The low PPO activity indicates that enzymatic browning reactions are unlikely to contribute significantly to cantaloupe melon product deterioration, and that there is no need for the use of a treatment aimed at controlling PPO activity in this fruit. In addition, PPO activity is not necessarily related to the total phenol content of fruits and vegetables. Only a relatively small part of food phenolic compounds can serve as substrates for PPO. Common fruit PPO substrates are flavonoid and related compounds such as catechins, cinnamic acid, leucoanthocyanidins and flavonols (33). The total phenol content of cantaloupe melon determined was 5.16 mg/100 g of fresh fruit (32). These phenolic compounds were all non-flavonoid compounds. The absence of oxidizable phenolic compounds, coupled with the relatively low PPO activity in the fruit, thus accounts for the lack of browning reactions in fresh-cut cantaloupe melon.

Our studies on cantaloupe melon (22,32) indicate that POD activity is consistent with that of ascorbate POD based on the relatively higher affinity for ascorbate over guaiacol. The sensitivity of ascorbate POD to thiol reagents (34) and p-chloromercuribenzoate (17) distinguishes it from guaiacol POD. The ascorbate-specific nature of cantaloupe melon POD was further indicated by the inhibitive effects of β-mercaptoethanol (2-ME), L-cysteine and p-chloromercuribenzoate . POD activity was highest in the freshly cut fruit, and there was a gradual decrease in enzyme activity with storage time at 4 °C. Optimum activity temperature of enzyme extract was 50-55 °C. The enzyme was stable at temperatures below 40 °C and at 50 °C for up to 10 min. Over 90% of total activity was lost at 80 °C within 5 min. Broad pH optima, 5.5-7.5 at 50 °C and 6-7 at 30 °C, were obtained. Broad pH optima are often associated with the presence of multiple isozymes with different pH optima (18,35). Consistent with the pH profile, two POD bands of approximate molecular weights of 240 and

165 kDa respectively, were obtained when the native-PAGE gel was stained with acidic benzidine and H_2O_2. A similar stain for the IEF gel, however, indicates 6 acidic POD isozymes (pI 5.1-6.0) suggesting that the band (mol wt = 240 kDa) obtained by native electrophoresis are comprised of six subunits that were unseparated, but pulled apart by the higher electrical charge in the IEF electrophoretic run. The lower molecular weight protein (mol. wt. = 165 kDa) would then correspond to correlate to the first band (mol. Wt. = 240 kDa) that lost two of the protein subunits either endogenously or during the electrophoretic analysis.

Increased ascorbate POD activity has been linked to increase in oxidative stress in plant cells (36-39). Such oxidative stress conditions that will reduce product shelf life would also cause a concurrent loss of carotenoids (40-42). In a study where cut cantaloupe slices were kept at 4 °C for 20 days, a considerable loss of carotenoids occurred as evidenced by changes in color measurements (Figure 2). POD activity also decreased with storage time at this temperature. Hue values, and to a smaller extent, the chroma values seem to correlate well with the observed bleaching of the cut fruit pieces. Dipping cut fruits in AA (500 ppm) was effective in maintaining cantaloupe color.

Ascorbic acid, when present in dip solutions also reduces POD activity in fresh-cut cantaloupe. Apparently, AA reduced tissue oxidative stress and consequently the need for POD production (32). The involvement of trace quantities of metal ions in the antioxidative action of AA is suggested by the increased hue change when EDTA was added to the dipping solution that contained AA. Metal ions such as manganese are able to act as cofactors in antioxidant enzymes (43), as in the ascorbate-POD complex (15). Thus, an enhanced antioxidative action of the ascorbate-POD complex and trace metal ion cofactors could account for the preservative action of AA. Increasing the concentration of Mn^{2+}, however, caused considerable changes in chroma and L* values.

Peroxidase enzymes in honeydew, kiwifruit, pineapple and watermelon also exhibit ascorbate POD characteristics. Ascorbic acid and 2-ME effectively inhibited POD activity when guaiacol was used as substrate (Table 3). Addition of EDTA had no significant effect on POD activity in honeydew, pineapple and watermelon, but inhibited enzymatic activity in kiwifruit. In kiwifruit, unlike the other fruits, the addition of Mn^{2+} also caused a decreased POD activity, an indication of the fact that metal ion catalysis of POD activity in kiwifruit would only occur when ions are present in trace quantities. Tiron and DETC, two compounds with the ability to bind with POD molecular iron, inactivated POD activity in the fruits. Tiron also has the ability to scavenge free radicals, including POD mediated free radicals. With the exception of kiwifruit where EDTA inactivation might indicate metal ion involvement in autocatalytic reactions, the inhibitory effects of the metal chelator on POD activities in the
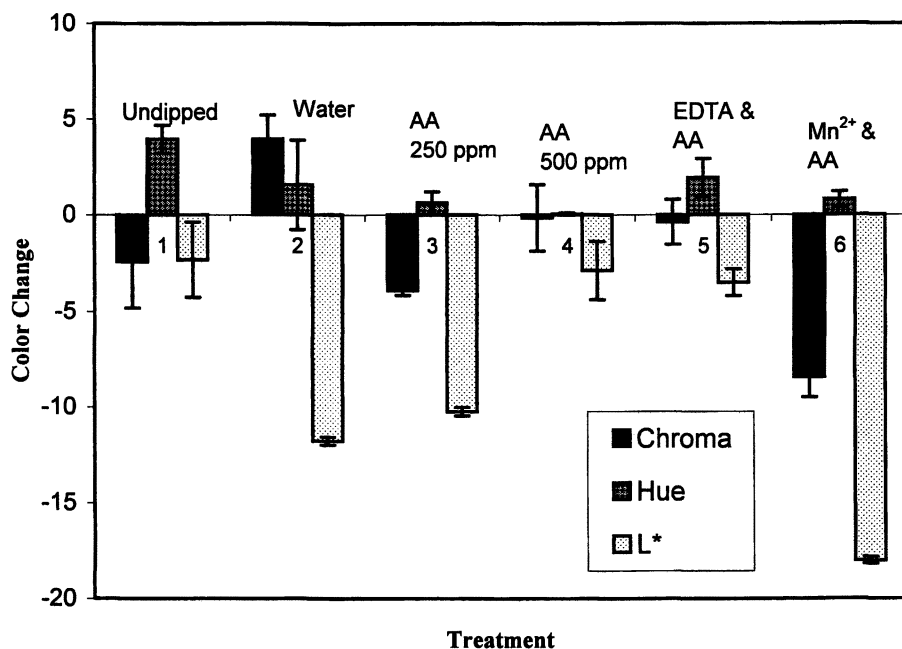
Figure 2. Effect of AA on the color of cut cantaloupe stored at 4 oC for 20 days. Reproduced with permission from ref. 47.
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other fruits and their lack of sensitivity to superoxide dismutase suggest heterolytic reaction pathways.

The ability of the fruit enzyme extracts to oxidize ascorbate to monodehydroascorbate, as would be expected of ascorbate POD was demonstrated. Reactivity of the enzymes with ascorbate followed the order honeydew>cantaloupe>watermelon>pineapple=kiwifruit (Table 4). Reactivity in the presence of POD extracts from watermelon and pineapple with guaiacol, relative to enzymatic activity of POD extracts in the other fruits were higher than their corresponding order when AA was the substrate. The order of relative activity when guaiacol was the substrate was cantaloupe=watermelon>honeydew>pineapple>kiwifruit. (Table 4). Wounding, as in fresh-cut processing, generally causes an increase in ascorbate biosynthesis and ascorbate POD (36-39,44). Ascorbate contents in plant tissues are known to regulate ascorbate POD activity (40). Ascorbate POD production in response to oxidative stress could help reduce deteriorative reactions (45-47). In the fruits, POD production is obviously a part of the cell adaptation to stress

Table III. Relative POD activity in 100g of fruit in the presence of guaiacol and H₂O₂ (control), ethylenediaminetetraacetic acid (10 mM), tiron (1.0 mM), CaCl₂ (0.5 mM), superoxide dismutase (100 units/mL), diethyldithiocarbamic acid (0.5 mM), 2-mercaptoethanol (0.5 mM), and ascorbic acid (0.5 mM).

POD Inhibitors	Fruit Tissue POD Activities			
	Honeydew	Kiwifruit	Pineapple	Watermelon
Control	1.0a	1.0a	1.0a	1.0a
EDTA	0.84a	0.35b	0.82a	0.80a
Tiron	0.03b	0.01c	0.12b	0.04b
CaCl₂	0.82a	0.86a	0.89a	0.93a
Mn²⁺	0.82a	0.42b	1.03a	0.98a
SOD	0.82a	0.98a	1.05a	0.90a
DETC	0	0	0	0
2-ME	0	0	0	0
AA	0	0	0	0

*Values in each row without the same letters are significantly different (P< 0.05).

Table IV. Relative POD activity in 100 g of fresh-cut fruit with AA and guaiacol substrates

POD Substrates	Relative POD Activity in Fruit Tissue				
	Cantaloupe	Honeydew	Kiwifruit	Pineapple	Watermelon
Ascorbic Acid	0.73b	1.00a	0.01d	0.02d	0.40c
Guaiacol	1.00a	0.76b	0.08d	0.51c	0.96a

*Values in each row without the same letters are significantly different (P< 0.05).

conditions created by surface tissue exposure. The POD activities in the fruits could also be indicative of their relative fresh-cut oxidative stress activity, with cantaloupe melon having the highest stress level. When honeydew was dipped in AA solution, it caused a 42% decrease in POD activity. The same was observed for cantaloupe melon (32). The effect of AA treatment in reducing fruit POD activity was attributed to an ascorbate preservative role by way of an enhanced antioxidative action of the enzyme-hydrogen donor complex and trace metal ion cofactors either because of the antioxidant nature of molecular AA, or the utilization of POD enzyme-hydrogen donor complex formation. Ascorbate treatment of fresh-cut kiwifruit and watermelon, however increased fruit POD activity five and two fold respectively. Peroxidase activity in pineapple was unaffected by ascorbate treatment. It is unclear why the effects of ascorbate treatment on production of POD enzyme in the other fruits differ from those of the two muskmelon fruit. This could be related to the much higher activities of POD from the muskmelons with AA substrate than enzyme extracts from the other fruits. The results, however, appear to indicate a product specific nature ascorbate-POD interactions in fruits, and the need for further research in this area.

The optimum pH activity for kiwifruit POD was considerably lower than those of the other fruits (Figure 3).
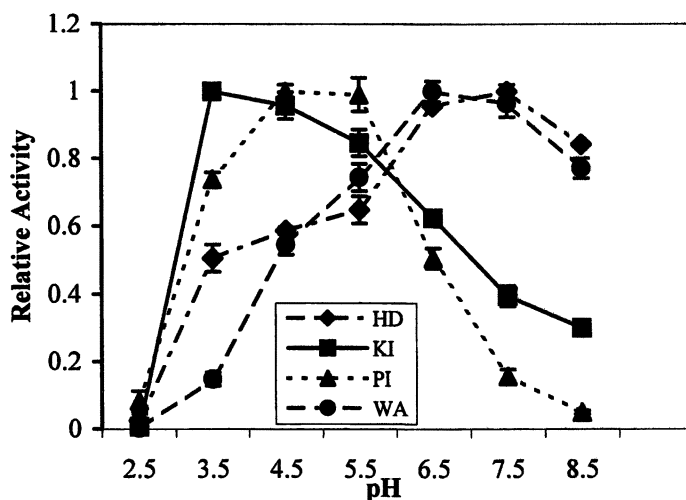
Figure 3. Effect of pH on POD activity in honeydew (HD), kiwifruit (KI), pineapple (PI) and watermelon (WA).
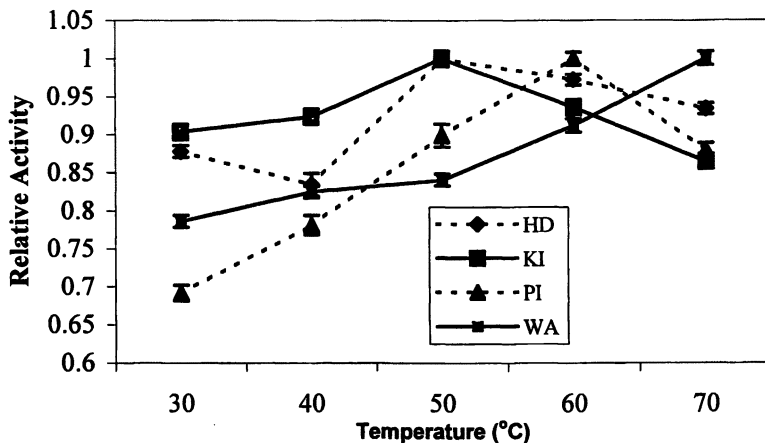
*Figure 4. Effect of temperature on POD activity in honeydew (HD), kiwifruit
(KI), pineapple (PI) and watermelon (WA).*

Kiwifruit (pH 3.5) and pineapple (pH 4.5-5.5) both exhibited acidic optimum pH
activities while that of watermelon (pH 6.5) was near neutral. Optimum pH
activity for honeydew was the highest at pH 7.5. The POD pH activity profile
indicates that enzymatic activities in these fruits are optimum during their
periods of normal harvest maturities. The more acidic kiwifruit and pineapple
have their optimum activities at lower pH values than the relatively less acidic
honeydew and watermelon. Honeydew and kiwifruit POD were most active at 50
°C, and pineapple had an optimum, temperature activity at 60 °C (Figure 4).
  The optimum temperature for watermelon POD activity was higher than those of
the other fruits, occurring after 70 °C. Watermelon POD was also the most
stable after thermal treatments (30 to 70 °C) for 5 and 20 min. (Figure 5). Over
90% of enzymatic activity was retained after 20 min of incubation at 70 °C.
Peroxidase stabilities in kiwifruit and honeydew followed a similar pattern.
Activities, as illustrated by kiwifruit POD stability illustrated in Figure 5,
decreased significantly after incubation at temperatures above 50 °C for 20 min.
POD isozymes in pineapple were relatively stable up to 60 °C. The heat
stability, pH and temperature activity optima for honeydew are quite similar to
those of cantaloupe melon (22). Residual POD after blanching and other forms
of temperature treatments could be used as an index for enzyme inactivation
because of their relative heat stability. The use of mild heat treatments for short
periods of time (~50 °C for less than 5 min) as a minimal processing step for the
cut fruits are not expected to affect POD activity levels in the fruit significantly.
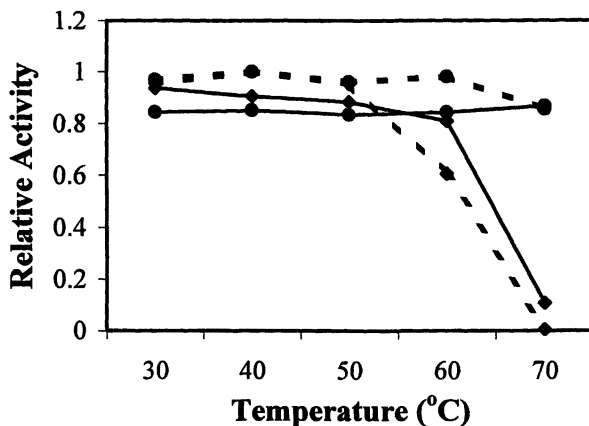
Figure 5. Residual POD activity after thermal treatment of watermelon for 5 (– • –) and 20 min (··· • ···), and kiwifruit after 5 (– ♦ –) and 20 min. (·· ♦ ··).

Peroxidase catalysis of cell wall activities such as the cross-linking of ferulic acid substituents of pectin and the synthesis of lignin and suberin polymers are mainly ascribed to anionic isozymes (48). Six acidic POD isozymes (pI 4.4 - 6.1) were separated by isoelectric focusing in pineapple while three were obtained for both honeydew and watermelon (Figure 6). The enzymes in honeydew (pI 5.5 - 6.0) and watermelon (pI 5.0 -5.8) are also acidic. Kiwifruit POD enzyme was undetectable with benzidine hence it was not possible to determine its isoelectric property. The anionic POD isozymes in the fruits would normally play significant roles in the firmness and texture of their fresh-cut products. Their role in the firmness and texture of fresh-cut fruits is, however, likely to be diminished by the preference of ascorbate POD for ascorbate as the electron donor (17).

Conclusions

Appearance and quality parameters (titratable acidity, pH, soluble solids and organic acid composition) cannot be used as reliable determinants of the freshness of refrigerated fresh-cut fruits. While these parameters do not change appreciably with proper cold storage, enzymatic and microbial activities that affect quality continue. Quality changes that result from temperature abuse are, however, easier to detect.
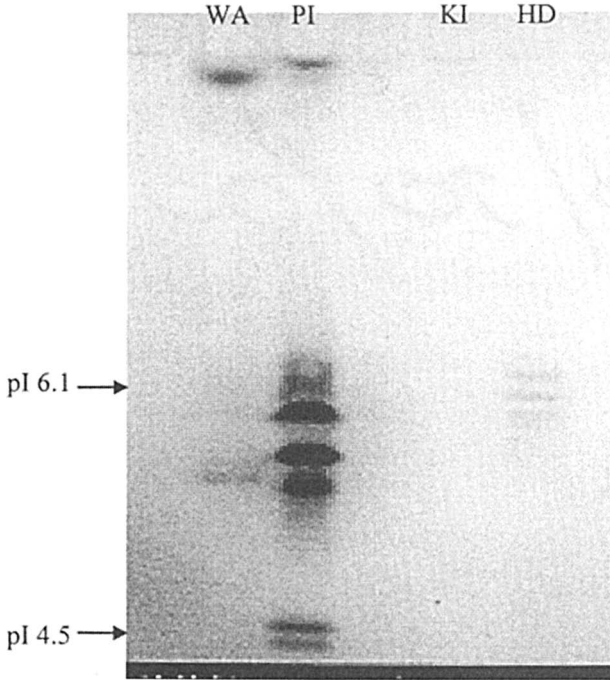
Figure6: Benzidine stained isoelectric focusing of POD in honeydew (HD), kiwifruit (KI), pineapple (PI) and watermelon (WA).

The presence and amount of lactic acid and Gram-positive microflora could serve as indicators of temperature abuse during transportation and storage. In commonly fresh-cut processed fruits such as cantaloupe melon, honeydew, kiwifruit, pineapple and watermelon, POD isozymes are anionic ascorbate POD enzymes. Although anionic POD isozymes are involved in a number of cell wall activities, their effects on firmness and texture of fresh-cut fruits is likely to be diminished by the preference of ascorbate POD for ascorbate as the electron donor. The enzymes appear to be produced as a response to fresh-cut processing induced oxidative stress. Ascorbic acid enhances the antioxidative action that involves metal ion cofactors in muskmelons apparently through ascorbate-POD complex formation. The nature of ascorbate-POD interactions in kiwifruit, pineapple and watermelon is unclear. Peroxidase activity profiles

indicate that enzymatic activities in the fruits are optimum during their normal harvest periods. Mild heat (minimal processing treatments) for short periods of time will have no effect on POD activity.

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Chapter 5

Changes in Key Aroma Compounds during Natural Beer Aging

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Application of Aroma Extract Dilution Analysis on a flavor
extract isolated from a fresh pale lager beer revealed 41 odor-
active compounds among which 2-phenylethanol (sweet,
flowery), 4-hydroxy-2,5-dimethyl-2(5H)-furanone (caramel-
like), 3-hydroxy-4,5-dimethyl-2(5H)-furanone (seasoning-like)
and (E)-ß-damascenone (cooked apple-like) predominated with
the highest Flavor Dilution (FD)-factors. In a beer sample of
the same batch stored for 34 month at 20°C in the dark, the
concentrations of ten of the most odor-active compounds did
not change significantly. However, four compounds, namely
ethyl 2-methylpropanoate and ethyl 2-methylbutanoate with
fruity notes as well as the Strecker aldehydes methional
(cooked potato-like) and phenylacetaldehyde (sweet, honey-
like) increased by factors of 3 to 10, respectively. Based on a
very precise method developed for the quantitation of (E)-2-
nonenal, it could clearly be ruled out that this aldehyde
contributes to off-flavor formation in the beer investigated.

Introduction

Flavor staling of beer has been, and still is, one of the greatest challenges in
the brewing industry. A great number of sensory studies have shown that during
storage the beer flavor is shifted to more sweet, toffee-like notes, while the
typical bitter, hoppy aroma is decreased. Furthermore, cardboard-like and ribes-
like aromas may develop, in particular, when higher concentrations of oxygen are
dissolved in beer (1-4). It is, however, obvious from most of the studies that the

type of off-flavor generated clearly depends on the storage conditions (e.g. light, temperature, storage time).

Up to now, more than 630 volatile compounds have been identified in various types of beer (5). Among them short-chain fatty acids, carbonyl compounds and esters (in total 330 compounds) predominate.

Within the past five decades different compounds have been suggested as the cause for the development of less desired aromas in beer. Burger et al. (6, 7) have suggested acetaldehyde and furfural to generate cardboard-like aromas in beer, when added in concentrations of 30 mg/L or 2 mg/L, respectively. These findings, however, could not be confirmed in later studies (8, 9). In addition, (E)-ß-damascenone (10) and 2-aminoacetophenone (11) have been proposed as further contributors to beer staling and also the Strecker aldehydes 2-methylpropanal as well as 2- and 3-methylbutanal have been suggested to increase the malty, cereal-like aromas in beer during storage (12). Furthermore, 4-methyl-4-mercaptopentan-2-one was identified as cause of a ribes-type off-flavor (13), and another sulfur compound, 3-methyl-2-butene-1-thiol, is long-known as the cause for the so-called "sunstruck" off-flavor in beer (14).

As mentioned above, oxygen has longbeen suggested as an important factor reducing beer stability. Based on the observation that the redox potential in beer decreases during aging, De Clerck (15) already sixty-eight years ago has proposed this crucial role of oxygen in beer flavor degradation. Because oxygen is an important substrate in lipid peroxidation, many studies have focused on this parameter. Jamieson and Gheluwe (8) identified (E)-2-nonenal, a well known degradation product of unsaturated fatty acids, in beer and have proved by means of sensory experiments that concentrations in the ppb-range were able to generate a cardboard-like off-flavor. The odor threshold of (E)-2-nonenal in beer lies between 0.04 and 0.5 µg/L (cf. review by Collin (16)). However, its role in beer off-flavor is not yet clear, because some authors found increased amounts of this aldehyde in stored beers (17, 18,) while others did not find an increase (19, 20).

By application of Aroma Extract Dilution Analysis, a method based on sniffing of GC eluates (21), we could previously show that the Flavor Dilution (FD) factors of, in particular, phenylacetaldehyde, 3-methyl-3-mercaptobutyl-formate, (E,E)-2,4-nonadienal, (E)-2-nonenal and an unknown compound with an aniseed-like odor quality were increased in a forced aged beer (14 days at 37°C) compared to the respective fresh beer (22). Evans et al. (23), applying GC/Olfactometry, later on confirmed the role of phenylacetaldehyde in beer staling and suggested methional and 4-methoxybenzaldehyde as further off-flavor contributors.

This brief literature survey shows that it is not yet clear, which compounds can be regarded as the main contributors to beer staling. There is, therefore, no reliable method available to objectify flavor changes occurring during beer storage. Several reasons can be given for this lack in information: (i) Depending on the storage parameters different off-odorants may be generated, (ii) the methods of quantitation applied in the older studies, e.g. derivatization of carbonyls with dinitrophenyl hydrazine, may result in degradation of precursors by the conditions applied in the derivatization procedure, (iii) a combination of sensory and analytical methods, e.g. the Aroma Extract Dilution Analysis, has

only been applied in very few studies and (iv) most of the studies have used forced aged beers by application of a thermal treatment and/or an oxygen administration.

The objectives of the present study were, therefore, to show the contribution of single odorants to beer flavor aging by application of a comparative Aroma Extract Dilution Analysis on a fresh and a naturally aged beer, and by subsequent identification and quantitation of the most odor-active compounds.

Materials and methods

The beer samples (pale lager beer) were supplied by a Dutch brewery. Storage conditions applied were 20°C in the original bottle, i.e. in the dark (naturally aged beer: NAB). For the isolation of the volatiles, the beer sample (500 mL) was extracted with diethyl ether and the volatile compounds were separated from the non-volatile material by a SAFE-distillation (24). Further separation steps and the application of the Aroma Extract Dilution Analysis were performed as described elsewhere (25). The most important odorants were quantified by stable isotope dilution assays (SIDA) (25). For the quantitation of (E)-2-nonenal a very selective and sensitive procedure of coupling the SIDA with high resolution mass spectrometry was used (26).

Results

Odorants in a fresh pale lager beer

The volatile compounds in a fresh pale lager beer were isolated by extraction with diethyl ether followed by high vacuum distillation. Application of the Aroma Extract Dilution Analysis on the concentrated extract (500 mL beer → 100 µL of extract; concentration factor 1:5000) revealed a total of 23 odor-active compounds in the fraction containing the neutral and basic volatile compounds and an additional number of 18 odorants in the fraction containing the acidic volatile compounds (25). In the Flavor Dilution (FD) factor range of 16 to 8192 thirty-eight odorants were identified. Among them, the ten aroma compound displayed in Fig. 1 showed the highest FD-factors. The results revealed, especially 2-phenylethanol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-hydroxy-4,5-dimethyl-2(5H)-furanone and (E)-ß-damascenone as the most odor-active compounds in the aroma of the fresh beer, thereby confirming data of our previous study (22).

With the exception of ethyl3-phenylpropanoate (FD 1024) and 2,3-dihydro-5-hydroxy-6-methyl-4(H)-pyran-4-one (FD 64) all other compounds have previously been reported in the literature as beer volatiles although their flavor contribution had not been studied.
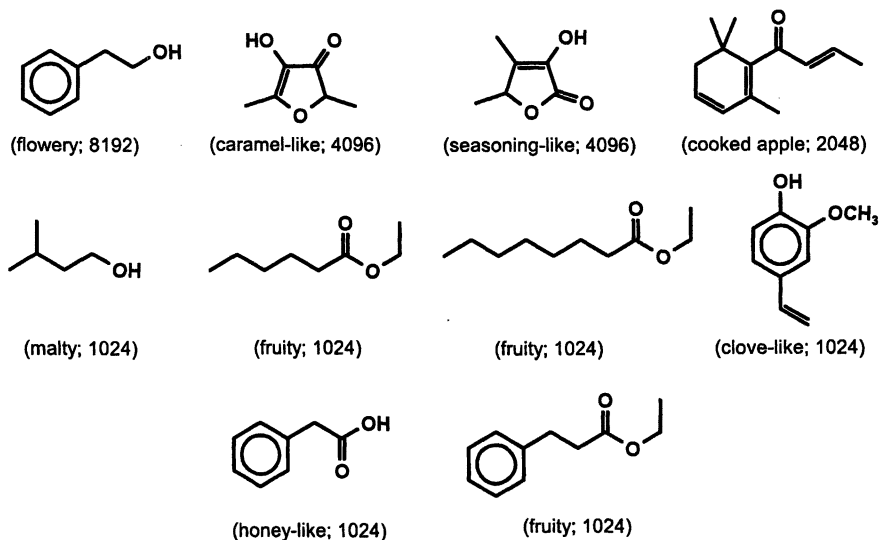
Figure 1. Structures of ten key odorants identified in fresh pale lager beer after application of an Aroma Extract Dilution Analysis (Odor quality, FD-factor)

Key odorants in naturally aged beer

Beer samples of the same batch were stored for a maximum of 34 months at 20°C in the dark in the original brown glass bottles. Every month, an overall sensory evaluation was performed by means of the triangle test and by flavor profile analysis. Fresh beer of the same type was supplied by the brewery every 2 months for comparison.

In general, even after 34 months, the beer was drinkable, but significant differences in the overall flavor were observed after about 12 months. The flavor impression clearly shifted to a more sweet-malty, honey- and cider-like aroma with a decrease in the hoppy, flowery aroma. Application of AEDA on a flavor extract isolated from the beer stored for 34 months at 20°C in the dark revealed the same odorants as identified in the fresh sample. The FD-factors of most of the odor-active odorants (cf. Fig. 1) were nearly identical with the FD-factors determined in the fresh sample indicating that neither a decomposition nor a formation of these odorants occurred during storage.

By contrast, in particular four compounds, namely ethyl 2-methylpropanoate (fruity), ethyl 2-methylbutanoate (fruity), 3-(methylthio)propanal (potato-like) and phenylacetaldehyde (sweet, honey-like) were significantly increased during storage (Table 1).

Table 1. Important odorants showing clear differences in their Flavor Dilution (FD) factors in naturally aged beer (NAB) compared to fresh beer (FB)

Odorant	Odor quality	FD in	
		NAB	FB
Ethyl 2-methylpropanoate	Fruity	1024	64
Ethyl 2-methylbutanoate	Fruity	64	16
3-(Methylthio)propanal	Potato-like	128	16
Phenylacetaldehyde	Honey-like	128	16

Quantitation of selected odorants

The data given above have shown that the flavor changes occurring during natural beer aging are not caused by formation of new aroma compounds, but by an increase of odorants already present in the fresh beer. To study such changes in more detail, twenty-seven of the odorants were quantified in the fresh and the naturally aged beer by application of stable isotope dilution assays (25).

As already suggested by the results of the AEDA, most of the odorants identified as key odorants in the fresh beer (cf. Fig. 1) were not much influenced by the storage (Table 2). Although their concentrations differed slightly in both samples, their "relative odor activity value" (ratio of concentration to odor threshold) was only by a factor of 1.5 higher in the stored sample, e.g. for 4-hydroxy-2,5-dimethyl-3(2H)-furanone.

Table 2. Comparison of the concentrations of five important beer odorants in the naturally aged (NAB) and in the fresh beer (FB)

Odorant	Conc. (μg/L)	
	FB	NAB
3-Methylbutanol	54000	49300
2-Phenylethanol	41400	39100
Phenylacetic acid	314	427
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	170	284
4-Vinyl-2-methoxyphenol	86	72

However, a completely different result was obtained for the six compounds given in Table 3. In particular, phenylacetaldehyde and 2-methylpropanal were increased by a factor of five, and ethyl 3-methylbutanoate showed a more than ten times higher concentration in the stored sample.

Quantitative experiments aimed at determining the time course of the formation of these odorants during storage revealed that ethyl 2-methylbutanoate (Fig. 2) and also phenylacetaldehyde (Fig. 3) clearly increased with storage time. Consequently, both compounds can be suggested as indicator aroma compounds to assess the flavor stability of beer.

Table 3. Comparison of the concentrations of six aroma compounds, which were clearly increased in naturally aged beer (NAB) compared to fresh beer (FB)

Odorant	Conc. (µg/L)	
	FB	NAB
2-Methylpropanal	11	62
3-Methylbutanal	12	32
Methional	1	3
Phenylacetaldehyde	5	25
Ethyl 2-methylpropanoate	67	367
Ethyl 3-methylbutanoate	20	233
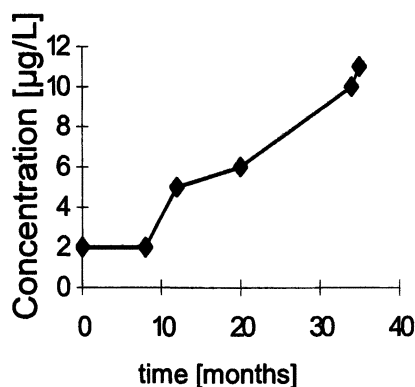
Figure 2. Time course of the formation of ethyl 2-methylpropanoate in beer during "natural" aging at 20°C in the dark

The role of (E)-2-Nonenal

As detailed in the "Introduction", (E)-2-nonenal has been suggested many times as the key "off-flavor" compound in beer, although the role of this aldehyde in beer staling is discussed quite controversely. As shown by other groups and confirmed also in the author's own studies (19, 26), by addition of 1 µg/L of (E)-2-nonenal to fresh beer, a cardboard-like off-flavor is generated. The crucial question, however, is whether enough (E)-2-nonenal is generated during storage to exceed this "breakthrough" value. By definition, this is the threshold at which a

single compound can clearly influence a food aroma in the presence of the whole set of food volatiles.
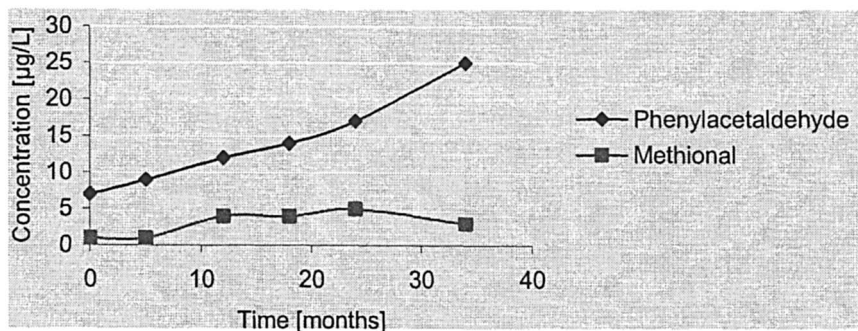
Figure 3. Time course of the formation of methional and phenylacetaldehyde during natural beer aging

It is a challenge in the analysis of such aroma-active compounds occurring in the sub-ppb level to get reliable quantitative data. In many of the studies published, no internal standard has been used. Furthermore, to increase sensitivity, very often derivatization procedures are used in which the application of heat and or low pH values is necessary. Such procedures, however, may generate (E)-2-nonenal from precursors present in the beer or extract, respectively, thereby yielding higher amounts than originally present in the beer.

Stable isotope dilution assays using internal standards labeled with either Deuterium or Carbon 13 (100 % labeling) have been successfully applied in the analysis of aroma compounds which are unstable and/or occur in trace amounts (21). Using [^{2}H$_2$]-(E)-2-nonenal as the internal standard, two different isotope dilution assays were developed and applied to quantify this odorant in beer (26). One is based on the enrichment with two dimensional gas chromatography before mass spectral measurement, the other uses a direct measurement by means of high resolution mass spectrometry (26). For the latter approach, a beer sample (1200 mL) was spiked with [^{2}H$_2$]-(E)-2-nonenal (0.2 µg) and, after isolation of the volatile fraction, directly analyzed by HRGC/high resolution mass spectrometry.

As indicated in Fig. 4, monitoring of the respective mass of the labeled and the unlabeled aldehyde allowed an exact quantitation directly from the extract without any enrichment step. By application of this method, the concentration of (E)-2-nonenal could reliably be followed during storage.

The results summarized in Table 4 clearly showed that there is no correlation in (E)-2-nonenal concentration with time, because even higher amounts were detected in the 1 month old beer compared to a beer stored for 34 month. In no case, the breakthrough threshold of 0.1 µg/L was matched. The data were in good agreement with the results of the sensory evaluations, because in no sample a cardboard-like off-flavor could be detected sensorially. The data clearly

reveal that in the beer type investigated, (E)-2-nonenal is not a contributor to the flavor changes occurring during storage.
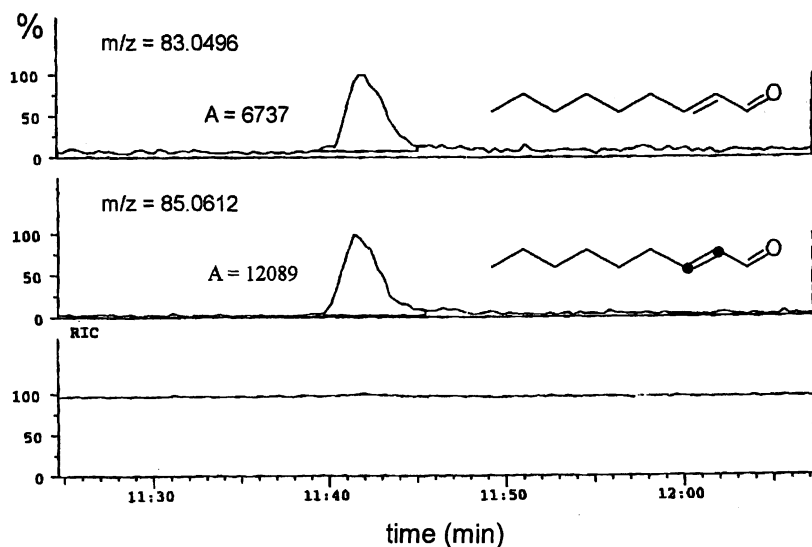
Figure 4. High resolution gas chromatography/high resolution mass chromatography of (E)-2-nonenal and [²H₂]-(E)-2-nonenal. Quantitation in a naturally aged beer (23 months, 20°C) containing 0.07 µg (E)-2-nonenal per L

Table 4. Concentrations of (E)-2-nonenal[a] determined by a stable isotope dilution assay in naturally aged beer

Storage time (months)	Conc. (µg/L)[b]
0	0.01
1	0.05
4	0.02
12	0.03
23	0.07
34	0.04

[a] The breakthrough threshold was determined to be 0.1 µg/L.
[b] Limit of confidence ± 10 %.

Conclusions

The results confirm that significant flavor changes do occur even if a beer is stored in the dark at room temperature. (E)-2-Nonenal was clearly excluded as an

important contributor to the sweet, cider- and honey-like flavor of the aged beer, but an increase in the Strecker aldehydes and in some esters clearly reflected this flavor change. Flavor recombination studies and dotation experiments using reference odorants are underway to prove the increase in Strecker aldehydes and esters as crucial for beer flavor stability. Preliminary studies already revealed that the classical way of Strecker aldehyde formation (27) does not apply in the formation pathway of these aldehydes in beer, but that other mechanisms are operating to generate these off-odorants during storage.

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Chapter 6

SPME–GC/MS Testing for Monitoring Off-Flavors in Processed Milk: Application of Odor Wheel Plots

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SPME-GC/MS is a powerful analytical system for deciphering causes of off-flavors in processed milk. Previous work has demonstrated how SPME-GC/MS can be used as an electronic-nose (e-nose) instrument to classify milk samples according to type of off-flavor and to predict the shelf life of processed milk. This paper describes how a new data presentation technique can be used in conjunction with e-nose results to better understand the actual causes of off-flavor development in milk samples. Radar plots of concentrations and odor units of "indicator" volatiles reveal which specific chemicals are the most likely contributors to off-flavors in samples and likely mechanisms involved in their formation.

An analytical technique using solid-phase microextraction, mass spectrometry, and multivariate analysis (SPME-MS-MVA) has been shown to be a useful tool for predicting the shelf life of processed milk samples (1,2). In this work, GC/MS was used as an electronic nose (e-nose). Principal component analysis (PCA) was used to show clustering patterns of milk samples with similar types of off-flavors, and partial least squares (PLS) analysis was used to provide a

quantitative estimate of processed milks' shelf life — i.e., the period between processing and the time when milk becomes unacceptable to consume because of odor or taste.

One problem with estimating shelf life with the e-nose approach is that results are statistical estimates, and it is difficult to make critical quality control decisions (e.g., whether or not to discard a day's production of fluid milk because of possible short shelf life) based solely on statistical estimates from SPME-MS-MVA results. Another problem with the e-nose approach is that it doesn't provide details about which specific chemicals are causing off-flavor. This type of supplementary information would be invaluable in making crucial quality control decisions regarding questionable samples.

Supplementing SPME-MS-MVA results with conventional GC/MS testing shows promise. While conventional GC/MS is a more time-consuming approach than the SPME-MS-MVA method, it does provide information that is difficult or impossible to derive from e-nose experiments — namely, the amount and identity of the specific off-flavor chemicals that are present. This paper describes how a new graphical technique can be applied to GC/MS peak area data to assist in elucidating the reasons for off-flavor development in milk.

Experimental

Sampling and Sensory Evaluation

All samples were commercially pasteurized and homogenized reduced-fat milk (2% milkfat) free of off-flavors at time of manufacture. Samples were packaged in either pint or half-pint high density polyethylene (HDPE) contoured bottles with screw caps. Thirty samples of reduced-fat milk were sampled consecutively from the production line at a dairy plant on the day of processing. This sampling scheme was conducted on five occasions over a seven-month period.

Samples were immediately taken from the dairy plant and refrigerated in a walk-in cooler at 7.2±0.5 °C until the end of shelf life was reached as determined by sensory paneling. During refrigerated storage, a sample was removed from the cooler on a daily basis and evaluated by a taste panel to determine when the samples first developed off-flavors. The fresh initial samples (controls) and the samples at the end of shelf life were subjected to SPME-GC/MS analysis. Prior to GC/MS analysis, samples were preincubated to allow bacteria to grow and produce enough metabolites to be detected. The preincubation procedure involved placing the sample in a 19±1 °C incubator for 16 h. After 16 h, the preincubated sample was subjected to SPME-GC/MS analysis.

Four judges experienced in tasting dairy products were used for sensory evaluation of milk samples. The method used for sensory scoring was based on

a 10-point scale according to the scoring guide of the American Dairy Science Association. Shelf life was ended when a score of 5 or lower was recorded by three of the four judges, and the day before was considered the end of shelf life.

SPME Analysis

A Saturn 3 GC/MS was used (Varian Analytical Systems, San Fernando, CA). The GC was equipped with a split/splitless model 1078 injector. The injector was operated in the split mode (6:1 split ratio) at a temperature of 275 °C. The SPME fiber used was 75-μm Carboxen/PDMS (Supelco, Bellefonte, PA). For thermal desorption, the SPME fiber remained in the injector for 3 min. Helium was used as the carrier gas. A 30 m x 0.25 mm i.d. DB-5 fused-silica capillary column with a film thickness of 1 μm (J&W Scientific, Folsom, CA) was used, and the flow rate of the helium carrier gas was 1.0 mL/min.

The following column temperature programming sequence was used: An initial temperature of 50 °C was maintained for 0.5 min, increased to 180 °C at a rate of 6 °C/min, and held at 180 °C for an additional 4 min.

Three milliliters of milk sample, 5 μL of internal standard solution (10 μg/mL chlorobenzene), and a micro-stirring bar (Fisher Chemical Co., Cat. No. 09-312-102) were placed in a 6 mL glass GC vial (38 mm high and 22 mm in diameter) and capped with 20 mm PTFE/silicone septa (Wheaton Scientific Products, Cat. No. 224173). The stock chlorobenzene solution that was used to prepare the 10 μg/mL working internal standard solution was purchased as a 5000 μg/mL in methanol standard solution from Supelco (Cat. No. 4-0006). Clogging the 10 μL syringe after internal standard addition to milk was common. This was prevented by rinsing the syringe with distilled water after each addition of internal standard to milk.

The setting on the SPME holder assembly scale was adjusted to 0.8 scale units to ensure that the fiber was positioned in the headspace above the sample in exactly the same way from run to run. With the fiber exposed, the sample vial was placed in a 50 °C water bath (fiber exposure started immediately with sample at 19 °C), and the sample was stirred at 350 rpm. Excessive stirring speed (i.e., greater than 1,000 rpm) sometimes caused milk to splash onto the fiber. When fibers contaminated with small amounts of milk were injected, unusual peaks from the thermal decomposition of lactose were observed. Examples included 2-furanmethanol, 5-methyl-2(3H)-furanone, 5-hydroxymethyl-2-furan carboxaldehyde, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

After a 15 min exposure time, the fiber was retracted into the needle assembly and removed from the vial. The setting on the SPME holder assembly was changed to 4.0 scale units prior to injection into the GC injector port, which was fitted with a special insert for SPME analysis (Varian, Cat. No. 03-925330-00).

MS Analysis

The Varian Saturn MS detector was used in the electron impact (EI) mode with a 1 sec scan time and a 1 count peak threshold. The mass range used was m/z 40 to m/z 350. The temperature of the ion trap manifold was 180 °C.

Generation of Graphs: Indicator Volatiles and Odor Wheel

Samples at the beginning (day of manufacture) and end of shelf life were used to prepare graphs. All graphs were made using the radar plot option with Microsoft® Excel 97. Radar plots display changes in values relative to a center point. Two general types of radar plots based on GC/MS analyte data were made:

(a) *Plots of indicator volatiles.* Indicator volatiles are the chromatograhic peaks that indicate a potential off-flavor problem and provide clues as to mechanisms of off-flavor formation. Indicator volatiles (IV) graphs are radar plots of the area of the analyte peak divided by the area of the internal standard peak (chlorobenzene) multiplied by a scaling factor. The scaling factor is a value between 0.1 and 10. Multiplication by a scaling factor allows for analytes of widely varying concentrations to be plotted and visualized on the same graph.

(b) *Odor Wheel plots.* Odor Wheels are radar plots of the log of the odor unit value for each analyte. These plots show which indicator volatiles are most likely to contribute to a sample's off-flavor and malodor.

Quantitation of Volatiles

Quantitation of analytes, which was necessary for computing odor unit values, was accomplished using the method of additions technique in which fresh, control milk samples were spiked with internal standard and various levels of standard analyte. Standard response factors for each series of analytes were used to quantitate levels of off-flavor volatiles in the samples tested.

Results and Discussion

An example of a graphical IV presentation is shown in Figure 1 for a fresh, reduced-fat milk sample (sampled at day of production) and for another sample from the same day's production but after storage in a 7.2±0.5 °C cooler for 18

days when the first sign of off-flavors was noted. The primary metabolites produced in this particular sample are 2-pentanone, hexanoic and octanoic acids, pentanal, hexanal, and dimethyl sulfide.

Based on the chromatographic profiles for the five end-of-shelf-life samples and numerous results from spoiled milk samples tested over the past 20 years in our laboratory, 25 volatiles plotted in the IV graphs were selected as "indicator volatiles" — i.e., volatiles that most consistently appear in samples with off-flavor (OF). Identification of the presence of these indicator volatiles can help in the determination of the mechanism involved in OF formation — for example, whether the OF originates from microbiological causes, sanitizer that hasn't been properly flushed from processing lines, lipid oxidation (of linoleic and linolenic acids in the butterfat) caused by exposure to light or prooxidant metals (e.g., copper or iron), or residual organic solvents from packaging materials. Furthermore, in some cases it may be possible to identify the type of organism (psychrotrophic bacteria) involved in spoilage. For example, the production of 3-methyl butanal, which contributes a malty OF to milk, is produced by *Streptococcus lactis maltigenes*. Determination of the mechanism of OF formation, however, is not always straightforward because many of the indicator volatiles can be produced by multiple mechanisms. Table 1 illustrates a few examples (3).

Figure 2 shows IV plots for the other four end-of-shelf-life samples tested. Plots for the four control samples are not shown because they appear essentially identical to the control sample in Figure 1. While control samples contain numerous volatiles (e.g., acetone, 2-butanone, limonene, etc.), these compounds are usually present in good-tasting milk and impart imperceptible flavors to the milk at concentrations normally present. These non-indicator volatiles are not plotted in IV graphs because they provide no insight into what is causing OF.

The primary metabolites detected in the end-of-shelf-life samples shown in Figure 2 include dimethyl sulfide, 1-butanol, 1-propanol, ethyl acetate, methyl butyrate, 2-heptanone, 2-nonanone, and free fatty acids (C4-C10). It is interesting that, while many of the plots contain similar types of indicator volatiles, no two end-of-shelf-life plots are identical.

Additional reduced-fat milk samples with nonmicrobiological OF were prepared to evaluate how well the IV plots depict the specific indicator volatiles produced. One sample of reduced-fat milk was spiked (under sterile conditions) with 1300 ppm of Matrixx sanitizer (Ecolab, St. Paul, MN). This level of sanitizer was chosen since it represents the lowest level that could be detected by sensory analysis. A second sample was spiked (under sterile conditions) with 5 ppm copper. Both samples were preincubated at 19 ± 1 °C for 16 h as previously described. After 16 h, the preincubated samples were subjected to SPME-GC/MS analysis, and the IV plots were made from peak area data. The same multiplication factors for each indicator volatile were used in this set of samples as was used for the microbially spoiled samples. Figure 3 shows the expected

Control (Fresh, 0 Days)
Reduced-Fat Milk
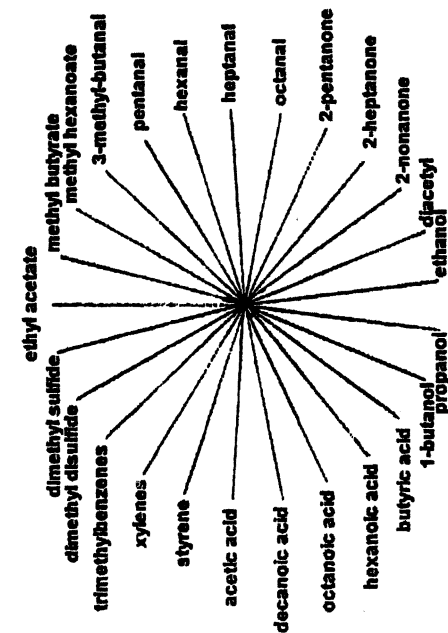
End of Shelf Life (18 Days Later)
Reduced-Fat Milk
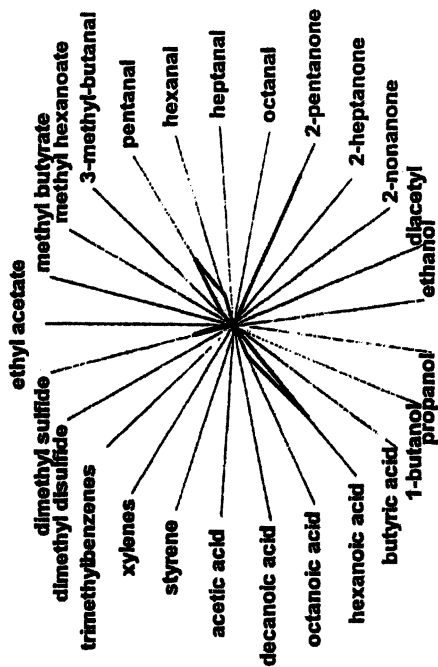
Figure 1: IV plots of control (fresh, no off-flavor) reduced-fat milk and another sample from the same production day at end of shelf life (i.e., first day with detectable off-flavor development).
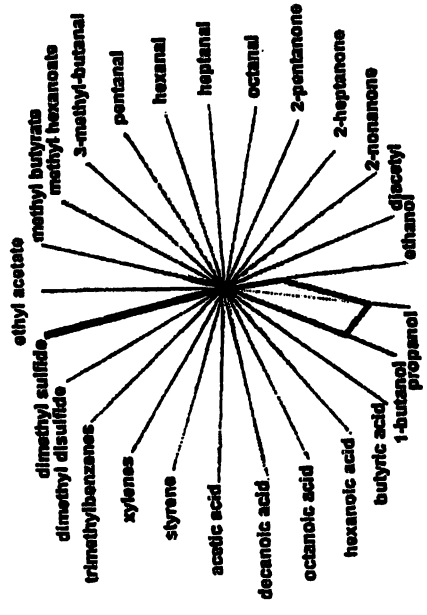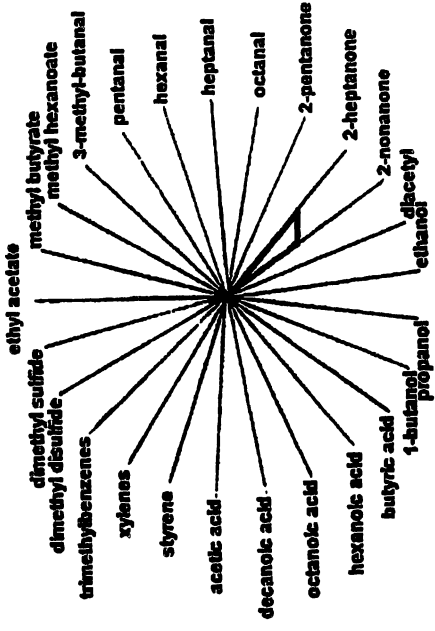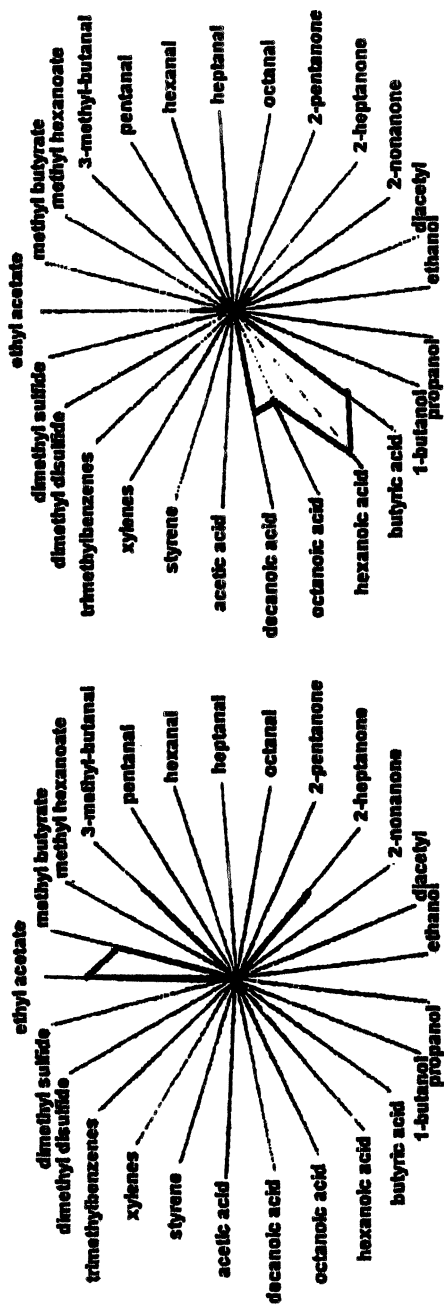
Figure 2: IV plots of the remaining four microbially-spoiled, reduced-fat milk samples at end of shelf life. Chemicals shown are psychrotrophic metabolites. All samples tested at first detection of OF by trained sensory panel (from 17 to 25 days after production).

