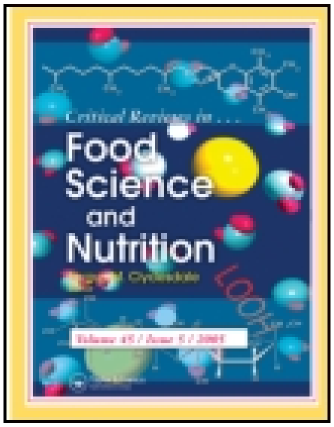

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Nonthermal Food Processing Alternatives and Their Effects on Taste and Flavor Compounds of Beverages

Enrique Ortega-Rivas [a] & Iván Salmerón-Ochoa

[a] Graduate Program in Food Technology, The Graduate School, Autonomous University of Chihuahua, University Circuit 1, New University Campus , Chihuahua , 31125 , México

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ENRIQUE ORTEGA-RIVAS and IVÁN SALMERÓN-OCHOA

Graduate Program in Food Technology, The Graduate School, Autonomous University of Chihuahua, University Circuit 1, New University Campus, Chihuahua 31125, México

Food drinks are normally processed to increase their shelf-life and facilitate distribution before consumption. Thermal pasteurization is quite efficient in preventing microbial spoilage of many types of beverages, but the applied heat may also cause undesirable biochemical and nutritious changes that may affect sensory attributes of the final product. Alternative methods of pasteurization that do not include direct heat have been investigated in order to obtain products safe for consumption, but with sensory attributes maintained as unchanged as possible. Food scientists interested in nonthermal food preservation technologies have claimed that such methods of preserving foods are equally efficient in microbial inactivation as compared with conventional thermal means of food processing. Researchers in the nonthermal food preservation area also affirm that alternative preservation technologies will not affect, as much as thermal processes, nutritional and sensory attributes of processed foods. This article reviews research in nonthermal food preservation, focusing on effects of processing of food drinks such as fruit juices and dairy products. Analytical techniques used to identify volatile flavor-aroma compounds will be reviewed and comparative effects for both thermal and nonthermal preservation technologies will be discussed.

Keywords Nonthermal food preservation, food drinks and beverages, flavor and aroma, sensory attributes, volatile compounds.

INTRODUCTION

Traditional and Alternative Food Preservation

With increasing demand to obtain processed foods with better attributes than have been available to date, food researchers have pursued the discovery and development of improved preservation processes with minimal impact on the fresh taste, texture, and nutritional value of food products. In food preservation, thermal treatment has proven to be very effective in terms of microbial inactivation, but may also cause loss of nutrients and impairment of desirable quality properties (Lado and Yousef, 2002). Combined methods can be used in food treatment to reduce textural and nutrient damage and obtain microbiologically safe products. Preservation of foods by combined methods consists of applying a combination of stress factors, often simultaneously, to minimize processing. By avoiding any single preservation technique, the synergistic effect allows the production of high-quality food products (Alzamora et al., 1998). There are also a series of individual nonthermal methods investigated recently in food processing that do not involve direct heat and are said to be able to produce safe foods with improved quality attributes. These methods include ultrahigh hydrostatic pressure (HPP) and high-voltage pulsed electric fields (PEF), and could have an enormous impact on the food industry (Flickinger, 1999). Some other nonthermal food processing techniques comprise ultraviolet (UV) radiation, gamma irradiation, ultrasound, and nonconventional chemical reagents. Commercial applications of some alternative technologies are varied and include purification of water, pasteurization of fruit juices, processing of milk, etc. UV radiation, for example, is making an impact in many of these applications (Koutchma, 2009).

When microorganisms are treated with heat, the logarithm of cell population decreases, linearly, with the treatment time, for constant treatment intensity. Alternative technologies are

Address correspondence to Enrique Ortega-Rivas, Graduate Program in Food Technology, The Graduate School, Autonomous University of Chihuahua, University Circuit 1, New University Campus, Chihuahua 31125, Mexico. E-mail: eortegar@uach.mx

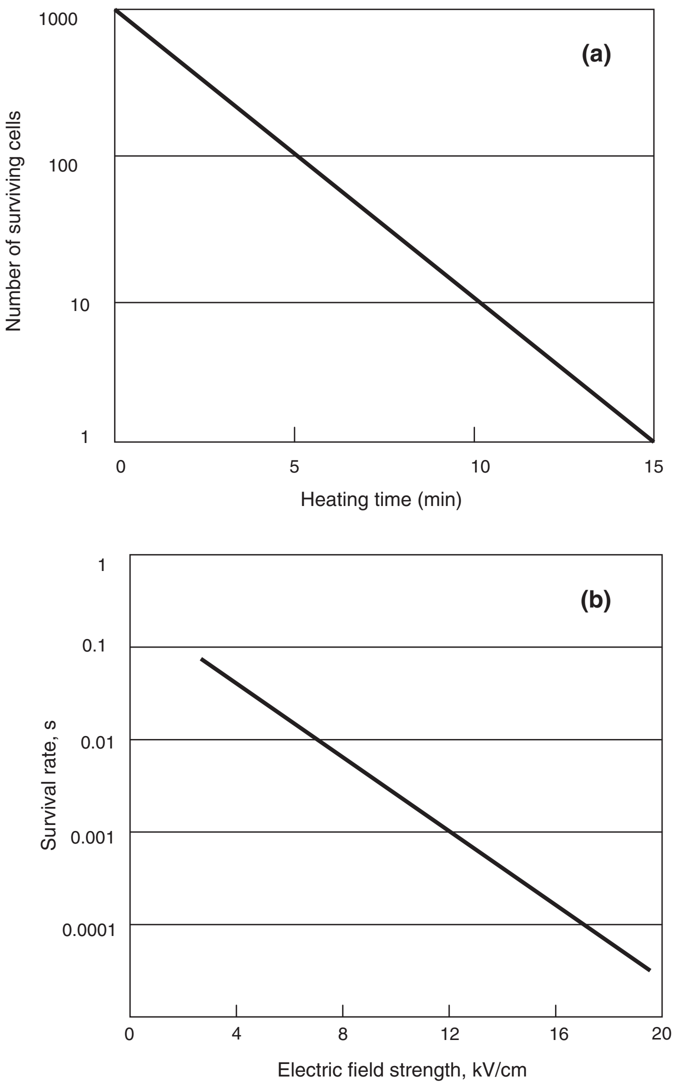

Figure 1 Examples of microbial decreasing population by: (a) conventional thermal pasteurization, (b) pasteurization by high-voltage pulsed electric fields.

also believed to inactivate microorganisms logarithmically. Dose–response models are derived from kinetic data to predict efficiency of variables of alternative processes (Van Gerwen and Zwietering, 1998). These models depict the relationship between treatment intensity and a microbial population resistance parameter. Treatment intensity corresponds to temperature, pressure, electric field intensity, or radiation dose, for the case of thermal, HPP, PEF, or radiation treatments, respectively. The intensity of the deleterious agent (e.g., heat or electrical discharge) must exceed a threshold value before microbial inactivation happens. Fig. 1 shows examples of microbial population decrease by thermal processing and by PEF.

Conventional Pasteurization of Food Drinks

Heat processing below 100°C is usually termed pasteurization and is generally designed to kill all pathogenic organisms and some of the spoilage organisms that would be capable of growing in beverages under defined storage conditions. Both, batch and continuous methods of pasteurization can be used for food drinks, and the treatment may be carried out before or after placing the product in the container. In batch pasteurization, individual volumes are treated in jacketed stainless steel vessels that may be used both for heating (with steam or hot water) and cooling (with chilled water or brine). Continuous pasteurization may be carried out by passage through plate heat exchangers, which usually comprise the stages of preheating, heating, holding, and cooling (Fig. 2). Traditional pasteurization methods for food drinks include thermal processing either as a low–temperature long-time (LTLT) method or a high-temperature short-time (HTST) technique. The method known as ultrahigh temperature (UHT), or aseptic processing, has been also employed for treatment of beverages. Currently, HTST pasteurization is the mode commonly used for heat treatment of beverages. HTST pasteurization is a continuous process that shows several advantages over batch pasteurization. UHT processing involves the production of a sterile product by rapid heating to high temperatures, followed by a short holding time and ending with a rapid cooling. The processed product is filled into a presterilized container within a sterile environment, to provide a prolonged shelf-life.