Table I. Indicator Volatiles in Milk: Multiple Mechanisms of Formation

Chemical/Chemical Type	Mechanisms of Formation
Free fatty acids (C4-C10)	Microbial, enzymatic, sanitizers
Acetic acid	Microbial, sanitizers
Methyl ketones	Microbial, heating
Toluene	Packaging solvent, decomposition of β-carotene
Dimethyl sulfide	Microbial, heat induced
Dimethyl disulfide	Microbial, light with methionine
3-Methyl butanal	Microbial, Strecker degradation reaction involving leucine
Hexanal	Microbial, lipid oxidation (caused by light, copper, etc.)
Lactones	Microbial, animal feed, heating

indicator volatiles for milk contaminated with sanitizer and for milk contaminated with copper. In the case of Matrixx sanitizer contamination, peroxyacetic acid decomposes in milk to form acetic acid and hydrogen peroxide. The hydrogen peroxide is capable of oxidizing milkfat components; hexanal and heptanal are produced by this mechanism. Octanoic acid, a component used in the Matrixx formulation, also appears in the IV plot. The IV plot for the sample contaminated with copper shows hexanal as the major indicator volatile (from oxidation of linoleic acid), with lesser quantities of pentanal and octanal produced.

Enhanced IV Plots That Incorporate More Information

The IV plots shown in Figures 1, 2, and 3 provide a quick visual indication of what types of volatiles are being generated in milk during shelf life. However, they fail to show how the chemicals were formed or the extent that each chemical actually contributes to the sample OF detected.

IV Plots That Show Likely Mechanisms of OF Formation

Figure 4 is one way more useful information can be incorporated into IV plots. The addition of color-coded balls at the terminus of each axis (or in this case, balls with differing patterns) provides a simple way of indicating possible mechanisms of formation. Figure 4 is a replot of the end-of-shelf-life sample in Figure 1 but includes the addition of coded balls to provide insights into how the off-flavors were formed.
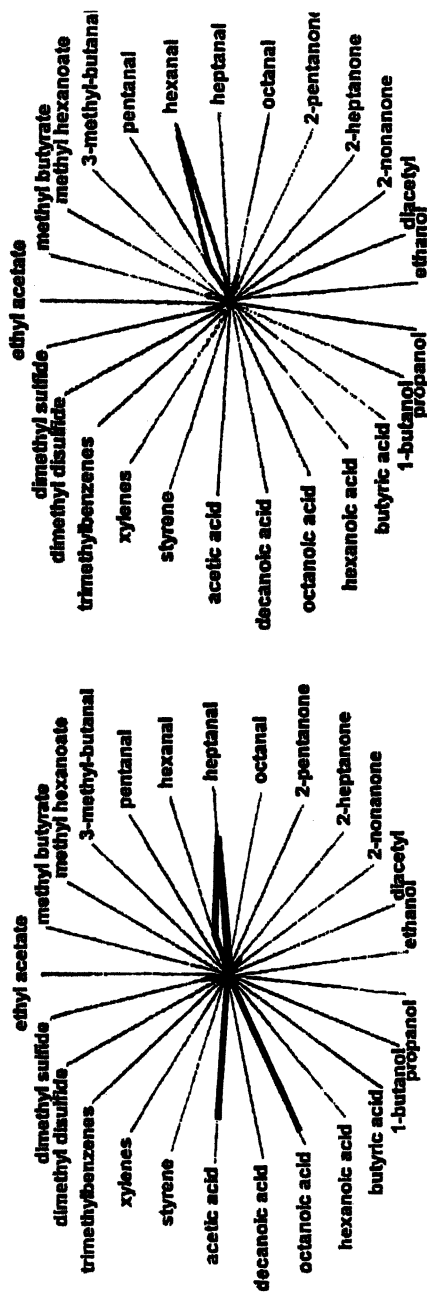
Contamination with Sanitizer

Contamination with Copper

Figure 3: *IV plots of fresh, normal-tasting, reduced-fat milk spiked with Matrixx sanitizer (1300 ppm) or copper (5 ppm). Samples preincubated at 19±1 °C for 16 h prior to SPME-GC/MS analysis.*
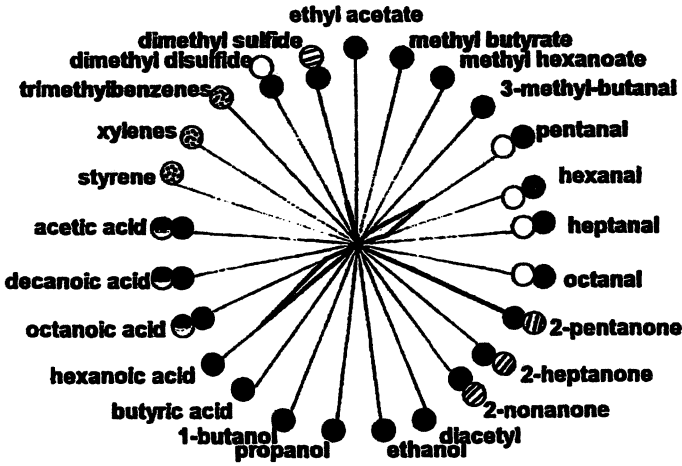
Figure 4: IV plot showing bacterial metabolites produced for the same end-of-shelf-life reduced-fat milk sample shown in Figure 1, with the addition of coded circles to show possible mechanisms of formation.

Table II. Threshold Odor Units (U₀) for Indicator Volatiles in Milk

Indicator Volatile	Threshold Odor Unit[a] (ppb)	Indicator Volatile	Threshold Odor Unit[a] (ppb)
ethyl acetate	2500	diacetyl	5
methyl butyrate	68	ethanol	100000
methyl hexanoate	77	propanol	9000
3-methyl-butanal	5	1-butanol	500
pentanal	27	butyric acid	240
hexanal	5	hexanoic acid	3000
heptanal	3	octanoic acid	3000
octanal	0.7	decanoic acid	3500
2-pentanone	70000	acetic acid	22000
2-heptanone	1600	dimethyl disulfide	1.3
2-nonanone	100	dimethyl sulfide	0.7

[a]Source: L.J. van Gemert. *Compliations of Odour Threshold Values in Air and Water*; TNO Nutrition and Food Research Institute: Zeist, The Netherlands, 2000.

Odor Wheel Plots Provide Quantitative Estimates of OF Contributions

Determining the degree that each indicator volatile contributes to the sample's OF could be useful. GC-olfactometry (GC-O) technqiues such as aroma extraction dilution analysis (AEDA) or CharmAnalysis are often applied to elucidate the key odorants in sample extracts. However, these techniques require repetitive analyses and are quite time-consuming, typically requiring at least two days to perform on one sample. Obviously, the standard GC-O tests are not appropriate to apply to quality control testing where dozens of samples are tested each day and results are needed quickly.

Information about the extent of off-flavors contributed by each indicator volatile can also be estimated by the use of odor units (U_0). This approach requires access to published odor threshold values (in water) for specific compounds or, for unpublished compounds, experimental determination of individual odor thresholds obtained by diluting standard solutions in water. The threshold odor values of the indicator volatiles appear in Table II and were taken from published tabulations (4).

An odor unit is defined as a flavor compound's concentration divided by its odor threshold:

$$U_0 = \text{Compound Concentration/Odor Threshold Concentration}$$

The logarithm of the odor unit (log U_0) can be calculated for each volatile and represents a value which is significant for olfactometry discrimination. Since odor activity follows a sigmoidal dose-response curve in that significant responses require order-of-magnitude changes in concentration, logarithmic functions more significantly represent meaningful sensory differences. Aroma unit values >1 are indicative of chemicals that are present at concentrations that greatly exceed their thresholds and are likely to contribute signficant flavor impact (5).

An example of an Odor Wheel plotted with log U_0 values is shown in Figure 5(a). The sample plotted in Figure 5(a) is the same sample plotted in Figures 1 and 4. By examining the IV plot in Figure 4 and the Odor Wheel in Figure 5(a), the following type of information is readily revealed about the sample: The primary chemicals produced during shelf life include 2-pentanone, hexanoic acid, octanoic acid, pentanal, hexanal, dimethylsulfide, and 3-methylbutanal; these chemicals are most likely produced as metabolites by psychrotrophic bacteria; and, of these chemicals, dimethylsulfide and 3-methylbutanal are the biggest contributors to the sample's OF. It is noteworthy that this useful information is readily obtained from the two types of plots and is acquired with little or no interpretation of data by the dairy plant Q.C. technician.

Another example of a Odor Wheel plot based on log U_0 values is shown in Figure 5(b). The sample used for this plot is the same Matrixx-contaminated sample used in Figure 3. Figure 5(b) shows that the primary contributers to off-

flavor in milk contaminated with Matrixx sanitizer are hexanal and pentanal (from oxidation of unsaturated fatty acids by hydrogen perioxide in the Matrixx). The octanoic and acetic acids, which are present at higher concentration levels than either pentanal or hexanal, do not contribute detectable OF.

It should be noted that Odor Wheels based on log U_0 values are always plotted with the axis values labeled. This is useful for two reasons. First, it helps to distinguish this type of plot from IV plots based on peak area ratios. Second, knowledge of the actual log U_0 value of an indicator volatile is important for assessing its odor/flavor contribution.

A GC-MS Strategy for Off-Flavor Testing in Dairy Q.C. Labs

Figure 6 illustrates a possible analytical strategy for Q.C. monitoring of processed milk. Initially, GC/MS would be used as an e-nose instrument to conduct high throughput screening. Instrumentation could include a less expensive benchtop GC/MS system (e.g., a Varian 2100) equipped with a SPME autosampler (e.g., the Combi PAL from Leap Technologies). With such an analytical system, minimal involvement would be required by the Q.C. technician.

It would be advantageous to apply a fast GC technique that would provide resolution of all chromatographic peaks but would require only 2-5 minutes of chromatography time. The GC/MS data file could be subjected to PLS in order to predict the sample's shelf life as previously described.

Samples that are predicted to have an unusually short shelf life could be subjected to further scrutiny by a corporate analytical research laboratory. Since a complete high-resolution total ion chromatogram is obtained by fast-GC/MS, the sample would not have to be retested. The data file could simply be e-mailed to the corporate lab, which could then perform further data analysis such as PCA or, most importantly, IV plots and Odor Wheels of individual chromatographic peaks. Based on additional detailed information about which indicator volatiles are present, their likely mechanism of formation, and their specific contribution to OF, a more intelligent decision could be made concerning whether production lots of suspect processed milk should be discarded to waste.

Future developments may include the addition of more types of indicator volatiles and more types of OF mechanisms in Figure 4-type plots.

Conclusion

A major advantage of SPME for studying OF in milk is its ability to extract a wide range of potential OF chemicals, including alcohols, aldehydes, ketones, free fatty acids, lactones, and volatile sulfur compounds. A significant problem is how to manage all the chemical data generated by GC/MS testing. The graphical techniques reported here are a good supplement to e-nose information
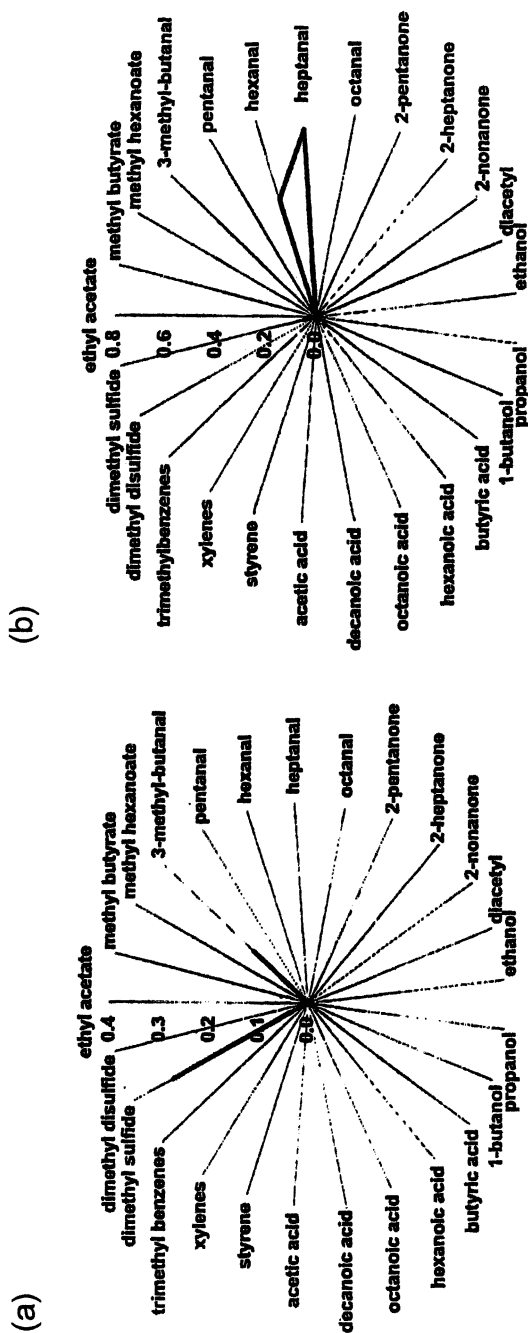
Figure 5: Odor Wheels of log U_o values (rather than peak area ratios) of indicator volatiles showing: (a) most odiferous bacterial metabolites produced for the same end-of-shelf-life reduced-fat milk sample shown in Figures 1 and 4 and (b) most odiferous indicator volatiles contributed by Matrixx sanitizer when spiked in reduced-fat milk (same sample as shown in Figure 3).

GC-MS Based Strategy for Off-Flavor Testing in Dairy Q.C. Lab
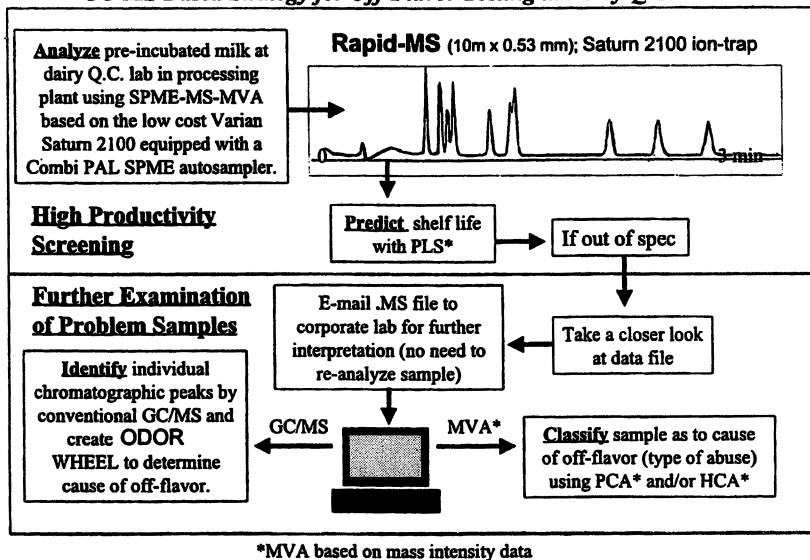
Figure 6: How Odor Wheel plots fit into a dairy Q.C. testing program.

obtained by SPME-GC/MS and provide insights into which chemicals are caus-
ing OF in a particular sample and their mechanism of formation.

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Chapter 7

Relating Analytical and Sensory Data To Predict Flavor Quality in Dairy Products

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Taste is highly decisive in whether or not a dairy product is successful. In order to be able to control and predict flavor quality, key-aroma components should be determined for the product of choice. Based on these key-aroma components, models can be developed that are able to predict flavor quality. In this manuscript, a general approach will be discussed that aims to relate sensory data with key-aroma components by statistical methods. This approach has been used for a number of dairy products. Two specific examples will be presented in detail: 1) A statistical model was developed that successfully predicts the flavor quality of (aged) UHT milk based solely on the concentrations of key-aroma components. UHT milk has a distinct flavor that changes upon aging. This change in flavor is perceived as negative by the consumer and has therefore a direct influence on the shelf life of UHT milk. 2) Key-aroma component analysis, free choice profiling and statistical methods were used to understand and predict the effects of processing on the flavor quality and stability of protein-hydrolysates of dairy origin.

Taste plays an important part in the consumer's choice of food products and is defined for 80% by volatile compounds that are perceived by the receptors in the upper part of the human nose cavity (*regio olfactoria*). The volatile compounds are perceived both by the nasal route (before the product is taken in the mouth) and by the retronasal route (during the eating of a product). The remaining non-volatile basal tastes, bitter, sweet, acid and salt, define the flavor perception for about 20% and are perceived by the receptors on the tongue. In order to control the sensory quality of a product during aging or during processing steps, many food industries use sensory panels. Although these sensory panels can be very successful in determining the sensory quality of a specific product, the fact is that they are labor- and cost-intensive. Therefore, there is a general desire to develop analytical alternatives for the sensory tests in order to be able to predict sensory quality from concentrations of key-aroma compounds, *i.e.* to make taste analytically measurable.

At NIZO food research, the SOIR procedure (Sensory evaluation, Olfactometry, Identification and Recombination, Figure 1) is used to link the sensory attributes of a product to key-aroma compounds.

SOIR

The SOIR procedure consists of four steps. In step one a test panel gives a description of the product or one of its attributes (flavor profile). A notion like 'tasting good' or 'tasting bad' is split up into different features, which are judged separately. In this way a good description of the product or a specific aspect is generated in 'common language'.

In step two, the sensory attributes are related to specific flavor compounds. To determine the most important aroma components of a product gas chromatography is linked to olfactometry (GC/O), which means that the nose of a trained person is used as a detector instead of a measuring instrument. In the eighties two techniques were developed simultaneously for separating the key aroma components from the less important aroma components by means of olfactometry: Aroma Extract Analysis (AEDA) (1) and CHARM analysis (2).

NIZO food research makes use of the AEDA method or uses an alternative method based on GC/O in combination with time intensity measurements. When the key aroma compounds have been identified in step 3 (MS, RI index), their role can be confirmed in a recombination experiment. This means that the key aroma components are added proportionally to a matrix equal or similar to that of the product examined (e.g., water in the case of coffee, or a gelatin gel in the case of custard). This mixture has to confirm the GC/O analysis and should result in a comparable flavor sensation as was perceived earlier in the original product.
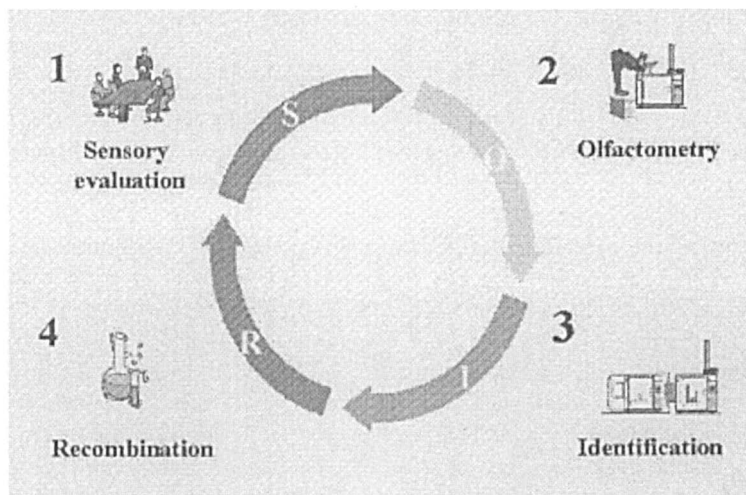
Figure 1: SOIR procedure

Once the key-aroma compounds have been determined, they can be used as markers for following aging or for evaluation of changes in processing. Combining sensory results and analysis data of key-aroma compounds using statistical methods will lead to the development of predictive models. Since differences in flavors often relate to complex balances between volatiles rather than a major change in one or two constituents, complex correlation techniques and multivariate approaches are frequently necessary to be able to extract useful information. Examples of this have been reported before (for instance in reference 3 and 4).The success of predictive models is strongly dependent on whether the key-aroma compounds (the markers!) have been chosen wisely and whether enough samples have been analyzed to provide a stable model. The benefit of successful models is that the sensory quality can be predicted based on the analysis of specific key-aroma components and that one needs to invest less effort in the organization and training of sensory panels.

In this manuscript the described procedures (SOIR together with data collection and multivariate statistics) will be illustrated by two examples:

- A statistical model that successfully predicts the flavor quality of (aged) UHT milk based solely on the concentrations of key-aroma components (5).

- Key-aroma component analysis, free choice profiling and statistical methods were used to understand the effects that processing has on the flavor quality and stability of protein-hydrolysates of dairy origin (6).

1) Predicting the flavor of UHT milk in terms from analytical data

The main reason for giving milk a heat treatment is prolonging the shelf life of milk. Heat treatments have a major effect on the flavor of milk. Different heat treatments will lead to different flavor profiles and therefore result in different types of milk. Over the last decades a lot of effort has been devoted to the issue of extending the shelf life of milk as much as possible with a minimal effect on flavor. The process that has been most successful from a commercial and qualitative point of view is the UHT process. In this process the milk is heated for a short time (3-15 s) at a high temperature (140-150 °C). Two types of continuous flow UHT processes are common (7): 1) "direct heating": the milk is heated directly by a steam injection and 2) "indirect heating": the milk is heated indirectly by tubular or plate-heat exchangers. Depending on the dispersion in residence-time in the heating system and the heating temperature, UHT-milk can be produced by relatively mild treatment (e.g. 4 s, 142 °C, directly) or by a relatively harsh treatment (e.g. 15 s, 150 °C, indirectly). As a consequence of these different heat treatments, UHT-milk can be produced with flavor profiles that vary between wide limits, *i.e.*, close to pasteurized milk with a mild treatment or close to sterilized milk with a harsh treatment.

Three important attributes dominate the sensory perception of UHT-milk (8): "cooked" (sometimes also called "sulfur" or "cabbage"), "sterile" (sometimes also called "Maillard", "caramel" or even "gluey") and "oxidation" (sometimes also called "stale" or "ketone") flavor. A fourth attribute that is sometimes mentioned is the intrinsic UHT-flavor that seems to be a combination flavor of "ketone", "oxidation" or "rich" attributes. In Table 1 a summary is given of the chemical volatiles that are responsible for the different sensory attributes in UHT-milk and the processes that have an effect on the strength of the attributes (8,9,12). Of course some of these volatiles can also be generated as metabolies in milk. However, in this project only UHT samples were used of which the microbial integrity was unquestionable. Badings *et al* have been able to develop a synthetic flavor mix that is able to give a "UHT-taste" to pasteurized milk (10). The synthetic mix consists of a mixture of methylketones, diacetyl, H$_2$S, methanethiol, methylisothiocyanate, ethylisothiocyanate, benzathiazole, several lactones, maltol, isobutylmercaptan and dimethylsulfide.

The taste of UHT milk is important for its consumer acceptability. Its change in flavor during aging is perceived as negative and has therefore a direct influence on the shelf life of UHT milk. In this project a mathematical model has been developed that is able to predict the taste of UHT milk based only on the chemical analyses of key-aroma components. The sensory attributes that have been linked to chemical analyses are "overall flavor quality", "cooked flavor" and "sterile flavor".

Table 1: Aromatic volatiles that are responsible for the main attributes of UHT-milk and the factors that govern them

Sensory attribute	Aromatic volatile	Factors of influence
Cooked flavor	Free SH groups	Amount of vitamin C
	Volatile sulfides	Sulphydryl oxidase
	H_2S	O_2 permeability of carton
	Methanethiol	Volume of headspace in carton
	Dimethylsulfide	Concentration O_2 in milk
	Carboxylsulfide	Aging conditions
Sterile flavor	2-alkanones	Extent of Maillard reaction
	Lactones	Aging conditions
	Maltol	
	Furanones	
Oxidation flavor	Aldehydes	Extent of auto-oxidation
(also called	(C2, C3, C5, C6)	Extent of Maillard reaction
"stale")	Ketones	Aging conditions
	(C5, C7, C8, C9)	

Experimental

Key-aroma compounds (SOIR): For the identification of the key-aroma compounds responsible for the characteristic UHT flavor, an UHT milk sample that does not exhibit a 'cooked' or 'sterile' taste was selected. These samples were analyzed according to the SOIR procedure.

Sensory analysis: Sensory evaluation of 144 UHT samples, that were aged under varying conditions, was carried out using a 8-membered trained panel. The following attributes were determined: overall quality score, cooked flavor, UHT flavor and sterile flavor. The samples were also divided in three quality groups: good, medium and bad.

Instrumental analysis: The same UHT samples were analyzed for the specified key-aroma compounds using headspace-GC-FPD and purge & trap procedures followed by GC/MS with selected ion recording (SIR). (10,11)

Statistics: Both sensory and analytical data were analyzed using multivariate statistics analysis. Linear Discriminant Analysis (LDA) was applied for the classifications.

Results

Based on olfactometry results and literature data (12,13), a list of key-aroma compounds of UHT milk was determined (Table 2). There can be some discussion about whether it is appropriate to have limonene in the Table 2. Feed and pasture forage are normal sources for limonene in milk and since it has quite a high odor/taste treshold iw ould not be expected to be a key-aromacomponent of milk. However, the AEDA procedure clearly demonstrates that limonene is an important flavour compound in the UHT milk that has been used in this project.

Table 2: Key-aroma compounds of UHT milk

Key-aroma compounds	
H_2S	Aldehydes (C $_{2,6,7}$)
Methanethiol	3-methylbutanal
Dimethylsulfide	Ketones ($C_{5-9, 11}$)
Dimethyldisulfide	Limonene
Dimethylsulfone	Diacetyl

The sensory data showed trends in attributes that were expected. Cooked flavor decreases with time, while sterile flavor increases with time. Higher aging temperatures result in faster overall quality decline. Figure 2 gives an example for the sterile flavor attribute.

The following relations were found between sensory attributes and aging conditions ($0.6 < R^2 < 0.8$).

Overall quality scores = 6.2+ 0.05*AGE+0.003*TEMP+0.136 * AGE x TEMP
Cooked flavor scores = 1.5-0.027*AGE-0.024*TEMP+0.156*AGExTEMP
Sterile flavor scores = 0.0-0.034*AGE +0.13 *AGExTEMP

Multiple linear regression of the key-aroma components on the sensory attributes proved that the analytical results were season dependent. Each season has therefore its own quantitative regression equations. The cause for this season dependency was undetermined. Here the qualitative model for the total data set is given:

Overall quality score = constant – K5 – K7 – DMS
Cooked flavor score = constant – K7 – K11 + Methanethiol (or H_2S) – DMS
$\qquad$ - K5
Sterile flavor score = constant + K5 + K7 + DMS

Quadratic Surface
AGE vs. TEMP vs. STERILE
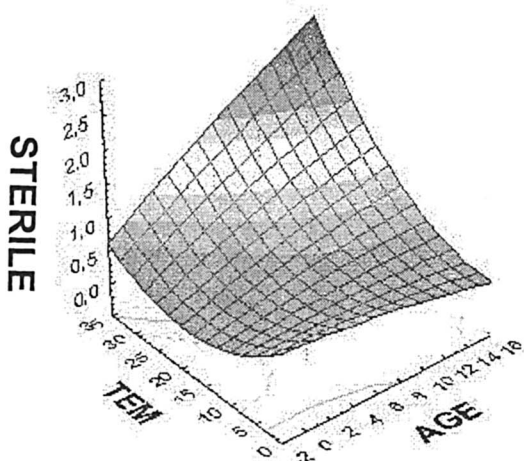
Figure 2: Effect of aging on the "Sterile" flavor score. Scale: 0 (no sterile flavur –4(extreme sterile flavor) in UHT milk (AGE in weeks, TEMP in °C)

Table 3: Percentage correct classification obtained by LDA based on key-aroma compounds only and between brackets, based on key-aroma compounds and aging condition parameters (AGE and Temp)

LDA	% Correct Classification			
Class	Spring	Summer	Fall	Winter
Good	100(100)	100(100)	88(94)	85(92)
Medium	75(92)	85(92)	67(91)	75(87)
Bad	100(100)	100(100)	100(100)	100(100)
Total	91(97)	93(97)	81(93)	86(93)

For the model based on the key-aroma compounds the UHT samples were divided in three quality groups (Good, Medium and Bad). Since the data was dependent on season, disciminant analysis was performed separately for the samples of each season. Statistic analysis was performed to select suitable discriminators from table 1. The obtained classifications are summarized in

Table 3. Figure 3 illustrates the discriminating power of the "spring" model. Currently the model is validated with new UHT milk samples from spring, summer, fall and winter.
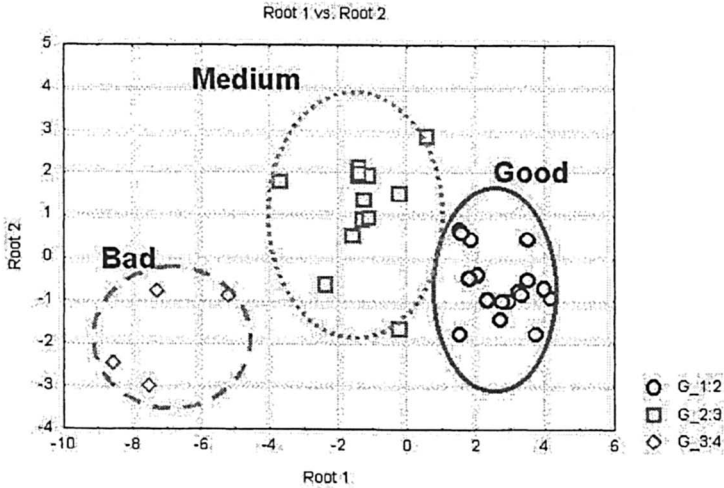
Figure 3: Biplot showing the ability of the model to cluster the (aged)UHT milk samples according to quality based on the analytical measurements of key-flavors

2. Defining Flavor Quality and Shelf Life of Protein Hydrolysate Concentrates During Processing.

Casein and whey protein hydrolysates are used in nutritional products within sports, health, clinical, infant and functional applications. The product applications consist of use in tablets, bars, instant drinks and heat processed neutral formulas.

For the commercial success of high-quality protein hydrolysate concentrates, it is important that these products have good organoleptic properties. This research is part of a project to improve and control the manufacturing process of these protein hydrolysate concentrates related to its sensory properties. In the present study, the development of an analytical method is described, which in combination with multivariate statistics allows predicting

the sensory quality of casein-protein hydrolysate concentrates. In order to develop such a model the key-aroma compounds involved in the generation of unwanted flavors were identified with Sensory evaluation, Olfactometry, Identification and Recombination studies (SOIR). After the development of a straightforward analytical method, based upon static headspace and GC/MS-SIR, samples were analyzed and evaluated organoleptically by means of Free Choice Profiling (FCP) and Generalized Procustus Analysis (GPA). In order to obtain a predictive model, both the analytical and sensory data were linked using multivariate statistics.

Experimental

Key-aroma compounds (SOIR): For the identification of the key-aroma compounds responsible for the unwanted flavor properties two good and two bad tasting samples were selected. These four samples were analyzed according to the SOIR procedure: *Sensory Flavor Profile* test, *GC/O (AEDA), Identification and* sensory evaluation of a *Recombination* mixture of added key-flavor compounds to the good sample matrix.

Sensory analysis: Sensory evaluation of 20 protein hydrolysate samples (learning set) was carried out using a panel of 15 members. Using Free Choice Profiling (FCP) and Generalized Procustus Analysis (GPA) the samples were divided into three different classes (Good, Doubtful and Bad).

Instrumental analysis: The same set of 20 samples (learning set) and 30 new samples were analyzed upon the defined key-aroma compounds using static headspace followed by GC/MS with selected ion recording (SIR). (11)

Statistics: Both sensory – and analytical data were analyzed with multivariate statistics analysis. Linear Discriminant Analysis (LDA) was applied for classifications.

Results

The marker key-aroma compounds responsible for the unwanted flavor selected by the SOIR procedure and statistic analysis are given in Table 4. The results showed that most off-flavor compounds are formed as a result of Maillard reactions.

The sensory evaluation of the samples started with QDA sessions with a trained panel. But because of the complex sensory aroma description of the unwanted flavors, sensory evaluation with this classically trained panel on well-defined attributes failed. The approach with free choice profiling (FCP) and generalized procrustus analysis (GPA) was more successful. Figure 4 and Figure 5 show the consensus space and the attributes of the sensory analyses.

Table 4: Key-aroma compounds of the protein hydrolysate product

Key-aroma compounds
Acetone
2-methylbutanal
3-methylbutanal
Dimethyldisulfide
2,6-dimethylpyrazine
Tetramethylpyrazine

Figure 4: Free choice profiling results, consensus space depicting the three quality clusters

set 4 Noot — set 3 Aangebrand
set 4 Chocolade — set 5 Kuilvoer — set 5 Pepton. — set 1 Zurig
set 1 Chocolade
set 1 Stro — set 1 Kool — set 7 Rot
set 5 Kool Bloemen

| putrid |
| sewer |
| sulphury |
| stable |
| manure |
| ammonia |
| liq. manure |
| burnt |
| media |

Figure 5: Different attributes generated in Free choice profiling (only first quarter is shown). The bad quality samples are characterized by attributes as sewer, sulfury, manure etc.

In order to obtain a predictive model, both the analytical and sensory data of the training set were linked using multivariate statistics. Because more compounds were analyzed then those selected by the SOIR procedure, statistical analysis was performed to select suitable discriminators in LDA.

The results showed that when all marker key-aroma compounds from Table 4 were involved, 100% correct classification with high posterior probabilities was achieved in LDA. Again this result showed the significance of the selection of key compounds with GC/O.

Next a validation of the model was done with 30 new samples. Out of these 30 new samples, 28 samples were correctly classified. After validation these samples were used to extend the initial training set in order for the model to become even more stable. In Figure 6 a scatterplot of the individual scores for all cases are given. A relatively simple and fast analytical method has been developed, which, in combination with multivariate statistics, is very well suited for routine prediction of the sensory quality of high-quality protein hydrolysate concentrates.

General Conclusions

Optimal flavor and taste are dependent on the balance of many different flavor molecules in a product. This flavor balance can for instance be disturbed by processing conditions or aging. The purpose of this manuscript was to show that by combining sensory evaluations, key-aroma compound analysis and multivariate statistics, objective and rapid analytical models can be developed that are able to predict sensory quality from analytical measurements. This has been illustrated by two cases. The successful prediction of the sensory quality of

UHT samples that have been aged under different conditions and prediction of the sensory quality of protein hydolysate concentrates that were processed under different conditions.

Figure 6. Biplot showing the ability of the model to predict the protein hydrolysate concentrate groups of different quality based on the analytical measurements of key-flavors

Acknowledgements

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Chapter 8

Aroma Characterization of Fresh and Stored-Nonfat Dry Milk

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Determination of the chemical nature and sensory profiles of nonfat
dry milk (NDM) is necessary to improve processing methods and
storage conditions to maintain product freshness. Aroma-active
compounds of NDM were identified by gas
chromatography/olfactometry (GCO) and gas chromatography-mass
spectrometry (GC-MS). Thermally induced volatiles Furaneol[®],
methional, sotolon, and maltol, free fatty acids, lactones as well as
aldehydes and ketones were primary contributors to both desirable
fresh and undesirable stale/stored aromas of NDM.

The primary dry milk product manufactured in the U.S. is nonfat dry milk.
It is mainly produced by spray drying of milk. The main advantages of drying
are reduction in transport and storage costs, and convenience of use in
formulations. The drying process is a continuation of a concentration process to
produce a stable, low moisture product with minimum sensory changes and
specific functional properties (*1*). Based on pre-heat treatment prior to spray
drying, three types of NDM are produced: low heat (not over 71°C for 2 min),

medium heat (71-79 °C for 20 min), and high heat (88 °C for 30 min). Pre-heat treatment is an integral part of the drying process, because heat treatments affect the functional properties of the powder. These heat processes generate different degrees of protein denaturation distinguished by the level of soluble whey protein nitrogen. The product has a shelf life of 12-18 months (2).

Nonfat dry milk should ideally have a clean, sweet and pleasant taste and be free of off-flavor defects. Cooked flavors may be present and vary according to heat treatment of the milk prior to evaporation and drying (3). Processing and storage of milk may change the flavor properties of the final spray dried product. Since nonfat dry milk is widely used both as an ingredient and for direct consumption, flavor quality is one of the most important factors to determine consumer acceptance or preference of dairy products. Because of consumer complaints, off-flavors are a major concern to the food industry. Undesirable flavors reduce the sensory quality and economic value of foods. Off-flavors can originate from several sources such as oxidation of lipids, enzyme decomposition, microbial growth or environmental sources.

Studies on flavor volatiles in skim milk powder have been conducted. Volatile flavor and off-flavor compounds in spray-dried skim milk were determined by simultaneous distillation-extraction (SDE) (4, 5). Free fatty acids and lactones were major contributors to the flavor of skim milk powder. In addition, aldehydes, aromatic hydrocarbons and some heterocyclic compounds affected the flavor indirectly. B-Ionone, benzothiazole and tetradecanal were found to be responsible for a cowhouse-like off-flavor in skim milk powder (5). In other research, the contributors of sweet and milky odors of skim milk powder were investigated (6). Nonanoic, decanoic, and dodecanoic acids were found to be responsible for this attribute. Sensory properties were not addressed in these studies.

Instrumental analysis of specific off-flavors in milk powder have also been studied. The role of Maillard reactions was investigated as an indicator of staling in nonfat dry milk (7). Constituents such as 2-furaldehyde, 2-furfuryl butyrate, alkylpyrazines and N-ethyl-2-formylpyrrole originating from nonenzymatic browning may contribute to the stale flavor. Early researchers (8) isolated carbonyl compounds responsible for the cereal-type flavor from instant and non-instant types of NDM. The compounds identified from instant NDM were formaldehyde, acetaldehyde, acetone, butanone, methylpropanal, 3-methylbutanal, furfural, diacetyl, hexanal, and nonanal. Driscoll and coworkers (9) studied sensory properties of NDM during storage. Time, storage temperature and type of packaging were critical factors to provide desirable sensory qualities.

This study provides information on the chemical nature of predominant aroma components of fresh and stored NDM. The aimss of the present study were to identify and compare the chemical nature of aroma active compounds of fresh and stored NDMs and to compare them with sensory evaluation results.

Materials and Methods

Chemicals

Aroma compounds (listed in Tables II and III were perchased from the following commercial sources including Aldrich Chemical Co. (Milwaukee, WI), Bedoukian Research Inc. (Danbury, CT) and Sigma (St. Louis, MO). Internal standards, 2-methyl-3-heptanone and 2-methylpentanoic acid, were purchased from Aldrich Chemical Co. and Lancaster (Windham, NH) respectively. Odorant no. 11 was obtained from Dr. R. Buttery (USDA, ARS, WRRC, Albany, CA).

Milk Powders.

Nonfat dry milks (n=100) were obtained from domestic producers. Powders ranged in age from approximately 3 months to 2 years old. Based on sensory analysis, six samples were chosen for chemical analysis. Three of them represented fresh, fluid-milk-type flavors and included different heat treatments. The other three samples exhibited stale/storage types of flavors. Samples were stored frozen (-20 °C) in Qorpak clear standard wide mouth bottles sealed with Teflon-lined closures (VWR Scientific Products, St. Louis, MO) until analysis.

Preparation of Extracts for AEDA

Direct Solvent Extraction. Milk powders (100 g) were hydrated with odor free water (500 mL) and blended with an electric hand-held mixer. The compounds 2-methyl-3-heptanone (5.44 µg/µL) and 2-methylpentanoic acid (6.18 µg/µL) in methanol were added (10 µL) as internal standards for neutral/basic and acidic fractions, respectively. Each sample was extracted with diethyl ether (3 x 300 mL) in 250-mL Teflon bottles with Tefzel closures (NALGENE; Rochester, NY). Solid NaCl (180 g) was added to the milk to break the emulsion during extraction. Each sample was agitated on a Roto Mix (Thermolyne, Type 50800; Dubuque, IA) for 30 min at the highest speed, and then centrifuged at 3500 rpm for 30 min. After centrifugation, each sample was slowly stirred to break the emulsion.

High Vacuum Distillation. The solvent extract was dried over anhydrous sodium sulfate (Na_2SO_4), and then concentrated to 100 mL at 35 °C using a Vigreux column (150 x 15 mm; VWR Scientific Products). The extract was poured into

a 1-L round bottom flask and frozen in liquid nitrogen. The two receiving tubes were placed in liquid nitrogen. Vacuum was applied (ca. 10^{-5} Torr) to the system for 4 h (10). The sample flask was kept at room temperature for the first 2 h, then temperature was increased to 60 °C (in a water bath), and the process was continued for an additional 2 h. The distillate was washed with sodium bicarbonate ($NaHCO_3$) (0.5 M; 2 x 15 mL) and a saturated solution of sodium chloride in water (3 x 5 mL). The upper (ether) phase containing the neutral/basic volatiles was dried over anhydrous Na_2SO_4 and concentrated to 0.5 mL under a gentle stream of nitrogen gas. The aqueous phase (bottom layer) was acidified with hydrochloric acid (18 % v/v) to pH 1.5 to 2 and the acidic volatiles were extracted with diethyl ether three times, dried over anhydrous Na_2SO_4, and concentrated under a nitrogen gas stream.

Gas Chromatography-Olfactometry (GCO)

The GCO system consisted of a HP5890 series II GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector (FID), a sniffing port, and on-column injector. Each extract (2 μL) was injected into a polar capillary column (DB-WAX or DB-FFAP 30 m length x 0.25 mm i.d. x 0.25 μm film thickness (d_f)) and a nonpolar column (DB-5ms 30 m length x 0.32 mm i.d. x 0.25 μm d_f; J & W Scientific, Folson, CA). Column eluate was split 1:1 between the FID and sniffing port using deactivated fused silica capillaries (1 m length x 0.25 mm i.d.). The GC oven temperature was programmed from 35 to 200 °C at a rate of 10 °C/min with initial and final hold times of 5 and 30 min, respectively. The FID and sniffing port were maintained at a temperature of 250 °C. The sniffing port was supplied with humidified air at 30 mL/min. Two experienced panelists conducted GCO analysis. The extracts containing the neutral/basic and acidic volatiles were diluted stepwise with diethyl ether at a ratio of 1:3 (v/v). The dilution procedure was performed until no odorants were detected by GCO. The highest dilution was defined as flavor dilution (FD) factor (11).

Gas Chromatography-Mass Spectrometry (GC-MS)

The system consisted of an HP5890 Series II GC/ 5972 mass selective detector (MSD, Hewlett-Packard, Co.). Separations were performed on fused silica capillary columns (DB-WAX 60 m length x 0.25 mm i.d. x 0.25 μm d_f, J&W Scientific). The carrier gas was helium at a constant flow of 0.96 mL/min. Oven temperature was programmed from 35 to 200 °C at a rate of 3 °C/min with initial and final hold times of 5 and 45 min, respectively. The MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization

energy, 70 eV; mass range, 33 to 350 a.m.u; EM voltage (Atune+200 V); scan rate, 2.2 scans/s. Each extract (2µL) was injected in the on-column mode.

Identification of Odorants

Positive identifications were made by comparing retention indices (RI), mass spectra, and odor properties of unknowns with those of authentic standard compounds analyzed under identical conditions. Tentative identifications were based on comparing mass spectra of unknown compounds with those in the Wiley138 mass spectral database (John Wiley & Sons, Inc., 1990) and a database generated from authentic standards in the Department of Food Science and Technology-Flavor Laboratory (Mississippi State University, Mississippi State, MS) or on matching the RI values and odor properties of unknowns against those of authentic standards. Retention indices were calculated by using an n-alkane series (*12*). To identify methional, selected ion monitoring mode (SIM) of Mass Spectrometry was used. The selected ions for methional were m/z 104 and 76.

Quantification of Selected Compounds

Reconstituted milk, after deodorization by high vacuum distillation, or water was used as a matrix to prepare standard solutions. Calibration was accomplished by addition of 0 (blank), 2 µL, 5 µL or 20 µL from a stock solution. Each mixture was also spiked with 10 µL of internal standard solution. The same procedure for preparation of extracts from samples was followed to prepare the standard solutions. The standard stock solution contained 54.6 µg of no. 1, 15.48 mg of no.2, 11.6 mg of no. 3, 48.4 µg of no. 4, 1.22 mg of no. 5, 12.68 mg of no. 12, 12.94 mg of no. 14, 58.4 µg of no. 18, 51 µg of no. 21, 2.34 mg of no. 26, 22.2 µg of no.31, 56.6 µg of no. 34, 10.84 mg of no. 38, 34.6 µg of no. 40, 110.6 µg of no. 42, 3.9 mg of no. 44, 119.2 µg of no. 45, 46.4 µg of no. 51 per ml methanol. For quantification of these compounds, a DB-FFAP column was used.

Sensory Evaluation

Sample Preparation and Descriptive Sensory Analysis

For flavor evaluation, 10 g of NDM was suspended in 100 mL of odor-free water at 40 °C and mixed by electric mixer (Biospec Products, Inc.; Bartlesville, OK) at the lowest speed for 2 min. Samples were evaluated at 20 ± 2 °C.

Liquid samples for flavor evaluation were served in Styrofoam cups equipped with plastic lids.

Descriptive analysis of flavor was conducted on skim milk powders (*13*). Panelists (n=13) were asked to identify and define flavor terms for the milk powders. Terms identified and selected by the panelists are listed in Table III. Panelists marked responses on 10-point numerical intensity scales anchored on the left with "none" and on the right with "extreme". Powders were evaluated in duplicate in a randomized block design (*14*).

Table I. Preparation of Reference Materials for Descriptive Sensory Evaluation of Nonfat Dry Milk

Descriptor	Reference	Preparation
Cooked/sulfurous	- Heated milk	- heat pasteurized skim milk to 85°C for 45 min
Caramelized/ Burnt sugar	- Autoclaved milk - Caramel syrup	- autoclave whole milk at 121 °C for 30 min. - dilute small amount of caramel syrup in skim milk
Sweet aromatic/ Cake mix	-Pillsbury-White cake mix	
Cereal/grass-like	- breakfast cereals (corn flakes, oat and wheaties)	- soak one cup cereal into three cups milk for 30 min and filter to remove cereals
Brothy/potato-like	- Kroger-Canned white potato slices	- remove the sliced potatoes from the broth
Animal/gelatin-like/ Wet dog	- Knox-unflavored gelatin	- dissolve one bag of gelatin (28 g) in two cups of distilled water
Papery/cardboard	- cardboard paper	- soak pieces of cardboard paper in skim milk overnight
Sweet taste	- sucrose	- 5% sucrose solution
Astringent	- tea	- soak 6 tea bags in water for 10 min

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Table II. Aroma-Active Compounds (Log $_3$ FD Factor ≥ 2) of Low (L), Medium (M) and High (H) Heat-Treated Fresh Nonfat Dry Milks Detected During Aroma Extract Dilution Analysis

No.	Odorant	Fraction[a]	RI[b] DB-WAX	RI[b] DB-5	Odor[c]	Log $_3$ FD Factor[a] By Heat Treatment L	M	H
1	2,5-Dimethyl-4-hydroxy 3(2H)-furanone (Furaneol®)[A]	A	2027	1057	Caramelized, burnt sugar	5	5	6
2	Butanoic acid[A]	A	1606	802	Rancid, cheesy	5	5	6
3	Methional[A]	N/B, A	1443	904	Boiled potato	5	5	6
4	o-Aminoacetophenone[A]	N/B	2218	1308	Grape, foxy	5	5	6
5	δ-Decalactone[A]	N/B	2183	1502	Burnt, sweet, fatty	5	6	5
6	Unknown	N/B	2202	1560	Cilantro	5	6	5
7	(E)-4,5-Epoxy-(E)-2-decenal[B]	N/B	2000	1392	Metallic, green	3	5	6
8	Pentanoic acid[A]	A	1756	902	Cheesy, sweaty	4	3	5
9	4,5-Dimethyl 3-hydroxy-2(5H)-furanone (sotolon)[B]	A	2204	1118	Curry, butterscotch	3	4	5
10	Vanillin (3-Methoxy-4-hydroxy-benzaldehyde)[A]	A	2540	1401	Vanilla, pudding	4	5	5
11	2-Acetyl-1-pyrroline[B]	N/B	1331	919	Popcorn	4	5	4
12	Hexanoic acid[A]	A	1834	1008	Sour, vinegar, cheesy	4	4	4
13	Phenylacetic acid[A]	A	2568	1265	Rosy	3	4	5
14	Octanoic acid[A]	A	2048	1289	Waxy, soapy, sweaty	5	3	4
15	Nonanal[A]	N/B	1384	1099	Fatty, stale, soapy	4	3	5
16	1-Octen-3-one[B]	N/B	1294	977	Mushroom	3	3	5
17	2-Acetyl-2-thiazoline[A]	N/B	1762	1107	Popcorn	4	5	3

No.	Compound	Fraction	RI	RI	Odor description				
18	γ-Dodecalactone[A]	N/B	2398	1656	Cilantro, sweet	4	4	4	4
19	(E)-2-Nonenal[A]	N/B	1532	1162	Hay, cucumber	4	3	3	4
20	(E)-2-Undecenal[B]	N/B	1722	1367	Waxy, green	4	4	3	3
21	(E,E)-2,4-Decadienal[A]	N/B	1807	1320	Fried fatty	3	4	3	3
22	Unknown	N/B	2130	1582	Fresh fishy	4	3	3	4
23	Unknown	N/B	1361	981	Fresh air, milky	3	4	4	3
24	3-Phenylpropionic acid[A]	A	>2600	1356	Sour, rose-like	2	4	4	4
25	Unknown	N/B	2108	1345	Mint, green	3	3	3	4
26	Maltol[A]	A	1978	1088	Cotton candy	3	2	2	3
27	Isobutyric acid[A]	A	1556	959	Bug, Swiss cheese	1	2	2	1
28	β-Damascenone[B]	N	1822	1370	Apple sauce	3	3	3	1
29	Unknown	A		1199	Metallic, waxy	3	1	1	2
30	Unknown	N/B		1146	Fatty, waxy	2	<1	4	4
31	(E,E)-2,4-Nonadienal[A]	N/B	1701	1218	Stale, fatty, soapy	2	3	1	1
32	2-Acetylthiazole[B]	N/B	1416	1025	Popcorn	1	2	3	3
33	2-Isopropyl-3-methoxy pyrazine[B]	N/B	1420	1091	Earthy	2	<1	4	4
34	β-Ionone[B]	N/B	1945	1488	Stale, hay	1	2	1	1
35	3-Methylindole (skatole)[A]	N/B	2477	1395	Mothball, skatole	1	1	1	2
36	(E,Z)-2,6-Nonadienal[A]	N/B	1583	1153	Cucumber	2	3	3	1
37	Acetic acid[A]	A	1431	<700	Sour, vinegar	1	1	1	2
38	Decanoic acid[A]	A	2262	1404	Fatty, soapy				

[A] Compound positively identified (RI, odor, MS), [B] Compound tentatively identified (RI, odor). [a] Fraction in which most of the compound appeared after separation in neutral/basic (N/B) and acidic (A) fractions [b] Retention indices (RI) calculated from GCO results [c] Odor description at the GC-sniffing port during GCO. [d] Average log3 of flavor dilution factors on DB-5ms column for nos. 1, 2, 6–8, 10, 12–14, 17, 29, 37, 38.

Table III. Selected Aroma-Active Compounds (Log $_3$ FD Factor $\geq$ 3) of Stored Nonfat Dry Milks

No.	Odorant	RI ^{a} WAX/ FFAP	DB-5	Odor ^{b}	Log $_3$ FD Factor ^{c} I	II	III
1	Furaneol® ^{A}	1991	1083	Caramel	8	6	6
4	o-Aminoacetophenone ^{A}	2204	1308	Foxy	6	7	7
9	Sotolon ^{A}	2164	1111	Curry	5	6	6
20	(E)-2-Undecenal ^{B}	1976	1360	Metallic	5	5	6
3	Methional ^{A}	1427	899	Boiled potato	6	4	6
26	Maltol ^{A}	1943	1091	Burnt sugar	4	6	5
11	2-Acetyl-1-pyrroline ^{B}	1311	916	Popcorn	5	7	3
10	Vanillin ^{A}	2530	1406	Vanilla	3	7	6
16	1-Octen-3-one ^{B}	1279	973	Mushroom	4	4	5
38	Decanoic acid ^{A}	2250	2013	Waxy	4	5	6
39	(E)-2-octenal ^{A}	1416	1060	Fatty	6	3	3
21	(E,E)-2,4-Decadienal ^{A}	1793	1313	Fried fatty	4	4	4
40	Benzothiazole ^{A}	1838	1269	Rubber	4	4	4
41	p-cresol ^{A}	2057	1070	Cowy/barny	4	3	4
14	Octanoic acid ^{A}	2030	1289	Waxy	4	3	4
42	Dodecanoic acid ^{A}	2438	2156	Waxy	3	4	4
43	2,3-butanedione ^{B}	970	621	Buttery	<3	<3	5
44	Nonanoic acid ^{A}	2116	1762	Waxy	3	4	3
45	Hexanal ^{A}	1060	794	Green, grass	3	<3	4
46	3-methylthiophene ^{B}	1078	774	Plastic	<3	<3	4
47	3-methylbutanal ^{A}	906	624	Sweet, fruity	<3	<3	4
32	2-acetylthiazole ^{B}	1404	1018	Popcorn	<3	4	3
48	(E)-2-nonenal ^{B}	1509	1155	Hay	5	<3	<3
17	2-Acetyl-2-thiazoline ^{A}	1743	1103	Popcorn	3	3	4
49	(Z)-2-nonenal ^{B}	1478	1147	Hay	3	<3	3
36	(E,Z)-2,6-Nonadienal ^{B}	1568	1148	Cucumber	3	<3	<3
31	(E,E)-2,4-Nonadienal ^{A}	1681	1211	Fatty, soapy	3	<3	3
50	1-hexen-3-one ^{B}	1147	775	Rubbery	3	<3	<3
35	3-Methylindole ^{A}	2468	1391	Fecal, skatole	<3	<3	3
34	β-Ionone ^{A}	1954	1482	Hay	nd	<3	<3
51	Ethyl disulfide ^{A}	1192	768	Gasoline	3	Nd	<3
52	Dimethyl trisulfide ^{A}	1360	963	Cabbage	nd	<3	<3

^{A} Compound positively identified (RI, odor, MS), ^{B} Compound tentatively identified (RI, odor). ^{a} Retention indices (RI) calculated from GCO results ^{b} Odor description at the GC-sniffing port during GCO. ^{c} Average log$_3$ of flavor dilution factors on DB-WAX column. Roman numbers represent the samples analyzed: I: 3 months old, II: 1 years old, III: 2 years old, nd: not determined.