Beverages of all sorts may present a series of undesirable effects when processed by conventional, thermal methods of pasteurization. Nonalcoholic beverages, such as fruit juices, have been commercially treated by HTST pasteurization (Sun, 2005). For example, orange juice is pasteurized by HTST at 90–95°C for 15–30 sec (Braddock, 1999), while apple juice is processed at 77–88°C for 25–30 sec by the same method (Moyer and Aitken, 1980). Aseptic processing has gained popularity as a thermal pasteurization technique for both juices with temperatures as high as 138°C and holding times of at least 2 sec (Lewis and Heppell, 2000). Aseptic processing produces shelf-stable juices and other products with shelf-lives as high as 8 months without refrigeration (Ellis, 1982). There is, however, a typical cooked flavor detected in aseptically processed juices.

Pertaining dairy products, the conventional pasteurization method for milk is also the HTST technique applied at 72°C for 15 sec, while the UHT treatment has been carried out under similar conditions for orange and apple juice previously mentioned (Ortega-Rivas, 2005). Since thermal processing may have an adverse effect on biochemical composition, chemical sterilization and nonconventional methods have also been used to pasteurize milk. Chemical treatments include addition of antibiotics or hydrogen peroxide, while nonconventional techniques comprise use of ionic radiation, magnetic fields, microwave energy, ultrafiltration, and PEF (Ortega-Rivas, 2010).

FOOD QUALITY ASSESSMENT

In food processing and preservation, validation of quality assurance can be done by instrumental methods coupled with

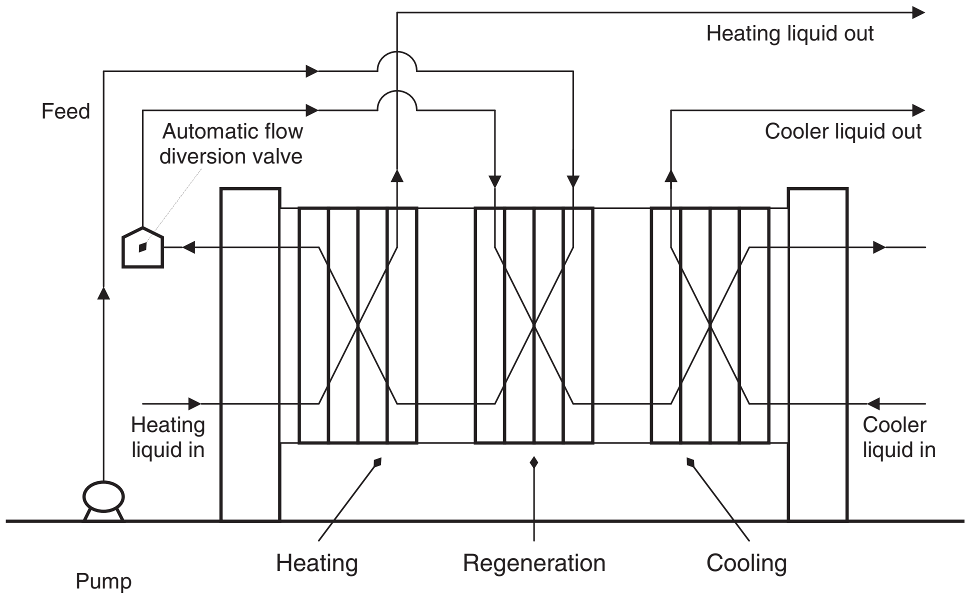

Figure 2 Diagram of continuous pasteurization using plate heat exchanger.

sensory techniques. Available instruments, such as gas chromatography, may quantify chemicals compounds related to sensory attributes. Sensory profiles, on the other hand, are useful tools to verify how ingredient and process modifications may affect some properties such as color, taste, and odor. Physicochemical properties of foods are important factors in processing, quality control, and consumer preference. In processing and product development, some of these properties are measured by means of instruments, but the key factor is to correlate them to sensory evaluation in order to make the crucial decisions for obtaining the desired overall quality. While shape, moisture, density, and viscosity are easy to define and an instrumental determination may be sufficient to meet quality requirements, properties that should be correlated with sensory attributes are those related to color, flavor, and texture, and are not easily measurable. Color and flavor attributes are said to be mainly due to organic chemical compounds, which may be properly determined by instrumental chemical methods such as chromatography and spectroscopy. Flavor is a characteristic feature of a particular product that can be used as an indicator of its freshness. Depending on the product, only a few compounds may be responsible for its flavor, but, often, food aroma is defined by the sum of hundreds or thousands of compounds. In most cases, food is a complex matrix and its constituents react with flavor molecules, influencing their binding and release. When analyzing flavor compounds, the issue of odor thresholds has to be considered. Odor thresholds are the lowest concentrations of a vapor in air that can be detected by the sense of smell. Often, strong odorants are present in very small amount, creating a challenge for instrumental analysis. The methods used for flavor analysis should be characterized by limits of detection comparable with those achieved by the human nose.

Sensory profiles are recognized as being very useful tools for verification of persistency of attributes such as flavor, taste, and aroma on processed foods. The sensory profile of a food product is considered to be the graphical representation of its sensory characteristics. Once established, it may be compared with a standard reference product in order to simulate it or to study the effects of minimal variations in some of the quality attributes of the product being developed. The feature most frequently studied by this methodology is texture profile (Brandt et al., 1963; Lawless and Heymann, 2010). Other profiles, such as the flavor profile, have also been used in food product development (Stone et al., 1974; Omobuwajo, 2007). The key component in establishing sensory profiles in food product development is a trained panel. It is recommended to train not less than 25 individuals in order to select a group of 10–14 persons. There are some good established guidelines to select and train testing panels (Civille and Szczesniak, 1973; Stone and Sidel, 2004).

STAGES OF DEVELOPMENT OF ALTERNATIVE TECHNOLOGIES

Conventional validation of nonthermal technologies has normally taken the path of proving, first, at least equally efficient to thermal methods in terms of inactivation of target microorganisms. A second part of the validation process could be considered the hard task of demonstrating the hypothesis suggested by workers in the area of nonthermal food processing, on their feasibility of preserving better than conventional methods the overall quality attributes of the treated products. This mentioned demonstrating process has also taken steps. Physicochemical characteristics of processed food drinks, such as acidity or pH, are easily determined by routine laboratory techniques or more sophisticated instrumental methods, and so they were initially related to testing efficiency of nonthermal technologies in preserving quality. Sensory attributes characterization however represent a more challenging procedure, since sensory properties are said to be the result of the combination of taste, texture, and astringency as perceived in the mouth, as well as aroma as perceived in the nose.

Sensory attributes can, therefore, only be detected satisfactorily by the human sense organs, so they have been studied, analyzed, and somewhat quantified, using sensory testing counting on panelists. Sensory properties are, nonetheless, the result of the biochemical composition of foods and their interactions with the environment, as well as the effects of their handling, their processing, and so on. For this reason, attributes as valued as the bitterness of certain drinks or the fragrant aroma of others are in the end caused by chemical compounds and their kinetics of reaction, degradation, etc. Sensory and flavor scientists have been working on coupling instrumental methods of chemical analysis to correlate chemical composition of foods with sensory panels responses.

AROMA COMPOUNDS IN FOODS AND BEVERAGES

The consumption of foods and beverages is intrinsically linked to the stimulation of the human senses, odor, and taste. The sensation of odor (smell) is triggered by highly complex mixtures of small hydrophobic molecules from many chemical compounds occurring in trace concentrations, and detected by receptor cells of the olfactory epithelium inside the nasal cavity (Goodenough, 1998). The chemical messengers of the sense of taste interact with receptors located in the tongue and impart four basic impressions: sweet, sour, salty, and bitter. There are also perception, inside the oral cavity but transmitted to the brain by nonspecific and trigeminal neurons, of pungent, cooling, and hot sensations.

It has been implied for some time that all sensory attributes, namely odor, taste, color, and texture, can be generally termed flavor. However, flavor is also said to be the blend of taste and smell sensations evoked by a substance in the mouth, and this definition would exclude color and texture. This apparent confusion has resulted in an often inconsistent usage of terms (Bauer et al., 2001), but convention among flavor scientists somewhat dictates that volatile flavor(s), odor(ous) compound(s), and aroma(s) can be considered synonyms. Fragrance substances, normally used in perfumes, cosmetics, and toiletries, are distinguished from volatile flavors mainly by their different range of applications.

The first comprehensive list of volatile compounds in foods comprised a few hundred constituents (Weurman, 1963). With the use of modern instrumental analysis, particularly gas chromatography coupled with mass spectrometry, more recent compilations (Maarse and Vischer, 1992) contained more than 6200 entries from about 400 different sources. Despite recent advances in sophistication of instrumental analysis, as well as capabilities for processing of data, the detection of new volatile flavors is getting more difficult. Extrapolating from the number of facultative aroma precursors from confidential industrial results and from recently published progress, it has been estimated that up to 10,000 volatiles may be present in food. An accurate estimate is not possible, however, since from 2000, most of the research into new flavoring substances by flavor companies and industries has been cancelled or significantly reduced, due to the impending provision of published positive lists, the loss of confidentiality, and the exorbitant costs of toxicological clearance of few food-related substances (Knights, 2010). Research in flavor science also indicates a considerable overlap of many volatiles in foods with independent flavor profiles. Many separation and enrichment procedures have been developed to allow the use of stable isotope-labeled internal standards for distillation/extraction, but each of the standard techniques shows different recoveries across the broad range of polarities and boiling points.

Aroma compounds in foods may originate from microbial or plant metabolisms or, to a much lesser extent, from animal metabolism. Natural aroma compounds are obtained directly from plan or animal sources by physical procedures. Nature-identical aroma compounds are produced synthetically, but are chemically identical to their natural counterparts. Artificial flavor substances are compounds that have not yet being identified in plant or animal products for human consumption. Nature-identical aroma compounds are the only synthetic compounds used in flavors, apart from natural products. The odors of single chemical compounds are extremely difficult to describe precisely. The odors of complex mixtures are virtually impossible to describe, unless one of the odors is so characteristic that practically dominates the odor or flavor of the composition. Although an objective classification of odors is not possible, some descriptors by adjectives such as flowery, fruity, spicy, or waxy can be used to relate the fragrances to natural or other known products with similar odors. Some conventional terms that have become of common usage are presented in Table 1.

Food handling and processing, including improper harvesting and thermal treatment, may cause loss of aroma, so further complementation should be needed in certain cases. The current trends toward light, less- and low-everything products and minimally processed foods have caused an increasing interest in flavor-related issues in food technology. Reducing fat, for example, will reduce its flavor content and appropriate flavor compensation could be needed to match the expectations created by flavor memory (Hatchwell, 1994). Aroma compounds are also used to substitute expensive natural extracts (vanillin or vanilla-type composed flavors, for instance), while undesirable natural odor notes can be masked not to hide spoilage but to improve acceptability of nutritious products such as soy milk. It has been argued that the sensory quality of food has been ranked higher by consumers, than nutritional value, price, or safety. Going far beyond simple acceptability of food items, the early development of chemical senses in human evolution has linked the perception of odorous compounds to emotional and behavioral responses not controlled by conscious brain functions. The ever-increasing interest in the food industry by

Table 1 Common terms to describe odors*

Term	Descriptor
Aldehydic	Odor note of the long-chain fatty aldehydes, e.g., fatty-sweaty, iron laundry, seawater
Animal(ic)	Typical notes from the animal kingdom, e.g., musk, castoreum
Balsamic	Heavy sweet odors, e.g., cocoa, vanilla, cinnamon
Camphoraceous	Reminiscent of camphor
Citrus	Fresh, stimulating odor of citrus fruits such as lemon or orange
Earthy	Humus-like, reminiscent of humid earth
Fatty	Reminiscent of animal fat and tallow
Floral, flowery	Generic term for odors of various flowers
Fruity	Generic term for odors of various fruits
Green	Typical odor of freshly cut grass and leaves
Herbaceous	Noncharacteristic, complex odor of green herbs, e.g., sage, minty, eucalyptus-like, or earthy nuances
Medicinal	Odor reminiscent of disinfectants, e.g., phenol, Lysol, methyl salicylate
Metallic	Typical odor perceived near metal surfaces, e.g., brass or steel
Minty	Peppermint-like odor
Mossy	Typical odor reminiscent of forest and seaweed
Powdery	Odor associated with toilet powders (talcum), diffusively sweet
Resinous	Aromatic odor of tree exudates
Spicy	Generic term for odors of various spices
Waxy	Odor resembling that of candle wax
Woody	Generic term for the odor of wood, e.g., cedar wood, sandalwood

*Adapted from Bauer et al. (2001).

common consumers may be explained by recognizing that volatile flavors act as chemical messengers in human beings. Most of the aroma-rich food items, such as candies and chewing gums, would simply not be consumed without a high-flavor dosage. It appears safe to assume that the share in the uptake of added volatile flavors is much smaller than the genuine aroma stock of foods. Not reliable data can be referred to, of course, but there seems also to be a growing trend to associate freshness of foods with perception of natural aroma compounds being retained by the use of mild food processing technologies. Flavor-related issues in food science can, therefore, be exploited in terms of the recent trends of alternative, nonthermal food processing technologies.

FOOD FLAVOR ANALYSIS

Food aroma compounds are, arguably, the most important food constituents, as consumers often base their choice of food products on flavor and appearance. The two most important approaches to flavor analysis are the use of the so-called "electronic nose" and the employment of techniques involving chromatography (Nollet et al., 2007). Only the latter approach, however, provides answers about the identity of key odorants and allows their quantification. Electronic noses were designed to mimic the human sense of olfaction and as tools to measure whole aroma, usually by transferring a headspace of the analyzed product into a chamber equipped with an array of nonspecific sensors. The response of the electronic nose to a specific flavor is to create a sort of flavor fingerprint. In contrast, chromatographic methods separate the volatile compounds responsible for a characteristic flavor, and then individually identify them using various detectors and the human nose.

In order to achieve the sensitivity required for analyzing food odorants, appropriate sample preparation and preconcentration steps are needed. As the food flavors perceived by the human nose are volatile compounds, methods based on distillation, solvent extraction, and headspace analysis are used. Headspace methods, i.e., those methods measuring the components in the gas present in the space above the sample, comprise static headspace, dynamic headspace (purge and trap), and solid-phase microextraction (SPME). Dynamic headspace and SPME methods provide lower detection limits as a result of their preconcentration and enrichment steps. These are the milestones in solventless extraction technology that have influenced most volatile compound analysis. Other sample preparation methods, such as solid-phase extraction (SPE), liquid–liquid extraction (LLE), and simultaneous distillation extraction (SDE) are used for flavor compound isolation and their use is determined by the type of compound to be isolated. SDE is the method used for analysis of many food products due to its versatility and ease of use. Vacuum distillation is a method providing convenient data for further analysis, as it allows low-temperature separation of flavor compounds from different matrices and does not affect the aroma of the extract of thermally labile compounds. Solvent-assisted flavor evaporation (SAFE) is a method easy to perform that uses vacuum to isolate flavor compounds from the matrix, being particularly useful for gas chromatography-olfactometry (Engel et al., 1999).