Results and Discussion

Total aroma-active compounds of fresh and some selected compounds of stored NDMs were presented in Tables II and III respectively. Aroma profiles (FD-chromatograms) of low, medium and high heat-treated NDM samples were similar, with only a few components differing markedly in $\log_3$ FD factors among the samples (Table II). The principal flavor of NDM originates from the native volatile compounds in milk, and chemical degradations during processing and storage of the product. As shown (Table II) the number and abundance of Furaneol®, methional, sotolon, free fatty acids, lactones and maltol in all heat treatment levels were high.

Thermally induced volatiles in skim milk powder included Furaneol®, methional, 2-acetyl-1-pyrroline, 2-acetyl-2-thiazoline and 2-acetylthiazole. Some thermally induced compounds such as Furaneol®, sotolon, maltol of high heat-treated NDM had higher $\log_3$ FD factors than medium and low heat-treated NDM powders (Table II). Many of the same compounds were also identified in the samples which exhibited off-flavors (Table III). However, the odor intensities of these compounds were higher in stored samples (Table III). Schieberle (*15*) isolated Furaneol® in popcorn and bread, and stated that thermal treatment of sugars generated this compound from fructose-1, 6-biphosphate via acetylformoine as the intermediate. Other related compounds maltol and sotolon were also detected in acidic fractions. It is possible that they are the contributors of the caramelized/burnt sugar-like and sweet aromatic flavors described by sensory analysis (Table IV). Schnermann and Schieberle (*16*) identified these furanones as key odorants of milk chocolate. Furaneol® (*17*) and sotolon (*18*) were indicated as causing an off-flavor in stored citrus juices.

Cooked/sulfurous, caramelized flavors and sweet taste were the most intense attributes for all powders. High heat-treated powders exhibited more intense cooked/sulfurous and caramelized flavors than low or medium heat-treated powders (Table IV).

Cereal/grassy attribute was indicated by sensory evaluation in stored samples (Table V). Higher intensities of diacetyl, hexanal, pyrroline and thiazole in stored samples (Table II) than fresh samples (Table I) may be the source of cereal/grassy flavor. Bassette and Keeney (*8*) isolated some compounds including formaldehyde, acetaldehyde, acetone, butanone, diacetyl, hexanal and nonanal as the contributor of cereal type flavor in instant/non-instant NDMs.

Shiratsuchi and coworkers (*4*) showed that lactones were key flavor compounds of NDM. Lactones contributed to sweet and fatty flavors in milk powder (Table II).

Table IV. Flavor Attributes of Fresh Nonfat Dry Milks

Attributes	Low	Medium	High
Cooked/sulfurous	3^c	3.61^b	4.25^a
Caramelized/burnt sugar	0.75^c	1.42^b	2.25^a
Sweet aromatic/cake mix	1.05^a	0.5^b	0.16^b
Sweet	1.45^a	1.46^a	1.05^b
Astringent	0.5^b	0.65^b	1.26^a

a,b,c Means within a row without a common superscript differ ($P < 0.05$)

Table V. Flavor Attributes of Stored Nonfat Dry Milks

Attributes	Sample		
	I	II	III
Cooked/sulfurous	2.3^a	2.3^a	2.5^a
Caramelized/burnt sugar	0.1^a	0.1^a	0^a
Sweet aromatic/ cake mix	1.4^a	1.8^a	0.95^b
Cereal/grassy	0.8^a	1.2^a	0.8^a
Animal/gelatin-like/wet dog	0.8^b	0.6^b	1.3^a
Brothy/potato-like	1.5^{ab}	1.3^b	1.9^a
Papery/cardboard	1.5^a	1.4^a	1.4^a
Sweet taste	1.6^a	1.7^a	1.6^a
Astringent	1.1^a	1.3^a	1.1^a

a,b Means within a row without a common superscript differ ($P < 0.05$).
Roman numbers represent the samples analyzed: I: 3 months old,
II: 1 year old, III: 2 years old.

The other major group of compounds that contributed to NDM flavor was free fatty acids liberated by hydrolysis from naturally occurring fats. Large amounts of free fatty acids were identified in all samples (Tables II, III). Milkfat is an important source of volatile flavor compounds in dairy foods. The short-chain fatty acids (C_4-C_{12}) are main flavor contributors of cheese and other dairy products (19). High concentrations of fatty acids were reported in both fresh and stored flavored milk powders (Tables VI, VII). Propionic, isobutyric, 2-/3-methylbutyric and pentanoic acids with sweaty or Swiss cheese-like aromanotes were positively identified in acidic fractions of samples (Table II). Free fatty acids including octanoic, nonanoic, decanoic, and dodecanoic acids were described as waxy. Specifically, decanoic and octanoic acids had high log_3 FD factors in all samples (Table III). Shiratsuchi and coworkers (6) also identified the same compounds as responsible for a sweet, fatty and butter-like odor.

Table VI. Concentration Ranges (mg/100g) of Selected Key Aroma-Active Compounds in Fresh Nonfat Dry Milks

Compounds	RI On DB-FFAP Column[a]	Concentration range (mg/100g)
Butanoic acid	1604	1150-1857
Hexanoic acid	1829	501-677
Maltol	1949	2.8-10.4
δ-Decalactone	2173	0.7-2.3
γ-Dodecalactone	2353	0.2-0.5
Furaneol®	2016	0.2-0.4

[a] Retention indices (RI) calculated from Mass Spectrometry (MS) results.

Table VII. Mean Concentration of Selected Flavor Compounds in Stored Nonfat Dry Milks

Compounds	RI On DB-FFAP Column[a]	Concentration (μg/100g)[b]		
		I	II	III
Octanoic acid	2044	154000	183000	252000
Decanoic acid	2261	147000	210000	286000
Nonanoic acid	2149	6000	4000	10000
Maltol	1949	8000	12000	400
Hexanal	<1100	5940	664	5060
Dodecanoic acid	2471	200	300	700
Furaneol®	2016	500	1000	40
Ethyl disulfide	1192	217	242	294
(E,E)-2,4-Nonadienal	1683	50	375	44
Methional	1443	56	26	110
(E,E)-2,4-Decadienal	1749	33	80	14
Benzothiazole	1932	5	26	24
o-Aminoacetophenone	>2600	5	7	13
β-ionone	1926	-	3	1

[a] Retention indices (RI) calculated from Mass Spectrometry (MS) results. [b] Roman numbers represent the samples analyzed: I: 3 months old, II: 1 year old, III: 2 years old
- : not determined.

Lipid oxidation of milk leads to flavors termed oxidized, cardboard, metallic, fatty (oily), painty and fishy. The contributors of oxidized flavor in dairy foods are not well known. The alkanals and alkenals with more than six carbon atoms as well as ketones are the typical volatiles generated by lipid oxidation (4,20). (E)-4,5-Epoxy-(E)-2-decenal was identified as an aroma-active compound with a metallic, green note in NDM (Table II). This compound was also identified as character impact odorant in other dairy products including butter (21), regular-fat and low-fat Cheddar cheeses (22). 2,4-Decadienal, a product of linoleic acid oxidation (20), imparted a fried fatty odor in NDM. Some other lipid oxidation products detected by GCO were nonanal, (E)-2-nonenal, (E,E)-2,4-nonadienal and (E,Z)-2,6-nonadienal with fatty, hay-like, stale and cucumber-like odor notes, respectively (Table II). These 'oxidized' flavors were not detected by sensory analysis of powders and were likely below sensory threshold in the milks. 1-Octen-3-one (mushroom-like) has been described as having a metallic odor (21). 1-Octen-3-one was reported as a responsible compound for metallic off-flavor in milk (23). It is formed by oxidation of arachidonic acid. Hammond and Hill (24) indicated that the reaction between 1-octen-3-one and an aldehyde such as 2-heptanal, octanal, or 2,4-heptadienal, caused oxidized flavor in milk. The other off-flavor that we determined was cardboard or papery/cardboard flavor (Table V). Grosch and coworkers (25) analyzed the off-flavor compounds of butter oil. They demonstrated that a mixture of (E)-2-nonenal and (Z)-2-nonenal contributed to development of the cardboard-like off-flavor in butter oil after a long storage period at room temperature. The sniffing data in our study agreed with these results. (E)- and (Z)-2-nonenal (hay-like) and 1-octen-3-one (mushroom) were identified in neutral/basic fractions (Table III). (E,E)-2,4-Decadienal and (E,E)-2,4-nonadienal were also identified as off-flavor compounds in stored NDM (Table III) and their odor intensities were greater than in fresh NDM. Concentrations of these compounds are presented in Table VII. These compounds might be the contributors of development of papery/cardboardy off-flavor in milk powders.

o-Aminoacetophenone, with a grape-like or foxy note was detected in the neutral/basic fraction at high intensity. This compound was identified as a prominent odorant in tortilla-type corn products (26). o-Aminoacetophenone is an important compound formed in stale dry milk (27) and in stale flavor fractions of sterilized concentrated milk (28). It was also identified as an off-flavor compound in micromilled and stored (12 months) milk powder (MMP) (29). The odor intensity of o-AAP was higher in off-flavored samples (Table III). Sensory panelists described an animal/wet-dog-like off-flavor in all samples, which may be due to the presence of o-AAP (Table V).

Methional, with a boiled potato-like aroma note, was identified in both fresh and stored samples. Sensory analysis also detected potato/brothy off-flavors in NDM (Table V). Methional is the first product of Strecker degradation of

methionine (*30*). It can also be formed from methionine when milk is exposed to light (*31*). It has been reported as a flavor compound of Cheddar cheese (*22*). The higher retronasal odor activity values (OAV) of homofuraneol, methional and Furaneol® were related to the meaty-brothy odor in low-fat Cheddar cheese (*22*).

Sweet odor and taste also contributed to the flavor of NDM (Tables IV, V). Sniffing results indicated that δ-decalactone, and γ-dodecalactone gave sweet odor properties to the milk powder (Table I). Sweet and milky odor properties were characterized by lactones including γ-undecalactone, γ-dodecalactone, γ-lactone, δ-decalactone and δ-undecalactone (*6*). Vanillin was also identified at high odor intensity (Table I). It was described as a vanilla/cake mix-like aroma. Vanillin may also contribute to sweet aroma in milk powders.

Sensory evaluation demonstrated that all milk powders had an astringent taste (Tables IV, V). Astringency is a common problem in high heat-treated or UHT-sterilized milk (*32*) and NDM (*33*). Josephson and coworkers (*34*) stated that astringency in high heat-treated milk was attributed to the interaction product involving whey proteins, calcium phosphate and caseins. It was also linked to the production of γ-caseins from β-casein by cleavage of peptide bound between residues 28-29, 105-106, and 107-108 (*35*).

In conclusion, based on comparative GCO and quantification results, a variety of aldehydes, ketones, alcohols and free fatty acids were found to be responsible for development of desirable and undesirable flavors in NDM. Mainly, Furaneol®, maltol and sotolon were the sources of burnt-sugar like or curry-like flavors. Methional and *o*-aminoacetophenone had high odor intensities in NDM. Other major compounds were free fatty acids including butanoic and hexanoic acids with cheesey notes, octanoic, nonanoic, decanoic and dodecanoic acids with waxy note.

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Chapter 9

Biochemical Manipulation of Flavor in Tomato Fruit

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The top notes of fresh tomato flavor are generated when fruit tissue is macerated and the lipid oxidation cascade is activated. This cascade occurs during eating and in the preparation of fruit for processing. The changes are rapid and a real time analysis was used to follow the concentrations of lipid oxidation products in the headspace above macerated fruit. As well as allowing measurement of lipid oxidation in living tissue, the response of the lipid oxidation reaction to added substrates, enzymes or cofactors was studied. When fatty acids were added, extra C6 compounds were generated but addition of lipids was not so effective. Mixtures of carotenoids and fatty acids produced more 6-methyl-5-hepten-2-one whereas addition of the amino acid precursors of 2- and 3-methyl butanal had no significant effect. If maceration occurred under anaerobic conditions and air was subsequently introduced, enzyme activity was significantly inhibited.

The role of the lipid oxidation pathway in the generation of flavor top notes in tomato and cucumber is well-documented (*1*). Many workers have studied the reaction in vitro where much useful fundamental data has been obtained. The

activity of the various enzymes has been examined in vitro along with substrate specificity (*2, 3*). From these studies, the presence of isoforms and their relative affinity for the different substrates has been determined (*4*). The situation in vivo, however, is not so clear. Do the enzymatic conversion rates measured in vitro relate to those found in vivo (i.e. in living whole tissue)? Does the environment in vivo promote better enzyme substrate interaction and therefore accelerate the reaction rate? In order to answer these questions, it is essential to be able to measure enzyme activity in vivo. Given the availability of a real time technique to follow the generation of volatile products from lipid oxidation, it is now possible to start such studies. In this paper, we investigate the feasibility of manipulating the lipid oxidation pathway in macerated tomatoes, as a first step towards comparing in vivo and in vitro activities of some key steps.

Materials and Methods

Tomato fruits

Tomatoes were obtained from local supermarkets throughout the season in batches of 5kg and stored at ambient temperature (21-22°C), then used when they were fully red ripe. Because of the known seasonal variation, each batch was used to study just one factor (e.g. the effect of added fatty acids or the effect of maceration under anaerobic conditions) to minimize variation within that experiment. Comparisons within one factor are therefore valid but comparison across factors needs caution as the changes noted may be due to different tomato quality. The average weight of fruits was 89 ± 4g and five fruit were analyzed separately to replicate each treatment (as described below).

Maceration device and real time monitoring of volatile production

Details of the device can be found in the previous paper and in Boukobza et al. (*5*). Briefly, an intact tomato was placed in a modified blender and the headspace swept with a stream of air or nitrogen. A portion of the headspace was sampled directly into an Atmospheric Pressure Ionization Mass Spectrometer (API-MS; (*6*)) where nine volatiles (identified as important components of tomato aroma) were monitored in Selected Ion mode. The fruit was then macerated and the headspace concentrations of the nine volatiles followed for 3 min with the API-MS. Since the experiments were designed to compare the amounts of compound formed under identical conditions, the amount of each compound was expressed at the ion intensity for the selected ion monitored.

Treatments

Treatments were applied by placing a concentrated solution of the substrate or enzyme on top of the intact fruit in the blender and monitoring the headspace for a short while (30 s) to ensure no reaction was occurring. The blender was then started (2-5 s) to macerate the fruit and thoroughly mix in the added compounds. Fatty acids were added as an emulsion in Tween 20 (*5*). Estimates of total linoleic acid (18:2) and linolenic acid (18:3) concentrations in tomato were 220 and 50ug/g fresh weight (*7*). The amounts added here corresponded to an additional 400ug fatty acid per g of tissue (fresh weight). Carotenoids (2mg) were added as emulsions with linoleic acid (1g) and Tween 20 (1g) in 0.1M phosphate buffer pH7 to give 0.09ug lycopene or beta carotene per g tomato (fresh weight). The total lycopene content of tomato was calculated as 62ug/g fresh weight. Free amino acids (leucine, isoleucine, and valine) were added in a phosphate buffer pH7 at levels of 5 or 10mg/g fruit

Some maceration experiments were performed under anaerobic conditions by flushing the blender containing the intact tomato with nitrogen for 20 min and then macerating the fruit. The macerate was maintained under nitrogen for periods of 5 and 50 min after which the nitrogen supply was switched to air.

Results and Discussion

Effect of added fatty acids

Figure 1a shows the traces obtained for hexanal production on maceration of five replicate fruit with no fatty acid added (control) or with extra (400ug/g) linoleic or linolenic acid. The control fruits show generation of hexanal with a plateau value of around 1×10^5. Addition of linoleic acid (the expected precursor of hexanal) increased the amount formed to around 6×10^5 while addition of linolenic acid (the precursor of hexenals) seems to cause a slight inhibition of hexanal production, may suggest substrate inhibition. Figure 1b shows the trace for hexenal from the same experiment (API-MS is incapable of resolving isomers so the technique measures both (*E*)-2-hexenal and (*Z*)-3-hexenal). In this experiment, linolenic acid caused an increase of around four times compared to the control whereas linoleic acid had a relatively small effect on hexenal production.
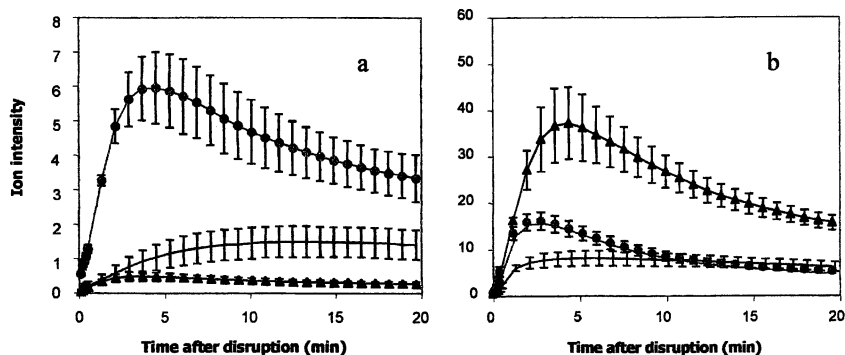
Figure 1. Headspace concentration of hexanal (a) and hexenal (b) above control macerated tomatoes (-) and above tomatoes with added linoleic acid (●) and added linolenic acid (▲). Values are the means of five replicates and the error bars show the standard deviation.

Monitoring of compounds that are not formed through the lipid oxidation pathway was also carried out in the presence of linoleic and linolenic acids. For isobutylthiazole, the amounts found in the presence of fatty acids decreased compared to the controls while, for methylbutanal, addition of both fatty acids caused a slight increase in headspace concentration (data not shown). The most likely explanation for these changes is that addition of emulsion to the macerate affects the partition of volatile compounds between the gas and liquid phase. This is a limitation of headspace sampling as it only provides an indirect measure of what is happening in the liquid phase and caution is needed when interpreting the data. Further experiments to measure the change in headspace caused by emulsions and methods to estimate the actual amounts formed in the tissue from the measured gas phase concentration are underway.

Maceration under anaerobic conditions

As mentioned in the Introduction to this paper, the ability to follow the lipid oxidation pathway on line allows an investigation of the sequence of reactions and their interdependence. The first step is lipolysis where the co factor for lipase is water but the next step, lipoxygenase, requires molecular oxygen to form hydroperoxides. These are then broken down by lyases (no cofactors) to give the C6 aldehydes in the case of tomatoes, as the lyase is specific for the 13-hydroperoxides. If tomatoes are macerated with no oxygen present, how does this affect the lipid oxidation pathway? This question has fundamental as well as practical implications as tomatoes destined for processing are often stored in

large containers where oxygen can become depleted. To study the effect, intact tomatoes were placed in the blender and kept under nitrogen for 5 and 50 min. During this period, the headspace was monitored but no detectable trace of C6 compounds could be seen. After the appropriate time under nitrogen, air was introduced into the system and the amounts of hexanal and hexenal produced compared to a control set from the same batch of fruits which had been kept and macerated in air (Figure 2).
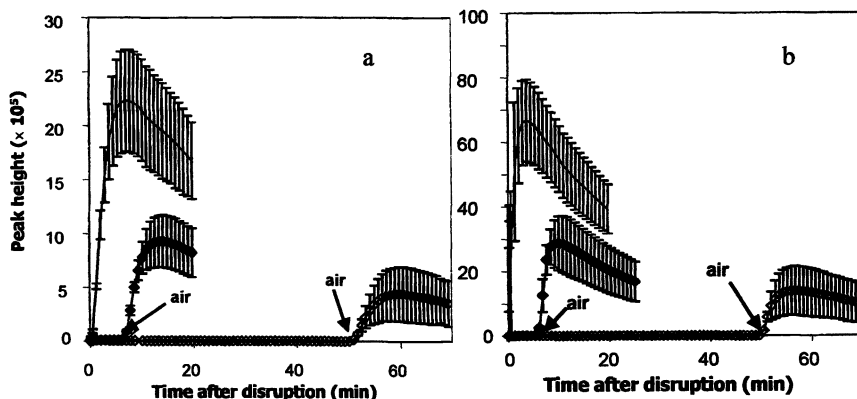
Figure 2. Release of hexanal (a) and hexenal (b) from tomatoes macerated under nitrogen, macerate held for 5 or 50 min and then exposed to air. Values are the means of five replicates and the error bars show the standard deviation

The results show that maceration under nitrogen produces no C6 compounds and the amounts formed when air is introduced into the system are decreased compared to control fruits. Also, the longer the time the macerated fruit is held under nitrogen, the greater the effect. This effect is specific to the products of the lipid oxidation pathway as other compounds showed no change in release pattern when held under nitrogen. A possible explanation for this observation is that the free radical part of the lipid oxidation pathway occurs but, in the absence of oxygen, the radicals cannot react along the usual path. Instead they react with whatever is at hand and this may be the active site of the enzyme which becomes changed to such an extent that it has reduced (or no) activity.

This explanation was supported by further work where additional fatty acids were added to the macerate after the macerate had been held under nitrogen. The hypothesis was that, if the enzyme had been damaged, no additional C6 compounds would be formed in the presence of extra fatty acid (Figures 1a and b show it is substrate not enzyme that is limiting in the production of the C6

compounds). This was indeed the case with no change in the amount of the C6 aldehydes suggesting that it was lack of enzyme (i.e. damage to the enzyme) that was the root cause of the effect.

Coupled lipid-carotenoid oxidation

6-Methyl-5-hepten-2-one has been identified as a carotenoid-derived aroma compound in tomato. It has been proposed that it is formed by coupled lipid oxidation and thus should be formed on maceration. To test this, batches of tomatoes were macerated with extra linoleic acid containing either lycopene or beta-carotene and the amounts of 6-methyl-5-hepten-2-one formed were compared with a batch of control fruits. Figure 3 shows the results where an immediate increase in 6-methyl-5-hepten-2-one is observed when extra fatty acid is added.
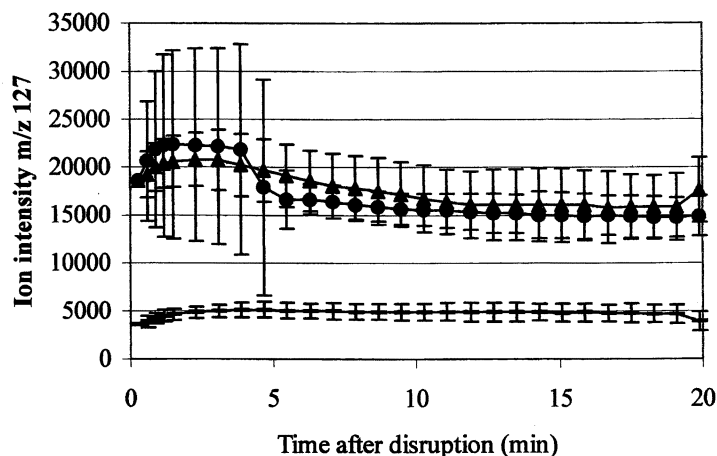
Figure 3. Headspace concentration of 6-methyl-5-hepten-2-one above control macerated tomatoes (-) and above tomatoes with added lycopene (●) and added beta-carotene (▲). Values are the means of five replicates and the error bars show the standard deviation.

This not only shows that the compound can be generated on maceration but it confirms the origin of this compound. The amounts of added carotenoid were small (0.09ug/g fresh weight) compared to the total lycopene content (62 ug/g) but much of the lycopene is present as crystals within the chromoplasts and is therefore not available for reaction.

Addition of amino acids and lipids

Leucine, isoleucine and valine are precursors for flavor compounds formed through a type of Strecker degradation, a process that commonly takes place in heated foods. Tomatoes were macerated in the presence an absence of 5 or 10 mg of amino acid (presented singly) and the volatile profile measured. Despite monitoring for extended periods of time, no significant changes in the methyl butanals or methyl butanols were observed, suggesting that this biosynthetic pathway operates in intact tissue rather than the macerate.

Addition of various lipids (triglycerides, phospholipids and galactolipids in a Tween 20 emulsion) caused no increase in C6 compounds. This is interesting considering the significant increases seen when fatty acids were added. The results may tell us something about the location of the enzymes and substrates in vivo. One hypothesis is that lipase is membrane bound and therefore access of added lipids is difficult while lipoxygenase is soluble and can interact readily with added fatty acids.

Summary

The data presented here demonstrate that the maceration device, coupled to the API-MS allows analyses of dynamic flavor formation through lipid oxidation in vivo. It can be used to study the relative rate of the enzyme cascade at those points where volatile compounds are formed to act as markers. Thus the activity of the lipoxygenase-lipase part can be monitored by the formation of C6 aldehydes and the alcohol dehydrogenase step through the formation of alcohols. By adding extra substrates the activity of each step can be partly decoupled from the overall lipid oxidation cascade (e.g. by adding fatty acids and measuring extra C6 generation, the activity of the lipoxygenase-lipase step can be determined). It should also be possible to compare enzyme activities in vivo and in vitro by using one half of a batch of tomatoes to obtain enzyme extracts and the other half for in vivo measurements. Given the speed of the API-MS technique, the chances of the fruits changing composition due to respiratory activity can be minimized. The use of the technique to screen large numbers of tomato fruits to provide data on pre- and post-harvest treatments is described elsewhere in this book.

Acknowledgements

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Chapter 10

Effect of Pre- and Post-Harvest Treatments on Fresh Tomato Quality

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To measure pre- and post-harvest effects on the flavor of individual tomato fruits, a controlled maceration device was coupled directly to an Atmospheric Pressure Chemical Ionization Mass Spectrometer to follow the release of nine volatiles associated with fresh tomato flavor. Throughput was rapid (100 fruit per day) and reproducibility ranged between 1.7 and 28%. The effect of pre-harvest treatments (variety, nutrients, season) on volatile content showed both varietal and seasonal effects on the amounts of some volatiles but the nutrient treatments used had no significant effect. Typical post-harvest factors (storage temperature and atmospheric composition) were applied to shop-bought fruits. Refrigerated storage caused an irreversible decrease in most volatiles tested as did short term high temperature storage (45°C for 15h). For lipid-derived volatiles, the decrease was due to lower enzyme activity within the lipid oxidation pathway.

Tomatoes are second only to potato in terms of world tonnage produced per year. Fresh tomato production has become highly organized involving the breeding of appropriate varieties, intensive production and refrigerated transport to the point of sale. These developments have increased yield per hectare as well as producing fruits which are of uniform color and size and have better resistance to softening. However, there have also been complaints about the lack of flavor in tomatoes. Previous work has identified the key aroma compounds in fresh tomatoes (*1, 2*) along with their biochemical origins. The precursors can be amino acids, carotenoids, sugars or lipids. It is generally true that aroma compounds from the first three classes are formed during the ripening period and are present in the fruit prior to eating. The lipid derived volatile compounds are actually generated when the fruit is macerated allowing enzyme and substrate to mix. The reaction is rapid with detectable amounts of various C6 compounds formed in a period of 10 to 30 seconds. In the analysis of tomato flavor, there is usually a maceration step followed by a set time period (to allow generation of the lipid-derived volatiles) before the flavor compounds are sampled and analyzed.

Tomato flavor analysis is characterized by other problems. One is that these climacteric fruit show rapid changes in their biochemistry during ripening and there are significant day to day changes. The other problem is that there appears to be considerable fruit to fruit variation (*3*). While this is often overcome analytically by macerating several fruits together and measuring the mean flavor composition, in practice, it can mean that the consumer is faced with variable quality in a batch of tomatoes even though the average quality is acceptable.

One of the limiting factors is the speed at which analysis of tomato flavor can be carried out. Typically, extraction is followed by GC-MS and a sample takes around 1 h to analyze. Thus daily throughput is limited to 6-8 fruit per GC-MS. Given the fruit to fruit variation, this throughput means that two different samples can be compared in one day (assuming 3 to 4 replicates of each sample). This analytical constraint has hampered work on fruit quality and limited the types of experiment that could be carried out and successfully analyzed. To address this problem, our laboratory has developed real time analyses using direct MS with no chromatography (*4*) and our method is based on Atmospheric Pressure Chemical Ionization Mass Spectrometry- (APCI-MS; (*5*)). APCI-MS has been combined with a controlled maceration device so that tomato analysis can be carried out rapidly with a throughput of around 100 fruits per day per MS. This opens new opportunities for studying the tomato crop pre- and post-harvest. This paper describes the technique and how it has been used to study both pre-harvest factors (effect of variety, growing conditions, seasonal effects) and some post-harvest factors (storage temperature and modified atmospheres) on the flavor volatiles of tomato fruits.

Materials and Methods

Maceration device

The maceration device and details of its operating characteristics have been described previously (*6*). In brief, a food blender was adapted so that the headspace could be swept with a carrier gas to transport the volatiles released from the tomato fruit to the sampling port of the API-MS (see Figure 1). A single fruit (about 50-80g) was placed in the blender, the lid sealed and headspace sampled into the API-MS. This gave the background level of volatile compounds above the intact tomato. The fruit was then macerated and the headspace concentration of nine selected compounds (see Table 1) monitored for periods of 2 to 5 min. The APCI-MS was calibrated with standards of known concentration and the headspace concentrations expressed as mg/m^3. The blender was equipped with a port through which solutions of substrates or enzymes could be added to study their effect on tomato flavor generation *in vivo*.
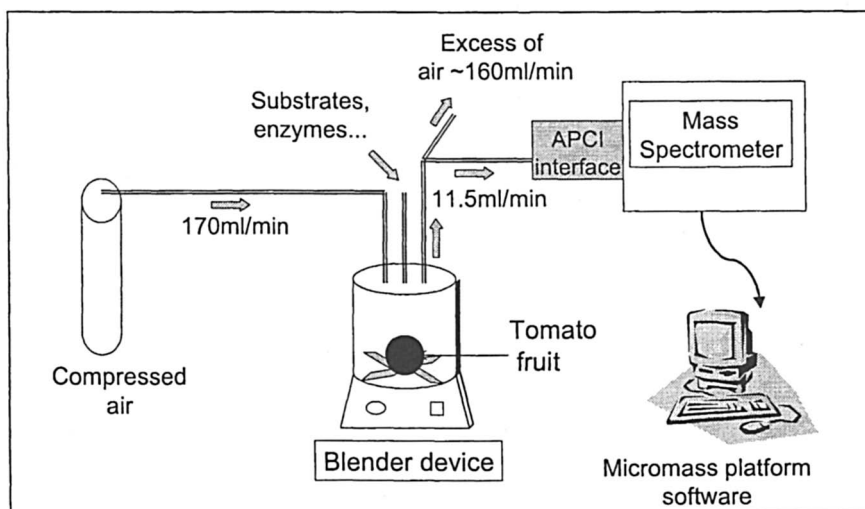
Figure 1. Schematic view of the modified blender, gas flows and APCI-MS

Tomato fruits

Seven varieties were grown (Plum, Espero, Aranca, Yellow cherry, Nectar, Solairo and Santa) by Horticulture Research International under commercial glasshouse conditions in a hydroponic system. To apply nutrient stress to the

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plants (var. Solairo and Espero), roots were split into two separate hydroponic streams so stress (addition of salt) could be applied to the plant solely on one half of the root (T3) or alternatively to one or the other half (T4). These treatments were compared to control plants with a single root using a control nutrient solution (control EC, T1) and to plants with a divided root using a control nutrient solution (T2). The plants were grown from May to September.

Table 1. Volatile compounds monitored by APCI-MS from macerated tomato fruits. "APCI ion" records which ion was used to monitor each compound. "Minimum amount detected" was calculated assuming a signal to noise ratio of 5:1 was needed for quantification. Comparison of this column with the odor threshold values indicates how well the APCI-MS analysis correlates with the sensitivity of the human nose.

Compound	Molecular weight (Da)	APCI ion (m/z)	Minimum amount detected (ug/m^3)	Odor threshold (ug/m^3)
Hexanal	100	101	420	40
Hexenal	98	99	12.3	0.09-480
Hexenol	100	83	16.7	4-16
Methylbutanal	86	87	161	3-6
Methylbutanol	88	71	1.0	100-200
Isobutylthiazole	141	142	2.7	No data
6-Methyl-5-hepten-2-one	126	127	1.9	300-500
Ethanol	46	47	1.7	20-76000
Acetaldehyde	44	45	2.0	41

Fruit were handpicked at point 4-5 on the HRI color scale (red fruit but still not fully ripe) with a weight range of 60 to 80g. Fruit were transported and stored under ambient conditions (21-22°C). Some fruit were stored at 4-6°C for 3 days and then allowed to recover at ambient temperature for 1 or 3 days prior to analysis. Another batch of fruit was stored at higher temperatures (35°C for 6h and 45°C for 6 or 15 h) and then allowed to recover at 21-22°C for 1 or 3 days prior to analysis. An atmosphere of nitrogen was used to store fruits for periods of 2, 6, 10, 15 and 35 hours followed by a recovery period between 4 and 6h under ambient conditions.

Results and Discussion

Maceration system for real time monitoring of volatile release from tomatoes

The APCI-MS was set to record simultaneously the ions listed in Table 1 and typical traces are shown in Figure 2. Since the technique resolves solely on a mass basis, resolution of isomers is not possible and "hexenal" represents the sum of *E*-2- and *Z*-3-hexenals, while methylbutanal(ol) is the sum of the 2- and 3-methyl isomers. For some compounds, there was a rapid rise in signal on maceration which represented release of pre-formed compounds upon maceration of the tomato tissue. For other compounds, the slow rise to a maximum value may be the result of slow, continuing enzyme activity (e.g hexenol) or slow equilibration of a hydrophilic compound between the liquid and gas phases of the system (e.g. ethanol).
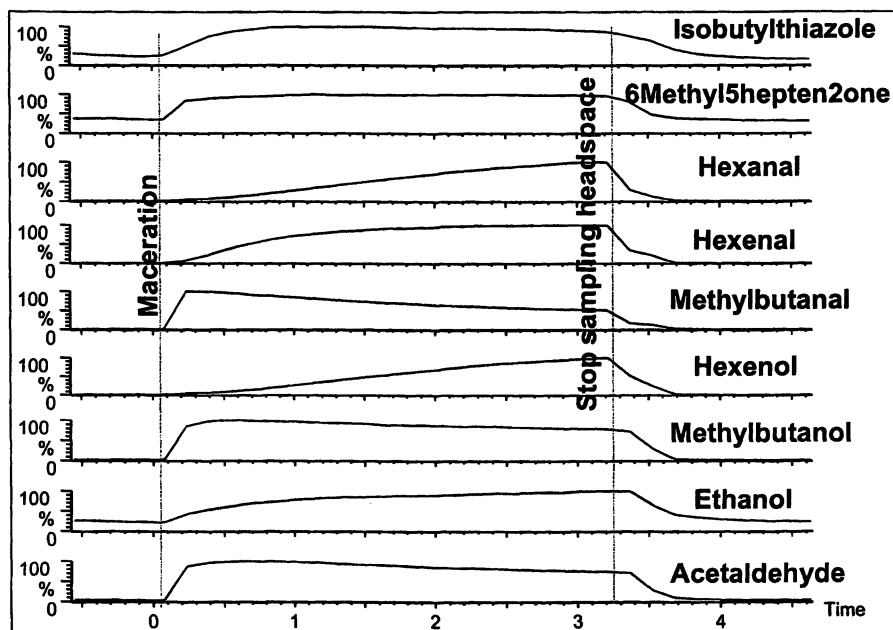
Figure 2. Relative concentrations of selected volatiles in the headspace above a tomato that was macerated at time 0min. Sampling and analysis conditions as described in Materials and Methods

The reproducibility of the device was tested using triplicate fruit portions cut from three large beef tomatoes. Table 2 shows the results with variation

ranging from 1.7% to 28% and a mean value of 13%. Previous work has demonstrated large variations in flavor composition between individual fruits. Because tomato fruits are harvested over periods of several months, this variation is not surprising as environmental factors (amount of sun, temperatures) will be different for each time period. In commercial glasshouses, the main stems become longer with each cropping and there may be changes in the amounts of nutrients that reach those fruits that crop late in the season.

Table 2. Variability in amounts of volatiles released from triplicate portions of three beef tomatoes . Values are the percentage coefficient of variation

Compound	Tomato 1	Tomato 2	Tomato 3
Hexanal	7.8	18	28
Hexenal	4.6	19	26
Methylbutanal	7.7	13	9.1
Hexenol	7.5	16	25
Methylbutanol	1.6	15	12
6-Methyl-5-hepten-2-one	3.3	13	6.6

The main advantage of the maceration method is that it allows rapid throughput of fruits (100 per day is possible) with variation that is acceptable given the fruit to fruit variation. For comparison of volatile compound release between fruits, it is valid to compare the release traces as the same sampling and analytical conditions were used. The maceration method relies on analysis of volatile compounds in the headspace, so the amounts measured are the net result of volatile generation and/or release as well as dilution in the gas which sweeps the volatiles out of the blender. Although calibration was carried out using solutions of authentic compounds to calculate the gas phase concentrations (in mg/m^3), it is not easy to relate these headspace concentrations to the concentrations in the macerate as the mass transfer conditions from tomato tissue to the aqueous phase are not known. To estimate the amount of volatile compound present in the aqueous phase, release of the target volatile compounds could be measured from aqueous solutions of the volatiles under the same conditions as used for tomato fruit. Then, assuming that the tomato slurry-to-air partition coefficient is the same as the air-water partition (K_{aw}) and that all the volatile is in solution, it should be possible to produce an estimate of actual volatile compound in the tissue, a value that is useful when considering biochemical conversion.

Effects of variety, nutrient stress and season on flavor profiles from tomato fruits

To make comparisons of the amounts of volatile compounds released from the different fruit samples, the maximum concentration of seven compounds was measured. Figures 3a and 3b show the maximum amounts of volatiles in the headspace after maceration, for the low concentration compounds (Fig 3a) and the higher concentration compounds (Fig 3b). These fruits were harvested on the same day from the same glasshouse trial on the same site, thus they should have experienced very similar environmental and nutritional growing conditions and the hypothesis is that the differences are due solely to variety.
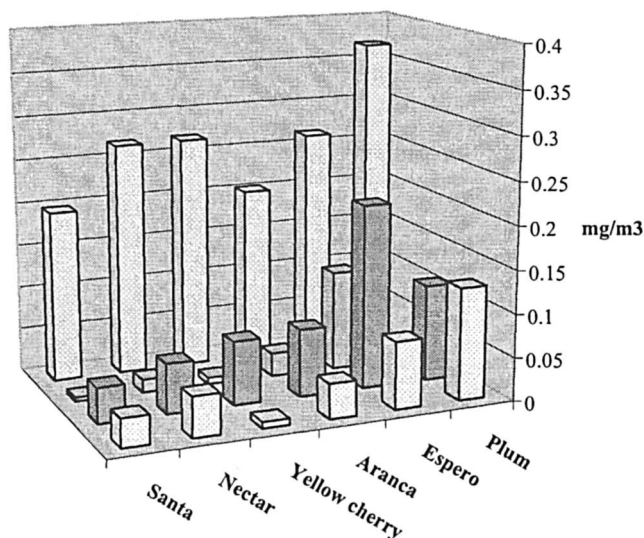
Figure 3a. Maximum amounts of volatile compounds released from different tomato varieties, grown and analyzed under identical conditions. From back to front hexenol, methylbutanol, isobutylthiazole and 6-methyl-5-hepten-2-one.

Figure 4 shows the release of hexanal from fruits (var. Solairo) harvested over the season from May to September as well as the effect of the different nutrient stress treatments. The behavior of hexanal typified the response of the
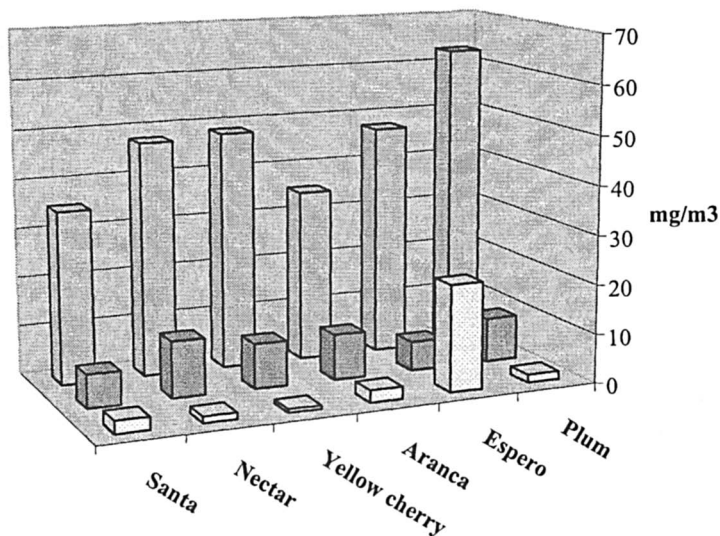
Figure 3b. Maximum amounts of hexanal (back row), hexenals (middle row) and methylbutanals (front row) released from different tomato varieties, grown and analyzed under identical conditions
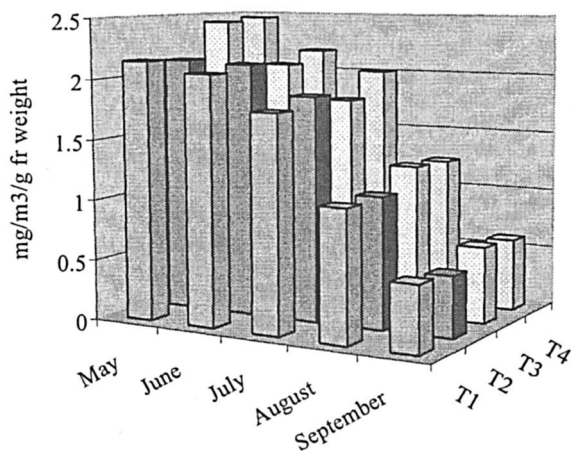
Figure 4. Maximum amount of hexanal released for Solairo grown under four different treatments: T1 (single slab, low control EC), T2 (split root, low control EC), T3 (split root, low/high EC) and T4 (split root, EC pulse).

other compounds over the season, namely a steady decrease in volatile release was observed. Since fruit weight varied over the season, the data in Figure 4 are expressed per g of tomato (fresh weight basis). There was no significant effect of nutrient treatment.

Post-harvest effects

Anaerobic conditions

Tomato fruits were stored under nitrogen for different times and then allowed to recover for 4-6 h. Under these anaerobic conditions, the maximum amount of ethanol found in the headspace increased steadily with anaerobic storage time while the amount of hexanal decreased steadily (Figures 5-6).
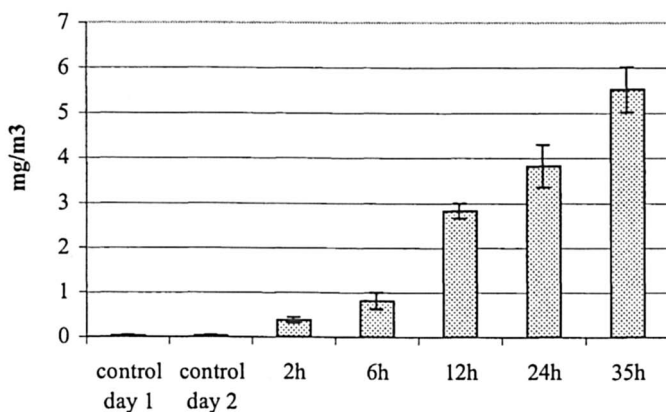
Figure 5. Maximum amount of ethanol released after different times of storage under nitrogen in tomato fruits allowed to recover for 4-6 h. Data are the mean of 5 replicates and error bars are ±SD.

Because the flavor composition of tomato fruits changes rapidly, control fruit were included for treatments up to 12h (control 1) and for treatments over 12h (control 2). These experiments show that anaerobic conditions have significant effects on the amount of ethanol present in the fruit (this might be a marker for poor storage conditions) but prolonged anaerobic storage (24h) is necessary to halve the hexanal content. Similar trends were seen for the other volatile flavor compounds in tomato.
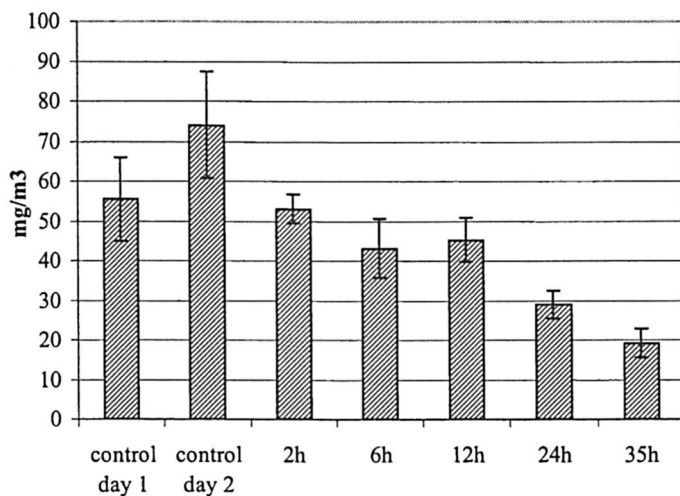
Figure 6. Maximum amount of hexanal released after different times of storage under nitrogen in tomato fruits allowed to recover for 4-6 h. Data are the mean of 5 replicates and error bars are ±SD.
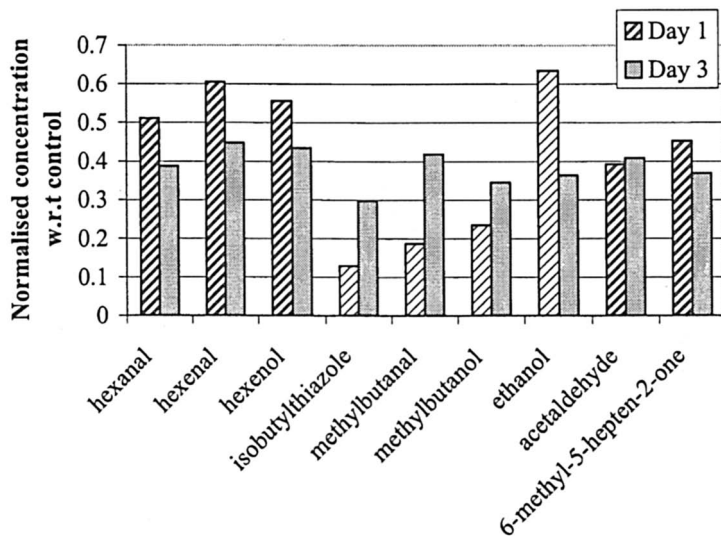
Figure 7. Effect of refrigerated storage (6°C for 3 days) on tomato fruits allowed to recover 4-6 h (Day 1) and 72 h (Day 3). The release of volatiles is expressed as the ratio of the maximum concentration for test fruits/control fruits.

Effect of storage temperature

Storage at 6°C for 3 days was followed by a period of 1 or 3 days at ambient temperature to study whether recovery took place. Again, because the experiments were carried out over 6 days, control fruit from the same batch were stored at ambient temperature and analyzed each day. The changes in volatile flavor compounds were expressed as a ratio of the test fruit content to the control fruit (a value of 1 indicates no change, <1 a decrease in the test fruit >1 indicates an increase in the test fruit). Figure 7 summarizes the changes for the nine compounds monitored. All the ratios were <1 showing a decrease in volatile content in the refrigerated fruits. Allowing fruits to recover for 1 or 3 days did not change the ratio significantly, with all fruit still showing a decreased volatile content.
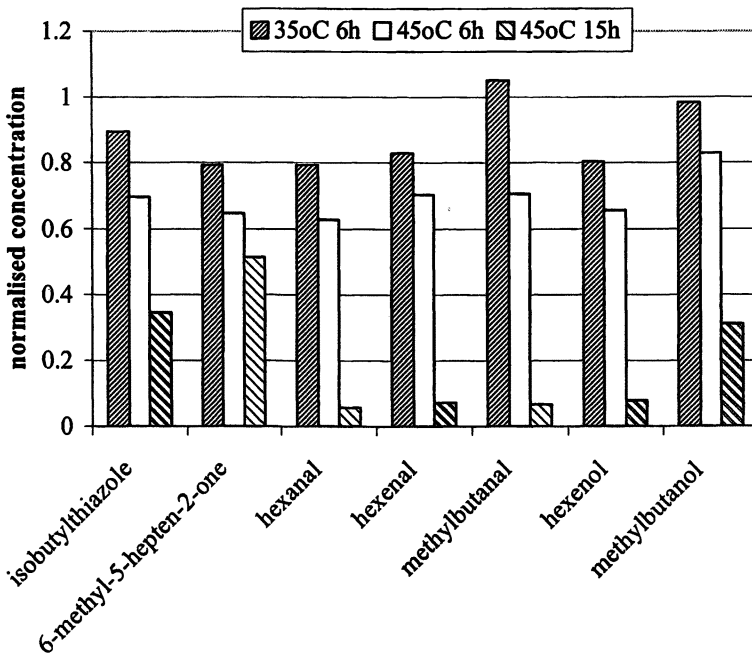
Figure 8. Effect of storage at elevated temperatures on tomato fruits allowed to recover for 4-6 h. Release of volatiles is expressed as the ratio of the maximum concentration for test fruits/control fruits

Summary

The results presented in this paper show the maceration technique can provide data on large number of tomato fruit and help build a full picture of the effects of pre- and post-harvest treatments on tomato fruit volatile compounds. From the experiments reported, it is clear that acceptable conditions for storage of tomato fruits could be determined by analyzing fruit over a wider range of conditions.

Acknowledgements

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Summary

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Chapter 11

Aroma of Fresh Field Tomatoes

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Although the flavor of tomatoes has been extensively studied over decades (*1, 2, 3*) it is not yet clear whether all odorants that are important contributors to fresh tomato aroma have been identified or whether the contribution of others has been overestimated. For the identification of the character impact odorants of fresh field tomatoes the method of aroma extract dilution analysis (AEDA, *4*) was applied to several different tomato cultivars. This resulted in finding 20 odorants with flavor dilution (FD)-factors larger than 1, which seem to contribute to the flavor of fresh tomato. Among these were (Z)-3-hexenal, hexanal, 1-penten-3-one, 2-phenylethanol, (E)-2-hexenal, phenylacetaldehyde and β-ionone, but also β-damascenone, 4-hydroxy-2,5-dimethyl-3-(2H)-furanone (Furaneol®), methional, 1-octen-3-one, guaiacol, (E,E)- and (E,Z)-2,4-decadienal and *trans*- and *cis*-4,5-epoxy-(E)-2-decenal, some of which have been underestimated so far. One previously unknown flavor compound in fresh tomato aroma was identified as (Z)-1,5-octadien-3-one. Different methods of sample preparation were employed. Solvent assisted flavor evaporation (SAFE, *5*) was found to be the most suitable method for the quantification of tomato odorants. The concentrations of all important odorants were determined in two different tomato cultivars and the odor units (ratio of the concentration of a flavor compound and its

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odor threshold) were calculated. The biggest differences between the two cultivars were found in the concentrations and odor units of methional, phenylacetaldehyde, 1-penten-3-one and 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone.

Introduction

The volatiles of fresh tomatoes have been investigated since the 1960s. About 400 compounds have been identified thus far (*6*). Not all of the volatiles actually contribute to the flavor of a product. The compounds that do contribute to fresh tomato flavor were first investigated in detail by Buttery and coworkers at the U. S. Department of Agriculture in the late 1980s and early 1990s. Buttery et al. identified 16 major contributors to fresh tomato aroma whose concentrations exceeded their odor thresholds (Table I, *7*). Buttery and coworkers were able to simulate the aroma of fresh tomato by mixing 10 of those 16 odorants in the concentrations found in fresh tomato (Table II, *8*). This model was very good and came very close to the aroma of fresh tomato, but still it was not perfect.

In the late 90s other researchers investigated fresh tomato aroma. They applied the method of aroma extract dilution analysis (AEDA, *4*) and mentioned some additional odorants, which might be important contributors to fresh tomato aroma (*2, 3*). In contrast to Buttery (*1, 7*), Krumbein and Auerswald (*2*) found that compounds such as 1-octen-3-one, methional, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and (*E,E*)-2,4-decadienal had quite high flavor dilution (FD)-factors (Table III). Other compounds, i.e., 2-isobutylthiazole, 6-methyl-5-hepten-2-one and (*Z*)-3-hexenol, had only small

Using the same method of AEDA, Guth and Grosch (*3*) found, apart from (*Z*)-3-hexenal, also acetic acid, methional, 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone, eugenol and *trans*-4,5-epoxy-(*E*)-2-decenal to be important contributors to fresh tomato aroma (Table IV). They also did not mention 2-isobutylthiazole, 6-methyl-5-hepten-2-one, (*Z*)-3-hexenol or methyl salicylate amongst the important odorants.

There are still some questions about the aroma of fresh tomatoes that have to be answered: Has the contribution of some odorants to fresh tomato aroma been overestimated? Have other odorants been underestimated, neglected or even overlooked? To answer these questions we investigated the flavor of fresh tomatoes again. The objectives of this investigation were the following:

- Identification of impact aroma compounds of fresh tomato
- Comparison of the concentrations of these odorants in different tomato cultivars
- Correlation of the analytical results with sensory results

FD-factors indicating that they are probably not as important as previously thought.

Table I. Most important odorants of fresh tomato

Compound	Log (Odor Units[a])
(Z)-3-hexenal	4.7
β-ionone	2.8
hexanal	2.8
β-damascenone	2.7
1-penten-3-one	2.7
3-methylbutanal	2.1
(E)-2-hexenal	1.2
2-isobutylthiazole	1.0
1-nitro-2-phenylethane	0.9
(E)-2-heptenal	0.7
phenylacetaldehyde	0.6
6-methyl-5-hepten-2-one	0.4
2-phenylethanol	0.3
(Z)-3-hexenol	0.3
3-methylbutanol	0.2
methyl salicylate	0.1

[a] Odor Unit = Concentration / Odor Threshold in water

SOURCE: Reproduced from reference 7. Copyright 1989 American Chemical Society.

Table II. Compounds used for simulating the aroma of fresh tomato

Compound	c (ppm) in H_2O
(Z)-3-hexenal	3.5
β-ionone	0.01
hexanal	0.6
1-penten-3-one	0.2
3-methylbutanal	0.2
(E)-2-hexenal	0.16
2-isobutylthiazole	0.01
6-methyl-5-hepten-2-one	0.1
(Z)-3-hexenol	1.5
methyl salicylate	0.05

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Table III. Most important odorants of fresh tomato as determined by aroma extract dilution analysis (AEDA)

Compound	Flavor Dilution (FD) - factor
(Z)-3-hexenal	4096
hexanal	2048
1-octen-3-one	1024
methional	256
unknown (RI on Supelcowax ca. 1370)	256
3-methylbutanal	128
1-penten-3-one	128
2-methyl-4-pentenal	64
unknown (RI on Supelcowax ca. 1230)	64
unknown (RI on Supelcowax ca. 1500)	64
4-hydroxy-2,5-dimethyl-3(2H)-furanone	64
unknown (RI on Supelcowax ca. 1400)	32
unknown (RI on Supelcowax ca. 1530)	32
(E,Z)-2,6-nonadienal	32
phenylacetaldehyde	32
(E,E)-2,4-decadienal	32
(E)-2-hexenal	16
linalool	16
methyl salicylate	16

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Table IV. Most important odorants of fresh tomato as determined by
aroma extract dilution analysis (AEDA)

Compound	Flavor Dilution (FD) factor
(Z)-3-hexenal	1000
acetic acid	100
methional	100
4-hydroxy-2,5-dimethyl-3(2H)-furanone	100
eugenol	100
(E)-2-hexenal	10
2-/3-methylbutyric acid	10
2-phenylethanol	10
trans-4,5-epoxy-(E)-2-decenal	10
5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone	10
unknown (RI on FFAP 2260)	10

Experimental Section

Tomatoes

Tomatoes, grown on a farm in the San Joaquin Valley, CA in 2000 and 2001, were picked regularly during the months of August through October.

Chemicals

All of the chemicals and most of the odorants were obtained commercially (Aldrich, Milwaukee, WI; Bedoukian, Danbury, CT). A few odorants had to be purified or synthesized according to the literature. (Z)-3-hexenal (50% in triacetin) was purified by preparative gas chromatography (Varian 3700 GC, Walnut Creek, CA) using a glass packed column (250 x 0.5 cm, i.d., packed with 1% Carbowax 20M on 120-140 mesh Chromosorb G). (E,E)- and (E,Z)-2,4-decadienal were purified by thin layer chromatography of the commercially available isomer mixture on silica 60 TLC plates with UV-indicator HF-254 (20 x 20 cm) using n-pentane/diethyl ether (7/3, v/v). Isomers were recovered by scratching off the two bands, rinsing the silica gel with diethyl ether and filtration. Trans- and cis-4,5-epoxy-(E)-2-decenal were synthesized by epoxidation of (E,E)- and (E,Z)-2,4-decadienal, respectively, using 3-chloro-

peroxybenzoic acid (9). (Z)-1,5-octadien-3-one was synthesized following procedures described by Swoboda and Peers (10). Briefly, (Z)-3-hexenol was oxidized to (Z)-3-hexenal with pyridinium chlorochromate. The resulting (Z)-3-hexenal was reacted with vinyl magnesium bromide to yield (Z)-1,5-octadien-3-ol. The latter alcohol was oxidized with pyridinium chlorochromate to (Z)-1,5-octadien-3-one. The target compound was finally purified by preparative gas chromatography on the same system as mentioned before.

Sensory Evaluation

Training of the Sensory Panel

The sensory panel consisted of 12 panelists (5 women and 7 men between the ages of 30 and 50) recruited from the Western Regional Research Center in Albany, CA. Most assessors had previous sensory panel experience. Nevertheless, all panelists were trained during a two-week period in daily sessions to make them familiar with different odor qualities and all of the important flavor compounds of fresh tomato. In the first session the panelists were asked to describe the smell of a fresh tomato in their own words using flavor attributes and odor qualities that came into their minds when smelling a fresh tomato sample. Of those odor qualities the most often mentioned ones were chosen for the following flavor profile analyses: sweet, green/grassy, fruity, floral/flowery, sour/acidic and smoky. Aqueous solutions of all important fresh tomato odorants at concentrations 100 times over their odor threshold in water (11) were presented together with an odor description to the panelists to get them acquainted with the odor qualities expected from a fresh tomato. The sample solutions together with their odor description were presented to the panelists, six at a time, at four sessions on four consecutive days. The panelists had to recognize these odors in the following sessions without being given the odor description. They had to recognize six odors at a time in five sessions on five consecutive days.

Flavor Profile Analysis

After completion of this training the panelists had to evaluate the flavor profiles of different freshly picked field tomatoes, about six times per season. The panelists were asked to rate the odor qualities they had chosen before (sweet, green/grassy, fruity, floral/flowery, sour/acidic and smoky) on a category scale from 0 (not perceptible) to 3 (strongly perceptible) in increments of 0.5, nasally and retronasally (smell and taste). They also had to rank the

tomatoes in order of preference from 1 (most preferred) to 5 (least preferred). The tomatoes were freshly cut in eighths immediately prior to evaluation and four pieces of each cultivar were presented in a glass or styrofoam container covered with aluminum foil.

Sample Preparation

Four tomatoes were cut in eighths and one eighth of each tomato was taken and weighed. The total amount of the four tomato pieces for analysis was about 35 g. The tomato pieces were blended for 30 sec in a Waring blender. The blended tomatoes were allowed to stand at room temperature for 3 min to give the enzymes time to generate some of the flavor compounds. After 3 min the enzyme activity was stopped by addition of saturated calcium chloride solution (volume [mL] = weight of tomato sample [g]) and blending for 10 sec (calcium chloride and SAFE method, see below) or by addition of 240 g of anhydrous sodium sulfate and thoroughly mixing by blending and by stirring with a glass rod (sodium sulfate method, see below). 1 mL of an aqueous solution of 3-hexanone, 2-octanone, anethole and maltol (internal standards) in a concentration range between 10 and 100 ppm was added to the mixture which was then blended again for 10 sec.

Calcium Chloride Method (CaCl₂ method)

The mixture of blended tomatoes, saturated calcium chloride solution and internal standards was added to a 1 L round bottomed flask. A Tenax trap (10 g of Tenax in a glass column 14 x 2.2 cm) was attached to the flask and an all Teflon diaphragm pump was connected (via Teflon tubing) after the trap. The system was flushed with nitrogen for 2 min and then the loop was closed by connecting the outlet of the pump to the 1 L flask. The pump circulated nitrogen through the system for three hours. The Tenax trap was removed and rinsed with 60 mL of diethyl ether. The eluate was concentrated to a final volume of about 100 μl using a Vigreux column (15 x 1 cm) and water bath at 40°C. The extract was then used for GC-O, GC-FID and GC-MS analyses.

Sodium Sulfate Method (Na₂SO₄ method)

The method is very similar to that described previously for the quantification of Furaneol® in tomatoes (12). The mixture of the blended

tomatoes, sodium sulfate and internal standards was added to a glass column (30 x 3 cm). A Tenax trap (10 g of Tenax in a glass column 14 x 2.2 cm) was attached and an all Teflon diaphragm pump was connected (via Teflon tubing) after the trap. The system was flushed with nitrogen for 2 min and then the loop was closed by connecting the outlet of the pump to the other end of the glass column. Nitrogen was pumped through the system for 3 h. The Tenax trap was removed and rinsed with diethyl ether (60 mL). The eluate was concentrated to a final volume of about 100 µl using a Vigreux column (15 x 1 cm) and water bath at 40°C. The extract was then subjected to GC-O, GC-FID and GC-MS analyses.

Solvent Assisted Flavor Evaporation (SAFE method, 5)

The mixture of blended tomatoes, saturated calcium chloride solution and internal standards was added to the dropping funnel of the SAFE apparatus, which was heated to 40°C with a circulating water bath. The distillation flask (500 mL) was heated to 40°C in a water bath. The receiving flask for the distillate as well as the safety-cooling trap of the SAFE apparatus were cooled with liquid nitrogen. The SAFE apparatus was connected to a high vacuum pump (<0.01 Pa) and then the mixture in the dropping funnel was added in small aliquots into the distillation flask over 20 min. The distillate was thawed at room temperature and then extracted with diethyl ether (2 x 30 mL). After addition of brine (25 mL) the distillate was again extracted with diethyl ether (2 x 30 mL). The combined ether extract was dried over anhydrous sodium sulfate and then concentrated to about 100 µL using a Vigreux column (15 x 1 cm) and water bath at 40°C. The extract was used for GC-O, GC-FID and GC-MS analyses.

Quantification of Fresh Tomato Odorants by GC-MS

Fresh tomato odorants in the aroma extracts were quantified by GC-MS analyses. Two different GC-MS systems were used. The first system consisted of an HP 6890 gas chromatograph coupled to an HP 5973 MSD (Hewlett-Packard, Avondale, PA). A 60 m X 0.25 mm (i.d.) DB-1 fused silica capillary column (d_f = 0.25 µm; J&W Scientific, Folsom, CA) was employed. The second system consisted of an Agilent Technologies 6890 gas chromatograph coupled to an Agilent Technologies 5973 Network MSD (Agilent Technologies, Palo Alto, CA). A 60 m X 0.25 mm (i.d.) DB-Wax fused silica capillary column was used (d_f = 0.25 µm; J&W Scientific, Folsom, CA). Both

systems utilized helium as the carrier gas. The concentration of each odorant was calculated by comparing the areas of certain characteristic fragment ions of the odorants with that of a certain internal standard (Table V). 3-Hexanone was used for the quantification of the C_4, C_5 and C_6 compounds while 2-octanone was used for 1-octen-3-one and methional. Maltol was used for the determination of the concentration of 4-hydroxy-2,5-dimethyl-3-(2H)-furanone and anethole was used for all of the other compounds. MS data were recorded in the electron impact mode with an ionization voltage of 70 eV. The differences in the ion intensities of the odorants and internal standards were corrected by determining MS response factors. Therefore, known amounts of the odorants and internal standards were mixed and injected into the GC-MS system. The response factors were calculated by comparing the area of the fragment ion of the odorant with the area of the fragment ion of the standard with their concentrations.

Table V. Fragment ions (m/z) used for quantification of selected fresh tomato odorants by GC-MS

Odorant	Ion m/z
3-methylbutanal	86
1-penten-3-one	84
3-hexanone (std.)	100
hexanal	82
(Z)-3-hexenal	83
(E)-2-hexenal	83
2-octanone (std.)	128
1-octen-3-one	70
methional	104
phenylacetaldehyde	120
3-methylbutyric acid	87
(E,Z)- / (E,E)-2,4-decadienal	81
ß-damascenone	121
anethole (std.)	148
guaiacol	124
2-phenylethanol	122
ß-ionone	177
cis- / trans-4,5-epoxy-(E)-2-decenal	68
eugenol	164
maltol (std.)	126
4-hydroxy-2,5-dimethyl-3-(2H)-furanone	128

Recovery Values

Recovery values of the fresh tomato odorants and internal standards were determined by adding known amounts of all of the compounds to blended green tomatoes and preparing this sample the same way as the fresh tomato samples. The concentrations of all odorants and internal standards were determined against 2-undecanone, which was added at end of sample preparation just before GC-MS analysis. Quantification was done by comparing the areas of certain characteristic mass fragment ions of the compounds (Table V) with that of 2-undecanone (m/z 170) including correction with the MS response factors between the compound ion and the 2-undecanone ion.

A background concentration of the odorants contained in the green tomato was also determined by analyzing the same green tomato mix without addition of odorants and standards. The concentrations were also calculated against 2-undecanone, which was added to the aroma extract at the end of sample preparation. These background concentrations were taken into consideration when calculating the recovery values by subtracting the background level from the concentration found after addition of the compounds. The recovery values were determined in this way for all odorants and internal standards for all three sample preparation methods (Table VI).

Aroma Extract Dilution Analysis (AEDA, 4)

An aliquot of the extract obtained from all three sample preparation methods (without addition of the internal standards) was analyzed by GC-O. The extract was then diluted to twice its starting volume with diethyl ether and analyzed again by GC-O. This procedure was repeated until no odor active compounds could be detected. GC-O analysis was performed on DB-Wax and DB-1 fused silica capillary columns (60 m x 0.32 mm (i.d.), d_f = 0.25 μm) installed into two HP 5890 gas chromatographs. At the end of the capillary column the effluent was split 1:1 between a flame ionization detector (FID) and a sniffing port using a fused silica "Y" connector (Supelco, Bellefonte, PA) and deactivated fused silica capillary tubing (30 cm x 0.25 mm (i.d.), J&W Scientific, Folsom, CA).

Identification of character impact odorants of fresh tomatoes

Flavor Profile Analysis of two different tomato cultivars

We first looked at the flavor profiles of several different tomato cultivars. Two of those are presented in Table VII. Sample A was rated less sweet and

Table VI. Recovery of selected odorants from three different sample preparation methods

Odorant	Recovery [%] [a]		
	CaCl$_2$	Na$_2$SO$_4$	SAFE
3-methylbutanal	33	1	24
1-penten-3-one	47	2	37
3-hexanone (std.)	54	4	48
hexanal	65	10	39
(Z)-3-hexenal	40	7	44
(E)-2-hexenal	69	22	68
2-octanone (std.)	64	19	46
1-octen-3-one	68	10	41
methional	44	30	46
phenylacetaldehyde	64	30	26
3-methylbutyric acid	28	34	83
(E,Z)-/ (E,E)-2,4-decadienal	65	40	34
ß-damascenone	65	43	28
anethole (std.)	54	52	21
guaiacol	68	56	79
2-phenylethanol	29	66	69
ß-ionone	49	51	18
cis-/ trans-4,5-epoxy-(E)-2-decenal	38	27	27
maltol (std.)	< 1	55	10
4-hydroxy-2,5-dimethyl-3-(2H)-furanone	5	32	23
eugenol	43	61	53

[a] determined in green tomatoes relative to 2-undecanone

less fruity than sample B. Sample A possessed a slight floral and smoky note. Retronasally, sample B was described to be more sweet and less green than sample A. The panelists always preferred sample B over sample A. What is the reason for this choice? Is it mainly a difference in the acid and sugar content of those two cultivars or also a difference in the concentrations of flavor compounds?

Table VII. Comparison of the flavor profiles of two different tomato cultivars

| Odor quality | Intensity[a] (standard deviation) | | | |
| | A | | B | |
	nasal	retronasal	nasal	retronasal
Sweet	0.6 (0.8)	1.1 (1.0)	1.2 (1.0)	1.9 (0.7)
Green / grassy	1.4 (1.0)	1.4 (1.0)	1.4 (1.0)	0.9 (0.8)
Fruity	0.4 (0.5)	0.7 (0.7)	1.0 (0.9)	0.8 (0.8)
Floral / flowery	0.7 (0.8)	0.3 (0.5)	0	0.5 (0.8)
Sour / acidic	0	0.4 (0.5)	0	0.4 (0.5)
Smoky	0.6 (0.8)	0	0	0
Preference	less		more	

[a] Intensity scale: 0 = not perceptible   3 = strongly perceptible

Aroma Extract Dilution Analysis (AEDA, 4)

To screen for important odorants the method of aroma extract dilution analysis (AEDA) introduced by Ullrich and Grosch (4) was applied. By analyzing up to ten different tomato cultivars using up to three different methods of sample preparation, the odorants listed in Table VIII representing one of the investigated tomatoes, seemed to have the biggest impact on the aroma of fresh tomatoes (flavor dilution (FD) factors are larger than 1).

The most important odorant for the aroma of fresh tomato was (Z)-3-hexenal with a FD-factor of 2048, followed by β-damascenone and trans-4,5-epoxy-(E)-2-decenal with 1024. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol®) and methional, as well as hexanal, 1-octen-3-one and an unknown compound with metallic odor had flavor-dilution (FD) factors between 32 and 128. Other contributors to fresh tomato flavor with FD-factors of 16 and 8, respectively, were 1-penten-3-one, guaiacol, 2-phenylethanol, cis-4,5-epoxy-(E)-2-decenal, as well as (E)-2-hexenal, 3-methylbutyric acid, phenylacetaldehyde, (E,Z)- and (E,E)-2,4-decadienal and β-ionone. Eugenol and 3-methylbutanal had FD-factors below 4. Compounds reported earlier as important odorants of fresh tomato such as 2-isobutylthiazole, 1-nitro-2-phenylethane, 6-methyl-5-hepten-2-one, (Z)-3-hexenol or methyl salicylate (7) were not perceived as important odorants, whereas the contribution of β-damascenone (7), 1-penten-3-one (2,7), methional, 4-hydroxy-2,5-dimethyl-

Table VIII. Most important odorants of fresh tomato by application of aroma extract dilution analysis (AEDA)

Compound	Flavor Dilution (FD) - factor
(Z)-3-hexenal	2048
β-damascenone	1024
trans-4,5-epoxy-(E)-2-decenal	1024
4-hydroxy-2,5-dimethyl-3(2H)-furanone	128
methional	128
hexanal	32
1-octen-3-one	32
unknown (metallic)	32
1-penten-3-one	16
guaiacol	16
2-phenylethanol	16
cis-4,5-epoxy-(E)-2-decenal	16
(E)-2-hexenal	8
3-methylbutyric acid	8
phenylacetaldehyde	8
(E,Z)-2,4-decadienal	8
(E,E)-2,4-decadienal	8
β-ionone	8
eugenol	<4
3-methylbutanal	<4

3-(2H)-furanone (2,3), 1-octen-3-one, (E,E)-2,4-decadienal (2) and trans-4,5-epoxy-(E)-2-decenal (3) to the aroma of fresh tomato could be confirmed.

Identification of the Unknown Compound in Fresh Tomato Extract

We were not able to get a clear mass spectrum of the unknown compound with a metallic odor (detected by AEDA) with the usual amount of tomato used for sample preparation. An extract from 900 g of tomatoes was prepared and subsequently purified by several runs on the preparative GC. The purified samples were combined and analyzed by GC-MS to yield a clean mass spectrum of the unknown compound. The MS data along with the retention indices on DB-Wax (I = 1381) and DB-1 columns (I = 961) and the odor impression (metallic, geranium-like) fit the literature data for (Z)-1,5-octadien-3-one. To verify its identity the reference compound was synthesized according to Swoboda and Peers (10). By comparing all of the analytical data for that compound, we confirmed that the unknown odorant in fresh tomato extract is (Z)-1,5-octadien-3-one. Considering the amount of sample needed to get a

mass spectrum and the sensitivity of our MS the estimated concentration of this compound in fresh tomato is approximately 0.1 ppb. However, with an odor threshold in water of 0.0012 ppb (*11*) its concentration exceeds the odor threshold by a factor of 83 so (*Z*)-1,5-octadien-3-one may contribute to fresh tomato flavor as well.

Quantification of fresh tomato odorants

Recovery Experiments

We first determined which of the three different sample preparation methods was best suited for quantification of the tomato odorants by comparing the recovery values for the different flavor compounds. The results are shown in Table VI. The calcium chloride method gave a good recovery of most of the compounds, only water soluble compounds such as 3-methylbutyric acid, 2-phenylethanol, 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone and the standard maltol showed very low recoveries with this method. The sodium sulfate method gave a good recovery of water soluble compounds, but highly volatile compounds showed very low recovery values due to evaporation while mixing the blended tomato sample with the sodium sulfate and then packing the glass column (procedure takes several minutes). The solvent assisted flavor evaporation (SAFE) method showed similar or slightly lower recovery values than the calcium chloride method for most of the odorants but better recoveries of the water-soluble compounds such as 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone and maltol. We decided that this method is the most suitable for the quantification of fresh tomato odorants, although we used the other methods for verification of some results.

Quantitative Results for Two Tomato Cultivars

Quantitative results for two tomato cultivars, A and B, obtained with the SAFE method are shown in Table IX. Some of the tomato odorants could be easily quantified by GC-FID, but for some minor compounds such as 1-octen-3-one, methional and β-damascenone, GC-MS detection is much more accurate since it is possible to clearly identify the peak by examining specific ions instead of quantifying an unresolved peak or even the wrong peak in the GC-FID chromatogram. The concentrations of fresh tomato odorants ranged from between 1 ppb to 10 ppm for (*Z*)-3-hexenal in sample B. Most of the odorants showed a similar concentration in both samples while big differences were found for 1-penten-3-one, methional, phenylacetaldehyde, guaiacol, 2-phenylethanol and 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone. Sample A

Table IX. Concentration [µg/kg] of selected odorants in two tomato cultivars

Odorant	concentration [µg/kg]	
	A	B
3-methylbutanal [a]	37	38
1-penten-3-one [a]	100	600
hexanal [a]	1240	1840
(Z)-3-hexenal [a]	3500	9700
(E)-2-hexenal [a]	80	130
1-octen-3-one [b]	4	5
methional [b]	31	1
phenylacetaldehyde [c]	580	120
3-methylbutyric acid [c]	70	120
(E,Z)-2,4-decadienal [c]	10	38
(E,E)-2,4-decadienal [c]	8	23
ß-damascenone [c]	6	3
guaiacol [c]	<1	-
2-phenylethanol [c]	1820	300
ß-ionone [c]	13	15
cis-4,5-epoxy-(E)-2-decenal [c]	93	90
trans-4,5-epoxy-(E)-2-decenal [c]	620	610
4-hydroxy-2,5-dimethyl-3-(2H)-furanone [d]	16	220
eugenol [c]	-	<1

[a] Quantified against 3-hexanone   [b] Quantified against 2-octanone
[c] Quantified against anethole   [d] Quantified against maltol

contained more methional, phenylacetaldehyde and 2-phenylethanol, whereas sample B had higher concentrations of 1-penten-3-one, (Z)-3-hexenal and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone. Guaiacol was only found in small amounts in sample A, but could not be detected in sample B. This might explain why sample A was rated higher in the floral/flowery and smoky notes while sample B was rated higher in the sweet odor quality in the flavor profile analysis (Table VII). Surprisingly the distinctly higher content of green smelling C_6-aldehydes in sample B could not be detected by the sensory panel in the flavor profile analysis. Other odorants also play a significant role in the overall tomato flavor and might influence the perception of the green odor quality (synergistic and antagonistic effects).

Odor Units – Most Important Odorants of Fresh Tomato

Odor units (also called odor activity values) were calculated by dividing the determined concentrations by their odor thresholds (*11*). The results are summarized in Table X, which shows a ranking of their sensory importance.

Table X. Odor Units of selected odorants in two tomato cultivars

Odorant	Odor Units [a]	
	A	B
(Z)-3-hexenal	14000	38800
trans-4,5-epoxy-(E)-2-decenal	5170	5080
ß-damascenone	3000	1500
ß-ionone	1860	2140
1-octen-3-one	800	1000
cis-4,5-epoxy-(E)-2-decenal	775*	750*
hexanal	276	409
3-methylbutanal	185	190
methional	155	5
phenylacetaldehyde	145	30
(E,Z)-2,4-decadienal	140*	540*
(E,E)-2,4-decadienal	110	330
1-penten-3-one	100	600
2-phenylethanol	27	4
(E)-2-hexenal	5	8
4-hydroxy-2,5-dimethyl-3-(2H)-furanone	<1	10
3-methylbutyric acid	<1	<1
guaiacol	<1	0
eugenol	0	<1

[a] Odor Unit = Concentration / Odor Threshold in water
* no odor threshold determined, estimated value based on the threshold of the other isomer

(Z)-3-Hexenal had the highest odor unit value, followed by *trans*-4,5-epoxy-(E)-2-decenal, β-damascenone, β-ionone and 1-octen-3-one, which seemed to be the most important odorants in fresh tomato. Further odorants with quite high odor units were hexanal, 3-methylbutanal, (E,E)-2,4-decadienal and 1-penten-3-one. *Cis*-4,5-epoxy-(E)-2-decenal and (E,Z)-2,4-decadienal probably have high odor units, but no values for their odor thresholds in water were found in the literature. We have not yet determined

their thresholds, so the numbers given were estimated from the thresholds of their corresponding isomers, i.e., *trans*-4,5-epoxy-(*E*)-2-decenal and (*E,E*)-2,4-decadienal, respectively (*11*). Methional, phenylacetaldehyde and 2-phenylethanol had higher odor units in sample A while 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone had higher odor units in sample B. The nasal odor threshold of 2-phenylethanol in water was suspected to be lower than previously stated (*11*). Using procedures described earlier (*13*), we determined an odor threshold of 68 ppb in contrast to the previously reported value of 1000 ppb. The odor units for (*E*)-2-hexenal, 3-methylbutyric acid, guaiacol and eugenol were very low, so these compounds do not seem to be as important for the flavor of these tomatoes.

Conclusion

Screening for potent odorants of fresh tomato by application of AEDA revealed that some flavor compounds previously have been underestimated or overlooked while others have been overestimated. In accordance with some other results (*2, 3*), odorants that do contribute to the flavor of fresh tomato, apart from (*Z*)-3-hexenal, hexanal, β-ionone, 1-penten-3-one, 3-methylbutanal, phenylacetaldehyde, are β-damascenone, 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone (Furaneol®), methional and 1-octen-3-one along with (*E,Z*)- and (*E,E*)-2,4-decadienal, *cis*- and *trans*-4,5-epoxy-(*E*)-2-decenal and (*Z*)-1,5-octadien-3-one, which is reported for the first time as a tomato odorant.

Outlook

To verify whether all important fresh tomato odorants have been identified and whether their concentrations have been determined correctly, an artificial aroma model will be made by combining the odorants in the concentrations found. The odor of this model mixture will be compared with a fresh tomato sample for similarity. This and other sensory experiments will be carried out in the near future.

Acknowledgement

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Chapter 12

Characterization of Aroma Compounds in Fresh and Processed Mandarin Oranges

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The most important odorants in freshly homogenized clementine segments were screened by application of GC/Olfactometry. A total of thirty-eight odorants was identified, thirty of which were quantified by means of stable isotope dilution assays. 3-sec-Butyl-2-methoxypyrazine, ethyl 2-methylbutanoate, 3-isopropyl-2-methoxypyrazine and (Z)-3-hexenal were characterized as most important aroma contributors showing OAVs >100 (OAV: ratio of concentration to odor threshold). In canned satsuma segments, 4-vinyl-2-methoxyphenol and 4-hydroxy-2,5-dimethyl-3(2H)-furanone were by factors of 85 and 40, respectively, higher than in the fresh clementine segments.

Mandarin oranges are a very diverse group of citrus fruits and are commonly named as mandarins, tangerines or satsumas. Up to now, investigations aimed at elucidating the volatile constituents in mandarin oranges have led to the identification of a total of 75 compounds in the juices of different mandarin oranges (*1*).

Among them, thymol and methyl N-methylanthranilate (*Figure 1*, a and b) have been suggested as key contributors to the typical aroma of mandarines. The amounts of both compounds were previously estimated from their concentrations in peel oil as well as from the amounts of peel oil present in the juice (*2*). A comparison with the flavor thresholds of both odorants in the juice has shown that methyl N-methylanthranilate is present at about five times its threshold level, while the concentration of thymol was far below its odor threshold (cf. *Table 1*). The data suggested the influence of methyl N-methylanthranilate on mandarine juice aroma. However, this assumption was never fully proven. Furthermore, it was also reported that addition of both odorants to either limonene or tangerine oil did not generate the

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characteristic aroma of mandarin peel oil and that compounds such as γ-terpinene and β-pinene seemed to be further important contributors (*3*).

(a)　　　　　　　(b)

(c)
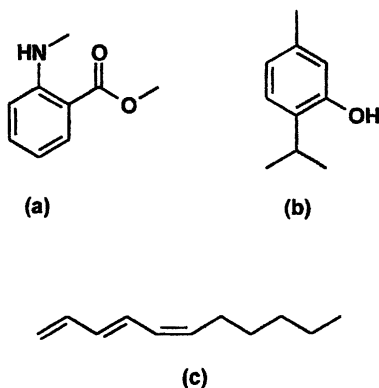
Figure 1. Compounds suggested as contributors to the aroma of either mandarin juice or mandarin peel oil. (a) Methyl N-methyl anthranilate (2), (b) thymol (3), (c) (E,Z)-1,3,5-undecatriene (4).

Table 1. Estimated concentrations and odor thresholds of volatile juice constituents.

Odorant	Estimated concentration in mandarin juice [µg/L][a]	Odor threshold in juice [µg/L][a]
Thymol	200	147000
Methyl N-methyl anthranilate	782	157

a)　Data from (*2,3*).

Using gas chromatography/olfactometry, (*E,Z*)-1,3,5-undecatriene (*Figure 1*, c) exhibiting an intense balsamic-fruity odor quality, was identified as another aroma active compound of clementine peel oil (*4*). Due to its very low threshold in the picogram range, the triene was suggested as key contributor to mandarine aroma.

Because no comprehensive study on the odorants of mandarin fruits aimed at evaluating the odor contribution of single volatiles has yet been reported, the aim of the present investigation was to characterize the important odorants of fresh clementine segments. Furthermore, a comparison with canned satsumas was performed.

Experimental Procedures

Materials

Fresh clementines (*Citrus reticulata blanco cv. Clementine*, from Argentina) were purchased at a local market and were used within two days. Fresh clementine juice (100 mL) was obtained by homogenization of carefully peeled fruit segments in a kitchen juicer. To inhibit enzymic reactions (*5*) the juice was poured into saturated $CaCl_2$-solution (200 mL). Canned satsuma segments (*Citrus reticulata blanco var. Unshiu*) were purchased from a local supplier. The segments were homogenized in the same way as reported above for the fresh fruits.

Cooked clementine juice

Freshly homogenized clementine segments (100 mL) were poured into a closed vessel (total volume: 250 mL) and immediately heated for 15 min at 80 °C. The material was then quickly cooled down by adding an aqueous, cold (4 °C), saturated $CaCl_2$-solution (100 mL) and by external cooling with ice water.

Isolation of the volatiles; characterization of aroma compounds

The juice volatiles, either from fresh or from cooked or canned samples, were isolated by solvent extraction followed by high vacuum distillation (*6*). Each of the three distillates was concentrated to the same volume (300 μL). At this concentration, the volatiles present in the extracts exhibited the full flavor quality of the original material when evaluated on a strip of filter paper.

The odorants were then screened in parallel by five panelists using gaschromatography/olfactometry (injection volume: 0.5 μL). Sniffing was repeated five times by each panelist. All odorants detected were identified by comparing them with reference substances on the basis of the following criteria: retention index (RI) on three stationary phases of different polarity (FFAP, DB-5; OV-1701), mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing-port.

High-Resolution Gas Chromatography/Olfactometry (HRGC/O) and Mass Spectrometry (HRGC/MS)

HRGC was performed using a Type 8000 gas chromatograph (Fisons Instruments, Mainz, Germany) equipped with the following fused silica capillaries: FFAP (free fatty acid phase; 30 m x 0.32 mm i.d., 0.25 μm d_f; Chrompack), DB-5 (30 m x 0.32 mm i.d., 0.25 μm d_f; J&W Scientific, Fisons Instruments), and DB-1701 (30 m x 0.32 mm i.d., 0.25 μm d_f; Chrompack). HRGC conditions were the same as described previously (*7*). MS analysis was performed by means of a mass spectrometer MS 8230 (Finnigan MAT, Bremen, Germany) in tandem with the capillaries described above.

Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) at 115 eV using isobutane as the reactant gas.

Quantitation of Flavor Compounds

Juices (100 mL) were poured into saturated $CaCl_2$-solution and spiked with known amounts of the following labeled internal standards [3,3,4,4-$^2H_4$]-hexanal (8), [3,3,4,4-$^2H_4$]-octanal (9), [5,6-$^2H_2$]-decanal (10), phenyl-[$^{13}C_2$]-acetaldehyde (11), [$^2H_5$]-ethyl cinnamate (11), [2,3-$^2H_2$]-(E)-non-2-enal (12), [7,7,8,8-$^2H_4$]-(E,E)-deca-2,4-dienal (12), [3,4-$^2H_2$]-(Z)-hex-3-enal (13), [2,2,2-$^2H_3$]-ethyl butanoate (13), [$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2H)-furanone (14), [$^2H_3$]-2-methoxyphenol (15), [$^2H_2$]-linalool (16), [$^2H_4$]-carvone (17), 3-isopropyl-2-[$^2H_3$]-methoxypyrazine (18), 3-sec-butyl-2-[$^2H_3$]-methoxypyrazine (19), 3-([$^2H_3$]-methylthio)-1-propanal (20), [$^2H_3$]-vanillin (21), [2,2,2-$^2H_3$]-ethyl 2-methylpropanoate (22), [2,2,2-$^2H_3$]-ethyl 2-methyl-butanoate (22) and [$^2H_4$]-β-damascenone (23). [$^{13}C_2$]-phenylacetic acid (99 atom %) was from Aldrich (Steinheim, Germany). Syntheses were performed as described in the literature given in brackets. Concentrations of the labeled compounds were determined gas chromatographically using FID response factors calculated in previous runs with defined mixtures of the unlabeled analyte and methyl octanoate as the internal standard. The MS-calibration factors were obtained based on mixtures of the labeled and the unlabeled compounds (23).

The solutions were then stirred at room temperature until equilibration (30 min), then extracted with dichloro methane (five times, total volume 500 mL) and the combined organic layers finally dried over anhydrous Na_2SO_4. The volatile fraction was subsequently isolated by high vacuum transfer and the aroma extract obtained was concentrated by careful distillation (6). At least four replicates were performed. Quantitation of the volatiles was done by two-dimensional gas chromatography/mass spectrometry (TD-HRGC/MS) using an ITD-800 (Finnigan, Bremen, Germany) running in the CI-mode with methanol as the reagent gas (21).

p-Menth-2-ene was used as internal standard for the quantitation of limonene, α-pinene and myrcene as reported in (24).

Sensory evaluation

Assessors (five male, five female) were recruited from the German Research Center of Food Chemistry and were trained with model solutions containing odorants at increasing suprathreshold levels. The odor intensities were scored using a seven-point intensity scale from 0.0 to 3.0. Sensory analyses were performed in a sensory panel room at 21±1 °C in three different sessions for each evaluation. All citrus juices were judged orthonasally.

Results and Discussion

Identification of odorants in freshly homogenized clementine segments
Isolation of the volatiles by solvent extraction and high vacuum distillation resulted in

an aroma distillate eliciting the full aroma of the fresh material when evaluated on a piece of filter paper. Evaluation of single volatiles in the extract by HRGC/Olfactometry on three different GC columns of different polarity revealed a total of 38 odor-active regions showing a variety of odor qualities such as fruity, citrus-like, green or coriander. However, none of the odorants revealed a characteristic clementine-like smell.

All compounds detected were subsequently identified using reference compounds (*Table 2*). The most dominating substance class by number were aldehydes/ketones with a total of 19 odorants, followed by esters (5 compounds). Interestingly, neither thymol nor methyl N-methyl anthranilate or (*E,Z*)-1,3,5-undecatriene were judged to be odor-active, although they were actively looked for.

Quantitation of 30 of the odorants identified and a calculation of the corresponding odor activity values (OAVs; ratio of concentration of the odorant divided by the corresponding orthonasal odor threshold in water) was performed to screen the compounds for their relative odor potencies. All odorants showing OAVs > 1 are summarized in *Table 3*.

The highest OAV was found for the pea-like smelling 3-sec-butyl-2-methoxy-pyrazine, followed by ethyl 2-methylbutanoate, 3-isopropyl-2-methoxypyrazine and (*Z*)-hex-3-enal with respectively fruity, earthy and green notes. High OAVs (OAV > 50) were also determined for ethyl cinnamate, ethyl 2-methylpropanoate, ethyl butanoate and (*E*)-β-damascenone contributing fruity and sweet odor qualities to the overall aroma.

Comparison between freshly homogenized clementine segments and canned satsuma segments

Next, an extract obtained from canned satsuma segments was evaluated using HRGC/olfactometry. *Table 4* displays those aroma compounds which were only detected in one of the two juices analyzed, thus being possible candidates causing the overall clear flavor differences observed. It became evident that 14 compounds detected in the fresh material were not sensorially active in the canned satsuma segments, such as linalool, ethyl butanoate or (Z)-3-hexenal. On the other hand, 4-vinyl-2-methoxyphenol, two furanones and phenylacetic acid were detected as potent odorants only in the canned satsuma sample.

To objectify the differences, the 18 odorants were quantified by stable isotope dilution assays in both samples. Representative data for eight compounds are given in *Table 5*. As already indicated by the GC/O results, the concentrations of the esters ethyl butanaote and ethyl cinnamate, as well as of (*Z*)-hex-3-enal and linalool were significantly higher in the fresh clementine fruits, thus giving an explanation for the more fresh and fruity aroma impression compared to the canned sample. On the other hand, the clove-like smelling 4-vinyl-2-methoxyphenol, the caramel-like 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone and the honey-like phenylacetic acid were increased by

factors of about five to ten in the canned sample. These findings agree with the overall sweet-honey and caramel-like odor impression of the canned satsuma segments, missing the freshness and fruityness of the fresh clementine fruits.

Table 2. Odorants identified[a] in freshly homogenized clementine segments based on GC/Olfactometry performed by five panellists.

Odorant		Odorant	
Aldehydes/Ketones	Odor quality	**Esters**	Odor quality
Hexanal	grassy	Ethyl 2-methyl-	fruity
Octanal	citrus-like	propanoate	
Decanal	green, fresh	Ethyl butanoate	fruity
Dodecanal	green, coriander	Ethyl 2-methylbutanoate	fruity
(Z)-Hex-3-enal	grassy	Ethyl cinnamate	sweet
(Z)-Non-2-enal	fatty, green	Ethyl phenylacetate	sweet
(E)-Non-2-enal	tallowy, green		
(E,E)-Deca-2,4-dienal	fatty	**Terpene hydrocarbons**	
Oct-1-en-3-one	mushroom-like	Myrcene	geranium-like
(Z)-Octa-1,5-dien-3-one	geranium-like	Limonene	citrus-like
tr-4,5-Epoxy-(E)-dec-2-enal	metallic	**Alcohols/Acids**	
		2-/3-Methylbutanol	malty
Carvone	caraway-like	Linalool	flovery
(E)-β-Damascenone	cooked apple	Acetic acid	vinegar-like
β-Ionone	violet-like	Butanoic acid	sweaty
α-Sinensal	metallic, green	2-/3-Methylbutanoic acid	rancid
β-Sinensal	metallic, green		
Phenylacetaldehyde	honey-like	Phenylacetic acid	honey-like
Methional	cooked potato		
Vanillin	vanilla-like	**Pyrazines**	
Furanones/Lactones		3-Isopropyl-2-methoxypyrazine	earthy
4-Hydroxy-2,5-dimethyl-3(2H) furanone	caramel-like	3-sec-Butyl-2-methoxypyrazine	pea-like
Winelactone	sweet		

[a] The compounds were identified by means of reference compounds based on the following criteria: mass spectra (MS/EI and MS/CI), retention indices on three GC columns of different polarity and odor threshold and odor quality at the sniffing port.

When simulating the cooking process on lab-scale using fresh clementine segments, a decrease in (Z)-hex-3-enal by a factor of two during cooking was observed, while (E)-non-2-enal, β-damascenone, phenylacetic acid and 4-vinyl-2-methoxyphenol increased by factors of about two to five. For ethyl butanoate, ethyl 2-methylbutanoate and 4-hydroxy-2,5-dimethyl-3(2H) furanone only slight increases were observed,

while the concentrations of methional and carvone remained more or less unchanged (*Table 6*).

Table 3. Odor activity values (OAV) of aroma compounds in freshly homogenized clementine fruits.

Odorant	Conc (µg/L)	OAV[a]
Hexanal	65.3	7
Decanal	163	33
(Z)-Hex-3-enal	29.2	117
(E)-Non-2-enal	4.4	6
(E,E)-Deca-2,4-dienal	1.9	10
(E)-β-Damascenone	0.1	50
Phenylacetaldehyde	17.1	4
Methional	4.3	2
Vanillin	17.1	2
Ethyl 2-methylpropanoate	1.0	50
Ethyl butanoate	55.2	55
Ethyl 2-methylbutanoate	2.4	400
Ethyl cinnamate	3.9	65
Limonene	6938	35
Linalool	57.3	10
3-Isopropyl-2-methoxypyrazine	0.8	400
3-sec-Butyl-2- methoxypyrazine	4.5	2250

[a] OAVs were calculated by dividing the concentration of the odorants by their odor thresholds in water (*25*).

A comparison of the data for the cooked clementine juice and the canned satsuma segments with freshly homogenized clementine fruits (cf. *Tables 5 and 6*) revealed similar tendencies for 4-vinyl-2-methoxyphenol, 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone or phenylacetic acid on the one hand and for (Z)-3-hexenal on the other hand, however, the changes were less pronounced in the cooked sample. The reason for this is undoubtedly the more careful thermal process applied in the model study compared with the canning process. Furthermore, differences in the fruit material used in both experiments have to be taken into account.

Differences between mandarin oranges and other citrus fruits

In a preliminary sensory experiment, freshly hand-squeezed juices of orange, grapefruit and clementine were evaluated sensorically by a trained panel. Subjects were told to rate the overall orange-, grapefruit- and clementine-like aroma impression in each of the three citrus juices. All three citrus juices were rated with the highest score (> 2.5) on their respective aroma quality, such as orange-like for orange juice etc. (*Figure 2*). However, the orange and the clementine juice samples were also rated relatively high on each others odor quality, e.g. orange juice was rated as clementine-

**Table 4. Aroma compounds which were detected by a comparative
GC/Olfactometry in one of two fruit samples: clementine fruits (CF) or the
canned satsuma segments (SS) (+: detected, -: not detected by GC/O).**

Odorant	Smelled in[a]	
	CF	SS
Ethyl 2-methylpropanoate	+	-
Ethyl butanoate	+	-
Ethyl 2-methylbutanoate	+	-
Ethyl cinnamate	+	-
Myrcene	+	-
(R)-Limonene	+	-
Linalool	+	-
Decanal	+	-
(Z)-Hex-3-enal	+	-
(E)-Non-2-enal	+	-
Dodecanal	+	-
(E,E)-Deca-2,4-dienal	+	-
4-Vinyl-2-methoxyphenol	-	+
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol)	-	+
3-Hydroxy-4,5-dimethyl-2(5H)-furanone (Sotolon)	-	+
α-Sinensal	+	-
β-Sinensal	+	-
Phenylacetic acid	-	+

[a] The comparison was done based on the same amount of fruits extracted and using the same
extract volume in GC/O experiments.

Table 5. Selected aroma compounds showing significant differences in their concentrations between freshly homogenized clementine fruits (CF) and canned satsuma segments (SS).

Odorant	Odor quality	Conc. [µg/kg]	
		CF	SS
Ethyl butanoate	fruity	55	0.4
Ethyl cinnamate	fruity	3.9	0.2
(Z)-Hex-3-enal	grassy	29	2.6
Linalool	flowery	57	1.8
4-Vinyl-2-methoxyphenol	clove-like	1.4	121
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	caramel-like	4.4	166
4-Hydroxy-3-methoxybenzaldehyde (Vanillin)	vanilla-like	46	105
Phenylacetic acid	honey-like	0.6	10

Table 6. Influence of a thermal treatment (15 min; 80°C) on the concentrations of selected clementine aroma compounds.

Odorant	Conc. [µg/kg]	
	fresh juice	cooked juice
Ethyl butanoate	55	66
Ethyl 2-methylbutanoate	2.4	3.5
(Z)-Hex-3-enal	29	13
(E)-non-2-enal	4.4	7.5
Methional	4.3	4.8
Carvone	4.5	4.7
β-Damascenone	0.1	0.5
Linalool	57	60
Phenylacetic acid	0.6	1.2
4-Vinyl-2-methoxyphenol	1.4	2.3
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	4.4	5.9
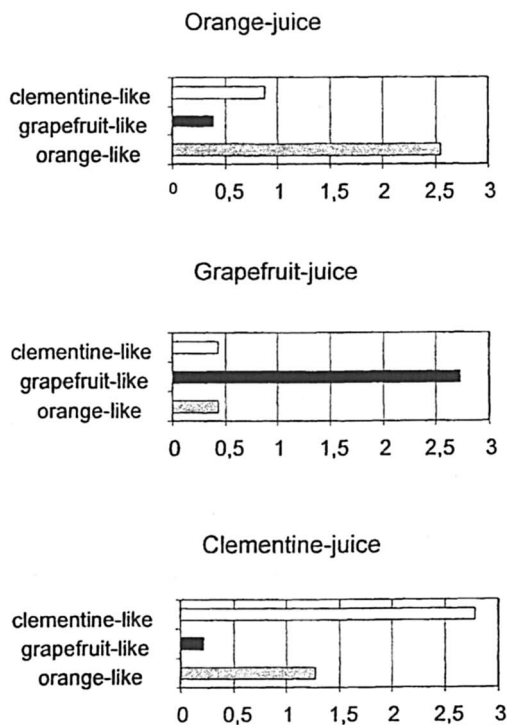
Figure 2. Overall sensory evaluation of the odor qualities clementine-, grapefruit- and orange-like in three citrus juices.

like, while clementine juice was said to elicit also an orange-like aroma quality. This phenomenon was not as pronounced in grapefruit juice, indicating that the aroma compositions of clementine and orange juice are more similar in comparison with grapefruit juice.

This observation is in agreement with the composition of the odor-active compounds responsible for the juice aromas. Grapefruit juice aroma is mainly characterized by two aroma impact compounds (*Figure 3*), the grapefruit-like smelling 1-p-menthen-8-thiol, as well as the blackcurrant-like smelling 4-mercapto-4-methylpentan-2-one which has recently been identified (*7, 27*) as a new grapefruit aroma constituent. In combination with several esters, terpene hydrocarbons and citrus-like and fresh aldehydes, these two compounds were found to be responsible for the characteristic sulfury, citrus-like and harsh aroma of grapefruits.
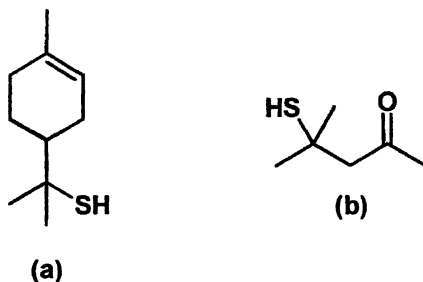
Figure 3. Character impact odorants in freshly squeezed grapefruit juice. (a) 1-p-Menthen-8-thiol (26) and (b) 4-mercapto-4-methylpentan-2-one (7, 27).

Thus, the significant difference between orange and clementine aroma compared to grapefruit aroma can first and foremost be explained by the absence of 4-mercapto-4-methylpentan-2-one, which was not detectable by GC/O in either the orange (*10*) or the clementine juice.

Many odorants detected in grapefruit, orange or mandarin juice by GC/O were identical but differed in their concentrations (*Table 7*). Especially high concentrations of ethyl butanoate, ethyl 2-methylbutanoate and limonene, but also of α-pinene and myrcene are characteristic for orange juice as compared to clementine and grapefruit (*Table 7*). This agrees with the very intense fruity-citrusy aroma of orange juice.

In clementines, β-damascenone, the sinensals, ethyl cinnamate, ethyl phenyl-acetate, 3-sec-butyl-2-methoxypyrazine and dodecanal were present as intense odorants, which were not detected in orange or grapefruit juices (*10; 27*). Their contribution to the overall clementine aroma, however, has to be investigated further, e.g., by aroma reconstitution experiments.

Conclusion

The results clearly show that clementine segments do not contain any specific flavor compound like, e.g., grapefruit, but possess a number of odorants which cannot be found in orange and grapefruit aroma. None of the odorants identified elicits a typical clementine-like odor quality. It might be therefore assumed that other odorants, being transfered from the peel to the segments during eating, might contribute more to the overall mandarin aroma than the "juice" odorants themself. Investigations on the peel odorants are under way.

Table 7. Comparison of the concentrations of selected odorants in freshly squeezed juices of different citrus fruits.

Odorant	Conc. [µg/kg]			
	Clementine	Grapefruit[a]	Orange	
			Valencia late[b]	Pera Rio[c]
(Z)-Hex-3-enal	29	108	187	463
Hexanal	65	33	197	146
Octanal	7	32	25	64
Decanal	163	89	45	126
(E)-Non-2-enal	4.4	0.5	0.6	n.a.
(E,E)-Deca-2,4-dienal	1.9	1.0	1.2	n.a.
Ethyl 2-methylpropanoate	1.0	5.8	8.8	1.3
Ethyl butanoate	55	70	1192	258
Ethyl (S)-2-methylbutanoate	2.4	3.9	48	3.2
α-Pinene	<3	42	308	375
Myrcene	24	94	594	1298
Limonene	8267	2308	85598	89900
Linalool	59	76	81	157

[a] Data from (27). [b] Data from (10). [c] Data from (28).

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Relating Sensory and Instrumental Data To Conduct an Accelerated Shelf-Life Testing of Whey–Protein-Coated Peanuts

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The predicted shelf-life of the whey-protein-coated peanuts was longer than that of the uncoated peanuts. The objectives of this study were, first, to correlate the sensory rancidity data and the instrumental gas chromatography (GC) data to validate the use of the GC data in accelerated shelf-life testing of peanuts, second, to determine coated and uncoated peanut oxidation at three accelerated shelf-life test temperatures, and third, to use this information to model the shelf-life of these peanuts at ambient conditions using the Arrhenius and the linear models. Four different formulations of WPI-based coatings were used to coat the peanuts. Four controls were used to investigate the effects of other ingredients in the coating formulation. The peanut samples were stored in duplicate for up to 31 weeks and then analyzed using static-headspace GC analysis of hexanal.

Peanuts have high susceptibility to oxidative rancidity when combined with high heat treatment of the roasting process due to the high content of unsaturated lipids (1-3). Due to the masking of desirable pyrazines by large amounts of low-molecular weight aldehyes such as hexanal, autoxidation in peanuts results in 'flavor-fade' and off-flavor development (4,5). Hexanal is an effective indicator

of lipid oxidation in peanuts (6), and it correlates well with sensory measures (7-10).

Films based on heat-denatured whey-protein-isolate (WPI) have been found to be excellent oxygen barriers (11). Moreover, whey protein coatings applied by a bench-scale coating method have been shown to provide significant protection against oxidative rancidity in peanuts when analyzed by chemical and instrumental methods (6,12).

Various innate factors such as maturity (13), fatty acid composition (14) and variety (15) may influence the shelf-life of peanuts. Shelf-life of peanuts can be significantly increased by special packaging techniques such as N_2 flushing, vacuum packaging, high oxygen-barrier packaging material, and oxygen-absorbing sachets or film ingredients. Shelf-life of peanuts can also be enhanced by direct external treatments such as oxygen-barrier edible coatings.

An accelerated shelf-life test is conducted by modifying the storage conditions such as increasing the temperature to accelerate the reaction in order to expedite obtaining the results. When the temperature is raised to conduct accelerated shelf-life testing in food systems, the results of such tests can be modeled using a temperature vs. reaction rate relationship (16) to predict the shelf-life at normal storage temperature.

Few studies have been devoted to investigating the effect of edible coatings on food products after storage using both sensory and instrumental measurements. Thus, the objectives of this study were threefold: first, to investigate the effects of WPI-based edible coating on sensory properties of peanuts after storage; second, to correlate sensory and instrumental measurements of peanut oxidation, and lastly, to determine the degree of WPI-coated and uncoated peanut oxidation using the instrumental measurements at three accelerated shelf-life test temperatures to extrapolate the shelf-life of these peanuts at ambient conditions. In this study, both the Arrhenius model (17) and the linear model (18) were used to predict the shelf-life of peanut samples at ambient conditions.

Materials and Methods

Raw materials

The variety of peanuts used for this experiment was "runner". The size of the peanut kernel was medium. The peanuts contained 48-52% fat, 22-30% protein, 3-5% sugar and less than 2% moisture. The peanuts were dry roasted.

The whey protein coatings included WPI (Bipro®, Davisco Foods International, Lesuer, Minn., U.S.A.), glycerol (USP/FCC, Fisher Scientific Inc., Fair Lawn, N.J., U.S.A.) as a plasticizer, lecithin (Centrolene® A, Central Soya Company, Fort Wayne, Ind., U.S.A.) as a surfactant and methyl paraben (NF/FCC, Fisher Scientific Inc., Fair Lawn, N.J., U.S.A.) as an antimicrobial agent. Vitamin E (Nature's Life, Gardengrove, Calif., U.S.A.) was added to

some of the coating formulations to test its antioxidant properties. The potency of vitamin E was 400 I.U. per capsule.

Sample treatments and coating solutions

Both native and heat-denatured WPI were included in the study, because they produce films with different solubility, tensile strength and oxygen-barrier properties (19,20). Table I shows the nine sample treatments investigated in this study.

All coating solutions contained 10% WPI (w/w). The heat-denatured solutions were prepared by heating 10% WPI solution (w/w) for 30 min in a water bath at 90°C (21). The denatured solutions were then cooled down to room temperature (~25°C) in an ice bath. Glycerol was added to all coating solutions at a 1 to 1 ratio of WPI to glycerol. Lecithin and methyl paraben were added to all coating solutions at 0.05% and 0.1% of the coating solution (w/w), respectively. When vitamin E was added, it was at 0.5% of the coating solution (w/w). The same amount of ingredient(s) was added to water (Arrowhead Mountain Spring Water Company, Brea, CA) to make each control. WPI-vitamin E emulsions were made using a Microfluidizer homogenizer (HC 5000, Microfluidics International Corp., Newbury, MA). The hot liquid was passed through the homogenizer 6 times using a homogenizing pressure of 6000 psig. The resulting emulsion had a normal particle-size distribution and the mean particle size was approximately 0.6 μm. After all the ingredients were mixed, the solution was strained with 2 layers of cheese cloth and stored for 1 to 3 d at a refrigeration temperature (5 – 10°C) until the coating process took place.

Table I. The composition of the nine sample treatments

Sample treatments	Composition
Heat-denatured WPI with vitamin E (DW)	heat-denatured WPI + vitamin E + glycerol + lecithin + methyl paraben + water
Heat-denatured WPI without vitamin E (DWO)	heat-denatured WPI + glycerol + lecithin + methyl paraben + water
Native WPI with vitamin E (NW)	native WPI + vitamin E + glycerol + lecithin + methyl paraben + water
Native WPI without vitamin E (NWO)	native WPI + glycerol + lecithin + methyl paraben + water
Control 4 (C4)	glycerol + lecithin + methyl paraben + water
Control 3 (C3)	lecithin + methyl paraben + water
Control 2 (C2)	methyl paraben + water
Control 1 (C1)	water
Reference (R)	untreated

The code used in Tables 2 and 3, and Figure 1 for each treatment is in parenthesis

A commercial coater (Labcoater II system, O'Hara Manufacturing, LTD., Toronto, Canada) was used to coat the peanuts with WPI solutions and control 4. This coater is a simultaneous spray-jog-dry type of coater which is mainly used to coat pharmaceuticals and nutritional supplements. For our peanut coating, we separated the spray phase and the dry phase of the process. The amount of coating solution applied was aimed at a 5% weight gain of the peanuts after the coating was completely dried. Close visual observation of the coated peanuts revealed smooth, glossy coatings without cracks or holes.