Developments in the analysis of flavor compounds have been necessarily associated with developments in gas chromatography (GC) and the combination of gas chromatography with mass spectrometry (GC-MS). In particular, the invention of capillary columns and their routine use has increased, generally, the number of food flavor-identified compounds. The next advance, after the introduction of the capillary columns, was the development of multidimensional chromatography. This technique allows the transfer of a particular fraction from one column into another, with the new column performing a different mechanism of separation and equipped with another detector. The development of comprehensive GC, especially in connection with high-speed time of flight mass spectrometers, has opened new horizons in compound separation and identification. This technique allows resolving of the most complicated mixtures based on simultaneous separation by orthogonal separation mechanisms. Mass spectrometers have become routine detectors for the identification of flavor compounds with abundant and relatively reliable information on compound identity. The use of the nose as detector in chromatography has become a basic tool for the sensory-oriented flavor analysis, particularly after the invention of quantitative methods such as the aroma extract dilution analysis (AEDA) or the charm method (Grosch, 1993).

The determination of key odorants responsible for the aroma of a particular food uses all the techniques described above to isolate, separate, and identify key odorants. Gas chromatography-olfactometry is considered particularly important as it allows the sensory identification of main volatile fractions. AEDA technique calculates a dilution factor for each compound as a last dilution in which the compounds can still be sensed and an "arogram" can be produced (Majcher and Jeleñ, 2005). The higher the dilution factor, the higher the contribution of that compound to the flavor of the food product. In order to determine the importance of a particular compound on the overall profile of a food product, the ratios of the compound concentration to its odor threshold (odor-aroma values) are often used. Flavor reconstitution experiments allow researchers to check out the results obtained using gas chromatography-olfactometry (Sollner and Schieberle, 2009). The techniques described are used for determination of key aroma compounds, but can also be used in the detection of compounds responsible for product taint or off-odor compounds.

The analysis of food aroma and volatile compounds can be used as a tool for monitoring chemical, enzymic, and microbial changes due to processing of different foods, such as beverages. Selected compounds can be used as indicators of undesirable effects, or a fingerprint of some volatiles can provide information on the degree of change in certain food products. To achieve these purposes, GC data peaks can be treated by multivariate statistical methods and sample results are normally presented in the form of polycomponent analysis graphs. Another approach makes use of the electronic nose principle of using a fingerprint of total volatile compounds forming in the headspace and subjected to statistical evaluation. In this field of research, a combination of SPME with mass spectrometry working as an electronic nose has shown promising results. This approach has been used for food authenticity testing, detection of microbial spoilage, and detection of chemical changes during processing and storage (Jeleñ et al., 2007).

CHANGES ON FOOD COMPOSITION INFLUENCED BY NONTHERMAL TECHNOLOGIES

Irradiation

The use of ionizing radiation for the preservation of food has been extensively studied for over 100 years, making it one of the most thoroughly researched means by which food can be treated in order to make it safer to eat and last longer (Diehl, 1995). The two main drivers for the use of food irradiation are the enhancement of food safety and of trade in agricultural products (Borsa, 2004). The process should, however, not be used as a substitute for good manufacturing practices but rather as a means of reducing risk. Although food irradiation is considered an authentic nonthermal process, and so it is supposed to keep quality attributes of processed foods, it has been often associated with development of off-flavors, as well as with softening and increasing permeability of tissue of some fresh fruits and vegetables (Stewart, 2009). Radiation can affect all the major components of food, i.e., carbohydrates, proteins, and lipids (Stewart, 2001). It should, however, be noted that even at the high doses used for sterilization, the changes are small and similar to those produced by other food processing technologies such as pasteurization. As for any other food processing method, irradiation has advantages and disadvantages. Some foods are sensitive to ionizing radiation and too high a dose may cause organoleptic changes, rendering the food unacceptable to the consumer. Therefore, the actual dose employed is a balance between what is needed and what can be tolerated by the product without unwanted changes (Farkas, 2006). Considerable amount of research has been performed about effects of ionizing radiation in sensory, nutritive, and functional properties of food drinks and beverages.

Wang et al. (2006) treated freshly squeezed cantaloupe juice with ^{60}Co irradiation at 1 and 2 kGy intensity. Enzyme activity and microorganism survival were measured and analyzed, and the irradiation off-odor intensity was sensory evaluated. The juice was found to show slight irradiation off-odor after treatment at 1 kGy, and strong off-odor at 2 kGy and above. Enzyme activity determination indicated that lipoxygenase (LOX) was the easiest one to be inactivated by irradiation, followed by polyphenoloxidase (PPO) and peroxidase (POD), but the three enzymes still remained active even at 5 kGy. Microorganism survival determination indicated that Escherichia coli was sensitive to irradiation, and was reduced by seven log-cycles at 1 kGy, whereas total colony and target spore bacteria in the juice had stronger endurance to the irradiation. The study indicated that gamma irradiation cannot completely inactivate the enzymes, total colony, and target spore bacteria in cantaloupe juice on the premise of acceptable off-odor.

It has been discussed that furan, a colorless chemical with potential carcinogenic effects to humans, may be released by ionizing radiation in orange and apple juices (Vranová and Ciesarová, 2009). Radiation doses up to 5 kGy may increase linearly furan levels possibly due to the residual effect of irradiation that generates primary radicals from radiolysis of water. These primary radicals can react with food components to form secondary radicals. Some stable radicals and reactive compounds might be present for days and may continue to induce formation of furan.

Pomegranate juice contains considerable amount of anthocyanins, so it is considered a functional product for health and dieting. The effects of gamma irradiation (0–10 kGy) on the stability of anthocyanins and inhibition of microbial growth in pomegranate juice during storage were investigated (Alighourchi et al., 2008). At higher doses of 3.5 to 10 kGy, an undesirable effect on the total content of anthocyanins was observed. Irradiation doses up to 2 kGy effectively diminished the total bacteria and fungi counts, retarded microbial growth during storage, and affected only slightly anthocyanins content.

Garcia de Oliveira et al. (2007) evaluated physicochemical, microbiological, and sensory stability of pure sugarcane juice and sugarcane juice mixed with fresh lemon and pineapple juice, subjected to a heat treatment (70°C for 25 min) and/or gamma radiation (2.5 kGy). They found out that processing of the sugarcane juice reduced the microorganism load without significantly altering the physicochemical composition, aroma, and flavor of the beverages in comparison with the control. Luminosity was higher in the product subjected to the heat treatment combined with gamma radiation than that resulting from the other treatments. The PPO activity in the processed beverages was significantly lower than in the control. The addition of fruit juice to the sugarcane juice did not modify the latter's physicochemical composition. However, the addition of 10% pineapple juice to the sugarcane juice increased the manganese and reduced sugars content when compared with pure sugarcane juice and with sugarcane juice mixed with 4% lemon juice.

Acyclic monoterpenes have found to be unstable in orange juice treated by radiation (Fan and Gates, 2001). Single-strength orange juice was irradiated at 0, 0.89, 2.24, 4.23, and 8.71 lGy of gamma irradiation at 5°C and then stored at 7°C for 21 days. Volatile compounds, isolated by SPME, were separated and identified using GC and a mass selector detector. Most of the volatiles present were terpenes, with ethanol and limonene showing high concentrations, and were practically stable during the 21-day storage period. Irradiation reduced the concentration of acyclic monoterpenes even for 1- and 7-day storage, following a linear decrease pattern with irradiation dose and correlated with an increase in thiobarbituric acid reactive substrates (TBARS).