For controls 1, 2 and 3, the coating procedures were done in a lab bench pan coater with pan diameter of 16 inches (LP16, LMC International, Elmhurst, IL). This coater is a conventional coater used in the confectionery industry. With the confectionery coater, the solution was ladled onto the samples, rather than sprayed on as in the pharmaceutical coater. After the control solutions were applied onto the samples they were dried for approximately 30 min, and then cooled down to room temperature before they were taken out of the pan.

After they were treated in the pan, all the peanut samples were laid out at room temperature for ~ 24 h prior to packaging them into oxygen-barrier bags. They were then held at −24°C until they were taken out of the freezer to be stored at various storage conditions for headspace GC analysis.

For storing at various conditions, peanut samples weighing 180 g were placed into wide-mouth 473 ml mason jars (Ball®, Alltrista Corp., Muncle, IN). The storage temperatures were 40°C, 50°C and 60°C; and the a_w values of the coated and the control peanuts were adjusted to the range 0.29 - 0.38 using moisture absorbent sachets (silica gel pillow pack, Desiccare Inc., Santa Fe Springs, CA). The number of moisture absorbent sachets added into the mason jars was calculated based on the initial a_w of the coated and the control peanuts and the capacity of the moisture absorbent sachets to absorb a certain amount of water. The a_w values of the peanuts were used to calculate the amount of moisture necessary to absorb in order to achieve the a_w of the reference sample, using a peanut moisture isotherm (22). The a_w of the reference was in the range 0.25 - 0.3. The temperatures and the relative humidities of the chambers were monitored using a data logger (Model TL 120, Dickson Company, Addison, IL). The samples were stored for up to 31 w at the three temperatures.

Sensory Evaluation Procedures

Judges

Fifteen judges (5 male, 10 female, age range 20 – 31) recruited from the university community, participated in this study. Thirteen out of fifteen judges were naïve to descriptive analysis method. The judges were advised not to eat, drink or smoke for one hour prior to the sessions.

Sample preparation

Samples were taken out from their storage chambers at least 4 h prior to evaluation in order to equilibrate to room temperature. Samples were served at room temperature in 29.6 mL plastic cups (Rykoff-Sexton, Inc., Lisle, Ill., U.S.A.) labeled with 3-digit random codes created by FIZZ software (Biosystèmes; Couternon, France).

Experimental Design

Samples were evaluated by descriptive analysis using a method derived from the quantitative descriptive analysis (QDA®) method (23) which provides quantitative descriptions of products based on the perceptions of a group of qualified subjects. In order to determine appropriate terms and procedures, the QDA® method utilizes statistical analysis extensively (24).

A total of 20 sessions were devoted to training the judges; and out of those 20 sessions, 4 were term-generation sessions, 6 were group-rating sessions and 10 were individual booth sessions. During the term generation sessions, the panel generated terms for the wide range of peanut samples. Fresh, oxidized, coated and uncoated peanut samples were prepared and provided to the judges to generate as many attributes as possible. The attributes were generated and defined by the panel, and for those attributes which share the same definition were grouped and renamed. During the term generation process, the judges were provided with the lexicon for peanut flavors (25) to compare our list of attributes to the lexicon. Most of the aroma and taste terms generated from the panel shared the same name and definition with the peanut lexicon. Those attributes in the lexicon that the panels could not detect from the samples used in this study were not included in the attribute list. Those attributes not in the peanut lexicon that the judges detected from the samples and were comfortable using, such as "rancid", were included in our attribute list. The judges were provided with oxidized and fresh peanuts to distinguish rancid- and roasted-peanut attributes.

In total, 144 samples (6 treatments x 4 storage times x 2 experimental reps x 3 temperatures) were evaluated in duplicate (sensory replication) over 18 sessions. Six treatments included all the WPI treatments, control 4 and the reference. Four storage times were 0, 5, 15 and 45 days. Each session was a subplot of a given treatment and a given temperature. The six treatments were subplots of three temperature blocks. These subplots were randomized with random numbers generated by Excel (Microsoft office 98 Version 8.0 for Apple® Macintosh® Series, Microsoft Corp.). The storage days and experimental reps were randomized within the subplots according to an incomplete Latin-Square design generated by the FIZZ system. Two sensory replications of the 8 samples (4 storage times x 2 experimental reps) were tested in a session with a 5 to 10 min break after the first sensory replication.

A 0 to 15 structured line scale was used to evaluate the intensity of the attributes, where 0 was no detection and 15 was the maximum intensity of the

attribute being assessed. Judges were instructed to taste and expectorate the samples. Rinsing protocol consisted of one deionized water followed by one carbonated water (Select, Safeway; Davis, Calif., U.S.A.) then one deionized water. The judges rinsed in between each sample. The samples were presented in multiple presentation, and the judges were allowed to retaste the samples. For taste, aroma and texture attributes, all the sessions were conducted under red light to mask any color difference. For appearance attributes, the assessments were done under normal lighting conditions. The total session length ranged from 18 to 55 min.

Headspace Gas Chromatography Analysis

Lipid oxidation was evaluated by measuring the hexanal content of the peanut samples by static headspace gas chromatography (GC) (Perkin-Elmer autosystem with HS-40 autosampler, Norwalk, CT). The GC analysis used a capillary DB-1701 column (30 m (l) x 0.32 mm (I.D.), 1μm thickness, J & W, Folsom, CA); HS sampler temperature, 60°C; oven temperature, 65°C; injector temperature, 180°C; detector temperature, 200°C. Peanut samples weighing 5g were ground for 8 s using a grinder (Braun coffee bean grinder KSM2(4), Braun Inc., Woburn, MA). Duplicate 0.5g ground peanut samples were placed into 22 ml headspace sample vials, which were immediately sealed with silicone rubber teflon caps. The vials were then inserted into the headspace sampler at 60°C for 15 min and pressurized with carrier gas (He) for 30 s. An aliquot of gas phase was injected directly into the GC through the stationary injection needle. The hexanal content of samples was measured for peanuts stored for 0, 5, 15, 28, 45, 56, 84, 112, 140, 161, 175, 189 and 217 d at 40°C, and for peanuts stored for 0, 5, 15, 28, 45, 56, 84, 112, 140 and 154 d at 50°C and 60°C.

Results and Discussion

The six coating treatments used in sensory evaluation differed significantly in all the attributes. The coated peanuts were judged darker, more glossy, less rancid, more burnt, less roasted, more dry cardboard, less brittle, more chewy, more rubbery and sweeter. The control 4 sample was generally characterized to be between the coated samples and the reference (uncoated).

The four different storage times (0, 5, 15 and 45 d) were significantly different in all the attributes. As the peanut samples were stored longer, the darkness, rancidity, burnt, dry cardboard and brittleness significantly increased; and gloss, roasted, chewiness and sweetness significantly decreased.

The effect of three storage temperatures (40°C, 50°C and 60°C) was significantly different in most of the attributes. For brittleness and chewiness, temperature was not a significant source of variation. Similar to storage time increase, darkness, rancidity, burnt, dry cardboard, rubbery and bitterness increased significantly as the temperature was increased; and roasted, initial

sweet and sweetness decreased significantly. These findings were also observed with peanut paste stored at different temperatures (26).

Principal Component Analysis (PCA) was performed on the matrix of mean sensory attribute ratings and hexanal content across the samples for each storage time.

Figure 1 shows the PCA biplot for storage day 45. PCI and PCII explained 48.0% and 25.4% of the total variance, respectively. The uncoated peanut samples were characterized by the rancid attribute and hexanal content as the storage time increased, whereas the coated samples were characterized more by dry cardboard and burnt attributes. As the storage days increased, the uncoated samples were mostly characterized by bitter, rancid, brittle and slippery attributes and hexanal content. The control was mostly characterized by roasted and smooth attributes. The coated samples were not different among themselves regarding the different coating treatments, indicating that the different formulations did not affect the characteristics of the coatings significantly. The coated samples stored for 45 d were mostly characterized by initial sweet, chewy, dry cardboard, rubbery, glossy, burnt and lightness. As suggested previously, the coated samples exhibited more of chewy and rubbery characteristics than the uncoated samples, likely due to higher moisture content.

The results of sensory rancid rating and instrumental hexanal measurement both indicated that the uncoated samples were definitely more oxidized than the coated samples, which in turn showed that WPI-based coatings played a significant role as a good oxygen barrier. As the storage time increased, the hexanal content significantly correlated with the sensory rancid attribute ($r = 0.77$, $p < 0.001$), which shows that the hexanal measurement by GC is a good indicator of rancidity in peanuts as assessed by human perception. Similar correlations have been reported for peanuts and peanut pastes (4,10,14,26). Hence, hexanal level was used as a deterioration factor to be monitored (quality attribute) in the next step of this study, accelerated shelf-life testing.

Most food quality deterioration has been found to fit either a zero- or first-order kinetics (27):

$$-(\frac{dA}{dt}) = k(A)^n$$

where, A = a quality attribute measured in some units, n = the reaction order, and k = the rate constant. For either zero- or first-order deterioration, it can be shown that (18): $k_1 t_{S1} = k_2 t_{S2}$, where, k_1 = rate constant at T_1, k_2 = rate constant at T_2, t_{S1} = shelf-life at T_1, and t_{S2} = shelf-life at T_2.

The Arrhenius relationship (27) or the linear model (18) can be utilized to describe the rate of a reaction (k) of the sample at different temperatures. These models can be used to extrapolate shelf-life results from accelerated tests at higher storage temperatures to ambient storage conditions.

The rate of oxidation for peanut samples was determined by plotting the hexanal level vs. storage time. From the hexanal vs. time plots, linear regressions were calculated from data points taken during the initiation and
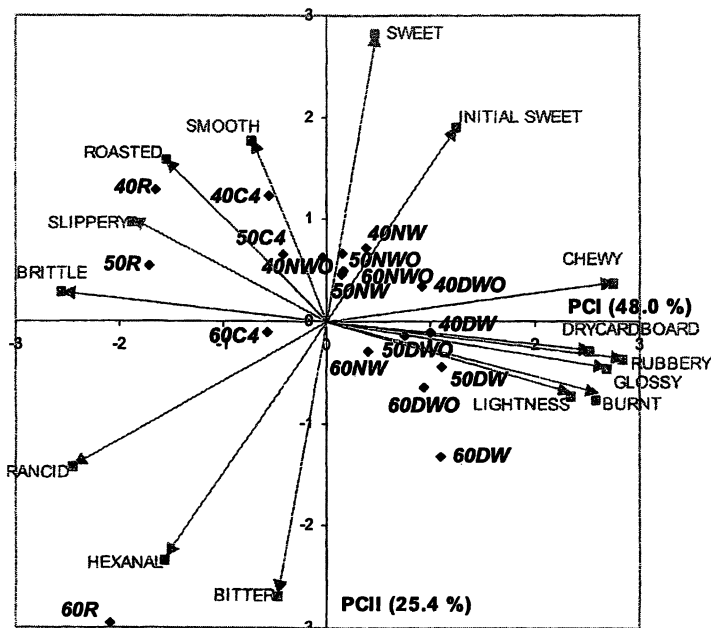
Figure 1. Principal Component Analysis (PCA) biplot of the matrix of mean sensory attribute ratings across the samples for storage day 45. Codes for the samples are in Table I.

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propagation periods. The x-value of the intercept of these linear regressions was determined to estimate induction period. This method of analysis was performed for all the samples at each temperature. Since the rate of oxidative rancidity accelerates starting from the end-point, the estimated induction period could be recognized as a conservative shelf-life (t_s) at the three accelerated-storage temperatures.

The general mathematical expression for the Arrhenius relationship is as follows (27):

$$k = k_0 \, e^{-EA/RT} \qquad [1]$$

where, k = rate constant for deteriorative reaction at temperature T, k_0 = constant, independent of temperature (also known as the Arrhenius, pre-exponential, collision or frequency factor), E_A = activation energy (J/ mole), R = ideal gas constant (8.314 JK^{-1} $mole^{-1}$), and T = absolute temperature (K). From equation [1], it can be seen that:

$$\log k = \log k_0 - \frac{E_A}{2.3RT} \qquad [2]$$

and, since $k_1 t_{S1} = k_2 t_{S2}$, it can be shown that:

$$\log\left(\frac{t_{S1}}{t_{S2}}\right) = \frac{E_A}{2.3R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad [3]$$

where, k_1 = rate constant at T_1, k_2 = rate constant at T_2, t_{S1} = shelf-life at T_1, and t_{S2} = shelf-life at T_2.

The plot of equation [3] was made by converting the estimated initiation period (t_s) at each of the three storage temperatures to log (t_s) and the storage temperature to 1/(absolute temperature of the storage temperature, T), and plotting log (t_s) vs. 1/T. Figure 2 shows this plot for peanuts coated with the denatured WPI with vitamin E treatment solution. From this plot, an Arrhenius shelf-life equation was determined using regression analysis, and the shelf-life for this treatment at 25°C was predicted. The same method of analysis was used to predict the shelf-life for each coating treatment at 25°C. This information is shown in Table II. This table shows that the Arrhenius shelf-life equation was a good fit for all the samples, with R^2 of above 0.99.

Table II. The Arrhenius shelf-life equation, R^2 and the estimated shelf–life (t_s) at 25°C.

Peanut Sample Codes [a]	Arrhenius equation	R^2	Estimated shelf-life at 25°C (Days)
DW	y = 2358.5x − 5.396	0.998	329.6
DWO	y = 1873.2x − 3.850	0.999	272.8
NW	y = 2129.9x − 4.690	1	286.6
NWO	y = 2131.2x − 4.665	0.999	306.6
C4	y = 2035.3x − 4.308	0.996	332.3
C3	y = 1870.1x − 3.750	1	327.9
C2	y = 1846.3x − 3.697	0.998	315.6
C1	y = 1749.2x − 3.409	0.996	288.8
R	y = 1788.2x − 3.867	0.999	136.1

[a] For peanut sample codes, see Table I.
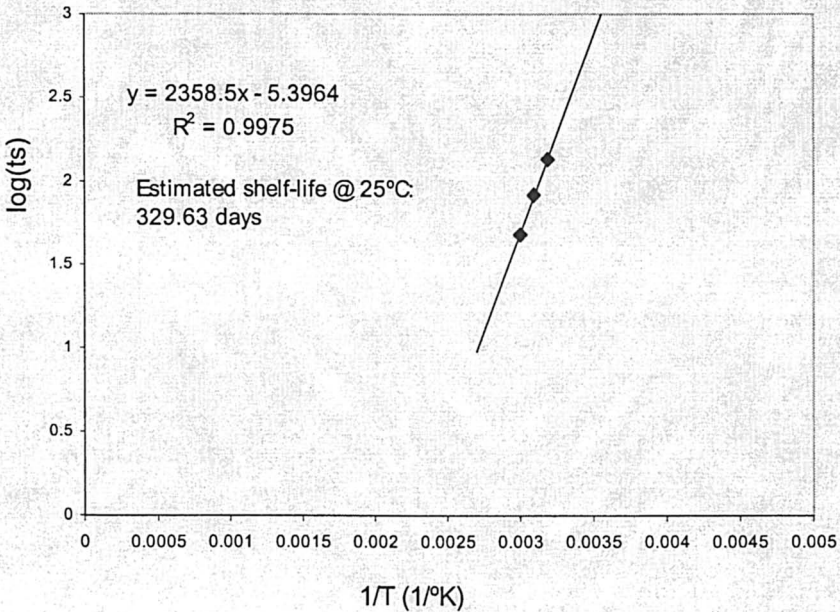
Figure 2. Arrhenius shelf-life plot and equation for peanuts coated with denatured WPI with Vit. E.

The linear model (18) can also be used to extrapolate the shelf-life to different temperatures. The general mathematical expression for the linear plot is as follows:

$$k = k_0 \, e^{\, b(T - T_0)} \qquad [4]$$

where, $k_0 =$ rate at temperature T_0 (°C), $k =$ rate at temperature T (°C) and b = a constant characteristic of the reaction (18). When only a small temperature range is used, there is little error in using the linear plot rather than the Arrhenius plot (27). Again, since $k_1 t_{S1} = k_2 t_{S2}$, it can be shown using equation [4] that:

$$\log(\frac{t_{S1}}{t_{S2}}) = \frac{b}{2.3}(T_1 - T_2) \qquad [5]$$

The linear model shelf-life plot, also known as the shelf-life plot (28) was made by plotting log (t_s) vs. T (°C). Figure 3 shows the linear model shelf-life plot for peanuts coated with the denatured WPI with vitamin E solution. The mathematical expressions and R^2 values for the linear model shelf-life equations for all the samples are shown in Table III, along with the predicted shelf-life at ambient temperature. A significant difference was not shown between the fit of the data to the linear model and that of the Arrhenius model. The data of this study fit the two models very well, with R^2 values exceeding 0.99 in each case.

Table III. The Linear model shelf-life equation, R^2 and the estimated shelf–life (t_s) at 25°C.

Peanut Sample Codes [a]	Linear model equation	R^2	Estimated shelf-life at 25°C (Days)
DW	y = -0.0226x + 3.043	0.999	300.3
DWO	y = -0.018x + 2.851	0.998	252.0
NW	y = -0.0204x + 2.930	1	263.3
NWO	y = -0.0204x + 2.959	0.998	281.3
C4	y = -0.0195x + 2.972	0.993	305.2
C3	y = -0.0179x + 2.931	0.998	304.1
C2	y = -0.0177x + 2.908	0.996	292.2
C1	y = -0.0168x + 2.848	0.993	267.8
R	y = -0.0172x + 2.531	1	126.3

[a] For peanut sample codes, see Table I.
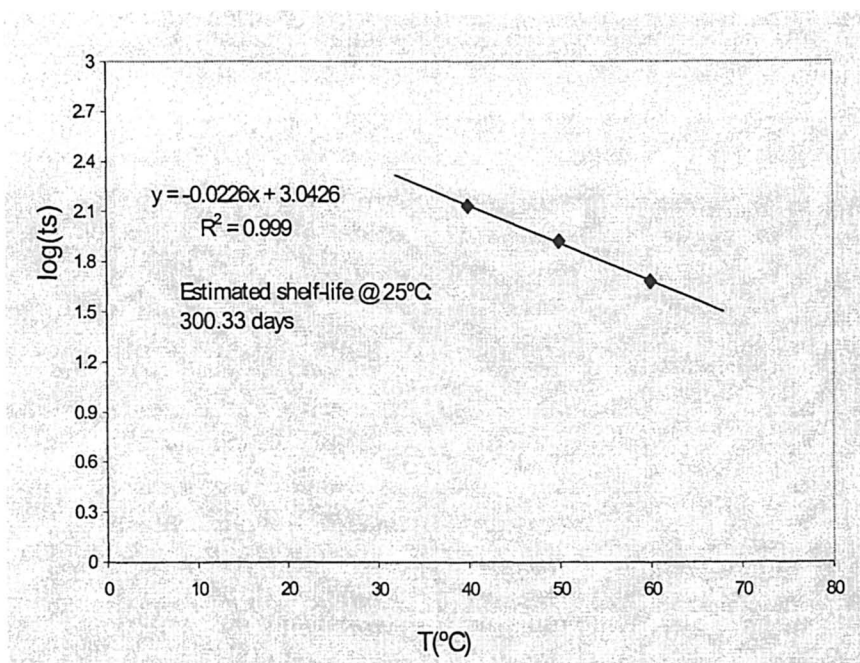
Figure 3. The linear shelf-life plot and equation for peanuts coated with denatured WPI with Vit. E.

Oxidation in the coated samples and the controls was delayed by two-fold compared to the reference, shown by increase in the shelf-life by two-fold. This result indicated that the WPI-based coatings provided protection against oxidation. However, the same degree of protection by the control treatments was also exhibited, even with control 1, which only had water treatment.

Conclusions

Instrumental headspace GC measurement was a good indicator of rancidity in agreement with sensory evaluation. The results from accelerated shelf-life testing conducted at higher temperatures can be extrapolated to predict the shelf-life at normal storage conditions. The Arrhenius and the linear models based on the accelerated test were both successful in predicting the shelf-life at room temperature (25°C) of the whey-protein-coated peanuts. The effect of the controls in delaying oxidation should be investigated in the further studies. In the future study, the predicted shelf-life of this study can be compared to the actual shelf-life of the peanut samples stored at ambient conditions to test the validity of the models used.

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Chapter 14

Lipid Oxidation in Muscle Foods

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The main factors governing the eating quality of muscle foods
are tenderness, color and flavor. Oxidation of lipids is a
major cause of deterioration in the quality of muscle foods and
can directly affect many quality characteristics such as flavor,
color, texture, nutritive value and safety of the food. It is
generally accepted that lipid oxidation in muscle foods is
initiated in the highly unsaturated phospholipid fraction in
subcellular membranes. Oxidative damage to lipids may
occur in the living animal because of an imbalance between
the production of reactive oxygen species and the animal's
antioxidant defence mechanisms. This may be brought about
by a high intake of polyunsaturated fatty acids, or by a
deficiency of nutrients involved in the antioxidant defence
system. Damage to lipids is accentuated in the immediate
post slaughter period and, in particular, during handling,
processing and storage. Dietary factors contribute to the
antioxidant defence system and protect biological membranes
against lipid oxidation. A variety of nutrients and non-
nutrients, including vitamin E, have been shown to affect the
prooxidant/antioxidant balance and ultimate quality of the
food. This review focuses on the effects of vitamin E and
other antioxidant micronutrients on lipid oxidation, color,
water-holding capacity and cholesterol oxidation in muscle
foods.

Introduction

Meat plays an important role in the diet of humans by contributing quality protein, essential minerals and trace elements and a range of B vitamins. In addition to its nutritive value meat has other important attributes, including its attractive sensory properties. The main factors governing the acceptability and eating quality of meat are tenderness, color and flavor. However, despite these important and highly acceptable attributes, meat consumption has come under close scrutiny in recent years. Emphasis on the role of fat and saturated fatty acids in health and disease and the greater sensitivity of people to environmental and animal welfare issues has had an influence on animal production, animal nutrition, food processing and food consumption patterns. There is now a greater demand than ever for foods perceived as natural, fresh tasting and more nutritious. In addition, changing lifestyles have a major impact on food purchasing, preparation, convenience and consumption. A recent pan-European Union survey of consumer attitudes to food, nutrition and health showed that the most important factors influencing consumer food choice were 'quality/freshness', 'price', 'taste', 'trying to eat healthy' and 'family preference' (1). One of the main factors limiting the quality and acceptability of meat and meat products is lipid oxidation, a process that leads to discoloration, drip loss, off-odor and off-flavor development and the production of potentially toxic compounds (2). Preventing lipid oxidation during storage and retail display is, therefore, essential in order to maintain the quality, wholesomeness and safety of meats, and to ensure that customers will make repeat purchases.

Lipid Oxidation *in vivo*

The production of free radicals *in vivo* is a critical determinant of animal health, and consequently food quality, wholesomeness and acceptability of muscle foods by the consumer. Lipid oxidation is a free radical-mediated chain reaction. The most important free radicals are reduced derivatives of oxygen called reactive oxygen species (ROS). These include free radicals having one or more unpaired electrons that can exist independently for a brief period (3). Examples are the hydroxyl radical (HO$^{\bullet}$) (the most potent oxidant encountered in biological systems with an estimated half-life of about 10^{-9}s), superoxide anion (O$_2^{-\bullet}$) and the oxygen-centered radicals of organic compounds (peroxyl, ROO$^{\bullet}$, and alkoxyl, RO$^{\bullet}$). Other ROS include hydrogen peroxide (H$_2$O$_2$), hypochlorous acid (HOCl) and hydroperoxides and epoxide metabolites of endogenous lipids. These are not free radicals but contain chemically reactive oxygen-containing groups (4).

ROS can be produced either accidentally or deliberately (3). Small amounts of ROS, including HO$^{\bullet}$, O$_2^{-\bullet}$ and H$_2$O$_2$, are produced during normal

aerobic mitochondrial metabolism. Peroxisomes that include fatty acyl CoA oxidase, dopamine-β-hydroxylase and urate oxidase among others produce H_2O_2 as a by-product, which is then degraded by catalase. Some H_2O_2 molecules escape degradation, leaks into other compartments and induces oxidative damage. The cytochrome P450 mixed-function oxidase system in animals constitutes a primary defence against various xenobiotics and endogenous substances and enhances production of free radicals. On the other hand, phagocytic cells deliberately generate $HO^\bullet$, $O_2^{-\bullet}$ and HOCl and use them to inactivate bacteria and viruses. A number of exogenous factors may also increase the endogenous free radical load. High intakes of iron and copper or 'misplaced' iron as a result of tissue breakdown promote the generation of oxidizing radicals from peroxides (5).

The initiation step in lipid oxidation involves the abstraction of a bis-allylic hydrogen from a polyunsaturated fatty acid (RH) by $HO^\bullet$ induced by ROS, heat, UV light or metal catalysts (6). The resulting fatty acid acyl radical ($R^\bullet$) reacts rapidly with O_2 to form a fatty acid peroxyl radical ($ROO^\bullet$), with a reaction rate constant (k_1) of 3×10^8 $mol^{-1}s^{-1}$. As $ROO^\bullet$ is more highly oxidized than $R^\bullet$, it will preferentially oxidize other unsaturated fatty acids, producing lipid hydroperoxides (ROOH) and $R^\bullet$, thereby propagating the chain reaction. The rate constant (k_2) for the reaction of $ROO^\bullet$ with an unsaturated fatty acid is relatively low ($10-10^2$ $mol^{-1}s^{-1}$). ROOH are both products of oxidation and substrates for further reactions with Fe^{2+} and Cu^+, yielding $ROO^\bullet$ and alkoxyl radicals (7). Fe^{2+} reductively cleaves ROOH as follows:

$$Fe^{2+} + ROOH \quad \rightarrow \quad ROO^\bullet + Fe^{3+} + OH^\bullet \qquad (1)$$
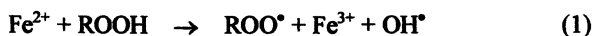
and Fe $^{2+}$ can be regenerated as follows:

$$Fe^{3+} + ROOH \quad \rightarrow \quad ROO^\bullet + Fe^{2+} + H^+ \qquad (2)$$
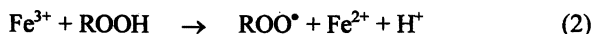
Other strong reductants such as ascorbic acid also reduce Fe^{3+} to Fe^{2+} (8). Both $ROO^\bullet$ and $RO^\bullet$ can initiate further reactions including:

$$RO^\bullet + RH \quad \rightarrow \quad ROH + R^\bullet \qquad (3)$$

$ROO^\bullet$, $RO^\bullet$ and ROOH degrade to alkyl radicals ($R'CH_2^\bullet$), ethane, pentane and a range of aldehydes including hexanal, malondialdehyde and 4-hydroxynonanal, which can react readily with ε-amino groups of proteins to yield Maillard type complexes.

Lipid Oxidation in Meat and Meat Products

Phase two of oxidative damage occurs in the immediate post-slaughter stage. Biochemical changes during the conversion to muscle to meat result in conditions where oxidation in the highly unsaturated phospholipid fraction in

subcellular membranes is no longer tightly controlled and the balance between prooxidative and antioxidative capacity favors oxidation. Post-slaughter factors such as early postmortem pH drop, carcass temperature and electrical stimulation are likely to disrupt cellular compartmentalization and release catalytic metal ions *(7)*.

The third phase of lipid peroxidation occurs during handling and processing and the mechanisms are likely to be similar to those that occur in stressed tissue *in vivo*. During handling, processing, cooking and storage, iron is released from high molecular weight molecules such as hemoglobin, myoglobin, ferritin and hemosiderin, resulting in the catalysis of lipid oxidation *(9)*. This may be compounded by dietary factors, particularly the degree of dietary fat unsaturation *(2)*.

Antioxidants and stability of meat and meat products

Lipid oxidation may be controlled, or at least minimized, through the use of antioxidants. Dietary supplementation allows vitamin E to be incorporated directly into phospholipid membranes where lipid oxidation is initiated *(10)*. It has been calculated that vitamin E can scavenge ROO° about 10^4 times faster than ROO° can react with unsaturated fatty acids *(6)*, so that relatively small amounts of vitamin E are required for effective antioxidant protection *(2)*. Numerous studies have demonstrated that dietary vitamin E supplementation (in the form of *all-rac-α*-tocopheryl acetate) consistently increases α-tocopherol levels in muscle and improves oxidative stability in meat from a number of species including pigs, chickens, turkeys, cattle and lambs *(11, 12, 13, 14, 15)*. The majority of studies have focused on measuring chemical indices of oxidation, most notably thiobarbituric acid-reacting substances (TBARS). However, in recent years emphasis has begun to shift towards sensory evaluation and the measurement of secondary oxidation products with odor and flavor impact. Other naturally occurring antioxidants have also been examined. Some, such as ascorbic acid, have been shown to produce inconsistent effects and their practical value as antioxidants in meats has been questioned *(2)*. However, plant extracts such as tea catechins and rosemary extracts have produced more consistent antioxidant effects.

Lipid oxidation

Dietary vitamin E supplementation inhibits lipid oxidation in pork chops *(16)* and ground pork *(17)*. Supplemental vitamin E also improves oxidative stability in cooked chops packed in modified atmospheres *(18)* and vacuum packaged precooked chops and roasts *(19, 20)*. The majority of studies have examined the effects of supplementation in the range of 100-200mg α-tocopheryl kg^{-1} feed. Based on the rate of uptake of dietary α-tocopherol by various tissues and the time required to achieve tissue saturation and optimal

resistance to oxidation, Morrissey *et al.* *(11)* proposed that pigs should be fed 200mg kg^{-1} feed for about 90 days.

Supplementation of broiler diets with 200mg α-tocopheryl acetate kg^{-1} feed for at least 4 weeks prior to slaughter has been recommended in order to provide adequate protection against lipid oxidation in broiler breast and thigh meat *(21)*. Turkey tissues accumulate α-tocopherol more slowly than other species such as chicken. Wen *et al.* *(22)* observed that at supplemental intakes (300 or 600 mg α-tocopheryl acetate kg^{-1} feed) 13 weeks were required for muscle α-tocopherol to reach its highest level. At these levels of supplementation, lipid oxidation was inhibited in raw and cooked ground breast meat during refrigerated and frozen storage. Higgins *et al.* *(23)* supplemented turkey poults with 600mg α-tocopheryl acetate kg^{-1} feed for 21 weeks. Supplementation inhibited lipid oxidation in vacuum-packaged and aerobic-packaged raw ground breast and thigh meat, with the inhibitory effect being greater in aerobic-packaged meat. Supplementation with 300 and 600mg kg^{-1} feed also reduced lipid oxidation in previously frozen turkey breast which was cooked, sliced and refrigerated in aerobic packaging *(24)*. Mercier *et al.* *(25)* supplemented turkey diets with 400mg kg^{-1} for 16 weeks, and reported inhibition of lipid oxidation in refrigerated raw meat, when diets contained saturated (tallow) or unsaturated (rapeseed and soya) fats.

Refined rosemary extract has been proposed as a natural antioxidant in several food systems, especially those containing animal fats and vegetable oils *(26)*. The addition of rosemary extracts has been shown to inhibit lipid oxidation in pork fat *(27)* and fresh and precooked, minced meat products stored under refrigerated and frozen conditions *(28)*. Recently, the optimum concentration of rosemary required to effectively inhibit oxidation in fresh and previously frozen pork patties was determined as 0.1% *(29)*. In addition, Murphy *et al.* *(30)* observed that rosemary oleoresin extract inhibited lipid oxidation in the presence of salt in pre-cooked roast beef slices during refrigerated, but not frozen, storage. Mixtures of α-tocopherol and rosemary extract have been shown to exert a stronger protective effect than either antioxidant alone *(31)*. The addition of antioxidants (Duralox, Herbalox and BHA/BHT) during processing was found to be more effective in preventing lipid oxidation than dietary α-tocopherol supplementation alone in modified atmosphere packaged (MAP) and aerobically packaged beef patties. Furthermore, the combination of dietary α-tocopherol and rosemary extracts (Duralox and Herbalox) was found to be as effective in inhibiting lipid oxidation as the combination of dietary α-tocopherol and BHA/BHT during processing *(32)*.

Tea catechins are a group of polyphenols present mainly in green tea (*Camellia sinensis*). They are reported to be efficient scavengers of the superoxide anion and hydrogen peroxide *(33)*, and singlet oxygen *(34)*. Tang *et al.* *(35)* supplemented broiler diets with 50, 100, 200 and 300mg tea catechins kg^{-1}. Tea catechins exhibited an antioxidant effect in breast and thigh meat that had been ground and refrigerated following frozen storage for up to 9 months,

with the greatest protection seen at the 200 and 300 level. Tea catechins offered a similar level of protection as an equivalent level of dietary vitamin E up to 3 months storage, while higher levels of tea catechins were required for longer storage periods. The antioxidant effects of added tea catechins in raw minced red meat (beef and pork), poultry (chicken, duck and ostrich) and fish (whiting and mackerel) muscle on susceptibility to lipid oxidation were compared to that of α-tocopherol during 10 days of refrigerated display *(36)*. The antioxidant potential of tea catechins was 2-4 fold greater than that of α-tocopherol at the same concentration and this potential was species dependent.

Flavor

Secondary oxidation products (volatiles) contribute significantly to the flavor, and hence acceptability of meat. However, uncontrolled oxidation results in the formation of off-odors and off-flavors and the phenomenon of warmed over flavor (WOF). Dirinck *et al. (37)* found that supplementing the finishing diet with 200mg α-tocopheryl acetate kg^{-1} resulted in fresher flavor in refrigerated, cooked pork compared to meat from animals fed 60mg kg^{-1}. Cava *et al. (38)* reported that supplementation of pig diets with 100mg α-tocopheryl acetate kg^{-1} feed reduced levels of saturated aldehydes (hexanal, pentanal and heptanal) in raw muscle.

Blum *et al. (39)* observed flavor deterioration in refrigerated meat from broilers fed 20mg α-tocopheryl acetate kg^{-1} compared to those fed 160mg kg^{-1}. However, no effect was observed in frozen samples. De Winne and Dirinck *(40)* found that vitamin E supplementation (200mg kg^{-1} feed) inhibited the formation of saturated and unsaturated aldehydes in raw chicken breast and thigh meat and reduced off-flavor in cooked meat. Vitamin E supplementation also reduced WOF development in cooked ground chicken thigh meat following refrigerated storage for up to 5 days, and in previously frozen meat (up to 10 weeks*) (41)*.

Wen *et al. (42)* reported lower hexanal levels in ground cooked turkey following storage for 7 days from birds supplemented with 600mg α-tocopheryl acetate kg^{-1} feed compared to an unsupplemented group, and Higgins *et al. (43)* reported that the same level of supplementation resulted in less WOF development in refrigerated ground cooked meat. Sheldon *et al. (44)* investigated the effect of supplementation with up to 25 times the normal vitamin E requirement on flavor and headspace volatiles in turkey. This level consistently produced higher turkey meat flavor and aftertaste scores and lower oxidized meat flavor and aftertaste scores in cooked ground meat during refrigerated storage for up to 8 days. Similar effects were observed in samples which had been previously frozen for up to 150 days. Supplementation with over 10 times the normal requirement reduced total headspace aldehydes (which contribute to flavor) in raw samples refrigerated for 7 days. Recent data from our laboratory also indicate that supplemental dietary vitamin E reduces the formation of aldehydes in cooked ground duck meat (unpublished data).

Irradiation is known to induce off-odors in meats. Patterson and Stevenson *(45)* reported that dietary vitamin E supplementation (800mg α-tocopheryl acetate kg^{-1}) resulted in a 39 and 44% reduction in total volatiles at doses of 2.5 and 10.0 kGy, respectively, in thigh meat.

Cholesterol oxidation

Cholesterol oxidation products (COPs) may be involved in atherogenesis, and their control is, therefore, of interest in muscle foods. Dietary α-tocopherol supplementation has been shown to reduce COPs in refrigerated (2 and 4 days) cooked ground pork *(46)*. COPs represented 1.6% of total cholesterol in pork from pigs supplemented with 200mg α-tocopheryl acetate kg^{-1} diet compared to 2.1% in pigs fed a basal diet (10mg α-tocopheryl acetate kg^{-1} feed). Recently supplementation of pig diets with 200mg α-tocopheryl acetate kg^{-1} feed was shown to reduce total COPs in cooked pork chops after 0 and 9 days refrigerated storage, when diets contained sunflower oil, olive oil or mixtures of both with linseed oil *(47)*. Supplementation with 500mg α-tocopheryl acetate kg^{-1} feed reduced COPs by 65% in cooked veal following refrigerated storage for 4 days *(48)*. In ground, cooked chicken breast and thigh, 25-hydroxycholesterol concentrations were reduced by vitamin E supplementation (200 or 800mg kg^{-1} feed) after 12 days refrigerated storage *(49)*. Similarly, supplementation with 200mg kg^{-1} reduced total COPs by approximately 60% in cooked ground chicken refrigerated for 4 days *(50)*. Grau *et al. (51)* reported that vitamin E supplementation (225 kg^{-1} feed) inhibited COPs formation, regardless of dietary fat source, (saturated or unsaturated) in raw and cooked vacuum packaged ground dark chicken meat following storage at -20°C for 7 months. In beef, Galvin *et al. (52)* found that supplementation with 3000mg α-tocopheryl acetate/head/day reduced 7-ketocholesterol concentrations in vacuum packaged cooked *M. psoas major* steaks during refrigerated and frozen storage, but not in *M. longissimus dorsi*. This was attributed to the greater lipid stability of *M. longissimus dorsi*.

Irradiation increases COPs in meat at doses permitted for food use *(53)*. Supplementation of broiler diets with 400mg α-tocopheryl acetate kg^{-1} feed reduced total COPs in irradiated ground breast and thigh meat after 5 days refrigerated storage, compared to supplementation with 100 and 200 kg^{-1} feed *(54)*.

Lopez-Bote *et al. (55)* reported that supplementation of diets with rosemary oleoresin (500mg kg^{-1} feed) reduced total COPs in white and dark cooked ground chicken meat following refrigerated storage for up to 4 days. Supplementation with α-tocopheryl acetate (200mg kg^{-1} feed) reduced cops to a greater extent.

Color

At the point of sale, color and color stability are the most important attributes of fresh meat quality and various approaches have been used to meet consumer expectation that an attractive bright-red color is compatible with long shelf-life and good eating quality *(56)*. There is no doubt that the willingness of consumers to purchase fresh meats is strongly influenced by the appearance of the meat on display. In red meats, particularly beef, a bright cherry-red color (bloom) is perceived by consumers as being indicative of freshness, while they discriminate against beef which has turned brown *(57)*. It is the oxidation of the fresh muscle pigment oxymyoglobin to the brown pigment metmyoglobin that leads to the discoloration of red meats. There is a general consensus that the processes of oxymyoglobin and lipid oxidation in muscle foods, while independent of each other, can be inter-related. However, the exact nature of this inter-relationship has not been established. For instance, one hypothesis is that oxymyoglobin oxidation initiates the first step in a sequence of chemical reactions leading to the production of radicals (porphyrin cation radicals) that lead to the initiation of lipid oxidation *(58)*. Conversely, another hypothesis is that muscle lipids and liposomes can catalyze oxymyoglobin oxidation *(59, 60)*. Irrespective of the process and inter-relationship between oxymyoglobin and lipid oxidation, dietary supplementation of α-tocopheryl acetate in bovine and ovine animals clearly stabilizes and extends the color shelf-life of meat cuts taken from these species. A comprehensive review of this area has been provided by Kerry *et al. (62)*. The majority of studies carried out on dietary supplementation with α-tocopheryl acetate to cattle have consistently shown improved color stability on subsequent retail storage of all meat cuts *(62, 63, 64, 65, 66, 67, 68, 69, 70)*. With the exception of the report by Strohecker *et al. (71)*, similar observations have been made for lamb meat color (*13, 14, 15*). Little has been reported on the effects of tea catechins and rosemary extracts on meat color stability. However, research carried out in our laboratory has shown that their direct addition to red meat during processing has improved and extended fresh meat color. Conversely, addition of tea catechins, in particular, to white processed meat systems can cause problematic discolorations in the final products.

Drip loss

Excessive drip loss from fresh meat signifies not only financial losses associated with such meat but losses in valuable vitamins, minerals, flavor compounds and water. Loss of the latter component can affect overall eating quality, producing meat that can be described as tough and having poor mouthfeel characteristics. Vitamin E may have beneficial effects on drip loss, as it is involved in stabilizing lipid membranes *(72)*. One of the first reports on the effect of vitamin E on drip loss in meat was provided by Asghar *et al. (73)*. They reported that pork chops from pigs receiving a control diet had significantly higher drip loss than pork chops from pigs which had received a

diet supplemented with 200 mg α-tocopheryl acetate kg^{-1}. In addition, pork chops with higher drip loss were found to be more favorable to the growth of spoilage microorganisms. Den Hertog-Meischke *et al. (74)* showed that vitamin E supplementation affected drip loss in various bovine muscles. However, the authors demonstrated that this effect was muscle dependent. They showed that while α-tocopheryl acetate supplementation reduced drip loss in *M. semitendinosus*, it increased drip loss in *M. psoas major*. This contrast in terms of vitamin E effects on drip loss from muscles, as well as the methodologies used to carry out drip loss analysis, may help explain the wide range of views and data that researchers have generated in relation to this particular meat quality parameter.

Conclusions

Lipid oxidation is one of the main factors limiting the quality and ultimately the acceptability of meat and meat products. There is considerable evidence that natural antioxidants, particularly vitamin E, are effective in reducing the extent of lipid oxidation. Dietary supplementation with vitamin E reduces lipid and cholesterol oxidation, oxymyoglobin oxidation, and, in some situations, drip loss. Supplementation also inhibits off-flavor formation. Based on current evidence, optimum levels of dietary vitamin E to effectively inhibit lipid oxidation can be suggested, as discussed previously *(11)*. However, the optimum levels of supplementation required to effectively maintain sensory quality are not clear. There is growing evidence to show that rosemary extracts and tea catechins are effective inhibitors of lipid oxidation. However, their impact on sensory quality also needs to be clarified.

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Chapter 15

Oxidative Stability of Edible Oils as Affected by Their Fatty Acid Composition and Minor Constituents

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Edible oils are composed of triacylyglycerols and minor constituents. The nutritional quality and stability of the oils is affected primarily by the chemical nature of the fatty acids involved, as reflected in their degree and type of unsaturation. However, minor components present in the oils also exert a major influence on their stability characteristics. The minor constituents of the oils include phospholipids, tocols, ubiquinones and other phenolics, phytosterols, carotenoids, chlorophylls, hydrocarbons, waxes and wax esters. During processing, the content of minor constituents is generally reduced by 35-95%. Thus, the Rancimat induction period of red palm oil was reduced by over 15 h at 100°C upon removal of its minor components, mainly tocols and carotenoids. Stability of other oils was also affected by the chemical nature of their minor constituents to different degrees. Under photooxidative conditions, the positive effects of minor constituents may be overwhelmed by the photosensitizing effect of chlorophylls, if present. Thus, proper storage and packaging conditions are deemed important for quality preservation and oxidative stability of edible oils.

Edible oils originate from plant, animal and algal sources. They provide a concentrated source of energy and essential fatty acids through daily dietary intake. Lipids also serve as an important constituent of cell walls and carrier of fat-soluble vitamins. In addition, lipids provide flavor, texture and mouthfeel to the food. Edible vegetable oils are also used for frying purposes and in this way serve as a

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heating medium which results in the generation of aroma, some of which are a direct consequence of the interaction of lipids and/or their degradation products with the constituents of food that is being fried.

Oilseeds and tropical fruits are a major source of food lipids although speciality fats and oils may also be obtained from a variety of unconventional source materials. The edible oils from oilseeds may be produced by pressing, solvent extraction or their combination. The seeds may first be subjected to a pretreatment heating to deactivate enzymes present. The oil after extraction is subsequently subjected to further processing of degumming, refining, bleaching and deodorization.

Edible oils from source materials are composed primarily of triacylyglycerols (TAG). In addition, phospholipids, glycolipids, waxes, wax esters, hydrocarbons, tocopherols and tocotrienols, other phenolics, carotenoids, sterols and chlorophylls, and hydrocarbons among others, may be present as minor constituents and these are collectively referred to as unsaponifiable matter (1). During processing, storage and use, edible oils undergo chemical and physical changes. Often, process-induced changes of lipids are necessary to manifest specific characters of food, however, such changes should not exceed a desirable limit. Both TAG and minor constituents of the oil exert a profound influence on the desirability of an oil source for nutrition and in health promotion and disease prevention.

In addition, in oilseeds and other oil sources, following oil extraction, the left over meal may serve as a source of fat-insoluble phytochemicals. Obviously, hulls, might be included in the meal if the seeds are not dehulled prior to oil extraction. The importance of bioactivities in processing by-products of oilseeds may thus require attention.

The quality characteristics of edible oils generally depend on the composition of their fatty acids, positional distribution of fatty acids, non-triacylglycerol components, presence/absence of antioxidants, the system in which the oil is present such as bulk oil versus emulsion and low-moisture foods, as well as the storage conditions and packaging material. An overview of the relevant topics is provided in this chapter.

Triacylyglycerols

Triacylyglycerols are generally the main fraction of neutral lipids and usually account for over 95% of edible oils. The fatty acids present are either saturated or unsaturated. Although saturated lipids were generally condemned because of their perceived negative effect on cardiovascular disease, more recent studies have shown that C18:0 is fairly benign while C14:0 may possess adverse health effects. Furthermore, the appreciation about detrimental effects of trans fats has served as

a catalyst for the return from using hydrogenated fats in place of naturally-occurring edible oils with a high degree of saturation.

Of the unsaturated lipids, monounsaturated fatty acids, similar to saturated fatty acids, are non-essential as they could be synthesized in the body *de novo*. However, polyunsaturated fatty acids (PUFA; containing 2 or more double bonds) could not be made in the body and must be acquired through dietary sources. The parent compounds in this group are linoleic acid (LA, C18:2ω6) and linolenic acid (LNA, C18:3ω3). The symbols ω6 (or n-6) and ω3 (or n-3) refer to the position of the first double bond from the methyl end group as this dictates the biological activity of the fatty acid molecules involved (2).

Over 80% of our dietary lipids are composed of C18 fatty acids and it is recommended that the ratio of ω6 to ω3 fatty acids be at least 5:1 to 10:1, but the western diet has a ratio of 20:1 or less. Enzymes in our body convert both groups of PUFA through a series of desaturation and elongation steps to C20 and C22 products, some of which are quite important for health and general well-being. The C20 compounds may subsequently produce a series of hormone-like molecules known as eicosanoids which are essential for maintenance of health. Obviously, elongation of LA and alpha-linolenic acid (ALA) to other fatty acids may be restricted by rate determining steps and lack of the required enzymes in the body (See Figure 1). Thus, pre-term infants and the elderly may not be able to effectively make these transformations and that production of docosahexaenoic acid (DHA) from eicosapentaenoic acid (EPA) is rather inefficient. Furthermore, supplementation with gamma-linolenic acid (GLA) as a precursor to arachidonic acid (AA) might also be necessary.

Lipids are generally highly stable in their natural environment; even the most unsaturated lipids from oilseeds are resistant to oxidative deterioration prior to extraction and processing. Nature appears to be able to protect itself as higher level of antioxidants are generally found in highly unsaturated oils. It is believed that unsaturated oils generally co-exist with antioxidants in order to protect themselves from oxidation; of course the natural capsule or seed coat provides a barrier to light and oxygen as well as compartmentalization of oil cells and inactivity of enzymes prior to crushing. However, upon crushing and oil extraction, the stability of edible oils is compromised and this is dictated by several factors, including the degree of unsaturation of fatty acid constituents. This topic will be discussed in further detail in a later section. In addition, the position of fatty acids in the triacylglycerol molecule (Sn-1, Sn-2 and Sn-3) would have a considerable effect on their assimilation into the body. Generally, fatty acids in the Sn-1 or Sn-3 position are hydrolyzed by pancreatic lipase and absorbed while those in the Sn-2 position are used for synthesis of new TAG and these might be deposited in the body.

Omega-6	Omega-3	
18:2 (LA)	18:3 (ALA)	
↓	↓	
18:3 (GLA)	18:4	
↓	↓	
20:3 (DGLA)	20:4	
↓	↓	
20:4 (AA)	20:5 (EPA)	
↓	↓	
22:4	22:5 (DPA) → 24:5	
↓	↓	↓
22:5	22:6 (DHA) ← 24:6	

Figure 1. Essential fatty acids (EFA) of the ω-6 and ω-3 families.

Non-triacylyglycerols Constituents

The non-triacylyglycerol or unsaponifiables matter content varies from one oil to another. Different classes of compounds belonging to this group are summarized in Table I. In most oils these constitute approximately 1%, but in others they may be present at 2-8%. Many of the unsaponifiable matter are recovered from oil during processing steps of degumming, refining, bleaching and deodorization. Thus, loss of sterols, tocopherols, carotenoids and related compounds during oil processing may range from 35 to 95%. These material may be collected as distillates during the deodorization process. Distillates that are rich in certain components may be separated and marketed for use in nutraceutical applications. Thus, mixed tocopherols, tocotrienols, carotenoids, lecithin and other constituents may be separated from soybean, palm oil, rice bran oil and barley oil, depending on their prevalence.

Table I. Non-tricylyglycerol Classes of Compounds in Edible Oils

Class of Compound	Example
Hydrocarbons	Squalene
Sterols	Phytosterols
Tocols	Tocopherols/Tocotrienols
Ubiquinones	Ubiquinone 9/Ubiquinone 10
Phenolic Compounds	Phenolic acids, Phenylpropanoids, Flavonoids/Isoflavonoids, Tannins
Carotenoids	Carotenes/Xanthophylls
Polar Compounds	Phospholipids/Glycolipids

Stability of edible oils is affected by their constituents and this is primarily dictated by the degree of unsaturation of fatty acids involved, the minor constituents of the oil and storage conditions. Thus as the degree of unsaturation of an oil increases, its susceptibility to oxidation, under similar conditions, increases. Furthermore, the condition in which the oil is present, e.g. bulk versus emulsion, has a profound effect on the stability of the oil. In addition, presence of antioxidants as well as metal ions, light, chlorophylls and other pigments might influence the stability of the oil. A cursory evaluation of the factors involved is given in the subsequent sections.

Degree of Unsaturation

As the number of double bonds in a fatty acid increases, its rate of oxidation increases. Thus, stability of fatty acid in edible oil sources follows the trend given below.

Docosahexaenoic acid > Eicosapentaenoic acid > Arachidonic acid >
    (C22:6ω3)   (C22:5ω3)   (C20:4ω6)
Linolenic acid > Linoleic acid > Oleic acid > Stearic acid
    (C18:3ω3)   (C18:2ω6)   (C18:1ω9)   (C18:0)

This trend is also reflected in the triacylyglycerols of different oils. Thus, as the iodine value (IV) of oils increases, their oxidation potential is also increased. Therefore, coconut oil with IV = 9 is far more stable than illipe oil with IV = 33 which is in turn more stable than canola oil with IV = 107 and black currant oil with IV = 143.

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Tocols and Ubiquinones

Tocols include both tocopherol (T) and tocotrienol (T3) family of compounds which are present in edible oils in different compositions and proportions. Eight different compounds exist; each series of T and T3 includes four components designated as α, β, γ and δ, depending on the number and position of methyl groups on a chromane ring. The α-isomer is 5, 7, 8-trimethyl; β-isomer, 5, 8-dimethyl; γ-isomer, 7,8-dimethyl; and δ is the 8-methyl isomer.

The occurrence of tocopherols in vegetable oils is diverse, but animal fats generally contain only α-tocopherol. However, absence of α-tocopherol in blood plasma and other body organs may not negate the importance of other tocopherols such as γ-tocopherol. In oilseed lipids, there appears to be a direct relationship between the degree of unsaturation as reflected in the IV, and the total content of tocopherols. Most vegetable oils contain α-, γ- and δ-tocopherols, while β-tocopherol is less prevalent, except for wheat germ oil. Meanwhile, tocotrienols are present mainly in palm and rice bran oils. The antioxidant activity of tocotrienols generally exceeds that of their corresponding tocopherols. Meanwhile, the antioxidant activity of tocopherols is generally in the order of δ 〉 γ〉 β 〉 α. The content of tocopherol/tocotrienol in selected oils is summarized in Table II. With respect to ubiquinones, also known as coenzyme Q, they occur as 6 to 10 isoprene unit compounds; that is Q_6 (UQ-6) to Q_{10} (UQ-10). Coenzymes Q_{10} (UQ-10) to a lesser extent Q_9 (UQ-9) are found in vegetable oils. Ubiquinone provides efficient protection *in-vivo* for mitochondria against oxidation, similar to vitamin E in the lipids and lipoproteins.

Phospholipids

Phospholipids possess fatty acids which are generally more unsaturated than their associated tricylglycerols. Therefore, phospholipids are more prone to oxidation than their associated triacylglycerols. However, this situation does not necessarily hold for marine oils such as seal blubber oil whose phospholipids are less unsaturated than its triacylglycerols. The role of phospholipids as pro- or antioxidants is, however, complex because in addition to their lipid moiety, they contain phosphorous - and nitrogen-containing groups that dictate their overall effect in food systems.

Extensive studies have demonstrated that phospholipids may exert an antioxidant effect in vegetable oils and animal fats. The exact mechanism of action of phospholipids in stabilizing fats and oils remains speculative; however, evidence points out to the possibility of their synergisms with tocopherols, chelation of pro-oxidant metal ions as well as their role in the formation of Maillard-type reaction products. King et al. (3) found a positive relationship between the presence and type of phospholipids and stability of salmon oil in the order given below.