Fan et al. (2004) reviewed effect of low-dose ionizing radiation on fruit juices, finding that irradiation at low doses effectively inactivated foodborne pathogens and reduced the levels of the mycotoxin patulin and of brownness. Irradiation induced, however, undesirable chemical changes such as the accumulation of malondialdehyde, formaldehyde, and tetrahydrofuran. Reports on the negative flavor changes of irradiated juice are contradictory in the literature, although there is some evidence concerning the involvement of volatile sulfur compounds in the development of off-flavor. It has been suggested that many of the undesirable effects of irradiation could be reduced by using low temperatures during treatment, by the addition of antioxidants to the product, and by combining irradiation with other techniques and treatments such as mild heating and the use of antimicrobials.

Song et al. (2007) carried out radiation pasteurization of fresh vegetable juice (carrot and kale) in order to improve the microbiological quality. All aerobic and coliform bacteria in the carrot juice were eliminated by a dose of 3 kGy, which was not the case for the kale juice, although it was noted that the cells that survived in the kale juice did not grow, whereas those of the nonirradiated juices increased upon storage. Amino acids in the juices were stable up to 5 kGy and there was a dose-dependent reduction in ascorbic acid content. It was observed, however, that total ascorbic acid (including dehydroascorbic acid) was stable up to a dose of 3 kGy. It was also found that at 3 days of storage, the sensory evaluation results of the irradiated juices were adequate, while the quality of the nonirradiated control had deteriorated.

Some studies have shown that ionizing radiation can cause the development of an off-odor, and volatile sulfur compounds have been suggested to be a possible cause of the off-odor. However, there is no clear evidence connecting sulfur compounds with the off-odor development. Fan (2005) reported on the contribution of the sulfur compounds to the sensory properties of irradiated orange juice. Freshly squeezed orange juice was irradiated using doses of 0, 0.5, 1.0, 2.0 and 3.0 kGy at 5°C. The juices were analyzed by GC-pulsed flame photometric detection and sensory evaluation. Results showed that methyl sulfide (MS) and methanethiol (MT) were induced most, followed by dimethyl disulfide and dimethyl trisulfide. Carbon disulfide was reduced by irradiation, while hydrogen sulfide was not consistently affected. Sensory evaluation indicated that the odor of irradiated juice differed from the nonirradiated samples at 0.5, 1, 2, and 3 kGy. Addition of the two major irradiation-induced sulfur compounds (MS and MT) into fresh juice made the juice smell different. The results suggested that volatile sulfur compounds were involved in the development of irradiation-induced off-odor.

Ultraviolet (UV) Energy Treatment

UV light in the food industry is used for disinfection, e.g., water or air, but irradiation of food with high-energy ionizing UV radiation is a used physical treatment of food. It can be used to prolong the shelf-life of food products and/or to reduce health hazards associated with certain products due to the presence of pathogenic microorganisms. UV is rapidly gaining acceptance across the whole spectrum of food and beverage industries as a highly efficient, nonchemical method of disinfection. UV kills all known pathogenic and food spoilage microorganisms, including bacteria, viruses, yeasts, and molds (and their spores). It is a low-maintenance, environmentally friendly technology that eliminates the need for chemical treatment while ensuring very high levels of disinfection. UV disinfection has many advantages over alternative methods. Unlike chemical biocides, UV does not introduce toxins or residues into the process and does not alter the chemical composition, taste, odor, or pH of the product. As UV has no residual effect, the best position for a treatment system is immediately prior to the point of use. This ensures incoming microbiological contaminants are destroyed and there is a minimal chance of post-treatment contamination. Pumpable fruit and vegetable products are generally very suitable for processing by UV light to reduce the microbial load. For example, UV radiation has been approved by the Food and Drug Administration (FDA) in the United States since 2000 (Lopez-Malo and Palou, 2005) for its use in treatment of juice products. UV light irradiation can be considered as a potential alternative to traditional thermal pasteurization for liquid foods and ingredients, fresh juices, soft drinks, and beverages, but its expanded use is still limited due to low UV transmittance of liquid foods (Koutchma, 2009). A number of studies are available in the literature, reporting on safety and quality effects of UV-treated food drinks and beverages.

Iwanami et al. (1997) studied effects of UV processing on a lemon-flavored drink prepared from lemon oil, water, and ethanol. Citral, which is one of the most important components responsible for the lemon-like odor, decreased significantly by a reaction of isomerization and new compounds of aldehyde were identified as photoreaction products of citral. Limonene, therpinolene, and nonanal decreased, while p-cymene increased after UV treatment. Their results suggested that citral is a more UV-unstable component in lemon flavor and that its photolysis could affect other components of lemon flavor during UV exposure.

Matak et al. (2007) reported on sensory properties of goat's milk treated with UV technology. Olfactory studies were conducted and a highly significant difference was determined between the odor of fresh goat's milk and that of UV processed milk. The extent of lipid oxidation and hydrolytic rancidity was measured by TBARS and acid degree values (ADVs). Results indicated that as the UV dose increased, there was a significant increase in TBARS values and ADVs of the milk samples. Milk samples were processed using the UV processor under the same conditions as previously described without exposure to the UV source to determine if the agitation from pumping was causing off-flavors by way of hydrolytic rancidity. The ADVs from these samples increased at the same rate as the UV-irradiated samples. However, sensory studies indicated that the increase in free fatty acids (FFA) was not enough to cause detectable off-odors in the milk. SPME-GC was used to quantify the production of volatile compounds that were formed due to UV processing. The formation of pentanal, hexanal, and heptanal was identified after as little as 1.3 mJ/cm^2 UV dose. Peak areas were measured and analyzed after exposure at 7.8 mJ/cm^2 and 15.6 mJ/cm^2, and were determined to increase significantly as the UV dose increased. The chemical analyses supported the findings from the olfactory studies. The outcome of this research showed that UV irradiation at the wavelength 254 nm was detrimental to certain chemical properties of fluid milk.

Noci et al. (2008) combined UV irradiation with PEF in treatment of apple juice and studied effects on microbial inactivation, quality attributes, enzymic activity, and functional properties. Apple juice samples thermally processed at 72°C and 94°C for 26 sec were used as control. Juice color and level of phenolic compounds were less affected by the alternative treatments than by heat pasteurization. Reduction in enzyme activity was greater in combined treatments than in thermal ones. The investigation showed the potential of a combination of UV irradiation and PEF to obtain satisfactory total microbial inactivation and improved product quality compared with heat pasteurization.

Valappil et al. (2009) investigated aroma composition and microbial quality of apple cider treated by UV irradiation and other treatments during storage at 4°C for 4 weeks. Main volatiles were quantified using GC-MS and odor activity values (OAD) were determined afterward. UV samples fermented after 2 weeks and lost completely two valuable volatiles, hexanal and 2-(E)-hexenal, after 4 weeks. Microbial spoilage of the UV-treated cider was also confirmed after the 4-week period.

Apple juices from different varieties were treated by UV irradiation and the effects on some quality attributes and enzymic activities were investigated (Falguera et al., 2011). The treatment was effective in the inactivation of the assayed enzymes. No variations were observed in pH, soluble solids content, formol index, total phenolics, and sugars. The color of the juice produces from King David apple variety was unchanged, while for the juices obtained from Golden, Starking, and Fuji apple varieties, UV radiation impaired their natural color. The content of vitamin C in juices from Golden, Starking, and Fuji apple varieties slightly changed, whereas in the juice from King David apple variety, the loss was significant.