Sphingomyelin, lysophosphatidylcholine ≈ phosphatidyl choline ≈ phosphatidyl-ethanolamine > phosphatidylserine > phosphatidylinositol > phosphatidylglycerol.

Phytosterols

Edible oils generally contain a variety of sterols which exist in the free form, as sterol ester of fatty acids and sterol glycosides or esters of sterol glycosides. Sterols are heat-stable molecules with no flavor of their own and exhibit antipolymerization activity during frying. Sterols serve as a means for fingerprinting of vegetable oils and lend themselves for detection of adultration of oils. Among sterols, Δ^5-avenasterol, fucosterol and citrostadienol have been shown to exhibit antioxidant properties. Donation of a hydrogen atom from the allylic methyl group in the side chain is contemplated. Most oils contain 100-800 mg/100g sterols (See Table II). Brassicasterol is specifically found in canola, rapeseed and mustard oils.

Carotenoids

Carotenoids are widespread in oilseeds, but are found in the highest amount in palm oil at 500-700 ppm levels. Both hydrocarbon-type carotenoids, namely α - and β-carotene, as well as xanthophylls are present. Carotenoids act as scavengers of singlet oxygen and hence are important in stability of oils exposed to light. While β-carotene, and α-carotene, are usually the dominant components, α-xanthophylls may be present in smaller amounts.

Chlorophylls

Chlorophylls are present in a variety of oils. In particular, extra virgin olive oil contains a large amount of chlorophylls and this is often associated with the high quality of this oil. However, in oils such as canola, immature seeds contain chlorophylls which end up in the oil and affect its stability. Meanwhile, grapeseed oil is generally green in color. Due to their photosensitizing effects, chlorophylls lead to oxidative deterioration of oils when exposed to light.

Phenolics

Phenolic compounds, other than tocols, may be present in edible oils. While tocols are always present in the free form phenolic acids, phenylpropanoids and flavonoids and related components occur in the free, esterified and glycosylated forms. These compounds reside mainly in the meal, but their presence in oils, such as olive oil, is well recognized. Olive oil contains a number of phenolics, including hydroxytyrosol. Furthermore, sesame oil contains sesamin, sesaminol and sesamol

Table II. Tocophenols (T) and Ubiquinones (UQ) and sterols in edible oils (mg/100g)

Oil	α-T	β-T	γ-T	δ-T	UQ-9	UQ-10	Sterols
Canola	17-26	-	35-61.2	0.4-1.2	0.2	7.3	350-840
Soybean	10.1-10.2	0.27	47.3-59.3	26.4-35.2	0.8	9.2	150-420
Sunflower	48.7-78.3	0.25	1.9-5.1	0.7-1.0	2.1	0.4	ND
Palm oil*	180	-	320	7.0	ND	ND	30-260

*Palm oil also contains 124, 30, 280 at 70 mg/100g of α-, β-, γ- and δ-tocotrienols.

which render stability to the oil. Meanwhile, oat oil contains a number of ferulates that affect its stability.

Hydrocarbons

Hydrocarbons are another group of unsaponifiable matter that may occur in edible oils. This class of compound includes squalene which constitutes one third of the unsaponifiables in olive oil. The effect of squalene in stabilization of oils at high temperatures and in the body for protection of skin is recognized. However, in an oil system, under Schaal oven conditions, we did not find it to be antioxidative in nature.

EFFECT OF PROCESSING ON NON-TRIACYLYGLYCEROLS COMPONENTS AND STABILITY OF OILS

As noted earlier, 35-95% of non-triacylyglyerol components of edible oils may be removed during different stages of degumming, refining, bleaching and deodorization process. The effects of processing, as determined by Ferrari et al. (4), on the contents of tocopherols and sterols are summarized in Table III. In addition, carotenoids in edible oils, especially in palm oil, might be depleted during processing. Bleaching of carotenoids might be carried out intentionally in order to remove the red color, however, carotenoids might be retained using cold pressing at temperatures of as low as 50°C.

In order to measure the effect of processing and contribution of minor components to the stability of edible oils, it is possible to strip the oil from its non-triacylyglycerols components. To achieve this, the oil is subjected to a multi-layered column separation. The procedure developed by Lampi et al. (5) may be employed. We used a column packed with activated silicic acid (bottom layer, 40 g) followed by a mixture of Celite 545 /activated charcoal (20 g, 1:2 (w/w)), a mixture of Celite 545/powdered sugar (80 g, 1:2 (w/w)) and activated silicic acid (40g) as the top layer. Oil was diluted with an equal volume of n-hexane and passed through the column that was attached to a water pump; the solvent was then removed. The characteristics of the oils before and after stripping indicated that while oxidative products and most of the minor components were removed, γ-tocopherol was somewhat retained in the oil.

Table III. Changes in the content and composition of minor components of edible oils during processing (mg/100g).

Constituent	Crude	Refined	Bleached	Deodorized
Canola (Rapeseed)				
Tocopherols	13.6	128.7	117.8	87.3
Sterols	820.6	797.8	650.4	393.0
Soybean				
Tocopherols	222.3	267.7	284.0	195.2
Sterols	359.5	313.9	288.8	295.4
Corn				
Tocopherols	194.6	203.8	201.9	76.7
Tocotrienols	7.9	10.2	10.0	6.1
Sterols	1113.9	859.2	848.8	715.3

The oils (olive, borage and evening primrose) were subjected to accelerated oxidation under Schaal oven condition at 60°C or under fluorescent lighting. Results indicated that oils were more stable, as such, than their stripped counterparts when subjected to heating, but under light, the oxidative stability of oils stripped of their minor components was higher. Examination of the spectral characteristics of oils indicated that chlorophylls were present in the original oils and hence might have acted as photosensitizers leading to enhanced oxidation of unstripped oils (6,7). In case of red palm oil, however, removal of carotenoids by stripping resulted in a decrease of near 15 h in this induction period as measured by Rancimat at 100°C (8). Therefore, minor components of edible oils and the nature of chemicals involved have a major influence on the stability of products during storage and food preparation.

NOVEL LIPIDS

Novel lipids may be prepared using blending and/or interesterification processes. While blending is an effective process to stabilize the oils due to high content of antioxidants in one component or to complement the fatty acid composition of an oil, interesterification places the desired fatty acids in the same molecule. Of particular interest is formulation of structured lipids in which medium-chain fatty acids are placed in the molecules together with other essential

fatty acids. In interesterification process, however, endogenous antioxidants in the oils might be eliminated and hence the stability of the final products compromised.

An innovative process for production of oils devoid of the Sn-2 fatty acids (ie. diacylglycerols) may be devised. These oils may further be supplemented with minor components of interests, such as phytosterols and tocols, among others.

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Chapter 16

Food Polymer Science Approach to Studies on Freshness and Shelf Life of Foods

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The usefulness of key concepts of the food polymer science (FPS) approach in studies on freshness and shelf-life has now been widely demonstrated and is discussed here. FPS, especially as it has been widely applied in the decade of the 90's, includes a practical understanding of the significance of the glass transition temperature, Tg, and its temperature range, how Tg is defined for multi-component, aqueous amorphous blends, and how Tg relates to the relative mobilities of individual components, including water, in such blends.

The science of sugar glasses has been studied for over 70 years [1-6]. Much more recently (22 years ago), the "food polymer science" (FPS) approach [7] was developed to study glasses and glass transitions, and their effects on processing, product quality and storage stability, in foods. Since 1980, this "new" approach to food research -- beyond that of the "water activity" concept of moisture management -- has been used to understand structure-function relationships, the effects of plasticization by water -- as illustrated by means of state diagrams -- on thermal, mechanical, rheological and textural properties, and physical (meta)stability in the non-equilibrium glassy solid state, vs. instability in the rubbery or viscous liquid state [8-12].

Blanshard [39] has stated that "there is no doubt that the perspectives and new understanding of food processing, storage and stability provided by a recognition of the existence and characteristics of the glassy and rubbery states have been one of the most stimulating and pervasive developments in food research in the past half century". We are proud to have been credited by Blanshard [39], and similarly by Roos et al. [40], with having played a pioneering role in this area of food research.

Recent applications and progress

Key concepts of the FPS approach, especially as it has been widely applied in the decade of the 90's, include a practical understanding of the significance of the glass transition temperature, Tg, and its temperature range, how Tg is defined for multi-component, aqueous amorphous blends, and how Tg relates to the relative mobilities of individual components, including water, in such blends [13]. The latter two aspects are illustrated in Figure 1 [13]. For a given glass-forming solute-water blend, Tg is determined by the weight-average molecular weight (Mw) of that blend. A probe molecule of the same Mw would be immobilized at the Tg of the blend, due to the dominance of local viscosity. In contrast, a solute molecule of higher molecular weight than Mw of the blend would be already immobilized at a higher Tg (determined by solute number-average molecular weight (Mn), due to the dominance of free volume), while water molecules, of lower molecular weight than Mw of the blend, would still be capable of showing some translational mobility (but much less than that of bulk liquid water) in the glassy blend at T < Tg of the blend [13].

Due to water's well-known plasticizing effect, Tg decreases monotonically (curvilinearly) with increasing moisture content, but the (inverse) linear relationship is between Tg and measured system relative humidity (% RH), rather than moisture content [11,14]. Like the glass transition itself, plasticization is a kinetic process, so the mere presence of water is not a guarantee that plasticization has already occurred [11,15,16].

In response to the question "what is the Tg of my system?", the following can be stated [13]. Across different glass-forming solute-water systems, Tg at a given RH increases with increasing solute(s) Mw, up to the so-called "entanglement molecular weight". This fact has been illustrated previously in terms of starch gelatinization temperatures and mold spore germination lag times [9]. If any ice can form in a system upon cooling, then the system is located above its Tg curve; i.e. Tsystem > Tg = Tg', Wsystem > Wg = Wg', and RHsystem > 0. For some solutes, Wg is more important than Tg, with respect to the location of the system relative to its Tg curve. Nevertheless, the controlling influence of the glass curve can extend to temperatures over 100 K above the glass curve. For example, the non-Arrhenius behavior of water itself extends to

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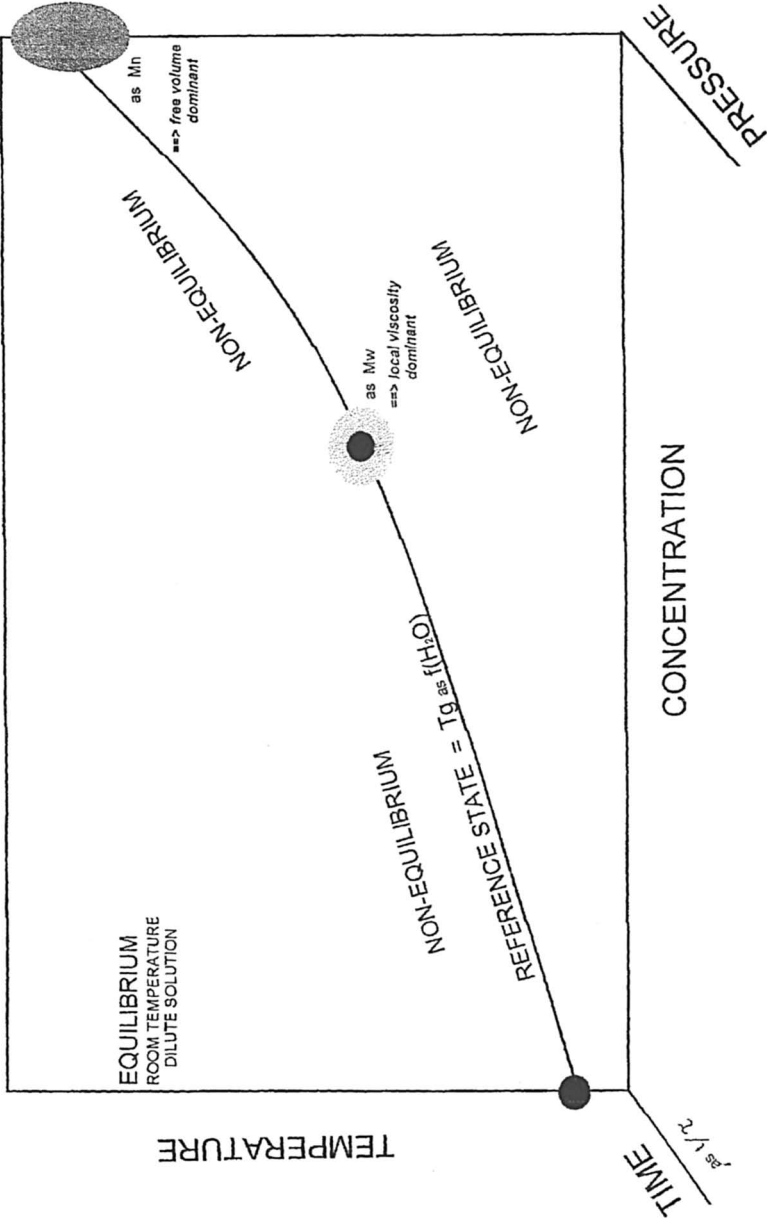
Fig. 1. Definition of Tg of a blend, illustrated by a glass curve plotted on a state diagram of temperature vs. solute concentration.

155 K above Tg of water alone. For single solutes, control by the glass curve extends from Tg up to Tm, and the ratio of Tm/Tg (in K) predicts the relative rigidity of a given glass.

An understanding of the effects of glass transition phenomena on freshness and shelf-life of various types of food systems starts with a description -- in terms of a "universal sorption isotherm" [37] (shown in Figure 2) -- of the general relationship between moisture content and relative vapor pressure (RVP) or % RH. For example, for different baked goods and other cereal-based and related food ingredients and products, it has been shown that low-moisture cookies (viewed as candy glasses), bread dough, and flour all exhibit typical ("universal") behavior, while baked bread does not [37]. Figure 2 illustrates the concept that multiple texture stabilization requires control of moisture content, sample RH, molecular Tg, and network Tg. Portions of the glass curves for sorbitol, for a non-networked biopolymer, and for a permanent network have been positioned relative to the "universal isotherm curve" on the diagram, as a way of illustrating that molecular Tg controls water vapor migration, while network Tg controls bulk liquid water migration. Figure 2 also points out that, for thermosets such as cheese and baked bread, it is relatively easy to effect surface dehydration, but difficult to remove bulk water. In contrast, it is easier to remove bulk water from a raisin, which typically exhibits desorption hysteresis.

Selected examples of applications of the FPS approach to food processing and to studies on product quality attributes -- e.g. crispness as a textural aspect of freshness over shelf-life -- can be described, which illustrate the significant progress made in recent years, based on amorphous product technology, as applied to methods for producing and then maintaining the freshness and improving the shelf-life of foods. For example, an earlier description of whole-grain processing, based on the state diagram and glass curve for starch-water [11], has led to a deeper understanding of the importance of Tg -- which controls the initial expansion [17,38] and subsequent collapse and resulting shrinkage of expanded products [18-20] -- in extrusion processing. Today, the FPS approach and state diagrams are explicitly used as a technological basis for describing pasta drying [21] and extrusion processing of cereal-based products [22,23]. [For completeness, we note that fresh orange juice is a good example of a food product for which Tg is not a relevant predictor of shelf-life, in contrast to frozen orange juice concentrate, for which Tg is the most relevant predictor.]

To consumers, texture is one of the most important quality attributes of food products. The creation of texture during product manufacture [24] and its stability during product storage [25,26] are subjects to which the FPS approach has been widely applied in the past five years. The direct relationship between product Tg -- in comparison to the ambient temperature of product storage or consumption -- and the textural property of crispness, which is a critical attribute

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MULTIPLE TEXTURE STABILIZATION REQUIRES CONTROL OF MOISTURE CONTENT, SAMPLE RH, $T_{g\ molecular}$, $T_{g\ network}$
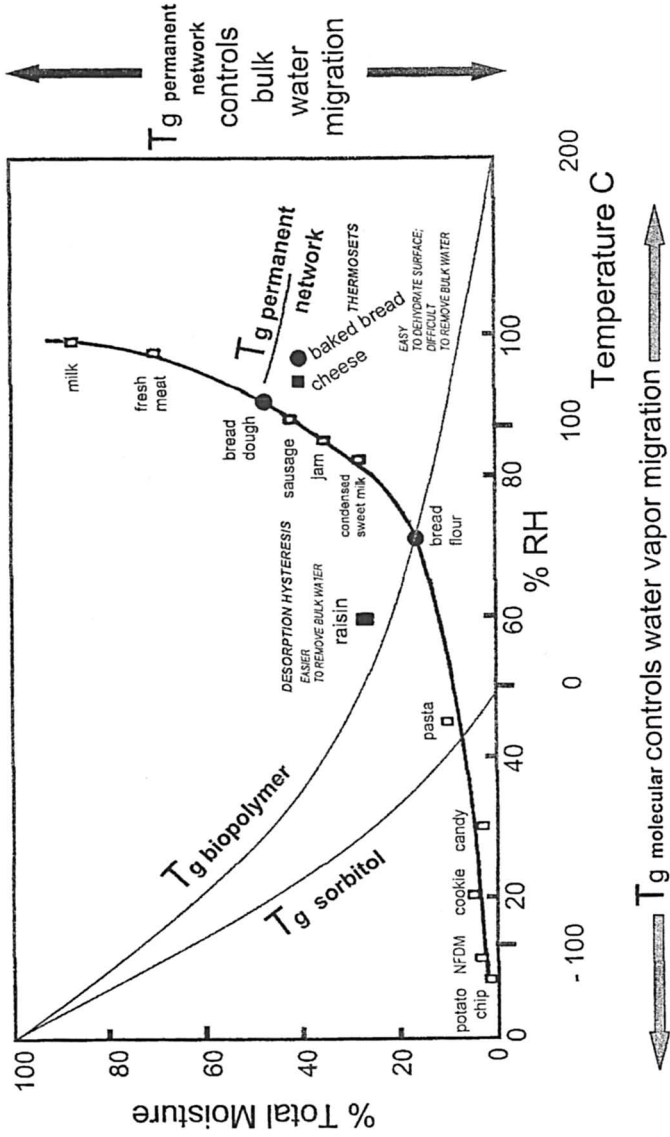
Fig. 2. % relative humidity of common foods at room temperature and typical steady-state moisture contents, plotted as a "universal sorption isotherm".

of many amorphous snack food products, has become increasingly recognized [11], as well as diagrammatically illustrated [27]. This recognition first arose from an intuitive realization and appreciation of the fundamental correlation between the glassy solid state (below Tg of the product matrix) and sensory crispness, and in contrast, between the rubbery liquid state (above Tg) and loss of crispness [11,25]. Intuitively, loss of crispness could be understood to often result from moisture uptake during product storage -- allowed by inferior barrier packaging material -- leading to plasticization (synonymous with "softening") and a consequent decrease in product Tg [13]. For amorphous, starch-based extrudates [27], it was shown that a sensory crispness score increased monotonically with increasing Tg. An early empirical correlation (inversely linear) between decreasing crispness intensity and increasing snack product % RH [28] was later explained on the basis of Tg and the effect of water uptake and resulting plasticization, to raise % RH, lower Tg and thereby reduce crispness [29]. Another early empirical finding confirmed the correlation between decreasing sensory crispness intensity and increasing % RH, resulting from moisture uptake, for commercial breakfast cereal products [30]. Later, this finding was explained as an effect of plasticization by water, to sharply reduce the mechanical modulus of the crisp, glassy solid matrix (at the "glassy plateau") to that of the soggy, rubbery liquid matrix [13]. It had been previously shown -- for a cereal-based solid food foam, equilibrated to various moisture contents, to achieve different extents of plasticization by water -- that modulus and sensory hardness score were directly linearly correlated [31]. In a study on cookies during commercial baking, it was found that crispness developed (i.e. sensory crispness score increased) as the modulus of elasticity increased with decreasing cookie moisture content [24]. In baked cookies [32], and in extruded amorphous model snack products [33,34], recent experimental evidence has further confirmed the direct relationship among Tg, water plasticization, and sensory crispness. In other recent studies on extruded glassy cereal-based foods [35] and crispy puffed extrudates [36], the effect of limited plasticization (resulting in antiplasticization) by water -- at moisture contents well below that at Tg -- on sensory crispness, sensory hardness, and mechanical properties was suggested to result from a secondary physical transition, from brittle to ductile [definition: deformable or plastic], which could occur in the glassy solid matrix at Tß < Tg, the possibility of which had been mentioned earlier [11].

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Chapter 17

Rehydration of Crispy Cereal Products: A Tentative Explanation of Texture Changes

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Crispness has been seen to be a source of pleasant textural contrasts and to be associated with a sensation of both freshness and quality. The texture deterioration of crispy products upon rehydration is thus known to cause the rejection of many cereal products by consumers. For many years, this evolution of texture was attributed to the glass transition of the material becoming lower than ambient temperature. For this work, a double approach of the texture evolution was chosen: on the one hand, sensory properties of cereal products at various hydrations were studied, while on the other hand, the physical basis of the phenomena controlling the texture were considered. Crispness loss was shown to coincide with a hardness increase of the products. Localized motions preceding the glass transition could be responsible for the texture changes, moreover, sugars could contribute to the stability of the products versus hydration.

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Introduction

Freshness is defined as the state of "being as if newly made". When freshness of food products is concerned, different scales of shelflife are available to the consumers : that of the fresh foods bought from producers and the more sophisticated, packaged manufactured foods. Whereas the first ones have a very limited shelflife, the manufactured products may be regarded as fresh as long as they remain protected from the outside environment in their original sealed packaging. Freshness covers a wide range of attributes from taste to flavor and texture, and shelflife relates to their stability over time.

Among the different components of food quality, the importance given to texture by the consumer has considerably increased in the last decades. In early consumers studies[1, 2] on food texture, crispness was revealed to be not only seen as a source of pleasant textural contrasts but to be associated with a sensation of both freshness and quality. For products such as breakfast cereal, chips, wafers, the crispy texture is the key attribute for the consumer appreciation.

The importance of crispness in the acceptability of a food has rapidly made the industry consider the necessity to control this characteristic as much at production level as at the storage or consumption one. Such an objective (controlling this texture attribute) implies to understand not only the sensory meaning of "crispy", but the physico-chemical parameters responsible for it.

For the present work, the interest will be focused on the possible effects of storage on crispy products rather than the different parameters that would possibly affect the texture during the products manufacture (processing or formulation parameters).

Influence of rehydration upon storage on texture of crispy cereal food products : an instrumental and sensory approach

Most low moisture fried, baked or extruded products such as breakfast cereals, wafers, biscuits, and snacks have a crispy texture resulting from a cellular structure. This attribute can be altered upon storage. As an example, when studying the effect of storage on potato and tortilla chips, Lee et al.[3] described fresh samples crispier than the stale ones.

Upon storage, and due to water sorption from the atmosphere or by mass transport from neighboring components (in absence of efficient moisture barriers), the moisture content of these products increases (Figure 1), and crispness is lost[4, 5, 6]. For this type of products, it is a major cause of consumer rejection. In spite of the economical impact of this loss, and although many

works have been dedicated to it, there has been yet no agreement on the molecular basis of these textural changes.

The texture changes upon rehydration can be characterized by both sensory and instrumental analysis. For this purpose, crispness of cereal products equilibrated at different humidities is evaluated at first bite by a trained sensory panel, whereas a puncture test is used to assess the fracture properties of the materials.
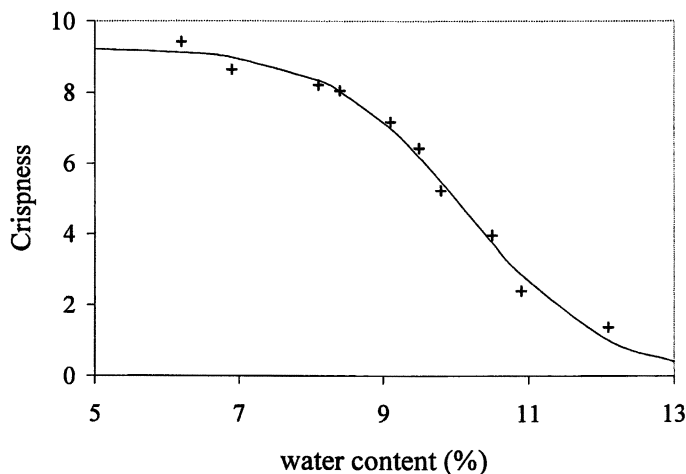
Figure 1: Hydration effect on sensory crispness of extruded flat bread.
(Reproduced with permission from reference 7. Copyright 1998.)

The influence of water on the texture properties is characterized (Figure 1) by an important decrease of the mark (between 0 and 10) given to crispness, the evolution can be described by a sigmoid curve[7] with a critical value (corresponding to half of the maximum value) at 9 % of water.
Similarly, the fracture properties exhibit important changes characterized by an attenuation of the jaggedness of the force-deformation curves (Figure 2). These patterns can be analyzed throughout the determination of the number of spatial ruptures (peak number per puncture distance). The logarithm of the latter is correlated to crispness[8] : the higher the number of peaks (corresponding to fracture of individual cells) the greater the crispness. It suggests that the panel evaluates crispness on the basis of the number of ruptures detected at first bite. Among the different sensory attributes evaluated by the panel, the interest was also called on the evolution of the hardness. Indeed, as shown on Figure 3, hardness is increasing upon hydration up to 9 % of water.
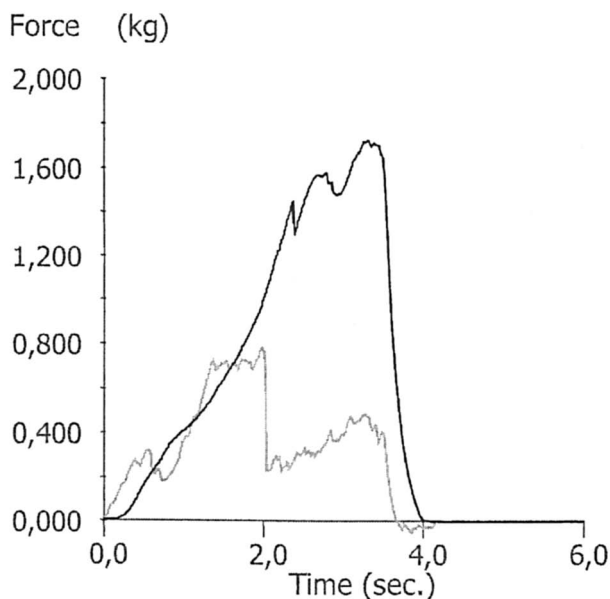
Figure 2 : Force-deformation curve obtained with extruded bread at 6 % (lower one) and 10 % moisture (higher).

It is interesting to compare it with the evolution of crispness: indeed while the latter decreases, hardness increases up to 9 % of water content, which is identical to the previously described critical water content for crispness.

Similar features were obtained with instrumental measurements. A analysis on a wider set of data revealed that such effects could be illustrated by a texture continuum[8, 9] describing the evolution of texture versus hydration from crispy, crackle to hard.

Further study aiming at the understanding of the molecular basis of texture changes requires the use of a physico-chemical approach based on techniques and concepts developed for material science. In material science, the loss of crispness upon rehydration could be described in terms of change in fracture mechanisms. Extensive studies performed on synthetic polymers have demonstrated that mechanical behavior is highly dependent on the physical state and organization of the polymer network[10]. Thus for further study, the physical state of the cereal products has to be considered.
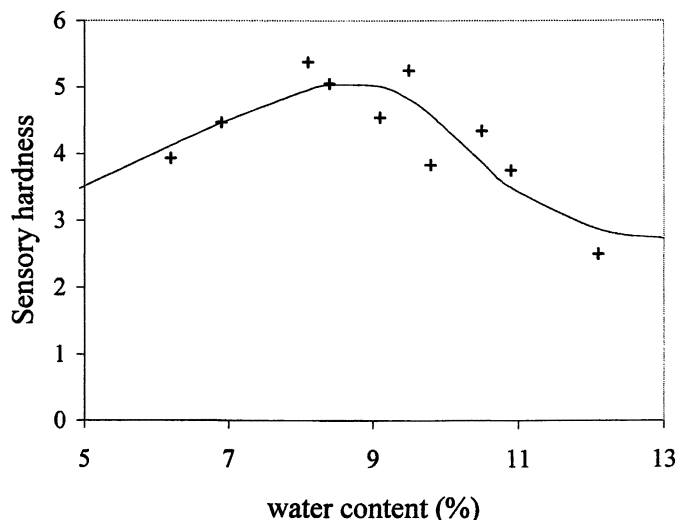
Figure 3: Influence of water content on the "hardness" descriptor for extruded flat bread), and the line is a modified sigmoid equation
(Reproduced with permission from reference 7. Copyright 1998.)

Physical state of cereal-based products

Baked or extruded cereal products are generally in the glassy state since cooking is accompanied by the disappearance of most crystalline structures of native starch[11]. The sensitivity of cereal products to the water content contributed to the proposal of Tg as a predictive parameter for the stability of low moisture products[12, 13, 14]. Indeed, stored above their glass transition temperature, amorphous samples exhibit mechanical changes; moreover due to collapse phenomena, cellular (a common feature to crispy foods) products could show densification which is also known to control mechanical/ texture properties[15, 16, 17]. Finally, above their glass transition temperature starch-based products are known to be subjected to retrogradation, this crystallization being able to affect the texture of the products[18, 19].

Differential Scanning Calorimetry (DSC) is classically used for the determination of the glass transition of simple systems (*e.g.* sugars). However, no event assignable to a glass transition was detected for the extruded bread described above; this is probably due to the heterogeneity of the system which causes a spreading of the transition over a very broad temperature range and thus an undetectable progressive increase of heat capacity.

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For complex systems, thermo-mechanical methods are more sensitive[20], they have been applied successfully to cereal-based products Thermo-Mechanical Analysis (TMA)[21], or Dynamic Mechanical Thermal Analysis (DMTA)[22, 23, 11] are more accurate/more sensitive for Tg measurement.

The glass transition temperature of extruded bread was determined by DMTA at several frequencies (from 5 to 40 Hz) from the drop of the storage modulus E', this feature was characterized by a frequency shift towards higher temperature with increasing frequency[11].

The plasticizing effect of water on extruded bread is shown on Figure 4 throughout the decreasing Tg plotted as a function of the water content of the material.
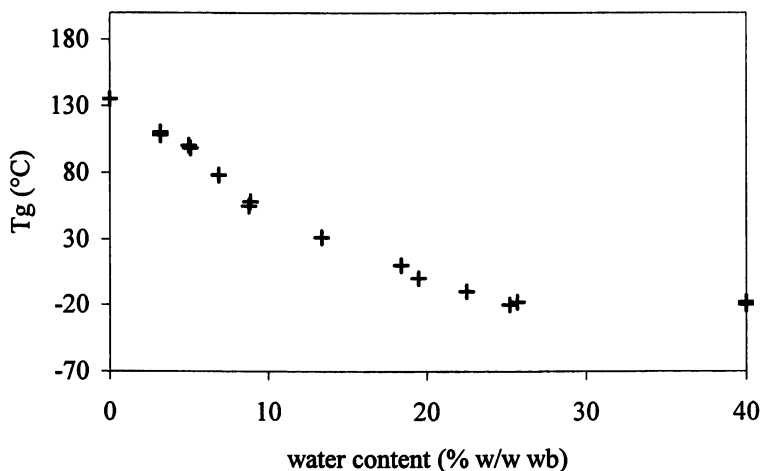
Figure 4 : Influence of water on the glass transition temperature (Tg) of extruded bread (determined at the onset of E' drop of DMTA at 5Hz

It is important to notice that according to these data, the glass transition takes place at room temperature when extruded bread contains a minimum of 15 % of moisture. Therefore, the texture changes observed around 9 % could not be attributed to the glass transition of the samples, but possibly to more localized motions occurring in the glassy state[24].

No marked macroscopic density changes were detected in this hydration range. No crystallinity could be detected with either DSC nor DMTA or X-rays diffraction, and therefore the hardening observed in the samples could not be attributed to retrogradation or to crystals remaining from the baking process.

The hardening observed upon hydration has been described as an "antiplasticizing" effect. Indeed, although the increasing water content still decreases the glass transition temperature, the effect observed for limited

hydration (< 9%) is opposite to the usual consequences of plasticization on mechanical properties, whereas the softening one only becomes dominant for higher hydration values. This antiplasticizing process has been observed in several systems but its molecular origin has no yet been clearly identified. It has been attributed to a densification at a molecular level through a decreasing amount of defects (lower density regions) present in the glass[25, 26], or to a strengthening of the interactions with the polymeric matrix[26, 27] or to a structural relaxation[28]. Finally, Fontanet et al.[29] suggest that the stiffening of the samples could result from a short range reorganization throughout H-bonding (at such a limited scale that undetected by DSC or X-rays) allowed by a greater mobility induced by the dilution.

Influence of solutes on the stability of cereal systems upon rehydration

For a further understanding, model systems were prepared, they consisted of expanded extrudates of waxy maize at different sucrose concentration. The effect of hydration was studied with the same techniques. Whereas the 0 % sucrose samples exhibit an important loss of crispness around 8 % of water, the critical water content for the 20 % sucrose samples is greater than 12 % (Figure 5). Moreover, it can be observed that although the crispness of sucrose-rich samples is affected at higher hydration, the effect is then more rapid than for the 0 % sucrose ones. The 10 % sucrose samples were rather heterogeneous which caused the irregular evolution of crispness versus hydration. However, the trend they follow places them in an intermediate position between the 0 % and the 20 % sucrose samples.

The 0 % sucrose samples exhibit a sensitivity to hydration comparable to the 0.1 % aspartame ones, which confirms that the panel was not biased in its judgement by the sweetness of the tasted products.

The influence of sugar content on sensory hardness is shown Figure 6.

Similarly to crispness, the influence of rehydration on the sensory hardness of extruded waxy starch depends also on the sucrose content of the samples. The lower the sucrose content the lower the hydration at which the stiffening starts. (due to possible effects of sugar on the structure of the extrudates, the values of hardness are not compared). Such results were confirmed by instrumental measurements where for a given moisture content the forces developed at fracture were greater at low sucrose concentration than at 20 %[8].
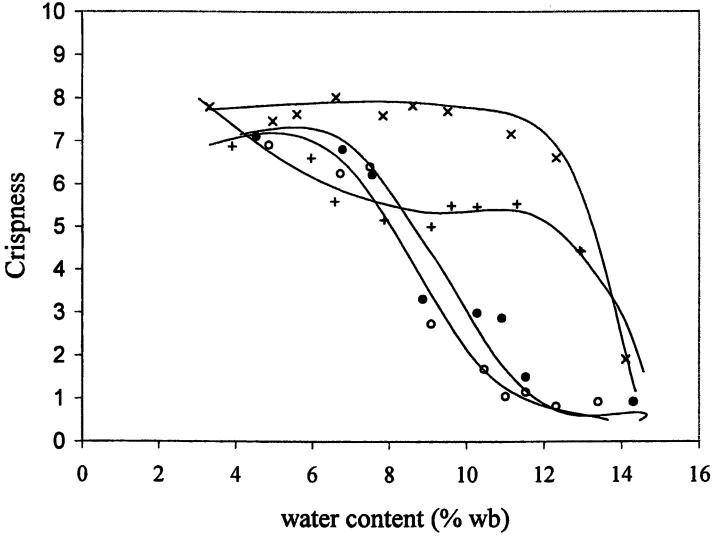
Figure 5: Influence of water content on crispness for extruded waxy maize with
(•) 0 %, (+) 10 % and (x) 20 % sucrose and (o) 0.1 % aspartame.
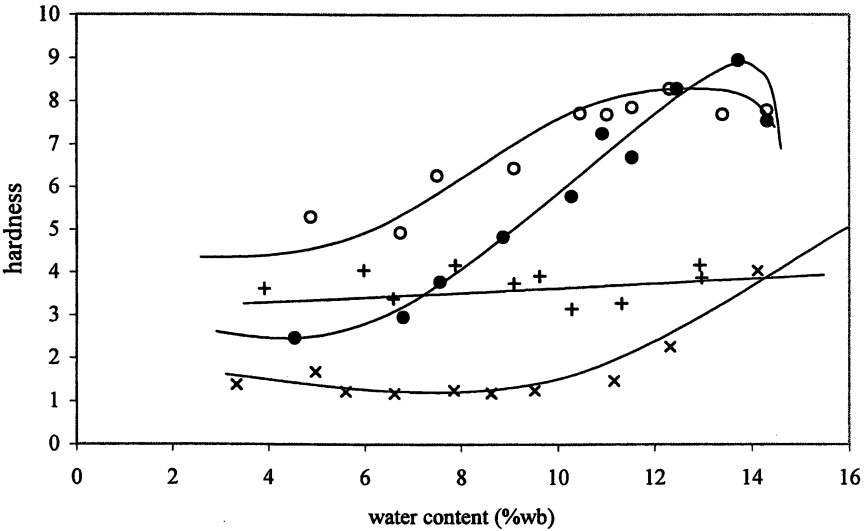
Figure 6 : Influence of water content on hardness for extruded waxy maize with
(•) 0 %, (+) 10 % and (x) 20 % sucrose and (o) 0.1 % aspartame.

Physical state of the starch-sucrose mixtures

When homogeneous ternary mixtures of sucrose-starch are prepared, the resulting Tg is decreased compared to that of starch alone[30]; in the absence of phase separation the greater the sucrose concentration the lower the Tg.

The plasticizing effect of both sucrose and water can be predicted using mathematical models such as the ten Brinke modified Karasz-Couchman model[31]:

$$Tg_{mixture} = \frac{W_1 \ddot{A}Cp_1 Tg_1 + W_2 \ddot{A}Cp_2 Tg_2 + W_3 \ddot{A}Cp_3 Tg_3}{W_1 \ddot{A}Cp_1 + W_2 \ddot{A}Cp_2 + W_3 \ddot{A}Cp_3}$$

where for the component x, W_x, is the weight fraction, ΔCp_x the heat capacity change at Tg and Tg_x (K) the glass transition temperature in Kelvin.

The values used for the prediction are given in Table I, and the calculated Tg as a function of water in Figure 7.

Table I: Parameters used for the prediction of Tg in ternary mixtures by Karaz Couchman model

Component	ΔCp (J/g/K)	Tg (K)
Starch[31]	0.47	502
Sucrose[30]	0.76	343
Water[30]	1.11	134

The applicability of the model assumes both a good miscibility of the 2 components and no crystallisation. These conditions were verified for the considered samples. The prediction suggests that the sucrose-starch mixtures remain glassy at room temperature at any water content or sucrose of the range considered. Therefore, as previously observed for the commercial extruded bread, the textures changes observed in the samples cannot be attributed to the glass transition.

Hydration of glassy material : suggested mechanism

The results obtained with the model products strengthen some of the hypothesis previously brought up. Glassy systems consist of both dense regions and lower density ones. The latter are characterized by the presence of voids
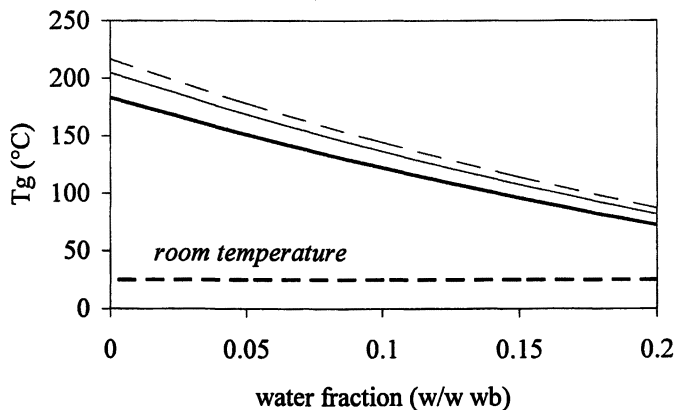
Figure 7 : Predicted Tg values for waxy maize starch as a function of water fraction for 0 % (dotted), 10 % (plain) and 20 % (bold) sucrose (g sucrose/g starch)

(also called defects or free volume) which are sometimes referred to as "mobility islands"[32] for the possible greater mobility they offer throughout the greater space. When considering hydration, the water molecules "first" added to the systems (below 9 % for extruded bread) would localize in the low density regions, filling the voids and therefore causing an increased density (at a molecular level) and thus an increased hardness of the glasses. Further hydration will see water interacting with polymers through hydrogen bonding in competition with the interpolymer H-bonding. When this level of hydration has been reached, the dominant effect of the water uptake will be a "softening" of the matrix by replacement of the interpolymer network by water-polymer interactions. When the matrix contains a mixture of starch and sucrose, the hydrogen-bonding could first establish between the water molecules and the solutes before affecting the biopolymer. Therefore crispness would be maintained up to a greater hydration level in presence of sucrose.

Moreover, the development of interactions reinforcing the polymer network, possibly throughout water bridges could also be suggested to interpret the observed phenomena. Indeed, several works based on relaxation studies have shown that localised motions such as secondary relaxations observed in dry systems were hindered when the water content was increased[27, 28, 29]. The susceptibility to this antiplasticization could be confered by the polarity of the polymer and solutes constituting the systems. The settlement of this network would inhibit the motions, and thus increase the rigidity of the systems and therefore contribute to the increase of both perceived and instrumental hardness accompanied with a change in the fracture properties and thus a crispness loss.

Throughout this work, the texture changes of crispy cereal products observed upon rehydration during storage have been considered with a particular focus on their physical origin. The loss of crispness occuring above 9 % of water was attributed to localised processes occuring in the glassy state, involving hydrogen bonding and building up of interactions between water and polymers. The role of polar solutes (such as sugars) is under investigation based on mixtures with solutes of different polarities. Preliminary studies showed that low resolution proton NMR was able to detect differences in the dipolar interactions between the samples at different sugar and water contents. Moreover, techniques such as RAMAN, or positron annihilation spectroscopy could be suitable to further investigate and test the hypothesis.

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Chapter 18

Staling of Bread: How To Counteract the Textural Changes and Improve the Flavor Aspects of Bread during Storage

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Staling is the common description of changes in the bread crumb during storage, and can be counteracted by anti-staling agents such as amylases and monoglycerides. In the present paper the most commonly used anti-staling agents, distilled monoglycerides and the maltogenic alpha-amylase, Novamyl, are compared by instrumental texture analysis and by sensory profiling. Both methods showed that Novamyl is superior to the monoglycerides in delaying the development of firmness. The sensory evaluation also showed that other characteristics such as moistness, crumbliness, crumb strength and flavor are highly influenced by anti-staling agents, and that Novamyl has an overall advantage over the monoglycerides in delaying staling. In order to explain the uniqueness of Novamyl, a classical bacterial amylase was included in the texture evaluations and in a chromatographic evaluation of the enzymatic degradation of amylopectin. Novamyl mainly modifies the outer branches of amylopectin, whereas the bacterial amylase liquefies the starch and induces gumminess.

Introduction

Staling is the common description of the decreasing consumer acceptance of bakery products as they age. The term covers changes in the breadcrumb other than those originating from the action of micro-organisms (*1*). Typical changes are hardening of the crumb, which also results in crumbliness, softening of the crust, which gives it a leathery texture, and loss of the flavor associated with fresh bread.

Several theories have been put forward on the factors involved in staling of bread, and although a lot of research is published, the mechanism behind the changes is still debated. A comprehensive staling model was proposed by Zobel & Kulp in 1996 (*2*). The model is based on the earlier bread staling models proposed by Schoch (*3*) and Lineback (*4*) and includes updated molecular views of starch and starch retrogradation. The model describes gluten as a continuous phase, which covers and bridges the surface of starch granules, but also as a phase being relatively inert to the changes taking place during storage of bread. According to this model, starch is the main component involved in changes over time. In opposition to the above theory Martin and Hoseney in 1991 suggested that bread firming is a result of hydrogen bonds between starch protruding from gelatinized starch and the imbedding protein (*5*). It is well known that several amylases inhibit staling, and Martin and Hoseney (*6*) believed that the dextrins released by the amylases prevented the interaction between starch and gluten, and thereby delayed staling. Gerrard et al. have later provided evidence that the dextrins produced by amylases do not inhibit staling directly, but rather reflects the changes taking place in the starch molecules (7). These findings have been confirmed by Spendler, who compared the changes taking place in normal wheat bread with changes in starch bread. From these studies it was concluded that gluten proteins are not needed to explain staling (*8*).

The inhibition of staling by use of anti-staling agents is of great importance in the bread making industry, where the manufacturers strive to satisfy consumer's expectations to convenience and long lasting product quality, and further strive to cut manufacturing and distribution costs by increasing the shelf life of the bread.

This paper will focus on the state-of-the art techniques to counteract staling: distilled monoglycerides and the maltogenic alpha amylase, Novamyl. Mechanistic and functional studies of the anti staling agents will be described and related to their impact on the changes in sensory characteristics of the bread during storage.

Role of starch in staling

As described above, the starch fraction of the flour plays a major role in the changes taking place during storage of bread, and the anti-staling effect of both amylases and distilled monoglycerides is related to their interaction with the starch fraction during the bread making process. Therefore, a basic description of the changes taking place in the starch fraction is needed for understanding the mechanism of the anti-staling agents.

The native starch granule contains both amorphous and crystalline regions. Amylose, which is almost completely amorphous, consists of single or double helices, whereas amylopectin has both amorphous and highly ordered crystalline regions; built from aggregates of double helices formed between the outer chains of the amylopectin molecules (2).

When the dough enters the oven and the temperature rises, the starch granules undergo a restricted swelling limited by the relatively small amount of water in the dough. The heating will dissolve the amylose and part of it will diffuse into the aqueous medium surrounding the starch granules. The crystalline parts of amylopectin will be disrupted and gelatinize, which allows part of the amylopectin to expand into the intergranular space (2). As the bread leaves the oven and the temperature decreases, amylose will quickly retrograde and form double helices. This results in an insoluble gel, which is important for "locking" the initial crumb structure. Amylopectin retrogrades more slowly. During storage of the bread, the double helical structures in amylopectin will be re-formed, resulting in firm granules and the formation of a rigid network within the granules, with starch in the intergranular space, or eventually between granules (2).

Counteracting staling

Monoglycerides served for many years as the best technical solution to counteract staling. Fungal and bacterial alpha-amylases were used to a limited extend. However, fungal amylases have only a marginal effect on extending crumb freshness, and the use of traditional bacterial amylases is limited, as they give a gummy and inelastic crumb when overdosed. The maltogenic alpha-amylase, Novamyl®, is today widely used to prolong shelf life.

Other ingredients such as fats, dough strengthening emulsifiers (e.g. Datem - diacetylated tartaric acid esters of monoglycerides and SSL – sodium stearoyl-2-lactylate), and other enzymes (e.g. xylanases and lipases) also posses some bread softening effect (9,10). However, the softening effect of these ingredients may be related to their ability to improve loaf volume, which results in a less

dense crumb. The effect of xylanase and lipase will be touched upon later in this paper.

The effect of distilled monoglycerides and Novamyl on bread texture is compared in Figure 1. Further, bread made with a bacterial alpha-amylase is included, as it serves to illustrate the importance of amylase specificity and temperature stability on the anti-staling effect. The comparison was made in bread baked by a lean straight dough recipe in lidded pans. Measurements of firmness and elasticity were made on a Texture Analyzer TA-TX2 over a storage period of 7 days. Firmness was measured according to a modified version of AACC method 74-09, and is the force required to compress a slice of bread by 25 %, whereas elasticity is the remaining force in the crumb after the slice has been further compressed to 30 % for 30 seconds (*11*).

It is seen from figure 1 that Novamyl is superior to the distilled monoglycerides in delaying the changes of crumb firmness and elasticity occurring during storage of the bread. 7 day old bread with Novamyl has the same textural properties as two-day old control bread without amylase, whereas the firmness of 7 day old bread with distilled monoglycerides has similar firmness as the control bread at day 4. When only considering firmness, the bacterial alpha-amylase is by far the most efficient of the tested antistaling agents. However, as seen from the elasticity curve, it is associated with a very gummy and inelastic crumb.

To better understand the differences between Novamyl and the classical bacterial alpha amylase, their reaction patterns were studied by Ion Moderated Partition Chromatography (IMPC). A watery suspension of amylopectin was hydrolyzed by the amylases for a period of 24 hours and the hydrolysates were subsequently analyzed by IMPC. The chromatograms are shown in Figure 2. The figure shows that the bacterial alpha-amylase hydrolyses or rather liquefies the amylopectin by a total degradation, whereas Novamyl primarily forms DP 1-7 by degrading the outer chains of the amylopectin molecules, and leaves the backbone of the structure intact.

The degradation pattern of the bacterial alpha-amylase and Novamyl on starch can be illustrated as shown in figure 3. The classical bacterial alpha-amylase, which has a temperature optimum at 70-80°C (*12*), aggressively degrades both amylopectin and amylose endo wise. This extensive degradation hinders proper settling of the crumb making the resulting bread gummy and inelastic. Novamyl, which is a purified maltogenic alpha-amylase produced by a genetically modified strain of Bacillus subtilis, possess both endo and exo activity. It has medium thermo stability with optimum at 60-70°C (*13*). As described above, Novamyl modifies amylopectin by reducing the outer branches of the amylopectin leaving behind a limit dextrin (an amylopectin molecule with shorter branches but with the backbone of the structure intact). The reduction of the length of the side chains prevents retrogradation of amylopectin and thereby counteracts staling of bread. As Novamyl only degrades amylose to a limited extent, the initial settling of the crumb is therefore not hindered.
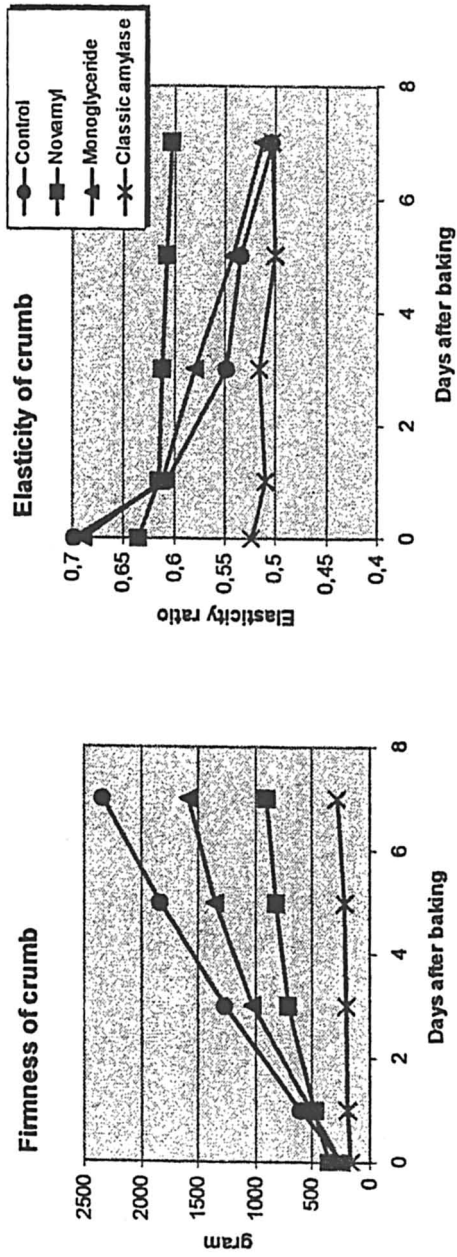
Figure 1. Firmness and elasticity curves comparing Novamyl® (750MANU/kg flour), Bacterial alpha-amylase (10KNU/kg flour), and distilled monoglycerides (0.5%)
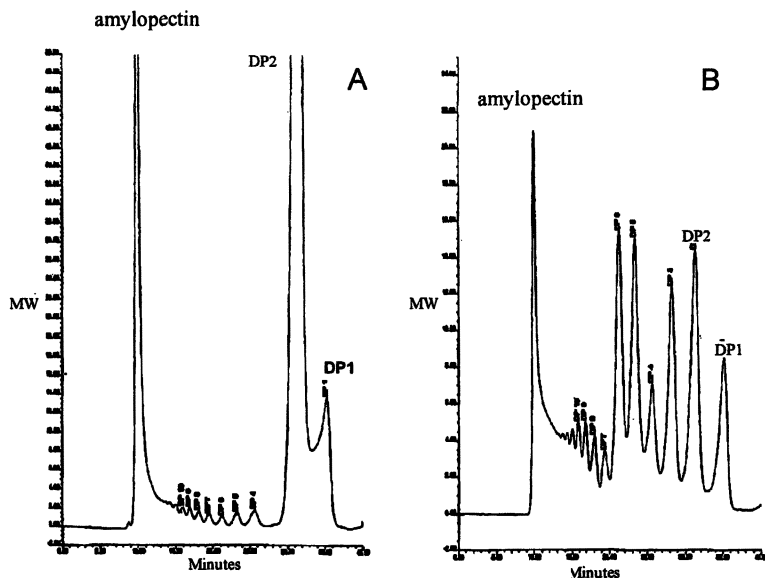
Figure 2. Reaction pattern of A) Novamyl and B) Bacterial alpha-amylase on amylopectin as determined by Ion Moderated Partition Chromatography (7).

Monoglycerides work through complexation with amylose, preventing it from entering stiff aggregates of double helical structures. It has been proposed that some complexing also may take place between monoglycerides and the outer branches of amylopectin (*14*). The limited effect observed with the distilled monoglycerides in figure 1, can be related to its complexation with amylose, which as described above mainly influences the initial texture of the bread.

Sensory perception of staling

The term staling covers the consumer's perception of the changes taking place in the bread as it ages. In order to get a step closer to consumer perception of the impact of the antistaling agents, a sensory profiling including texture- as well as flavor parameters was carried out.
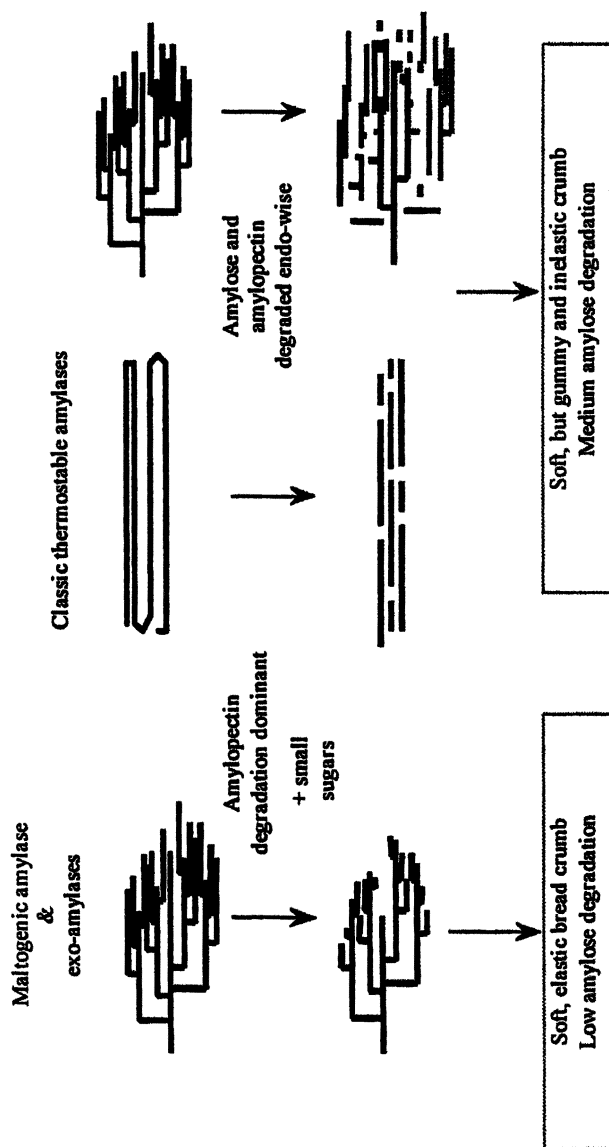
Figure 3. Schematic model of the degradation pattern of the maltogenic amylase, Novamyl and a classical bacterial alpha-amylase.

An in-house sensory panel consisting of 11 trained assessors was used for the sensory evaluations. The antistaling agents tested were A) distilled monoglycerides, B) Novamyl®, and C) Novamyl®, Fungmyl Super MA® (Fungal alpha-amylase + xylanase), and Lipopan® (Lipase). The enzyme combination C was included to evaluate the impact of other enzymes, which are commonly used in combination with Novamyl at the bakeries. The terms found by the assessors to describe the changes taking place in bread crumb during storage are shown in table I. These 15 terms were applied in the sensory profiling.

The descriptors were used to evaluate bread baked by a standard American sponge & dough procedure containing 3% oil, 6% sugar and 0.5% SSL in the base, after 1 and 5 days of storage. The results were evaluated by use of partial least square regression (PLS) in The Unscrambler programme (CAMO ASA). The reliability of replicates, assessors and descriptors was investigated by variance analysis. From this analysis it was chosen to include 4 replicates (based on high and comparable total variance/residual variance-ratio). Further, 6 descriptors were identified, which contained the most information, and which were used with most consistency among the assessors (not shown). The average response (assessors and replicates) for these descriptors are presented in figure 4, where only the firmness evaluated by mouth is included.

The day after baking, the loaves were all quite similar. However, the loaves with Novamyl and the enzyme combination were significantly less firm and had a moister crumb than the bread with distilled monoglycerides. Further, the bread baked with the enzyme combination was evaluated as less crumbly than the two other samples. For the other parameters the differences were not significant.

For all parameters changes took place within the 5 days of storage. However, the speed at which these changes occured differed among the antistaling agents evaluated.

When the bread was evaluated after 5 days of storage, the crumb of the loaves with distilled monoglycerides was significantly firmer to bite through, they were more crumbly in the mouth, and they had a drier crumb surface than loaves containing Novamyl and the enzyme combination. The bread with distilled monoglycerides also had less strength than the Novamyl containing loaves, which makes it more difficult to apply spreading.

Besides the textural changes, also flavor and odor were evaluated. As for the texture parameters, significant flavor changes took place during storage of the bread, whereas no differences were found between loaves with different antistaling agents at the same storage time.

No significant differences were observed between the Novamyl containing bread and the bread with the enzyme combination for any of the parameters at day 5, which means that the addition of xylanase, amylase and lipase did not give any perceivable changes compared to bread with only Novamyl in this trial.

Table I. Descriptors used for sensory profiling

Texture by mouth	Texture by hand	Flavor	Odor
Firmness	Moistness	Fresh bread	Fresh bread
Crumbliness	Firmness	Sour	Sour
	Springiness	Sweet	Sharp
	Crumb strength	Sharp	Mouldy
		Mouldy	
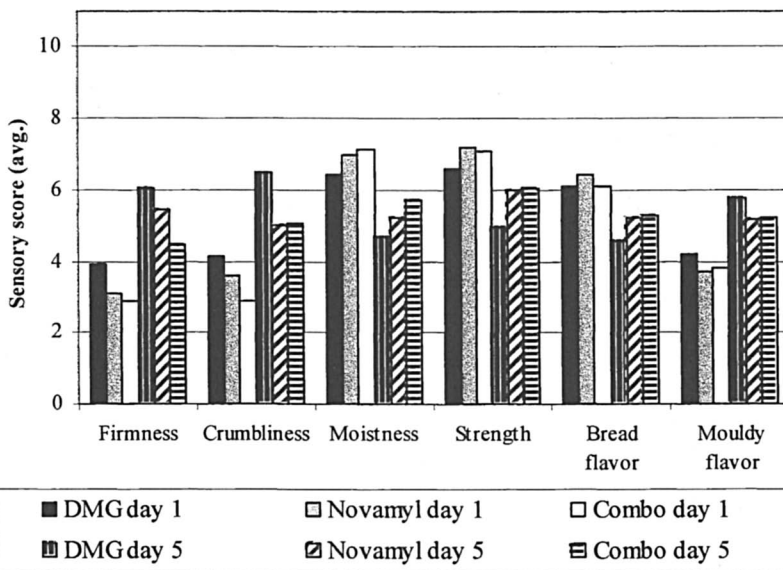
Figure 4. Sensory profile of American sponge & dough bread with 3% oil, 6% sugar, and 0.5% SSL after 1 and 5 days of storage. DMG = distilled monoglycerides 0.5%, Novamyl = 450MANU/kg flour, Combo=Novamyl 300MANU/kg flour, Fungamyl Super MA 50ppm, Lipopan 1000LU/kg flour. A high score is desirable for moistness, crumb strength and fresh bread flavor; a low score is desirable for firmness, crumbliness, and mouldy flavor.

A similar sensory evaluation was carried out in a European straight dough procedure where 3% solid fat was added as a base. The fat was added to give the antistaling agents the most difficult working conditions, as it is well known that fat has a softening effect (e.g. *15*). The results are not presented in detail. But in general the responses were quite similar to what was observed in the sponge & dough bread, although the differences were more pronounced in the straight dough bread. It is noteworthy that in the straight dough bread, the Novamyl containing loaves had significantly more fresh bread flavor than loaves with distilled monoglycerides after 5 days of storage.

The sensory evaluations of firmness confirmed the results obtained in the instrumental evaluation of firmness (figure 1). Due to high variation among the assessors, the evaluation of springiness did not result in significant differences between the products, wherefore a direct correlation to the instrumental evaluation of elasticity can not be made. The sensory evaluation further gave a description of product properties, which are much more difficult to evaluate by instrument, such as the feeling of moistness, crumbliness, crumb strength and flavor. This description is valuable as it provides evidence that the overall perception of staleness is delayed more efficiently by Novamyl than by the distilled monoglycerides, under the conditions applied.

Sensitivity of human senses versus instrument

As described earlier, texture of bread has traditionally been measured on a texture analyzer or similar instruments. Instrumental evaluations are still commonly used when evaluating anti-staling agents, as they serve as quick and easy tools to evaluate the basic textural properties of the bread. From the study presented in figure 4, it was indicated that the instrumental evaluation of firmness corresponded to the sensory response. But this type of evaluation does not clarify how big a difference between instrumental responses is needed to give a sensory perceivable difference. This issue was therefore addressed in another study, where sponge & dough pan bread, made by the same procedure as described above, were made with 5 different dosages of Novamyl. A sensory profiling was carried out after 3 and 7 days of storage based on the descriptors presented in table 1. Again 11 trained assessors participated in the evaluations. 6 replicated sessions were carried out, and the variance analysis in The Unscrambler (CAMO ASA) revealed that all six replicates had similar total variance/residual variance-ratio Therefore; all replicates are included in the calculations.

In Figure 5 the results from firmness evaluations made on the Texture analyzer (TA-TX2) is plotted against the average sensory score of firmness evaluated by mouth. The figure shows that there is a good correlation between the sensory perception of firmness and the texture analyzer evaluations (R^2=0.94).

However, a good correlation does not guarantee that differences seen on the texture analyzer can actually be perceived and vice versa. Therefore, the numbers are looked at in more details in figure 6. This figure shows that the addition of Novamyl reduces firmness of the crumb and that the reduction is dosage-dependent. The sensory evaluation and the texture analyses indicate that increasing the Novamyl dosage does not continuously reduce crumb firmness. At some point, further addition of Novamyl to the dough does not result in further reduction of bread crumb firmness. However, the critical Novamyl dosage is dependent of the storage period of the bread. After three days of storage, the difference between bread with 450MANU/kg flour and 900MANU/kg flour was not significant when measured by sensory evaluation, whereas after 7 days of storage, an obvious advantage was obtained by using 750MANU/kg flour instead of 450MANU/kg flour. This means that the bread manufacturers can balance the dosage of Novamyl to be applied in a specific process with the intended storage period of the bread.

The results indicate that the sensory panel evaluated firmness with at least the same sensitivity as the texture analyzer. Small differences were observed in
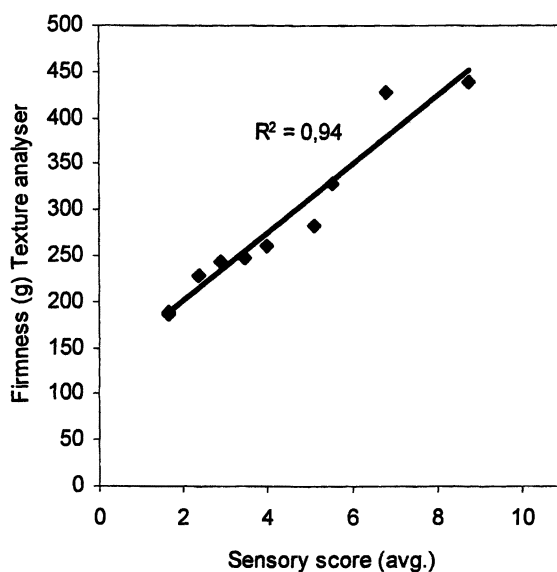
Figure 5. Correlation between firmness perceived by sensory evaluation and evaluations of firmness on the Texture Analyser
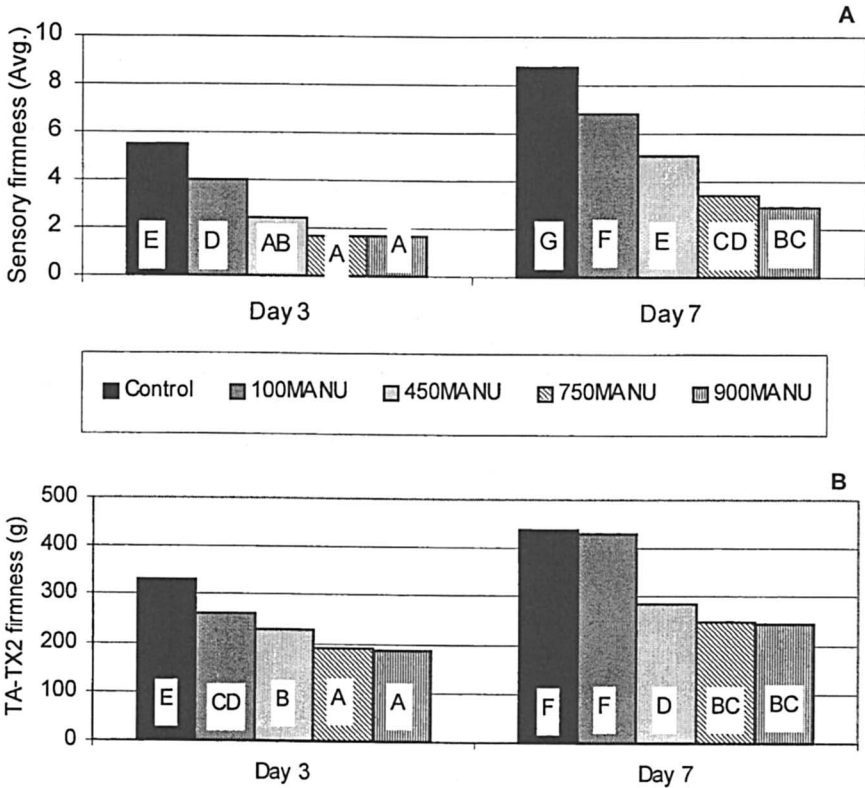
Figure 6. A. Sensory evaluation of firmness, B. Texture analyzer measurements of firmness. Samples with the same letter are not significantly different (95%). Dosages are given in MANU/kg flour.

the sensitivity of instrument and human, but the results show that the texture analyzer will give a good indication of the sensory perceived bread firmness.

Concluding remarks

Staling cannot be described simply as firming of bread. A full range of changes takes place, including both texture and flavor. All these aspects need to be considered when evaluating anti-staling agents. Sensory evaluation is a powerful tool for investigating anti-staling agents, as it gives a complex picture of the impact of these agents on the sensory perception of bread. The sensory profiling brings us a step closer to the consumer's experience when eating bread. It thereby supports the understanding of the link between mechanism of the anti-

staling agents and consumer's choise of baked goods. Further, sensory evaluations strongly enhance the understanding of instrumental information, and can be used to ensure that applied instrumental methods provide sensory relevant information.

Novamyl is found to counteract staling very efficiently. In a sensory profiling this enzyme outperformed distilled monoglycerides on all texture parameters important for the changes taking place during storage of the bread.

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Chapter 19

Rheological Methods for Assessment of Food Freshness and Stability

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Food rheology is considered the material science for food systems. Rheological approaches to shelf-life and freshness may range from a simple squeezing technique to advanced oscillatory methodologies probing material microstructure. As rheology relates to consumer perceptions of quality and freshness, a more common sensory term, texture, is employed. Changes in texture and stability during storage have been recognized as important factors influencing consumer acceptability of many food products. Texture is a complex attribute of food quality and can only be measured directly by sensory evaluation. However, many instrumental methods are used to measure mechanical properties of foods that are, up to a certain extent, related to sensory characteristics. Large strain methods such as puncture, penetration, bending, tension, shear, compression, and texture profile analysis are commonly used to evaluate freshness and textural changes of foods with respect to storage conditions. For fluid foods, various rheological techniques evaluate yield stress and shear viscosity, providing information about pourability, thickness, and dispersion or emulsion stability over time. Small strain methods, specifically dynamic oscillatory shear and mechanical analyses, are useful in probing microstructure, viscoelastic properties, and phase transitions in food materials. This chapter reviews rheological methods applicable to assessment of freshness and textural stability of foods.

Introduction

Changing texture and stability during storage is an important factor influencing consumer acceptability of many food products. However, the effect of storage on food texture, with few exceptions (bread and muscle meat), has not been studied extensively because texture changes are generally considered quality attributes rather than potential health hazards. Moreover, the importance of texture in consumer perception has only recently gained general recognition (1). Texture has been defined as a "group of physical characteristics that arise from the structural elements of the food, are sensed primarily by the feeling of touch, are related to the deformation, disintegration and flow of the food under a force, and are measured objectively by functions of mass, time, and length" (2). This definition indicates texture: 1) is a multidimensional property comprising a number of sensory characteristics, and 2) has roots in food structure and the manner by which this structure responds to applied conditions. In practice, the term texture is used primarily for solid and semi-solid foods, while viscosity and consistency describe the flow behavior of pourable foods. Being a complex sensory property, food texture can be measured directly by sensory evaluation. During the past few decades, many instrumental methods have been used to measure mechanical characteristics of solid- and liquid- type foods that are, up to a certain extent, related to sensory attributes. More details on textural measurements are available from several excellent sources (2-5).

Food rheology, the material science for food, investigates the mechanical response of food systems during flow or deformation. Rheological analyses benefit scientists and engineers by providing a quantitative assessment of ingredient interactions, processing conditions, and shelf-life effects on food texture, consistency, and integrity. Essentially all rheological tests attempt to relate a magnitude of force with a corresponding shape change in a material. This simplistic view of rheology can be further extended to categorize measurements as either fundamental or empirical in nature.

Fundamental

Fundamental procedures are derived from basic, physical relationships where *stress* is a force term and *strain* is the deformation parameter. Rheological properties, like *viscosity* and *moduli*, are important material parameters relating stress with strain. A stress is the force applied across a given area: force per area; and the relative deformation or strain describes the degree of shape change a material undergoes. The unique aspect of fundamental rheology is that these constitutive relationships between stress and strain are independent of sample volume or physical dimensions. Accordingly, these

basic properties transcend instrumentation selection and should be obtained regardless of the measuring system.

Empirical

Empirical measurements, on the other hand, simply record a measurement of force and the corresponding sample deflection. Unfortunately, many extenuating circumstances tend to confound empirical measurements, being strongly dependent upon instrumentation, sample volume and geometry. Nevertheless, empirical techniques have a secure place in the food industry, providing a relatively quick indication of quality and texture for process control and assurance practices.

Large Strain Methods

As the name indicates, large strain techniques subject samples to large deformation, resulting in structural breakdown and/or fracture. Depending on the test conditions and material characteristics, various degrees of correlation between the results of large strain tests and sensory data have been reported (6-8). The general principle of large strain tests is to promote contact between a probe and food sample, while measuring a force and/ or deformation during the process. Most methods are based on force measurements and can be performed with a single point device or universal testing machines. These techniques may be classified according to the type of action and/or probe involved.

Penetration and Puncture Tests

Penetration and puncture are considered empirical tests, wherein both normal and shear forces may be involved (Fig. 1) during the procedure. Penetration and puncture techniques are widely used in texture measurement of many foods including fruits, vegetables, butter, hydrocolloid gels (2, 4, 9), cheese (10), chips (11), and sea foods (12). In addtion, this simple procedure is used to evaluate different parts of a food on the overall product texture, such as the crust and inner portion of a french fry (13) or the exocarp, mesocarp, and endocarp of a tomato (14). During penetration testing, the depth or time to reach a certain distance is measured under a constant force, while puncture tests measure the force resulting during probe penetration (2). Probes with

various shapes, such as a flat-ended cylinder (Bloom Gelometer), cone, sphere (penetrometer for firmness, consistency and spreadability of fat-based products) or needle (Magness-Taylor fruit firmness tester, multiple pea attachment of TA.XT2 Texture Analyzer) are made to penetrate into the test sample (Fig. 1). The acquired force-deformation data (Fig. 2) are used to calculate indices of hardness, firmness, and toughness. These indices have been documented to decrease with storage of fruits, vegetables (15), fresh fish (16), and increase with ageing of other foods such as bread and carbohydrate-based products (17). Attempts have been made to relate the penetration forces with fundamental parameter such as Young's modulus (18) and absolute shear modulus using finite element analysis (19, 20).

Shear and Cutting Tests

Shear tests can be performed with single (Warner-Bratzler shear device) or multiple blades (Kramer shear cell) that slice through samples (Fig. 3). The maximum force and energy required to shear are taken as an index of firmness, tenderness, or toughness of fibrous foods (2, 21-23). As shown in Fig. 4, the shear force of minimally processed sweetpotato strips decreased with storage time (25). For texture measurement of raw fish, the shear test was recommended because it was more sensitive than puncture using cylindrical or spherical probes (26).

Although these tests are called "shear," forces due to friction, compression, and tension are also encountered. The magnitude of these forces depends on the structural properties and rupture mechanism of the tested materials and should be considered when interpreting results (27, 28). Therefore, shear devices can only provide empirical comparisons under specified conditions. Detailed discussion and interpretation of the force-deformation patterns of the shear tests are available in the literature (2, 28).

Cutting with a sharp blade or wire has also been used to measure the cutting forces of fruits, vegetables, meat, and dairy products (29-31). The wire cutting test involves fracture, deformation, and friction (Fig. 5). These parameters have been used to derive relationships among fundamental properties (32),

$$\frac{F}{B} = G_c + (1+\mu)\sigma_y d \qquad (3)$$

where F is the total force pushed during cutting, B is the sample thickness, G_c represents the energy necessary to create new surfaces, μ is the coefficient of
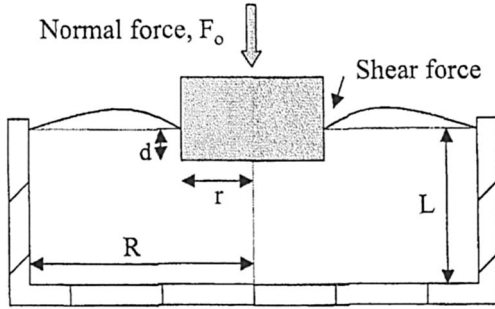
Figure 1- Geometry of a penetration test.
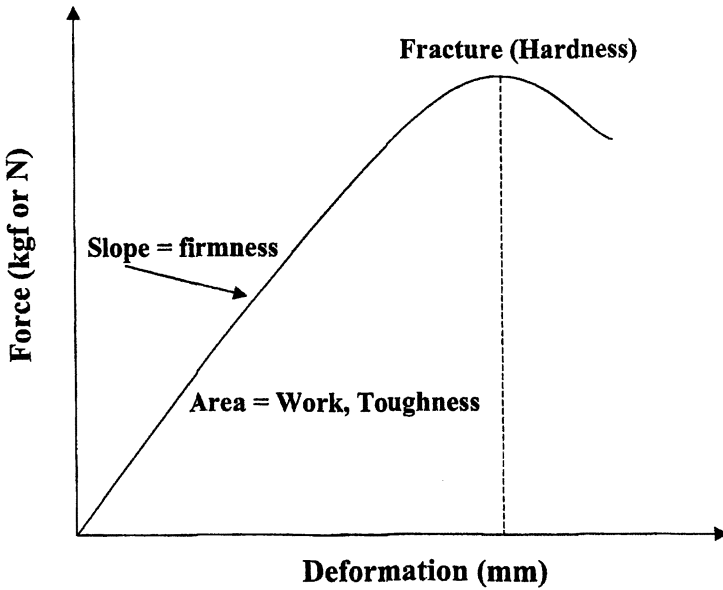
Figure 2- A typical force-deformation curve.
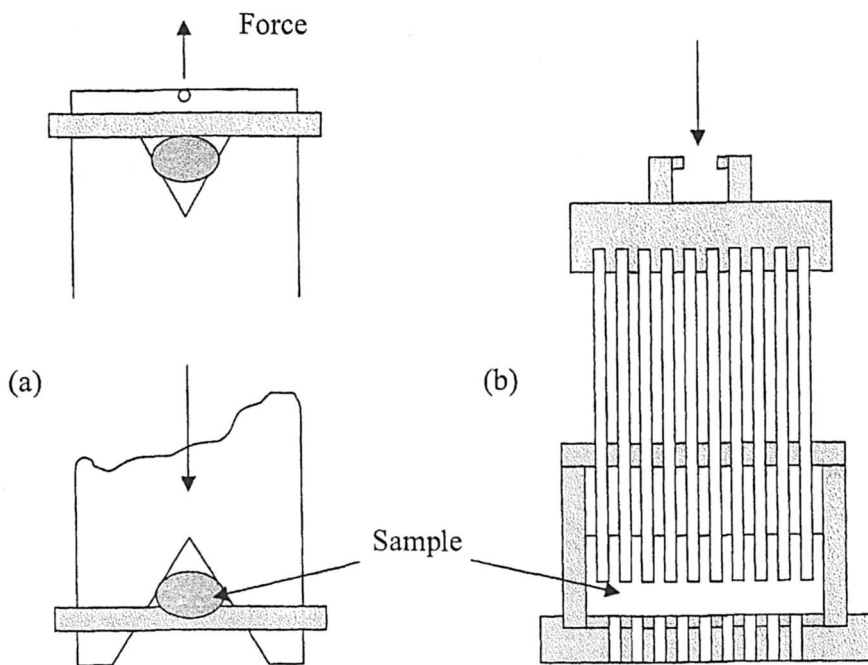
Figure 3- Cross-sectional view of (a) Warner-Bratzler shearing
device and (b) Kramer shear cell.
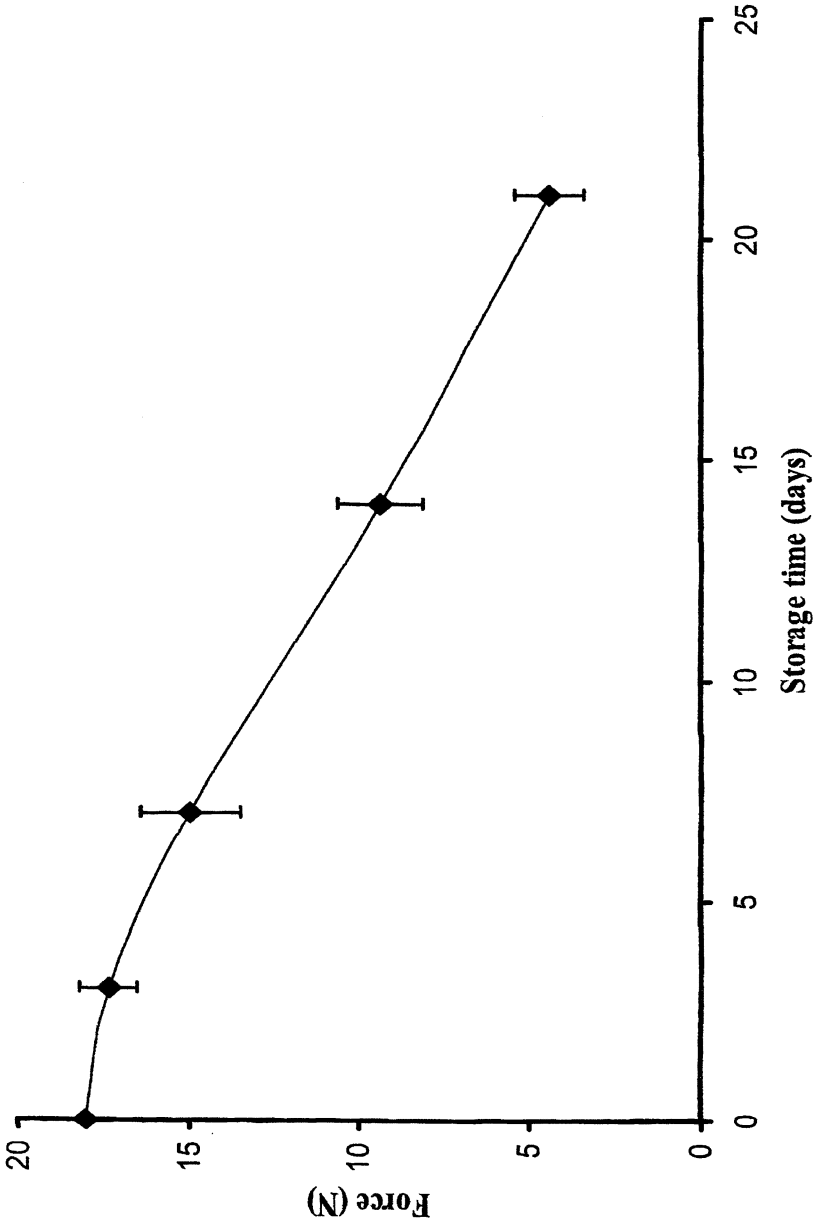
Figure 4- Change in force values with storage time of minimally processed sweetpotato strips tested in Kramer shear cell (adapted from 25).
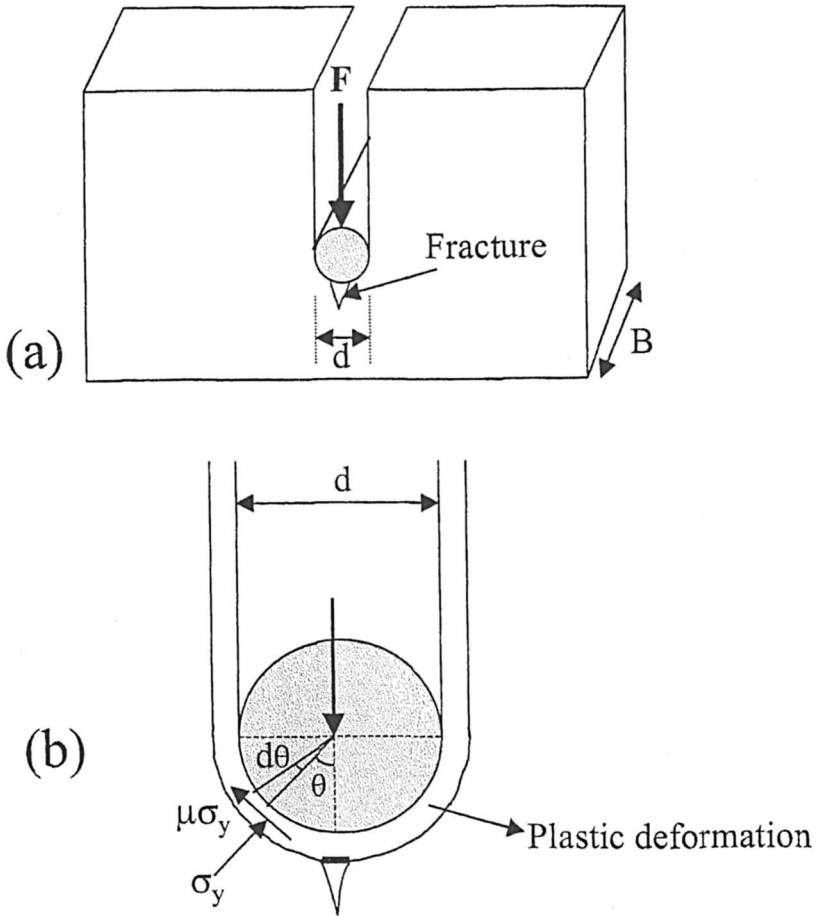
Figure 5–Wire cutting: (a) wire in a sample block; (b) plastic deformation and frictional forces (adapted from 32).

friction, σ_y is a yield stress, and d is the wire diameter. For steady state cutting, the constant force per unit width, F/B, is proportional to d with a slope of $(1+\mu)$ σ_y and an intercept of G_c. The G_c values obtained from the wire-cutting test on cheeses were very comparable with those of the single-edge notch bending test (SENB, see below), and the slopes, $(1+\mu)$ σ_y, were highly correlated with μ and σ_y of compression and friction tests (32). The cutting test is easy to perform and generates fundamental variables. Alvarez et al. (33) applied the cutting test with a single-edge razor blade to monitor the textural changes of apples during storage. They reported that cutting energy decreased linearly with storage time and appeared to be a more precise measure than fracture toughness of the SENB test.

Bending/Snapping

This class of large strain tests measures the force required to bend or snap a food with a well defined shape, usually a bar, cylinder, or sheet (34, 13). The bending technique detected changes to tortilla texture during storage (35). The fundamental, bending parameters, namely fracture strain, fracture stress, and flexural modulus can be derived from the standard beam-bending equations adapted from material science (34, 36),

$$\sigma^f = \frac{3F_fL}{2} \tag{4}$$

$$\varepsilon_f = \frac{6Y_fB}{L^2} \tag{5}$$

where σ_f = fracture stress (N m^{-2}), ε_f = fracture strain, F_f = force at fracture (N), L = span distance (m), W = width of specimen (m), B = thickness of specimen (m), Y_f = deformation of the beam at fracture (m).

A major disadvantage of the bending test is that the upper surface will be in compression, and the lower curved surface will be in tension, resulting in the non-uniform strain and stress state in different parts of the specimen. These problems can be overcome in the notched bending tests such as SENB.

The SENB, a type of three-point bending test, is widely used in studying the fracture of metals and plastics (37). In this test, the sample is notched with a razor blade to give a sharp starter crack with a dimension a, loaded on a three-point bending fixture (Fig. 6), and deformed at a given crosshead speed to generate the force-deformation data. Assuming a linear elastic behavior, the fracture toughness, G_c, can be computed from

$$G_c = \frac{U}{BW\phi} \tag{6}$$

where U = energy to fracture (area under the load-distance curve), B = sample thickness, W = sample width and ϕ (a/W) = a calibration factor. The crack propagated in the SENB test is somehow similar to the crack propagation leading to fracture during biting of a piece of food with the incisors in the mouth. This test was applied in studying the crispness of fruits and vegetables (33, 38), and the influence of ageing on fracture properties of Cheddar cheeses (39).

Compression, tension, and vane tests

Compression is similar to puncture testing except that the area of the probe is equal or larger than the area of the sample surface. Several compression devices are widely used in assessing texture of baked goods (Baker Compressimeter), cheeses (Ball Compressor), and fruits and vegetables (Firmness Meter). Uniaxial compression tests have been documented to assess the storage effects on the texture of baked goods (40, 41), cheeses (39), fruits and vegetables (2, 42, 43). The American Association of Cereal Chemists approved the compression method for testing firmness of bread and other baked goods (44). Baker and Ponte (45) reported consistent increasing patterns of firmness as function of bread staling time. Earlier work of Bashford and Hartung (46) established a relationship between the force at 25% compression and bread freshness. Guinot and Mathlouthi (40) applied the compression test in studying sponge cake firmness as a function of additive and storage conditions.

Uniaxial compression methods generate several mechanical parameters (Fig. 7) namely stress, strain, moduli, and work at fracture to describe the textural properties of many food materials. For compressible materials, e.g. bread and other products with a spongy structure, the cross-sectional area of compression remains releatively unchanged, and the engineering stress (σ_E) and strain (ε_E) can be used (47):

$$\sigma_E = \frac{F}{A_o} \tag{7}$$

$$\varepsilon_E = \frac{\Delta H}{H_o} \tag{8}$$

where F = force, A_o = initial specimen area, H_o = initial specimen height, ΔH = absolute deformation.
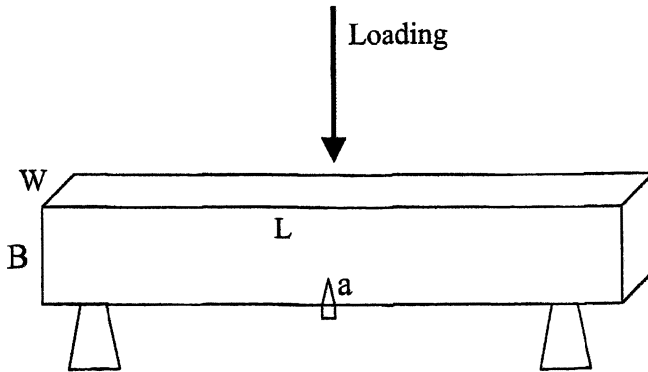
Fig. 6 – Notched 3-point bending.
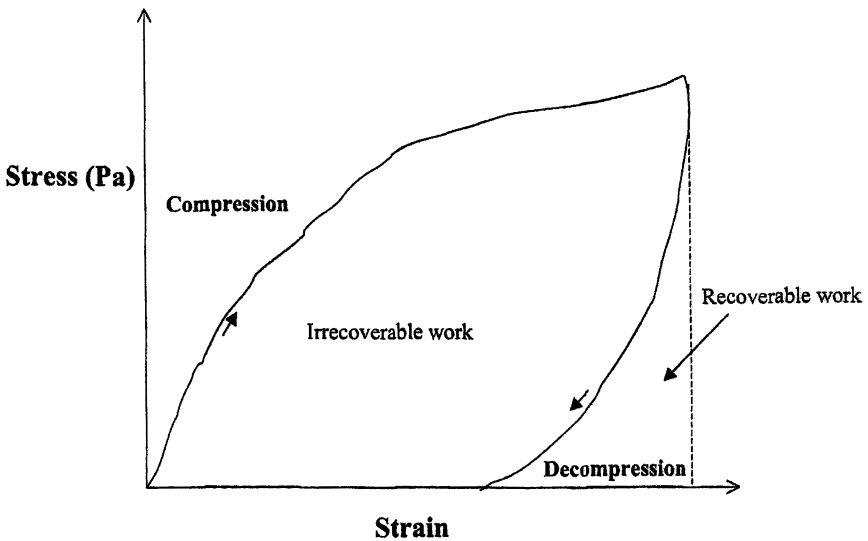
Figure 7-Schematic view of stress-strain relationships in a compression-decompression cycle (adapted from 48).

In addition, the area under the compression-decompression curves (Fig. 7) relates to the recoverable work that describes the degree of elasticity of the materials (48). For incompressible materials or large strain conditions, adjustments were made to stress and strain calculations, correcting for changing area during specimen compression (49, 50):

$$\sigma_c = \frac{F(H_o - \Delta H)}{A_o H_o} \qquad (9)$$

$$\varepsilon_c = -\ln\left[1 - \frac{\Delta H}{H_o}\right] \qquad (10)$$

The problems associated with the compression test especially in studying the fracture properties of foods in relation to their sensory characteristics during mastication are: 1) fracture may not occur for highly deformable materials, and 2) compressive forces may dictate the failure mode and cause specimen slumping due to water excretion. Therefore, other techniques have been recommended to improve upon the limitations of compression testing, such as vane (51, 52), torsion (49), and tensile testing (47, 50, 53, 54). Plotting stress and strain at failure from these test modes generates "texture maps" (Fig. 8) describing characteristics of food products as affected by formulations, processing or storage conditions.

Texture Profile Analysis

Instrumental texture profile analysis (TPA), an imitative method using a Universal Testing Machine (55), has been widely adapted to characterize textural properties of many food products. In a TPA test, a sample of specific dimensions is uniaxially compressed two times in a reciprocating motion, and the compressive force is recorded as a function of the degree of compression (distance). Several instrumental texture profile parameters can be derived from a TPA curve (Fig. 9): the force at the first significant break is called "fracturability (not all foods exhibit this peak); the maximum force at the end of the first compression cycle equates to "hardness;" the work done to compress the sample during the first and second cycles is the area (A_1 and A_2) under the respective curves, and the ratio A_2/A_1 is called cohesiveness. Other textural terms have been defined from the TPA data including, springiness, adhesiveness, gumminess, and chewiness (56). Basically, TPA is a compression test, therefore, the force-deformation relationship of the initial
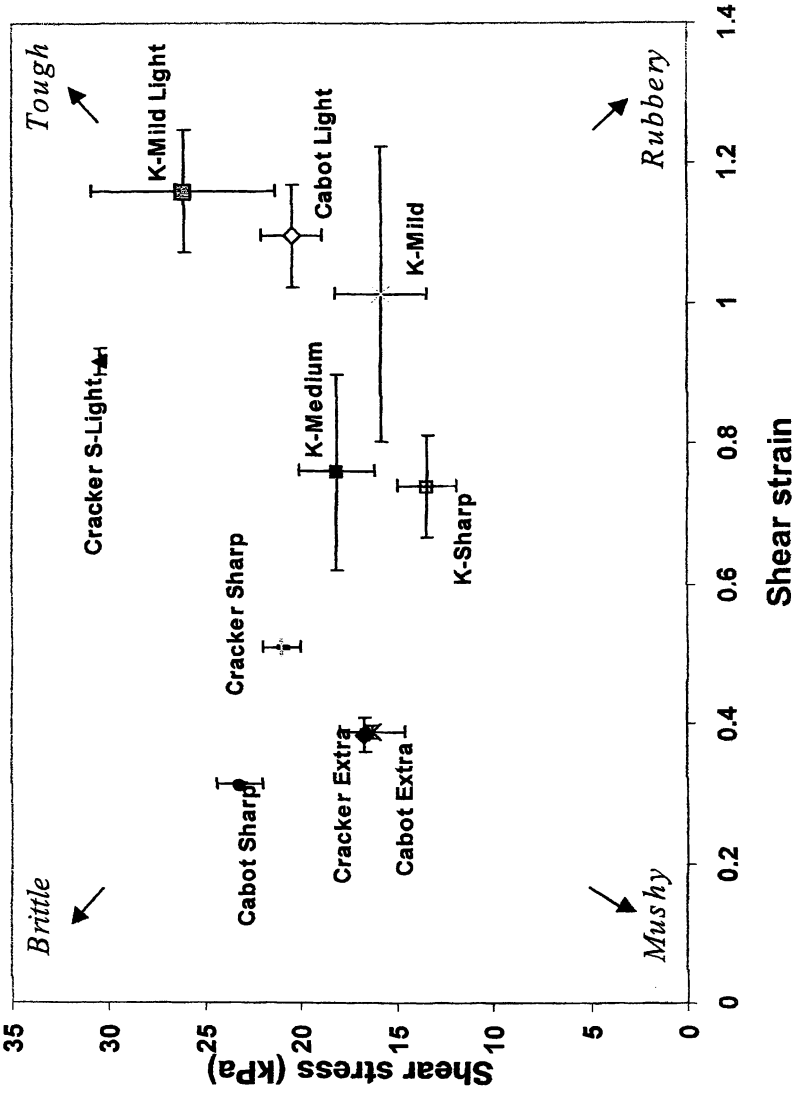
Figure 8- Texture Map of Cheddar Cheeses: Compression Test (adapted from 57).
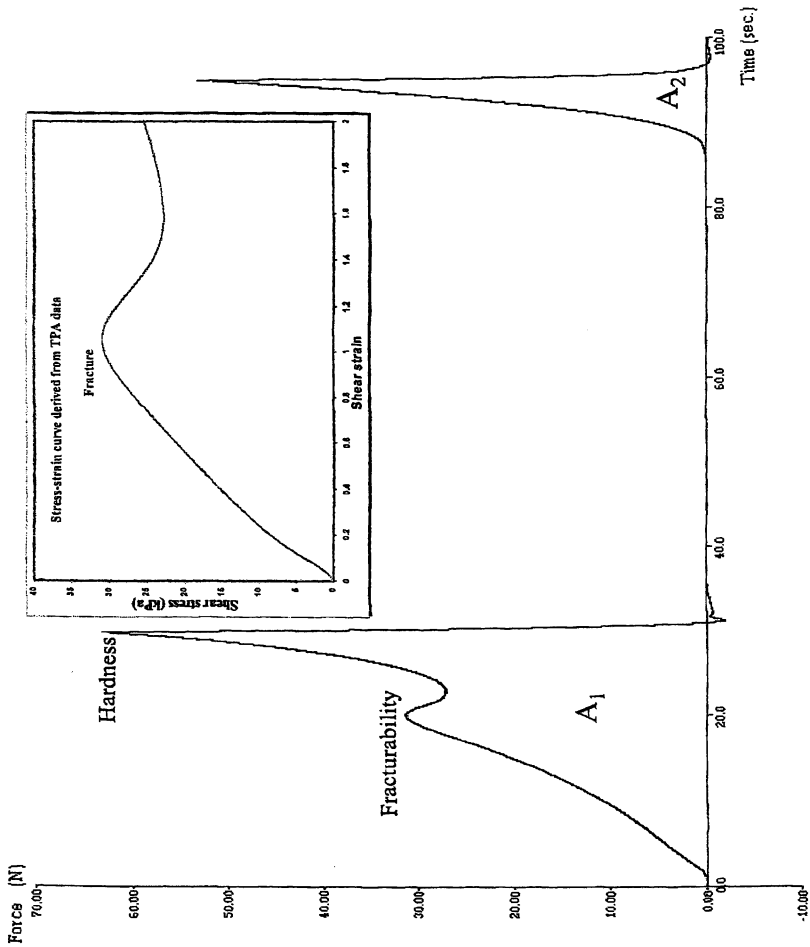
Figure 9- Typical TPA curve of Cheddar cheeses and derived stress-strain plot (adapted from 57).

compression cycle can be converted, by applying equations 9 and 10, into a texture map (Fig. 9), providing the sample dimensions are well defined (57). With advancing instrumentation and data acquisition capabilities, TPA is gaining renewed popularity for food texture characterization. However, depending on the type of sample, data obtained from such automation should be interpreted with cautions. A comprehensive review on terminology and testing conditions affecting TPA results was recently presented by Pons and Fiszman (58). Fiszman and Damasio (59) pointed out that adhesiveness may not properly be measured by TPA especially for a material with high instantaneous recoverable springiness wherein specimen separation from the probe surface during the upstroke may not be completed. Nevertheless, the TPA test has been used in assessing the changes in textural quality during storage of various food products such as bread and cakes (34, 60), cheeses (61, 62), raw and cooked potatoes (43, 63), starch gels (64), fruits (65), nuts (66), beef patties (67), and seafoods (23, 68, 69). Depending on the foods, changes of TPA parameters with storage time as well as their correlations with other instrumental and sensory variables vary with product types.

Steady Shear Tests

Viscosity is perhaps the most common of all rheological terms and is defined as the resistance to shear flow. The apparent viscosity (η) is the ratio of shear stress (σ) to a corresponding shear strain rate ($\dot{\gamma}$).

$$\eta = \frac{\sigma}{\dot{\gamma}} \tag{11}$$

To acquire viscosity data, a viscometer will shear a sample using a specified configuration: couette, cone/plate, or parallel plate to name a few. For each attachment, geometrical factors are known. The viscometer records rotational speeds and torques during operation, and this information coupled with the system geometry permits a viscosity calculation. Steady shear tests for viscosity determination may be used to evaluate dispersion stability of colloids and emulsions. A thorough discussion and review may be obtained from Dickinson (70).

Small Strain Methods

Dynamic rheological testing applies a sinusoidally varying input function to a sample of known geometry at a controlled frequency. Data from these tests

may be used to monitor gelling, probe microstructure, or investigate stability. Essentially these tests evaluate the viscoelastic nature of materials, quantifying the phase relationship between viscous (G") and elastic (G') components (71). Modern instrumentation permits the application of oscillatory testing while samples are exposed to a variety of conditions, including changing temperatures, pressure, pH, frequency, or time. Another benefit to dynamic rheology is that all measurements are performed in the linear viscoelastic region, where essentially moduli remain independent of test condition. By obeying this constraint, material microstructure is assumed to remain undamaged during testing - a major difference in comparison with large strain procedures.

Dynamic rheology is a fundamental technique that may help explain textural differences between samples from the standpoint of a microstructural understanding, but the test should not be used independently to describe sensory texture. Mastication is a large strain process, and difficulties may arise when attempting relationships with small strain data. Nevertheless, microstructure does impact sample failure, but the rheology should be coupled with additional analtyical tools, such as microscopy, to further elucidate the role of structure in sensory texture.

While products remain in the store awaiting customer purchase, the foods are not subjected to large deformation practices. In fact with the exception of environmental conditions, products are exposed to constant forces associated with their own weight. For example, chocolate milk is a colloidal dispersion of chocolate particles suspended by a weak gel network created by stabilizers. In time, these particles may sediment to the bottom of a container as a result of gravity and density differences between particles and continuum. Dynamic testing provides a means to accelerate a sedimentation process by applying a constant stress condition to probe the weak gel network, thereby serving as an effective tool to perform shelf-life studies. In addition, small strain procedures may monitor the synersis of starch-based foods such as baby foods and pie fillings, proteolysis during cheese ripening, or the staling of bread. Figure 10 shows a typical spectra for Cheddar cheese during maturation from 1 to 5 months. At earlier periods of ageing, water tends to penetrate between protein interactions, creating a more viscous (less elastic) material. During cheese ripening, the casein matrix becomes more rigid due to moisture loss. This phenomenon is evident in figure 10, where increased months of maturation display a larger G' inidicating a firmer cheese. Many applications of dynamic testing to monitor shelf-life and investigate texture have been performed and may be further reviewed (17, 37, 73-78).
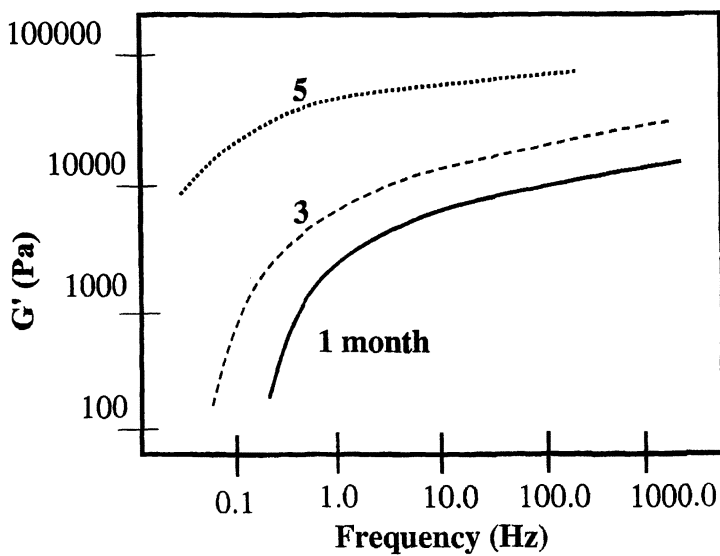
Figure 10 -Typical viscoelastic aata for an ageing Cheddar cheese at T = 25 °C (adapted from 72).

Conclusion

Rheological techniques have long served as useful tools to assess food freshness and stability. Many applications are simple and rapid, making them suitable for quality assurance. In addition, more advanced rheological methods provide insights into structural properties and interactions of food components during processing and storage. As new capabilities arise in the coming years, rheological methods will continue to play an important role in improving food texture and shelf stability to meet consumers' demand on better product quality.

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Chapter 20

Argon Packaging and Processing Preserves and Enhances Flavor, Freshness, and Shelf Life of Foods

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Extensive organoleptic and microbial assessment of food products packaged or processed using argon-based gas mixtures showed excellent preservation of flavor and other quality characteristics. Compared to classical nitrogen-based modified atmosphere packaging (MAP), argon displaces oxygen more efficiently, prevents oxidation, and yields superior results. Quantitative data obtained from in-house double-blind sensory panels, competing product quality challenges and testing at point of manufacture were integrated with microbial assays throughout shelf life to determine relative quality scores. Products tested include wine, cheese, potato chips, nuts, pizzas, processed meats, poultry, orange juice, fresh pasta, prepared meals, and produce. Comparative studies with these products show that argon yields both a significant extension of shelf life and an average of 25% improvement in quantifiable quality parameters such as flavor, appearance, aroma, color, texture, and overall customer acceptability. Argon preserves chemical flavor components better than other techniques, resulting in significant improvements in shelf life and product quality.

Introduction

We report here the first commercial development of the use of argon gas in the modified atmosphere packaging (MAP) preservation of a large variety of food products. Argon was found to be superior to MAP gases used previously. This is attributed to the differences in physical properties between argon and nitrogen, the commonly used MAP gas. We know that oxidation and microbe growth cause food deterioration and that MAP reduces the rate of deterioration. We also know a great deal about the mechanisms by which oxidation causes generation of off-flavors and degeneration of food products. This paper shows that, using argon, we can exclude oxygen better, remove oxygen more efficiently, minimize levels of CO_2 in packaging gas, and depress respiratory and other oxidative enzyme activity. This results in significant improvement in the quality and shelf life of food products.

First, the development of the use of argon in MAP is discussed. Next, the methods used in the current study are outlined, and finally, the results of argon use in MAP are presented for a number of food types.

Inhibition of Oxidation of Food Products by MAP

The three gases used previously in classical modified atmosphere packaging (MAP) are nitrogen, carbon dioxide, and oxygen. MAP for food products generally uses mixtures of these three gases (*1,2*). MAP is widely used in food preservation today (*3*), but is particularly well-developed in Europe. It is used in meats, poultry, produce, vegetables, fruits, bakery, and many other products (*2*). MAP has been found to increase food safety, and at the same time does not present added risk. While concerns have been expressed previously about packaging of foods in anaerobic environments created by using mixtures of nitrogen:carbon dioxide, this risk has been found to be not fundamentally greater than the risk posed by abuse in developing anaerobic conditions in any closed package.

Oxidation of Flavors in Foods

Oxidation of foods causes deleterious degradation of flavor principles. The mechanisms by which the flavor, aroma and color of foodstuffs are spoiled by air include enzymatic oxidation, autoxidation and photooxidation. The reaction

mechanisms responsible are well-understood (*4*). The initiation and continuation of autoxidation and photooxidation reactions themselves are exponentially dependent upon oxygen concentration (*5*); thus, exclusion of oxygen is key to flavor retention. Enzymatic oxidation, such as lipoxygenase and oxidase-catalyzed reactions, are even more sensitive to oxygen. These reactions are more important in solution and in respiring products than are autooxidations. Excluding air prevents ingress of oxygen into foodstuffs, thus inhibiting the rate of oxidation, although entrained oxygen, solubilized oxygen, and chemical sources of oxygen may remain to catalyze and to propel destructive reactions.

The oxidative development of rancidity in food products during storage proceeds as a delayed exponential curve (*6*). The measurement of oxidation in foods has become highly standardized. Standard measures include para-anisidine value (p-AV), peroxide value (PV), (thiobarbituric acid reactive substances) TBARS values, Totox value, Totox TBA value, oxirane value, fatty acid (FA) composition, refractive index (RI), and gas chromatography/ mass spectral (gc/ms) identification of reactant and product species (*7*). These can all be related directly to flavor loss. For example, the direct relationship of the development of sensory rancidity to chemical oxidation as measured by peroxide values has been measured by standard AOCS flavor scales (*8*). The progress of most cases of oxidative degradation of food flavor over time may be suitably approximated through measurement of organoleptic parameters.

The Chemistry of Flavor Preservation by MAP

Using nitrogen to exclude oxygen significantly inhibits the initiation and progression of oxidation as measured by the above standard means. As an example, blanketing or sparging of nitrogen to inhibit oxidation of highly labile products such as soybean oil is standard industry practice. Using peroxide values (*9*), the utility of nitrogen blanketing has been proved. Oxidative stability has been related to off-flavor generation (*10*), and extensive data has been generated showing that nitrogen improves flavor stability (*11*). The efficacy of nitrogen in stabilizing the oil is directly proportional to its ability to limit the ingress of oxygen, which is in turn limited by practical engineering considerations. The superior effect of argon vs nitrogen in inhibiting chemical oxidation of soybean oil has been outlined (*12*), and the mechanism of its effect in such products will be outlined below.

MAP has also been seen as having great potential to retain the fresh flavor and aroma of labile food products (*13*) through limitation of the growth of

spoilage microorganisms and the inhibition of biochemical degradation by enzymes. MAP is economical and avoids the use of additives. Basically, classical MAP involves the replacement of oxygen by inert nitrogen and/or carbon dioxide, where carbon dioxide is the antimicrobial active agent. Carbon dioxide is highly soluble in water, and acts by forming carbonic acid through dissociation, which lowers pH, inhibiting the growth of many microorganisms. Furthermore, carbon dioxide acts directly to inhibit respiratory pathways: decreases in oxygen levels and increases in carbon dioxide levels inhibit tissue respiration (14). While carbon dioxide is thus highly reactive, disrupting cell membrane function, suppressing respiratory pathways, and inhibiting respiratory and other enzymes, nitrogen has no such effects.