Ultrasound

Application of sound waves with frequencies above 20 kHz may induce cavitation in liquids that can lead to localized high temperature and pressure bursts, improved mass transport, and induction and acceleration of radical-driven reactions. These physical and chemical effects provide platforms for potential applications of ultrasound in food processing operations ranging from microbial inactivation and modification of texture, color, taste, and odor to enhancement in the functionality of food ingredients. Nowadays, power ultrasound is considered to be an emerging and promising technology for industrial food processing. Two possible applications of ultrasound are, first, the use of sound as a diagnostic tool, e.g., in nondestructive evaluation, and the second is the use of sound as a source of energy, e.g., in sonochemistry. These applications involve different frequency ranges of ultrasound and the uses of both ranges in the food industry are an active subject for research and development. The potential use of this novel technology to produce permanent changes in food materials in liquid systems is, as previously mentioned, through the generation of intense cavitation. This can lead to the inactivation of microorganisms and enzymes for food preservation or decontamination by ultrasonic irradiation demonstrates the benefits of ultrasound as a food preservation tool. In addition, there are an increasing number of industrial processes that employ power ultrasound as a processing aid, including the mixing materials, foam formation or destruction, agglomeration and precipitation of airborne powders; the improvement in efficiency of filtration, drying, and extraction techniques in solid materials; and the enhanced extraction of valuable compounds from vegetables and food products. Like any other nonthermal or alternative food preservation technology, ultrasound presents pros and cons, and some research on this aspect has been reported in the literature.

Xia et al. (2006) studied the effects of ultrasound-assisted extraction on the chemical and sensory quality of tea infusion.

Ultrasound-assisted extraction showed a better effect on the extraction efficiency of the main chemical components from tea at lower temperatures. The contents of tea polyphenols, amino acid, and caffeine in tea infusions were more than those obtained by conventional extraction. Moreover, ultrasound-assisted extraction inhibited the extraction of protein and pectin. The results also showed that ultrasound-assisted extraction could increase the extraction yields of aroma components and glycosidic aroma precursors as well as changing the Owuor index of tea infusions. Organoleptic evaluation indicated that the sensory quality of tea infusion with ultrasound-assisted extraction was better than that of tea infusion with conventional extraction.

In order to investigate the effect of different extracting methods on the quality of tea infusion for ready-to-drink tea production, Sun et al. (2005) experimented with high-temperature extraction, low-temperature extraction, and ultrasound extraction. The results show that the high-temperature extraction resulted in high extraction yield and low lightness of tea infusion. The ultrasound and low-temperature extraction gave high tea infusion lightness and the extraction yield by ultrasound extraction was higher than that by low temperature extraction. Thus, ultrasound extraction was considered to be beneficial for production of ready-to-drink tea.

The use of acoustic energy to secure apple cider safety was explored by Ugarte-Romero et al. (2006). Titratable acidity, pH, and °Brix of the ultrasound-treated cider were not affected. Minor changes in color and turbidity of samples sonicated at 40°C for 17.7 min were observed. The results of the study proved that ultrasound processing of apple cider can render a pasteurized product and should be considered a suitable alternative for commercial exploitation.

The effect of ultrasonic treatments on inactivation of *Alicyclobacillus acidoterrestris* in apple juice was investigated by Yuan et al. (2009). Effects on some quality attributes were also verified. Microbial inactivation was more pronounced at an elevated power level and as the processing time increased. Approximately 60% of the cells were inactivated after treating the apple juice with 300-W ultrasound for 30 min. The reduction reached more than 80% when the juice was processed for 60 min. Changes in sugar content, acidity, haze, and juice browning were noted after ultrasonic treatments but did not adversely alter the juice quality.

Tiwari et al. (2008) reviewed the effects of ultrasound processing on some quality and nutritional properties of fruit juices. They reported that such processing presented minimal effects on key characteristics of the juices such as color and ascorbic acid content. Gómez-López et al. (2010) investigated the influence of sonication on microbiological control and quality attributes of calcium-enriched orange juice. Ultrasonic treatments were applied at a frequency of 20 kHz and three wave amplitudes for 2, 4, 6, 8, and 10 min. Sonication affected color minimally and decreased ascorbic acid content slightly. The study, therefore, showed that ultrasonication may be useful to extend the shelf-life of orange juice.

Ultrahigh Hydrostatic Pressure

HPP, a relatively new technology to the food industry (Torres and Velazquez, 2008), inactivates microorganisms without causing significant flavor and nutritional changes to foods (Berlin et al., 1999). HPP is a method of food processing where food is subjected to elevated pressures (up to about 600 MPa), with or without the addition of heat, to achieve microbial inactivation or to alter the food attributes in order to achieve consumer-desired qualities. Pressure inactivates most vegetative bacteria at pressures above 400 MPa. HPP retains food quality, maintains natural freshness, and extends microbiological shelf-life. HPP causes minimal changes in the freshness characteristics of foods by eliminating thermal degradation. As compared with thermal processing, HPP produces foods with better appearance, texture, and nutrition. HPP can be conducted at ambient or refrigerated temperatures, thereby eliminating thermally induced cooked off-flavors. This technology is especially beneficial for heat-sensitive products.

Most processed foods today are heat treated to kill bacteria, which often diminishes product quality. HPP provides an alternative means of killing bacteria that can cause spoilage or food-borne disease without the loss of sensory quality or nutrients. In a typical HPP process, the product is packaged in a flexible container (usually a pouch or plastic bottle) and is loaded into a high-pressure chamber filled with a pressure-transmitting (hydraulic) fluid. The hydraulic fluid (normally water) in the chamber is pressurized with a pump, and this pressure is transmitted through the package into the food itself. Pressure is applied for a specific time, usually 3 to 5 min. A schematic diagram of a unit with direct pressure generation is shown in Fig. 3. The processed product is then removed and stored/distributed

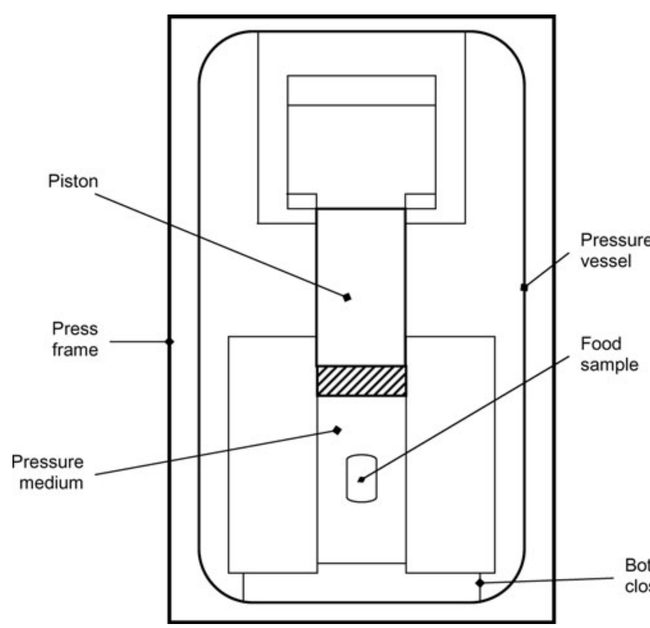

Figure 3 Principle of ultrahigh hydrostatic pressure equipment: diagram of direct pressure generation unit.

in the conventional manner. Because the pressure is transmitted uniformly (in all directions simultaneously), food retains its shape, even at extreme pressures. Since no heat is needed, the sensory characteristics of the food are retained without compromising microbial safety. Like any other processing method, HPP cannot be universally applied to all types of foods. HPP can be used to process both liquid and solid foods. Foods with a high acid content are particularly good candidates for HPP technology. HPP has being used in the United States, Europe, and Japan on a select variety of high-value foods either to extend shelf-life or to improve food safety. Some products that are commercially produced using HPP are cooked ready-to-eat meats, avocado products (guacamole), tomato salsa, applesauce, and orange juice. HPP can be considered an example of successful technology transfer from academia to industry, whose commercialization came into fruition in a relatively short time. There are, therefore, numerous reports in the literature on fundamental research, applied research, prospective research, and so on.

Oey et al. (2008) presented a review on the subject of treating fruit and vegetable food products, emphasizing about effects on sensory attributes. Their discussion focuses specifically on the effects of HPP treatment on color, flavor, and texture and tries to elucidate the mechanisms behind the observed changes in quality attributes. Possible impacts of HPP treatments at elevated temperatures on these sensory properties are also highlighted since the temperature regime used for research on high pressure has been extended to elevated temperatures in order to achieve spore inactivation, i.e., HPP sterilization.

An available review, by Norton and Sun (2008), gives an account of the different aspects associated with applying HPP as a processing method in the food industry, including operating principles, effects on food quality and safety, and most recent commercial and research applications. Among other features, they point out that the key advantages of HPP applications to food systems are the independence of size and geometry of the sample during processing, possibilities for low-temperature treatment and the availability of a waste-free environmentally friendly technology.

Heinz and Buckow (2010) discussed theory, development in equipment, and European legislation on HPP. According to them, juices and beverages represent 17% of the utilization of HPP technology in different segments of the food industry of a total annual volume of more than 200,000 tons of food products. They also point out that, among the different novel food preservation technologies, HPP has the potential to be used in the development of a whole new generation of value-added food products, including juices and beverages.

Black et al. (2007) focused a review on spores inactivation by HPP in different food products. Apart form spores of *Bacillus* species, spores of *Clostridium* and *Alicyclobacillus* species were also discussed, and a section of the review surveyed the responses of fungal spores to high-pressure processing. The mechanisms of the germination of bacterial spores were outlined in detail with regard to spore physiology and structure, along with molecular aspects of germinants and the interaction with spore receptors. They discussed pioneering studies by Hite (1899), in which, by using pressures ranging from 400 to 700 MPa at room temperature, a 4-log reduction in microbial counts was achieved, while maintaining product freshness. Hite had some success in creating shelf-stable HPP-treated fruit products with a pH that prevented growth of spores, but he never could achieve shelf-stability of HPP-treated milk due to its neutral pH and the presence of spores.

Pressure-assisted thermal sterilization (PATS) has been also discussed by Black et al. (2007) and other workers (Rovere et al., 1998; Rodriguez et al., 2004). PATS is a combined process in which both pressure and temperature contribute to the inactivation of spores and enzymes. Pressure alone has a remarkable ability to inactivate vegetative forms of bacteria; however, sole use of high pressure has been found to have little effect on bacterial spores and various enzymes. Since spores are a great concern to the food industry, specifically in regard to *Bacillus* species and *Clostridium* species (especially *C. botulinum*), it may represent a suitable option. In the production of low-acid and shelf-stable foods, microbiological safety is the single most important prerequisite, with spores of *C. botulinum* being the critical target for elimination. By combining heat treatment of the product with application of high pressures, inactivation of spores and enzymes can be achieved. Spore lethality from PATS can therefore be considered a combination of protein denaturation and enzyme inactivation by heat with the aggregation of proteins under pressure.

Valdramidis et al. (2009) reported on the design and optimization of an HPP process for apple juice treatment. An experimental approach was applied to determine the impact of HPP processing (350–550 MPa at 20°C and for 1–25 min of holding time) on the survival of *Issatchenkia orientalis* and the spoilage of apple juice (with 300 ppm added ascorbic acid) during different storage conditions, i.e., 4–12°C and 0–36 days of storage. Probabilistic modeling approaches based on logistic regression models were developed in order to describe quantitatively the spoilage/no spoilage and survival/death interfaces. For a microbially stable processed apple juice treated at 400 MPa, 10°C and a holding time of 15 min, the degradation kinetics of vitamin C were described quantitatively during subsequent storage at 4°C, 8°C, and 12°C. The rates of vitamin losses were highly reduced after the first 13 days of storage. The stability of the apple juice with respect to browning and cloudiness was evaluated by studying qualitatively the activity of PPO and pectin methyl esterase (PME) enzymes at combined treatments of HPP and temperature (10–50°C, HPP at 750 MPa, and holding time from 1 to 25 min). The highest achieved reduction in PPO and PME was 51.47% and 81.44%, respectively.

Ma et al. (2010) investigated the change in flavor, enzyme activities, and microorganism survival in cantaloupe juice after HPP treatments, and found the corresponding kinetic models. After the treatment at 500 MPa for 20 min, the pressurized juice attained the microbial safety standards stipulated for beverage by Chinese national standard, while some enzyme activities were significantly reduced. Sensory evaluation indicated that no significant change was observed after HPP treatments. The above findings indicated that HPP treatment is a promising way to process the cantaloupe juice.

Navel orange juices were subjected to HPP and temperature treatment and stored at 4 and 10°C for up to 12 weeks to establish the shelf-life of such products (Baxter et al., 2005). The processed juices and a control juice, stored at −20°C, were assessed by a trained sensory panel and a consumer acceptance panel at 0, 1, 2, 4, 8, and 12 weeks or until such time that the juices were considered unfit for consumption. Untreated juice stored at 4°C was similarly assessed for up to 2 weeks and untreated juice stored at 10°C was assessed for up to 1 week. The volatile components of corresponding juices were isolated by SPME and the extracts were analyzed by GC-MS. Twenty key aroma compounds were selected for quantification and these data were used to monitor the change in the volatile content of the juices during storage. The study showed that the odor and flavor of the HPP juice were acceptable to consumers after storage for 12 weeks at temperatures up to 10°C.

Vazquez-Landaverde et al. (2006) studied the effects of HPP on volatile content generation in milk. Raw milk samples were treated under different pressures (482, 586, and 620 MPa), temperatures (25°C and 60°C), and holding times (1, 3, and 5 min). Samples submitted to heat treatments alone (25°C, 60°C, and 80°C for 1, 3, and 5 min, respectively) were used for comparison. Trace volatile sulfur compounds were analyzed by SPME and GC with pulsed-flame photometric detection (PFPD), whereas the rest of the volatile compounds were analyzed using SPME-GC with flame ionization detection (FID). Multivariate analysis of variance (MANOVA) and principal component analysis (PCA) were used to study the effect of pressure, temperature, and time on volatile content generation. Relative concentration increases of 27 selected volatile compounds were compared with an untreated sample. It was found that pressure, temperature, and time, as well as their interactions, all had significant effects on volatile content generation in milk. Pressure and time effects were significant at 60°C, whereas their effects were almost negligible at 25°C. The PCA plot indicated that the volatile content generation of pressure-heated samples at 60°C was different from that of heated-alone samples. Heat treatment tended to promote the formation of methanethiol, hydrogen sulfide, methyl ketones, and aldehydes, whereas high-pressure treatment favored the formation of hydrogen sulfide and aldehydes.

The flavor characteristics of fresh and processed pennywort juices treated by pasteurization, sterilization, and HPP were investigated by Wongfhun et al. (2010) by using SPME combined with GC-MS. Sesquiterpene hydrocarbons comprise the major class of volatile components present and the juices had a characteristic smell due to the presence of volatile compounds, including β-caryophyllene, humulene, E-β-farnesene, α-copaene, alloaromadendrene, and β-elemene. All processing operations caused a reduction in the total volatile concentration, but HPP caused more volatile acyclic alcohols, aldehydes, and oxygenated monoterpenoids to be retained than pasteurization and sterilization. Ketones were not present in fresh pennywort juice, but 2-butanone and 3-nonen-2-one were generated in all processed juices, and 2-nonanone and 2-hexanone were present in pasteurized and sterilized juices. Other chemical changes, including isomerization, were also reduced by HPP as compared with pasteurization and sterilization.