Development of Argon for Use in MAP

Argon is a safe, inert gas which has been tested for use for modified atmosphere packaging and processing of a variety of foodstuffs (12,15,16). These reports indicate that excellent results were achieved with high concentrations of argon, and even better results for krypton, xenon and sometimes neon. Argon has GRAS status in the USA as does nitrogen, and like nitrogen has EU approval, permitting its free use as a MAP component.

Retailers are constantly challenged to improve own-brand quality and freshness throughout their preferred product range. Considerable effort is expended, working in partnership with suppliers, to improve in-store quality through extension of shelf-life at source. Being aware of the existence of research and patents for the use of argon and other noble gases for food preservation, we embarked upon a program to develop the application for noble gas preservation of food and drinks. Engineering novel gas systems for suppliers' plants, we successfully developed the methodology for application, and have installed argon systems at 24 food manufacturing sites, most of which are currently producing products packaged and/or processed in argon. Initiated at the end of 1998 under license, the business development program has recently been successfully completed, resulting in the roll-out of some two hundred argon MAP products. Safeway has transferred its interest in the UK patent rights to industrial gas companies, and continues to specify argon in these products.

Differences Between Nitrogen and Argon

The reasons that argon can remove and exclude oxygen better than nitrogen, and that argon is more effective in controlling enzymatic oxidations, are due to the several obvious differences in the gases' physical properties (Table I). Argon is much more dense than nitrogen, and is much more soluble than nitrogen in water and oils. Argon has a polarizability closer to oxygen than to nitrogen, and has a higher ionization potential than nitrogen. In addition, argon has a van der Waals' constant closer to oxygen than nitrogen and is closer in size to oxygen than is nitrogen. Finally, argon makes up only 0.934% of the atmosphere, compared to 20.946 for oxygen and 78.084 for nitrogen (*17*). Increasing its concentration to high levels such as those used in MAP creates a novel atmosphere for the product in question.

Table I. Important Physical Properties of Argon, Oxygen, and Nitrogen

	Polarizability (10^{-24} cm3)	Ionization Potential	Van der Waals' Constant	Diameter (10^{-8})
Ar	1.6411	15.759	1.47	2.94
O_2	1.5812	13.618	1.360	2.92
N_2	1.7403	14.534	1.390	3.15

While van der Waals' forces are known to be responsible for the inclusion of argon in stable clathrates, and unstable hydrates have been formed, argon forms no compounds under normal conditions.

Density Effects

Argon is more dense than nitrogen (1.650 vs 1.153 kg/m3, or a factor of 1.43). It can therefore be readily calculated that argon can fill void spaces and displace oxygen four times more efficiently than nitrogen. Since the density of nitrogen approximates that of air (which consists of 78% nitrogen and 20.9% oxygen), it will mix rapidly and completely with air in any given space. Thus, without added force, introducing 1 liter of nitrogen into a container of air of 1 liter volume will be expected to displace only about 50%, or 500 cc, of the air in

a random exchange of volumes. A second 1 liter of nitrogen will remove 50% of the remaining air, leaving 250 cc, etc. Following this iteration, the substantial removal of residual oxygen requires addition of eight volumes of nitrogen.

Since it is much more dense than air, it is possible to introduce argon gas in laminar flow similar to that found in the introduction of a liquid. The argon may be placed in the bottom of the container and made to fill upwards, displacing the air entirely through the introduction of a single 1 liter volume. Thus, in practice, while it requires up to 8 volumes of nitrogen to clear a void tank, vat or package space, it takes only 1 to 2 volumes of argon. Since argon generally costs 4 to 5 times as much as nitrogen, it is evident that argon can be profitably employed as a MAP gas. We have developed detailed know-how in practical application of argon and design of gas-handling systems to take advantage of these figures.

Solubility Effects

Argon is 3.1 times more soluble in water than nitrogen (60.88 vs   at 19.67ppm at 20 °C), 3.21 times more soluble at refrigerated temperatures (95.53 vs 29.76 ppm at 0 °C), and 1.97 times more soluble in oils (136 vs 69 ppm at 25 °C). (The figures for O_2 are 44.49, 69.99 and 116 ppm respectively.) It is therefore possible to introduce a higher volume of argon than nitrogen into a given liquid food. The removal and exclusion of oxygen from a liquid column depends upon the amount of inert gas present as well as its rate of introduction compared to oxygen, both of which are proportional to the solubility of the gas. In establishing the dynamic of introduction, it is possible to use a much lower gas pressure and total volume, thus preventing the stripping-off of essential aroma and flavor volatiles by outgassing. Thus, argon is superior to nitrogen for purposes of deoxygenating liquid foods.

Argon is chemically inert, as is nitrogen when used in food packaging. Carbon dioxide, while having greater density and costing less, might seem a good candidate for MAP inerting, but it is in fact reactive to most food products. Thus argon is the best gas for use in food MAP as an excluder of oxygen.

Microbiostatic/Microbiocidal Effects

Carbon dioxide mixed with nitrogen is used to control microbes, but has deleterious effects upon the product such as bleaching and generation of off-

colors and tastes. We have found that using argon instead of N_2 makes it possible to use less carbon dioxide to achieve the same or better level of microbial control, with less product damage.

Argon has been shown to inhibit oxidases. Figure 1 shows the inhibition of oxidase (tyrosinase = monophenol, dihydroxyphenylalanine: oxygen reductase;
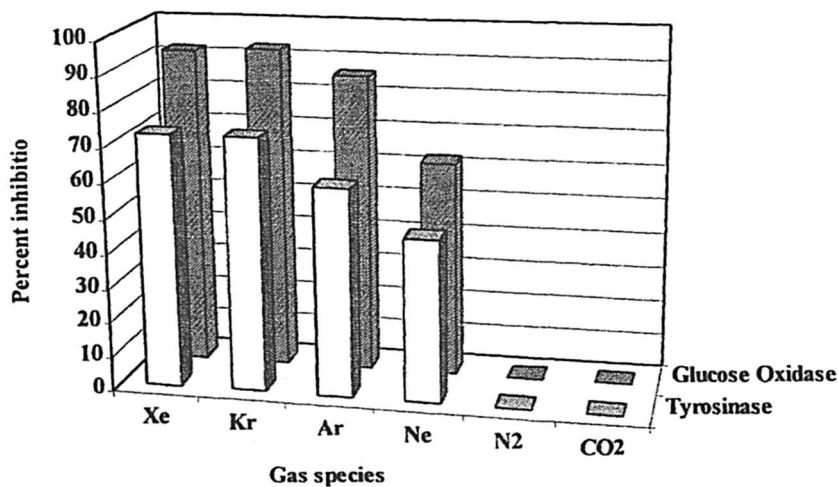
Figure 1. Inhibition of oxidase enzyme activity by noble gases.

EC 1.14.18.1) activity. It has been shown that noble gases, including argon, change enzyme reaction rates and yields (18, 19). The efficiency of this inhibition is seen to be proportional to the atomic size of the noble gas tested. This can only happen if the conformation of the protein has changed, affecting the active site, or if the gas is interacting at the active site. The same figure shows a much smaller apparent inhibition by nitrogen, which is wholly attributable to the complete deoxygenation of the medium: an identical result is obtained using vacuum depletion of oxygen. Argon inhibits oxidases better than nitrogen even when the same amount of oxygen is present. Thus argon has a molecular effect which nitrogen does not have, and is better at controlling microbial growth than nitrogen, particularly growth of yeasts and molds.

Methods

Sensory Assays

Three primary types of sensory discrimination tests were used in the present study: Paired comparison discrimination, paired comparison hedonic scale acceptance, and triangle discrimination tests. Samples were assessed as follows.

First, all products were assessed by independent paired comparison discrimination and paired comparison hedonic acceptance tests in-house using our dedicated product evaluation center. Naïve panelists were randomly selected and asked depending upon the test either to select or to score each product using a hedonic 5-point scale (20). (Occasionally, a 6-point scale was used, in which an additional scalar was included to indicate product was completely spoiled and unacceptable for testing.) We used a score of 3 as the cut-off for shelf life acceptability. Each of five organoleptic parameters (overall acceptability, flavor, aroma, texture, and appearance) was rated in randomized pairwise tests of treatment vs control samples. Sufficient numbers of panelists were used to attain a minimum significance level of $p < 0.05$ in replication of assays, usually $N = 30$ in a single panel or $N = 49$ in seven panels of 7 panelists were used, and the results are reported as the pooled average of the panels (the differences between the panels having been determined to be nonsignificant).

Second, all products were also assayed organoleptically by trained panelists in pair-wise difference tests at point-of-pack and post-distribution, using the same hedonic scale as above and sufficient replications to assure significance

Third, most of the products were subject to expert panels using triangle discrimination tests. These tests were used to discriminate between treatment and controls based upon strength of individual critical organoleptic product parameters. Results from these triangle tests are identified as such.

Calculation of significance was by chi-square, binomial (t), or reported probability tables (20, 21). The (alpha) $p < 0.05$ probability level was taken as the minimum for reporting purposes. All reported differences are highly replicable and robust across panel venue, test method, and date (replicant) of paneling.

Finally, post-distribution products and in-store packages were difference tested using both product evaluation center and expert paneling. Treatment vs

control packages of similar production date-code were taken off the shelf or received directly from distribution. These represent full-scale production sampling trials, and organoleptic differences so measured were found to have great statistical depth and discrimination.

All difference tests were conducted on samples prepared, stored and shipped according to standard protocols, reflecting normal treatment during distribution. Product specifications were held constant for both treatment and control samples. Seal integrities and precise gas measurements were made of all samples. Production, storage, and distribution temperatures were all specifed to standard and checked. It should be noted that separate studies of samples subjected to systematic, temperature, handling or microbial abuse were conducted, but are outside the scope of this report. However, we were able to conclude from the latter that products prepared in argon MAP are not subject to any greater rate of degradation nor hazard than those prepared in nitrogen MAP.

Microbial Assays

Assays were conducted in triplicate or pentuplicate for the indicated days post-packaging throughout the life of the study, using standard methods as required by regulatory oversight. Assays were conducted on all products in coordination with sensory paneling. Total viable count (TVC) was always assayed, and depending upon the product, we also assayed total anaerobes, pathogens, yeasts, and molds. All products were assayed blind-coded, using external, independent laboratories. During production, all products are routinely sampled and assayed microbiologically according to normal Good Manufacturing Practice, and in compliance with UK and EU regulations.

Results

Non-Respiring Products: (Oil-Containing) -Potato Chips and Nuts

As we have seen, oxygen-catalyzed oxidation of oils is responsible for degradation of flavors in oil-containing products. Additionally, argon removes and excludes oxygen more completely than nitrogen. Thus, we would expect for a typical oil-containing product such as potato chips, that use of argon MAP

would be highly effective We found that, by re-engineering potato chip packaging factories' process to utilize argon instead of nitrogen, we were able to obtain substantial improvements in flavor characteristics and other quality parameters, and that the residual oxygen levels in potato chip packages were far more controllable using argon than using nitrogen. Using argon, we are able to drop the average oxygen in packages by nearly half (58% in this example, from 1.46% to 0.86%), using the same or less amount of gas. Indeed, any final target residual oxygen level can be obtained, down to 0.0%. The critical factors in this process are cost and complexity of operational control which affect the amount of gas used. Figure 2 shows the frequency distribution of packages at decile residual oxygen levels from a typical production run.
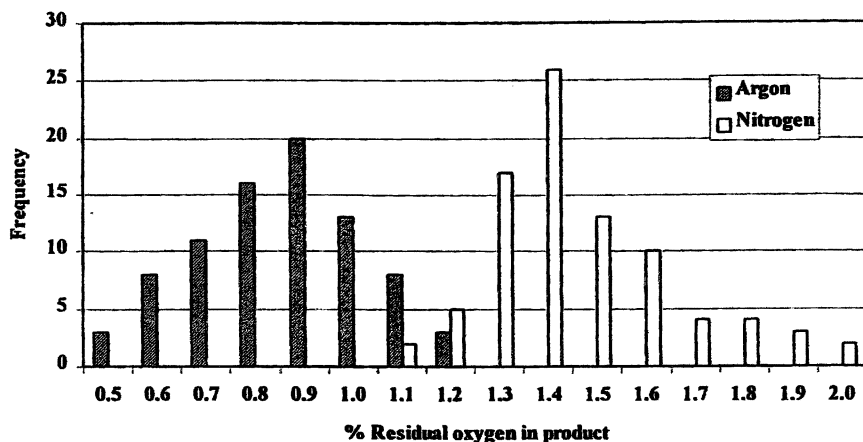
Figure 2. Residual oxygen levels in potato chips packaged in argon vs nitrogen.

The reduction in oxygen levels is directly responsible for better flavor retention in the packages. A paired comparison test for salted potato chips (Figure 3) shows a marked preference for the argon MAP product at six weeks preference in overall acceptability and flavor which increases over time. As we consider a more highly flavored product, the differences increase. This is due to the fact that the addition of such flavorings to the product increases the sensitivity of panelists to MAP differences because there are more flavors which can be adversely affected by the residual oxygen. Figure 4 shows the results of a triangle test for sensory parameter strength in flavored cheese and onion potato chips of similar age. Argon MAP product was selected by a ratio of two to one.
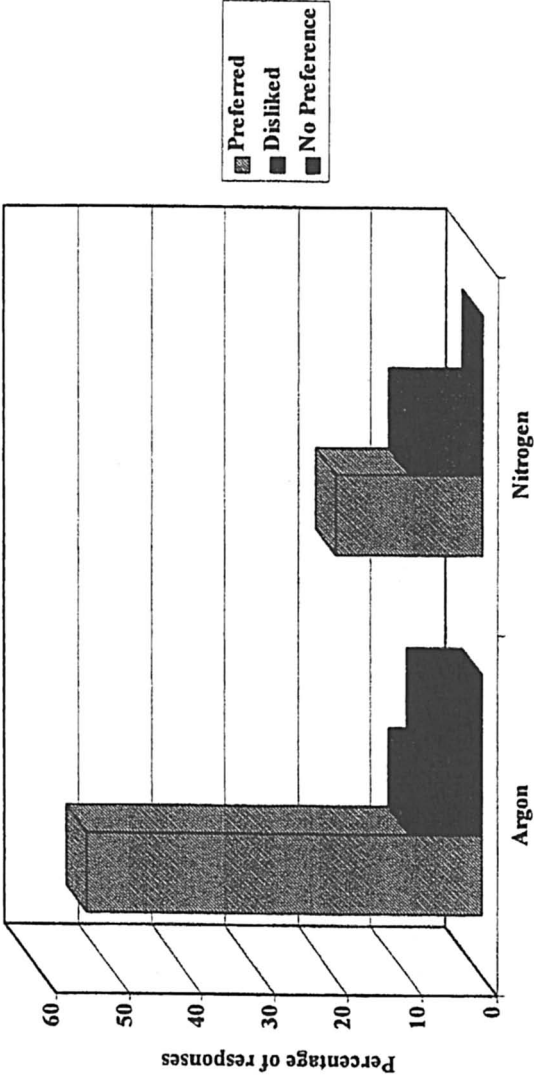
Figure 3. Paired Comparison test of preference for salted potato chips packaged in argon vs nitrogen MAP.
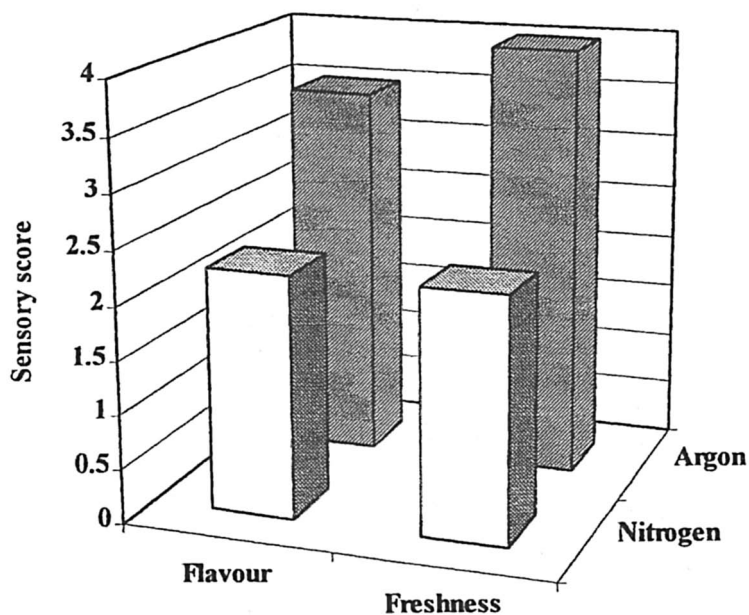
Figure 4. Triangle sensory test of strength of flavor and freshness parameters of cheese and onion flavored potato chips packaged in argon vs nitrogen MAP.

The significant preference for the argon MAP chips builds over time, as shown in a paired comparison test over shelf life (Figure 5). In this example, cheese and onion flavored chips were assayed over 12 weeks by fifteen trained panelists for appearance, texture, flavor, and freshness. Argon preference increased from 0 to 35 to 53% over the twelve-week period. We may attribute this difference to the development of oxidized flavors in the control samples. For very strong flavors, such as prawn cocktail (age 8 weeks), the effect is even more pronounced: of 19 panelists, 13 preferred argon, 3 nitrogen, and 1 had no preference, while 0 disliked argon, and 2 disliked nitrogen.

Sensory panel evaluation data (at age 6 weeks) for another oil-containing product, packaged peanuts, is given in Figure 6. Panelists found the argon
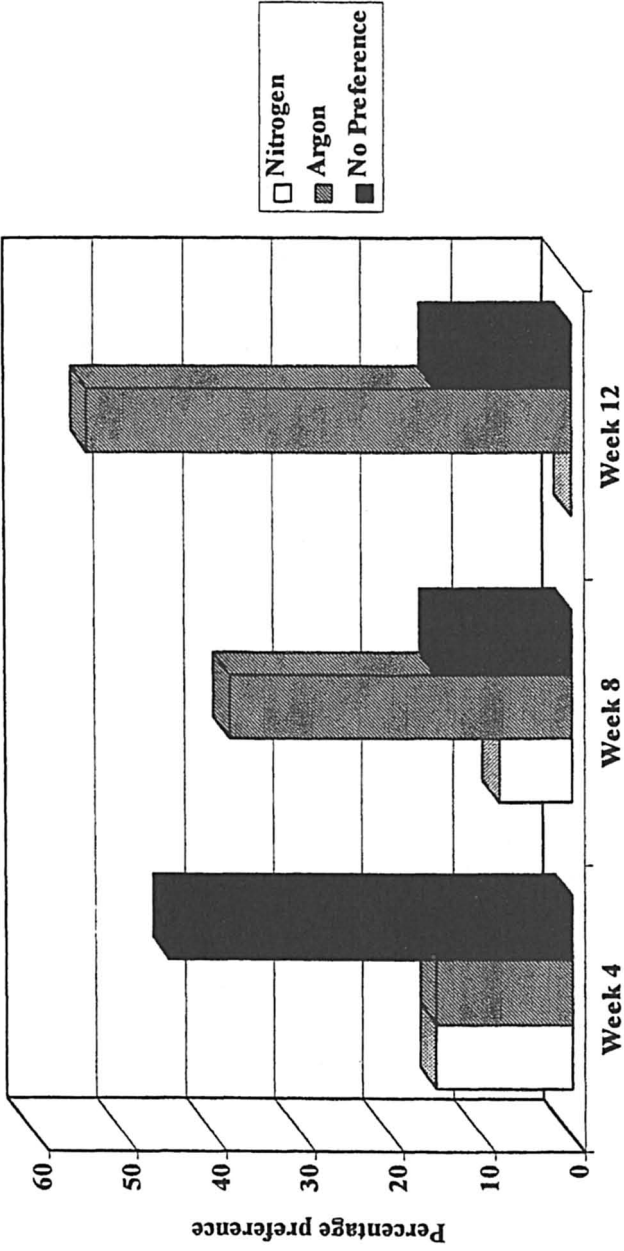
Figure 5. Preference over shelf life for cheese and onion flavored potato chips packaged in argon vs nitrogen MAP.

packaged product to be better in all five parameters, averaging a 14% improvement overall. In other panels with a variety of different nut products (almonds, pecans, walnuts, macadamias, mixes) ranging from 4 to 52 weeks in age, preference for the argon-packaged product exceeded 90% (n=120). These differences may be attributed to reduced oxygen levels in argon MAP packages, which were routinely less than 50% of those packed in nitrogen Analysis of free fatty acid (FFA) levels in chopped mixed nuts at 5 months after packing showed 0.46% for nitrogen MAP and 0.33% for argon MAP, confirming the relationship between oxygen level, flavor, and panel preference.

Chilled Products: Processed Meats, Fresh Pasta and Fresh Meats

Flavor components in processed meats are also sensitive to oxygen degradation. Oxidizable flavor components and color are adversely affected by oxygen concentration, which manufacturers try to minimize. In addressing flavor degradation, oxygen levels in meat packages made on thermoforming packaging machines can be easily be dropped by half using argon instead of nitrogen. Figure 7 shows oxygen residuals for an average of four product production runs at a typical efficient plant comparing product packaged in argon vs nitrogen MAP (the volume of argon used did not exceed the volume of nitrogen used). In this example, the oxygen residuals resulting from using argon average 37% less than in standard production. Results of sensory evaluation (pairwise comparison) are presented in Figure 8 for overall acceptability of this product. Similar results were obtained for flavor, aroma, appearance and texture.

Over the 32-day period, differences increased continually over time. Argon MAP product scored significantly higher over most of the shelf life. Panelists (N=116) in 4 blind tasting sessions concluded that the product was 25% better.

Meats as well as other packaged chilled products are primarily limited in shelf life due to microbial growth, which manufacturers attempt to control by adding carbon dioxide, typically up to 40% CO_2 in nitrogen MAP in the UK. Since CO_2 is a reactant which affects meat quality negatively, reducing its use improves the product further: 20-25% CO_2 in argon gives acceptable control of microbial growth. Thus, argon improves overall acceptability through the reduction of CO_2 levels in addition to the benefits of reduced oxygen level.
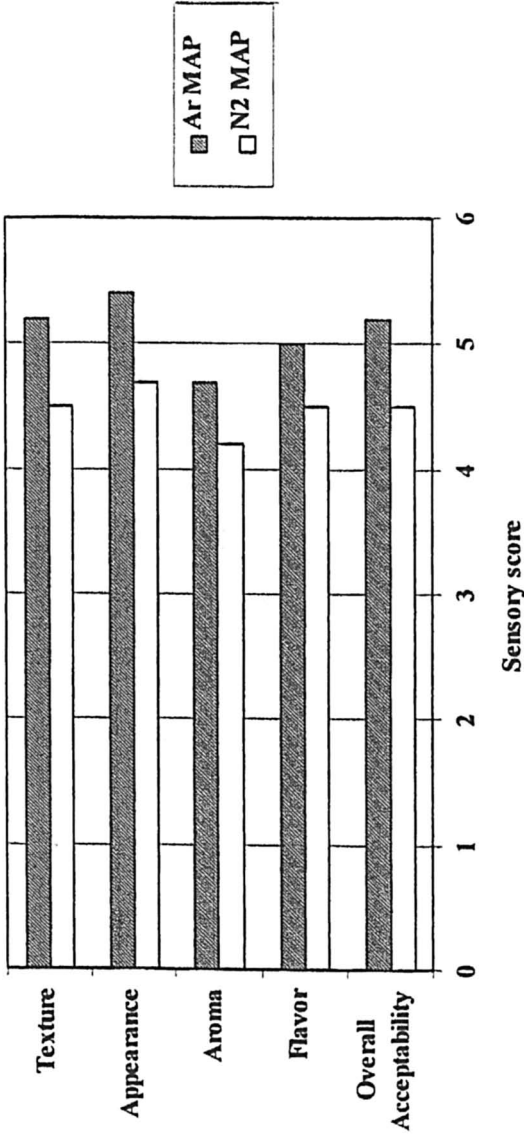
Figure 6. Sensory panel evaluation of five organoleptic parameters of peanuts packaged in argon vs nitrogen MAP.
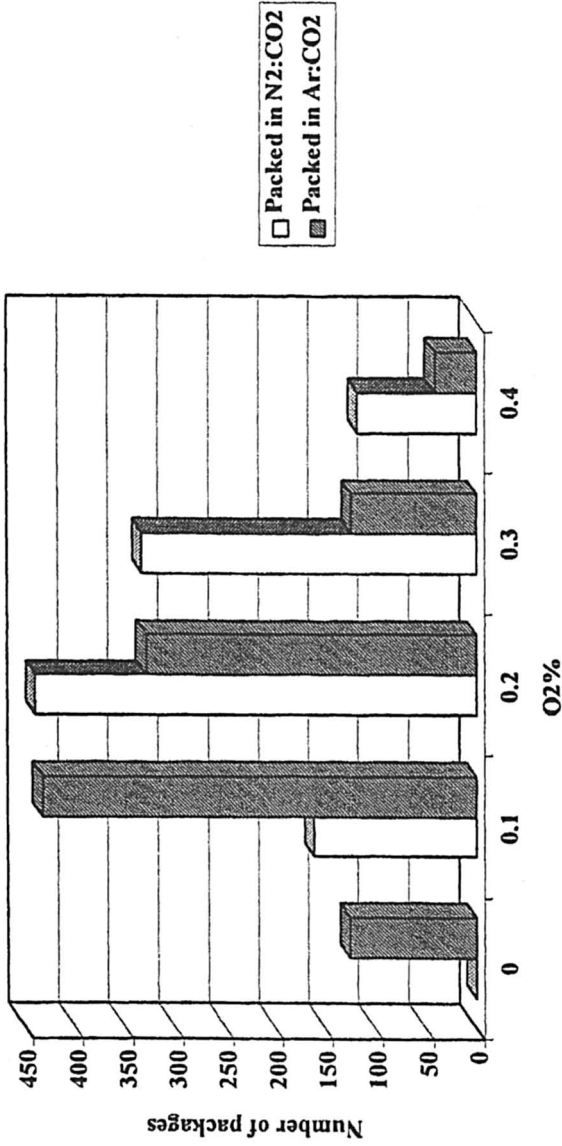
Figure 7. Average residual oxygen in processed meats packaged in argon vs nitrogen MAP.
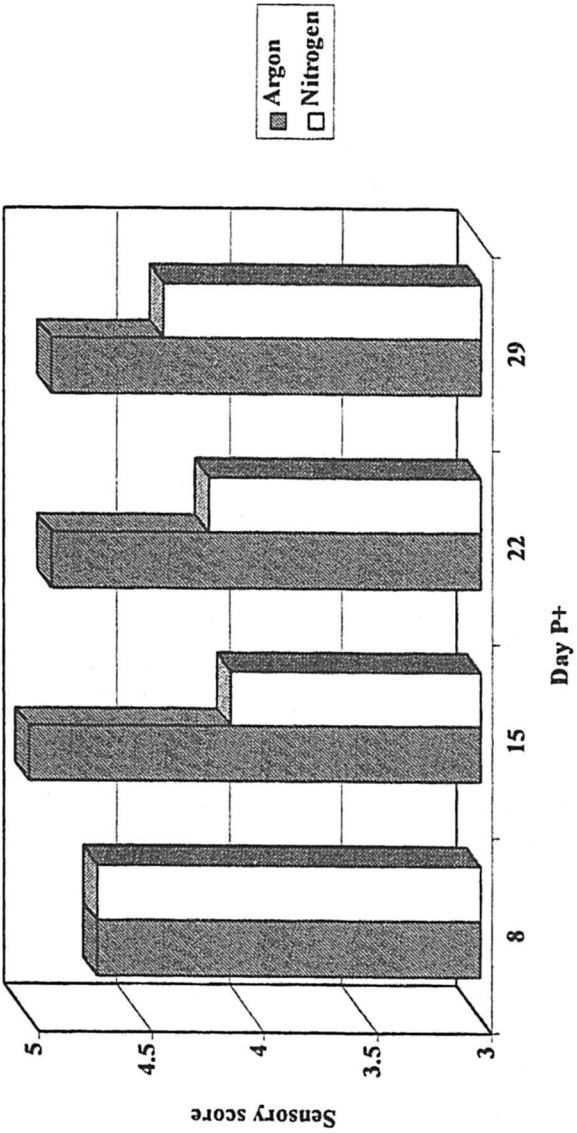
Figure 8. Organoleptic assessment of overall acceptability in processed meats packaged in argon vs nitrogen MAP.

The results of microassays of the above meat products are given in Figure 9. TVC were assayed in triplicate each day from day of pack to a shelf life cut-off of 5×10^6. Using argon MAP, shelf life was increased by 50%, from 20 to 32 days. Microbial growth was depressed by argon by one-half to one log.

Chilled fresh pasta products are sensitive to oxidation of flavor components, and green spinach or herb-containing pastas lose color in the presence of molecular oxygen, rapidly bleaching in the presence of carbon dioxide from N_2:CO_2 MAP. Lower oxygen and carbon dioxide levels were obtained using argon MAP, resulting in less bleaching and a significant extension of shelf life. Testing full-scale production, panelists found an average of 13% improvement in both color and flavor parameters. Microassay showed argon to be 15% better.

In fresh meats, such as poultry, we used argon to lower the amount of carbon dioxide required to reduce microbial loading, without changing the appearance of the product deleteriously. Sensory evaluation showed argon MAP product flavor scored 19% higher than nitrogen MAP product, and microassay (TVC) showed a shelf life extension from 9 to 14 days, or a 64% improvement.

Respiring Products: Salads and Produce

Inclusion of oxygen in MAP is necessary for respiring products. Salads in the UK are typically packaged in nitrogen-based MAP consisting of N_2:CO_2 O_2 70-90:10-30:5-20 (22, 23, 24). Recommended noble gas-based preservation mixtures (15, 16) include Ar:CO_2:O_2 70-90:0-20:0-15. As salads respire on the shelf, they typically degrade, consuming O_2 and producing CO_2. Over an average 5-day shelf life, O_2 levels decline steadily from their initial level of 10-15% toward 0%. The CO_2 levels resulting from respiration of product and entrained microbial flora rise by that amount, in precise correspondence, from an initial 5-15%, to 20-30% at end of life. Ultimately, the product becomes anaerobic and thereafter unacceptable for sale. Increasing CO_2 titers can slow this process, but the resulting product quality is severely affected as CO_2 in aqueous solution is a strong oxidant, causing bleaching of color, generation of off-tastes, and deliquescence, particularly in colored produce such as carrots or red cabbage.

Figure 10 gives the pooled results of a sensory trial (N=500) comparing argon vs nitrogen based MAP. Packaged salads from store were analyzed for gas content and presented pairwise, according to date code, to panels for preference choice. In 78% of the pairs, the consumer group chose argon MAP as superior, in 12% the group chose nitrogen, and in 10% no difference was found, the results correlated perfectly with the progress of respiration in the packages.
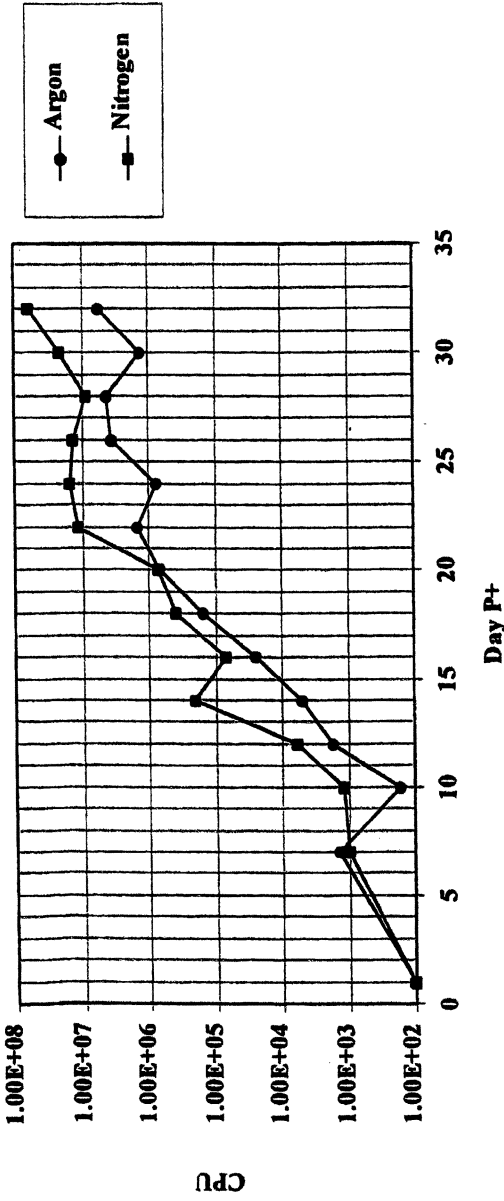
Figure 9. Microbial growth (TVC) in packaged processed meats packaged in argon vs nitrogen MAP.
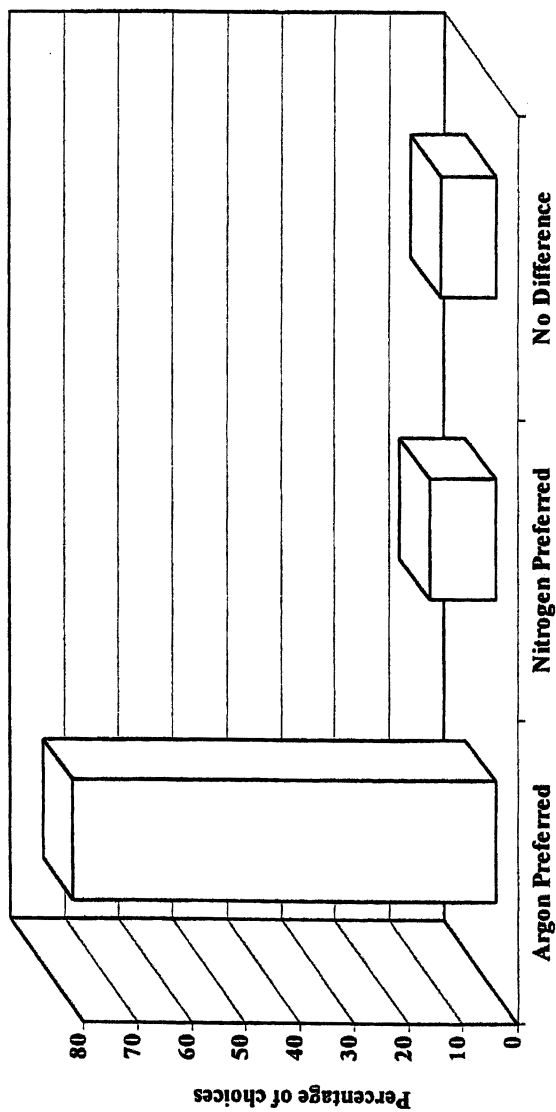
Figure 10. Sensory assay for preference for salads packaged in argon vs nitrogen MAP.

Microassay showed TVC in argon to be 40% less than in similar nitrogen based mixtures. As degradation of produce is catalyzed by oxidative enzymes, from either the product or from microbial sources, argon inhibition of oxidases slows degradation. Packaging produce in noble gas based mixtures results in a superior quality of product, which retains freshness over an extended period.

Conclusions

Oxygen catalyzes oxidative degradation of flavors and other quality components of foodstuffs, and modified atmosphere packaging lowers residual oxygen levels in packages. Because of the different physical properties of argon, it is far superior to nitrogen in removing and excluding oxygen from packages or during processing, and lower levels of residual oxygen may be obtained. Carbon dioxide is used in nitrogen-based MAP to control microbial growth, but using argon combined with CO_2 provides an added level of antimicrobial action as well as allowing the use of a smaller percentage inclusion of CO_2, resulting in less oxidation of flavor and color components. Since argon inhibits oxidase activity, argon-based MAP mixtures effectively depress respiration in both produce and microbes. Shelf life and quality over life of a wide range of products are significantly improved by packaging and/or processing in argon.

Safeway, in partnership with Air Liquide, has successfully developed argon MAP technology for an extensive range of improved products, profitably. The products are better. They are fresher and have more flavor. Applied as part of a systematic effort to improve product quality at source and maintain quality to store, we have found argon MAP to be a useful and cost-effective technology.

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Chapter 21

Processing Technologies To Enhance Fresh Flavor of Citrus Juice

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Thermal pasteurization of citrus juice has traditionally been required to inactivate enzymes and microbes and is a necessary adjunct to enable energy efficient evaporation, freeze- or reverse osmosis concentration and not-from-concentrate (NFC) juice production. Pasteurization avoidance by providing raw, unpasteurized juice products, high pressure processing and pulsed electric field technology has been proposed in order to improve flavor of commercial juices. Several important issues must be considered before one embraces such technologies as a panacea to replace thermally pasteurized juice. These include 1) properly defining the flavor of "fresh" juice, 2) acknowledgement of potential safety and handling problems associated with minimally processed or fresh juice, 3) understanding the large-scale efficiencies realized in current citrus juice thermal processing systems and, 4) utilization of continuing improvements in product flavor by commercial sector developments.

Fresh, Non-Pasteurized Juice

The aroma and flavor of freshly squeezed citrus juice is very distinctive and preferable to thermally processed juice for those consumers who have tasted it. Small-scale commercial distribution of this product in the locales of juice extraction

facilities is possible; however, microbial spoilage and food safety issues prevent broad commercialization. Although initial flavor of fresh juice is excellent, after a day or two at refrigerated temperatures, active juice enzymes and microbial growth result in significant quality degradation. Microbial growth in fresh juice, in a matter of a few hours, may alter the profile of flavor volatiles. Acetaldehyde, ethanol, isoamyl alcohol and isobutanol increased significantly within 10 hr, after inoculation of juice with strains of *Saccharomyces cerevisiae* (1). In this study, isoamyl alcohol reached a concentration of 10 ppm in orange juice incubated with yeast at 20 ˚C. This compound is a well-known off-flavor in many fermentation products and has an aroma threshold of 0.3 ppm in water (2).

Attempts to quantify "fresh" citrus juice flavor date to the 1940s, where single strength juice was hot-filled in cans and subjected to storage studies. It was also recognized as early as 1925 that peel oil was important to the flavor of concentrated orange juice, since aromas were lost during concentration. Early concentrates had poor flavor until it was recognized that addition of raw juice to the concentrate improved the flavor, if the product was kept frozen (3).

Although flavor is important, recent issues of microbial stability and safety need to be addressed, when considering commercial distribution of fresh, unpasteurized juices. The U.S. Food and Drug Administration (FDA) estimates there are between 16,000 and 48,000 cases of juice-related illnesses each year, with the most recent and severe outbreaks directly related to unpasteurized products. It is estimated that less than 2% of all fruit juice consumed in the US is in the fresh, unpasteurized form. Recent federal rulemaking forces all juice producers and packers to conform to manufacturing under the principles of HACCP (Hazard Analysis and Critical Control Point). Within this rule are several items specific to fresh juice processing and distribution (4). All juice producers (fresh and otherwise) must implement HACCP systems, fresh juice producers must demonstrate a validated system for removing at least 5-log cycles of pertinent microbial pathogens from the fruit surface (citrus processors, only), and all processors must demonstrate that their stabilization systems, including thermal pasteurizers, UV irradiation systems, and any others approved for use, can achieve that same 5-log (100,000) reduction of the key pathogen over that in the untreated juice.

Evaporation

The convenience and economy of large-scale storage and shipping of orange juice concentrate, followed by dilution to single-strength (ss) juice at the packaging location assures prominence of this traditional product in global commerce. The commercial process requires preheating the ss juice to 90–100 ˚C to inactivate pectinesterase, vacuum flashing to the evaporator 1st and succeeding stage tube nests until the product is pumped out at 65 ˚Brix. The heat required to perform the

evaporation results in some "cooked" notes recognized as off-flavors. Equally important is the loss of the most volatile aromas during the evaporation process. Partial recovery of these volatiles may be achieved by cold-water condensation and rectification in a still located on the evaporator, resulting in a concentrated oil- and aqueous-phase aroma mixture (essence). Essence by-products may be sold (similar to cold-pressed peel oils) by citrus processors to essential oil manufacturers. These companies modify the citrus oils and aroma products, selling blends suitable for use in the concentrates, juices and beverages products to citrus manufacturers. With addition of the appropriate oil and aroma blend, water and pulp, the 65 °Brix concentrate may be diluted to manufacture 42 °Brix frozen concentrated orange juice (FCOJ), ss chilled orange juice (COJ) and many juice-containing drinks and beverages.

Much has been written about the individual volatile components (e.g. acetaldehyde, ethyl butyrate, terpene thiols, etc.) contributing to the fresh aroma notes recognized in raw citrus juices. This chapter will not contribute to that body of literature. The difficulty of determining the impact of individual components separating fresh and processed juice aromas is inherent in the analytical methodology (5) and in the processes for commercial recovery of the compounds contributing to the aroma (6). Since individual flavor companies have extensive databases, the most practical approach to achieving fresh-like flavor in processed, packaged FCOJ, COJ and drinks requires juice manufacturers to work with flavor suppliers, selecting the most suitable flavor for the product. Once the flavor is added and properly blended, FCOJ will have better long-term flavor stability than COJ, because it is held below 0 °C. COJ is pasteurized at 80 °C after dilution prior to packaging, which means it has endured two thermal treatments, and the product is maintained at 6-10 °C, usually in a polyethylene-lined carton. The initial flavor of these products has improved dramatically in the last decade, as a result of commercial sophistication of the flavor systems. However, the fresh flavor notes have little staying power (less than a few weeks) in the finished consumer products. This actually is not so bad, because refrigerated raw juice has a fresh taste and aroma only for a few days.

Freeze Concentration

Since evaporative concentration of juice results in loss of volatiles, commercial freeze concentration systems, which preserve the volatiles during the water removal process, were put into place in the 1980s (7). The water removal technique by ice re-crystallization wash columns is efficient enough to concentrate even highly volatile molecules, such as ethanol and ethyl butyrate in orange juice (8). The process requires that the juice first be pasteurized to inactivate microbes and pectinesterase, but less total heat is required than for evaporation, resulting in a

product concentrate with excellent flavor quality (9). A limitation of the process for citrus juice application is that concentration above 50 °Brix is difficult and the world market for bulk transport is based on 65 °Brix concentrate, which can be pumped at -9 °C. Also, while it is possible to concentrate fresh, non-pasteurized juice and preserve the flavor, microbial and enzymatic degradation of the product during storage and upon reconstitution makes this unrealistic.

Reverse Osmosis

Attempts to use non-thermal concentration membrane technology to improve citrus juice concentrate flavor are generally successful, in principle. Commercial reverse osmosis (RO) membranes with high rejection efficiencies are available to allow separation of the smaller molecules constituting many volatile flavors. Compounds in orange juice aromas (aqueous essence) which may be concentrated by RO include acetaldehyde, ethanol, hexanal, ethyl butyrate, as well as terpene derivatives (10). Similarly, the technique applies to aroma from lemon juice (11). Concentration of the actual juice by RO is possible, although engineering and quality problems have limited commercial application. Like freeze concentration, juice freshness is preserved if concentration occurs without applying heat. In reality, pasteurization is necessary prior to RO for sanitary and quality purposes, defeating the purpose of membrane concentration. Also, it is difficult to achieve 65 °Brix due to the high osmotic pressures of the concentrate (12).

NFC Juices

Growth of not-from-concentrate (NFC) citrus juices has surpassed all other citrus products during the last decade. NFC is currently about 40% of orange juice sales in US retail stores (300 million gal.) and the amount has increased to 90 million gal. in the European market (13). To many consumers, NFC juice is perceived as a generally higher-value product, and they are willing to pay more for NFC juice than the equivalent volume of reconstituted from-concentrate juice. This premium quality, incidentally, may be real (as defined by sensory acceptance) or merely perception. In the US and Europe, NFC is perceived as definitely more natural and premium than reconstituted products, and the product packaging (i.e. reclosable pourspouts, chilled case, diary connotation) reinforces that perception. Certainly a properly handled juice product sold through chilled distribution is more likely to have a fresher flavor and aroma than a shelf-stable product, all other things being equal.

As stated previously for FCOJ, enhancing the fresh-like flavor of NFC is largely dependent on the processing and blending steps, requiring close cooperation

between the flavor system developer and the juice processor. The processor has the dilemma of achieving high juice yields from fruit during the extraction/finishing operations, without degrading juice quality by incorporating bitterness or excessive peel oils. During manufacture of NFC juice, a de-oiling step is required. This operation involves either vacuum flashing or passing the juice through hermetically sealed centrifuges to lower the peel oil terpene content to acceptable levels ($\leq$ 200 ppm). The juice may then have special aromas and flavors added to enhance the sensory quality, prior to the aseptic treatment/bulk storage process. Technology also exists to inject the flavor system through an aseptic filter into an aseptic juice stream, bypassing the heating step.

The objective of modifying the flavor is to provide the packaged juice with a stable flavor over a shelf life of about 60 days. Most NFC orange and grapefruit juice is consumer packaged non-aseptically in half-gallon polyethylene lined paper cartons, which are kept refrigerated. Polyethylene has well-documented affinity for d-limonene and other non-polar terpenes in citrus juice aroma. Scalping of these constituents occurs rapidly when the juice is in contact with the package (3). Manipulation of the flavor system to minimize neutral terpene content and provide more juice-like notes and oxygenates is standard in the industry. For NFC orange juices, low-molecular weight volatiles, esters, reduced terpenes and a mix of less volatile sesquiterpenes are desired. This is also true for grapefruit juice, with the additional knowledge of the contribution of certain sulfur compounds. Terpene thiols were established in the 1980s as key character-impact constituents of fresh grapefruit juice aroma (14); however, in practice they have short half-lives in packaged products and the characteristic aroma impact is extremely dependent on the concentration in the product (15).

High Pressure Processing

A number of reports in the last decade have stated the merits of high pressure processing (HPP) for preserving the flavor of citrus juices, while producing stable packaged products having few microbes and active enzymes. Initially, studies promoted completely non-thermal HPP of chilled juice as a best choice for processing conditions to retain quality (16). Citrus juice requires thermal treatment to completely inactivate pectinesterase (PE), which has also been shown to be sensitive to HPP at higher pressures and temperatures (17). While the actual mode of PE inactivation by HPP is not established, temperature probably is not involved, as the heat labile, but not the heat stable form of PE is inactivated by pressure (18), resulting in cloud stable juice (19). Performing HPP of juice at lower temperatures means less chance of thermal flavor and quality degradation due to the adiabatic heating from pressurization. Also, from close examination of a published report

combining heat, pressure and fractionation of juice components (20), few actual quality advantages can be achieved, compared with a typical pasteurization process.

It is evident that HPP treatment of raw, chilled juice produces a shelf stable product with good fresh-like flavor. Sensory scores of HPP juice were statistically different, but perceived closer to fresh orange juice than thermally processed juice (16). GC/MS analysis of volatile compounds in juice package headspace is difficult to interpret quantitatively and compare to sensory results. However, qualitative attempts to correlate certain compounds with juice processing by HPP, thermal treatment and fresh juice have been tried. One study used principal component analysis by dynamic purge-and-trap GC of 4 volatile terpenes and 2 oxygenates present in juice headspace to compare these process treatments (21). Two factors, factor 1 (pinene, myrcene, limonene) and factor 2 (hexenol, octanal) represented 73% of the variance for separating the treatment groups of fresh, HPP and heated juices (Figure 1). Although there was some overlap, the treatments of Figure 1 fell in distinctly different groups and the effects were more likely related to factor 2, as it is difficult to quantitate the headspace terpenes. A sensory panel (triangle test of 22 subjects) was unable to discern between fresh and HPP juice, which indicates the volatile profile changes measured were not correlated well with actual flavor. This confirms the complexity of citrus juice flavor and the difficulty of quantitatively correlating volatile profile analyses with actual sensory tests.

A process combining pressure with use of CO_2 claims to preserve the flavor qualities of citrus juices, while inactivating juice enzymes (22). This process raises questions of some significance in the citrus industry. Attempts to preserve the quality of fresh orange juice by CO_2 treatment similar to carbonated beverages were historically unsuccessful. It has been reported that CO_2 saturated orange juice failed to maintain fresh flavor, inactivate pectinesterase or stabilize juice cloud, although the lower pH did reduce PE activity slightly (23). Under supercritical pressure conditions, the pH would be lowered sufficiently to inactivate the heat labile form of PE, thus the cloud would be partially stabilized for a time period. If microbes were not inactivated, after storage, microbial haze could even account for increased cloud. The problem of volatile flavor loss when the CO_2 is removed needs to be addressed, because carbonated orange juice tends to give one the impression of fermentation off-flavor. Also, vacuum systems for degassing can be difficult to sanitize, especially since the CO_2–pressure step for sanitation precedes this process and the result would be recontamination of the juice prior to storage or packaging.

The current status of HPP of citrus juices could be described as a technical success and a commercial disappointment. Although HPP produces a product with initial flavor characteristics similar to fresh juice, the process has few commercial juice applications, but may show promise for other products (24). Large citrus processors have not adopted it due to high capital cost of the equipment and lack of a true high-speed continuous process capable of handling 10–20 million L juice/yr/plant. For potential installations, economics of HPP favors larger volumes,
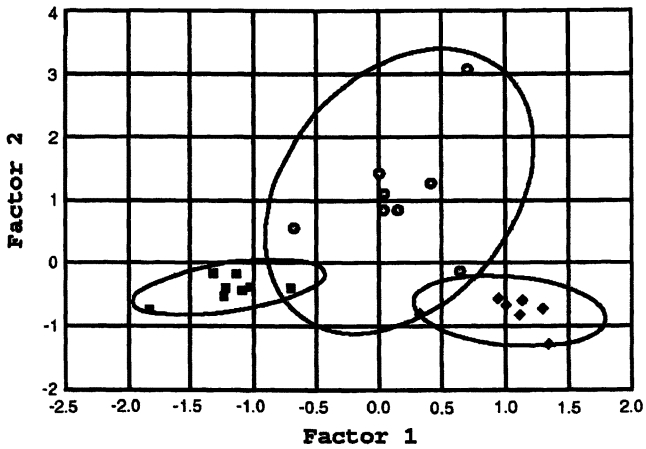
Figure 1. Principal component analysis of orange juice samples by dynamic purge-and-trap headspace volatile analysis using two factors (Factor 1 = pinene, myrcene and limonene; Factor 2 = hexenol and octanal). Calculated from published data (21). **O** = *Control* **□** = *HPP* **◇** = *Heated*

short dwell times and higher pressures over longer times and lower pressures (25). Some food safety questions also remain to be answered for citrus processors. e.g. Will sterility of the process system be achieved if NFC bulk storage aseptic juice is the target? How does one validate the process in a manner similar to thermal processing? Such questions will need addressing so that processors are in compliance with the FDA Juice HACCP regulations, as outlined in the discussion above. The processor will be responsible for providing the data that support the validation of HPP systems in the context of how the processor is utilizing them, i.e. flow rate, microbial loading, physical parameters of the juice, juice chemistry, including pH. This mandatory validation is no small barrier to implementing an alternative to thermal pasteurization, even if flavor quality differences are distinct.

Pulsed Electric Fields

The use of rapid pulsed electric fields (PEF) to inactivate microorganisms and enzymes in citrus juices and other foods has been described and research studies are continuing at a number of labs (26). There have been some reports that PEF-treated orange juice might retain certain flavor volatiles better than thermally processed juice (27,28). Studies comparing PEF (or other process) treatment of orange juice with thermal pasteurization must use proper control juices and sensory evaluation of products to be valid. For example, fresh orange juice frozen in drums, which must be thawed for 10-12 days before processing is not typical and probably has fermented off-flavors and enzymatic breakdown products. Performing GC analysis of headspace terpenes provides only minimal useful data, because quantitation from headspace concentration is questionable due to solubility limits and saturation in the headspace.

Some researchers have found a significant synergistic effect of thermal and PEF treatments on microbial destruction and have designed PEF systems expressly with this in mind (29). These researchers suggest that moderate heating of 45-55°C combined with pulsed fields could be expected to yield an equally microbially stable product with fewer heat effects than a liquid product subjected to thermal processing alone. This was not confirmed with sensory testing. Similar to HPP, researchers in the PEF field are beginning to discuss the PEF process as part of a combination method, i.e. heat + PEF (30). This is in part due to the difficulty in separating thermal effects from those of the electric fields on microbial cells, enzymes and the food itself, and the difficulty in applying research developed with one type of PEF chamber to other systems. Other researchers have investigated the inactivation of pathogenic bacteria by the combination of organic acids and PEF, again in an attempt to provide a properly processed product with minimum application of heat (31).

Commercial Adaptation of Thermal Processing Alternatives

Processing research that seeks to compare alternative processing schemes to the large-scale commercial systems currently in use must be based in actual industry practice, if the new processes are to ultimately replace the current systems. Large-scale citrus juice processing and handling is quite standardized; researchers must, in their work, handle the juice properly for their conclusions regarding process improvements to be valid (for example, methods for storing and thawing frozen raw juice are often sub-optimal, leading to possibly confounding factors in subsequent analysis). A fundamental difficulty in process development is defining the improvement desired; in the context of this paper researchers are seeking improved "fresh citrus juice character". Sensory analysis over commercial shelf life continues to be the most valid means of quantifying "fresh" character from consumers' perspective. This is not an easily-achieved objective. Volatile compound profiling, although both convenient and loosely correlated with "freshness" in some cases, serves as an estimate of the true character of the juice product. Non-volatile compounds, microbial and enzymatic changes, and even texture, rheology and mouthfeel of a juice product all impact overall acceptability and fresh character perception. Regulatory approval is also an important step in the commercialization of some alternative processes. Careful research on processing effects paves the way for regulatory approval if food safety issues are addressed and validated. Research must strive to control, quantify and elucidate the complex nature of product freshness. Research of alternative processes on the many factors that comprise freshness and other key issues such as safety, economics and quality, will be the research that leads to adaptation by industry and subsequent future commercialization.

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