Li et al. (2004) reported on the effect of HPP treatment on the sensory properties of mango juice, with special focus on the changes in volatile components. Mango juice was prepared from fresh mango fruit and sealed in plastic pouches. Samples were pressure-treated at 100–400 MPa for 10–40 min at room temperature or heat-treated at 80°C for 30 sec. All samples were analyzed for their color, pH, vitamin C, viscosity, and volatile aroma components. Pressurized and untreated samples gave similar color values, which were brighter than heated samples. Vitamin C loss of heated samples (29.1%) was greater than that of pressurized samples (2.1–8.3%). The pH values of all samples did not change over the course of study. There were small increases in viscosity of pressurized samples. The most abundant volatile compound, α-terpinolene, experienced some losses for the 400 MPa/30 min-pressurized sample (25.5%) and the heated sample (50.9%) when comparing with the untreated sample. Both the pressurized and the heated sample had more myristic acid than the untreated sample. The 400 MPa/30 min-pressurized and the heated sample gave a shelf-life greater than 16 weeks at 4°C. HPP pasteurization produced a high-quality mango juice that retained its freshness and nutritional values.

High-Voltage Pulsed Electric Fields

PEF processing involves the application of an externally generated electric field across a food product with the intent of inactivating pathogenic microorganisms, modify enzymes, intensifying some processes, or achieving some specific transformation in the product. The technology has long been used for cell hybridization and electrofusion in genetic engineering and biotechnology. Its application is based on the transformation or rupture of cells under a sufficiently high external electric field, resulting in increased permeability and electrical conductivity of the cellular material. This effect, named dielectric breakdown (Zimmermann et al., 1976) or electroplasmolysis (McLellan et al., 1991), can be explained by two main factors: (1) electroporation, i.e., electroinduced formation and growth of pores in biomembranes as a result of their polarization, and (2) denaturation of cell membranes as a result of their ohmic heating caused by the electric resistance of membranes, which is typically much higher than that of cell sap content. Besides these, the physiological impact and electro-osmotic effect may also influence the electroplasmolysis efficiency (Weaver and Chizmadzhev, 1996).

PEF technologies have been demonstrated to be a viable alternative to high-temperature inactivation of microbial load in liquid foods such as fruit juices and milk (Knorr et al., 1994; Barbosa-Canova et al., 1999). The majority of research effort on PEF has been on liquid food pasteurization. PEF processing involves a short burst of high-voltage application to a food placed between two electrodes. When high electric voltage is applied, a large flux of electric current flows through food materials, which may act as electrical conductors due to the presence of electrical charge carriers, such as large concentration of ions (Barbosa-Cánovas et al., 1999). In general, a PEF system consists of a high-voltage power source, an energy storage capacitor bank, a charging current limiting resistor, a switch to discharge energy from the capacitor across the food, and a treatment chamber. The bank of capacitors is charged by a direct current power source obtained from amplified and rectified regular alternative current main source. An electrical switch is used to discharge energy (instantaneously in millionth of a second) stored in the capacitor storage bank across the food held in the treatment chamber. Apart from these major components, some adjunct parts are also necessary. In the case of continuous systems, a pump is used to convey the food through the treatment chamber. A chamber cooling system may be used to diminish the ohmic heating effect and control food temperature during treatment. High-voltage and high-current probes are used to measure the voltage and current delivered to the chamber (Barbosa-Cánovas et al., 1999; Floury et al., 2005; Amiali et al., 2006). Fig. 4 shows a basic PEF treatment unit, while Fig. 5 presents different chamber designs. Researchers on PEF have been actively involved in a range of activities, and there have been diverse reports on safety and quality issues pertaining processing of food drinks and beverages.

PEF treatment may influence physical and chemical properties of products. The nature and extent of PEF influence on quality changes are still being actively discussed. Barsotti et al. (2002) indicated that PEF treatment of model emulsions and liquid dairy cream may result in dispersal of oil droplets and dissociation of fat globule aggregates. Dunn (1996) reported that milk treated with PEF (E = 20–80 kV/cm) suffered less flavor degradation. The author proposed the possibility of manufacturing dairy products such as cheese, butter, and icecream using PEF-treated milk, although no detailed information was given in his report. Qin et al. (1995) carried out a study of shelf-life, physicochemical properties, and sensory attributes of milk with 2% milk fat, treated with 40 kV/cm of electric field and 6–7 pulses. No physicochemical or sensory changes were observed after treatment, in comparison with a sample treated with thermal pasteurization.

The flavor of orange is due to more than 200 chemical compounds (Maarse, 1991), and is composed of hydrocarbons, aldehydes, esters, ketones, and alcohols. Limonene is the most important flavor compound in quantity, although not in quality (Siezer et al., 1988). It has been reported (Ahmed et al., 1978) that acetaldehyde, citral, ethyl butyrate, limonene, linalool, octanal, and α-pinene are the major contributors to orange juice flavor. The development of off-flavors in orange juice has been attributed to the degradation of limonene to α-terpineol and other compounds (Tatum et al., 1975). Effects of PEF processing on specific flavor compounds of orange juice have been

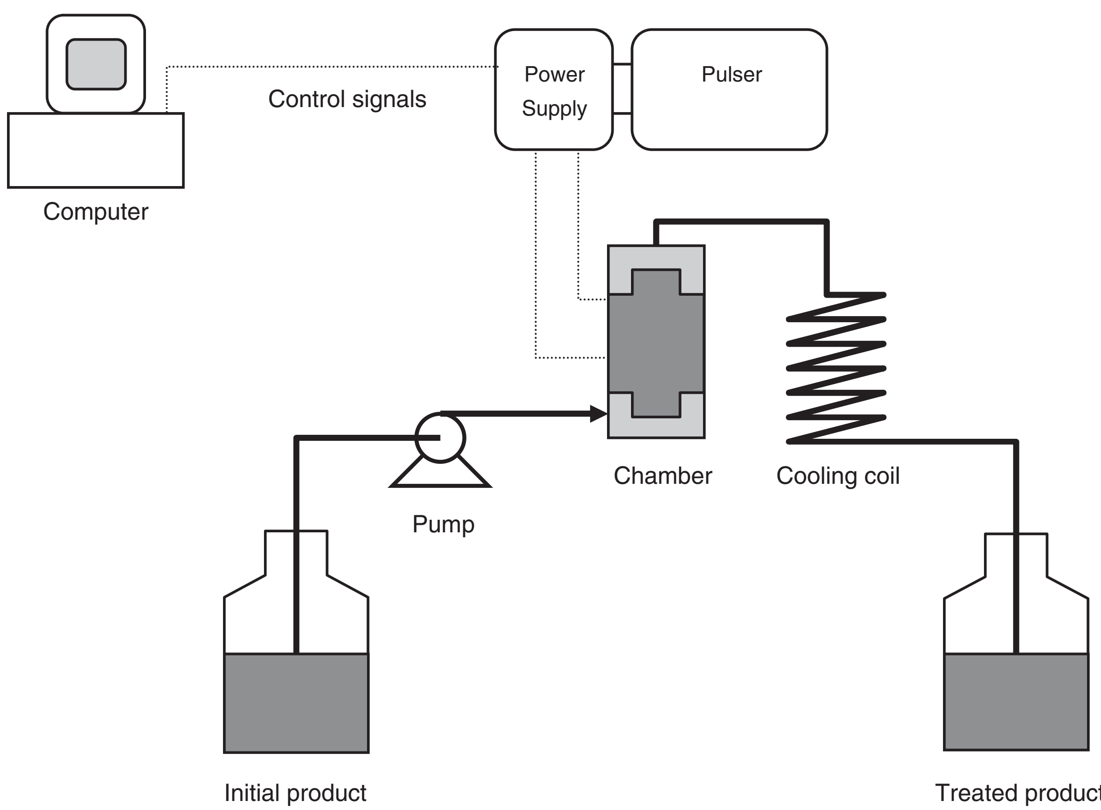

Figure 4 Schematic diagram of a pulsed electric field operation.

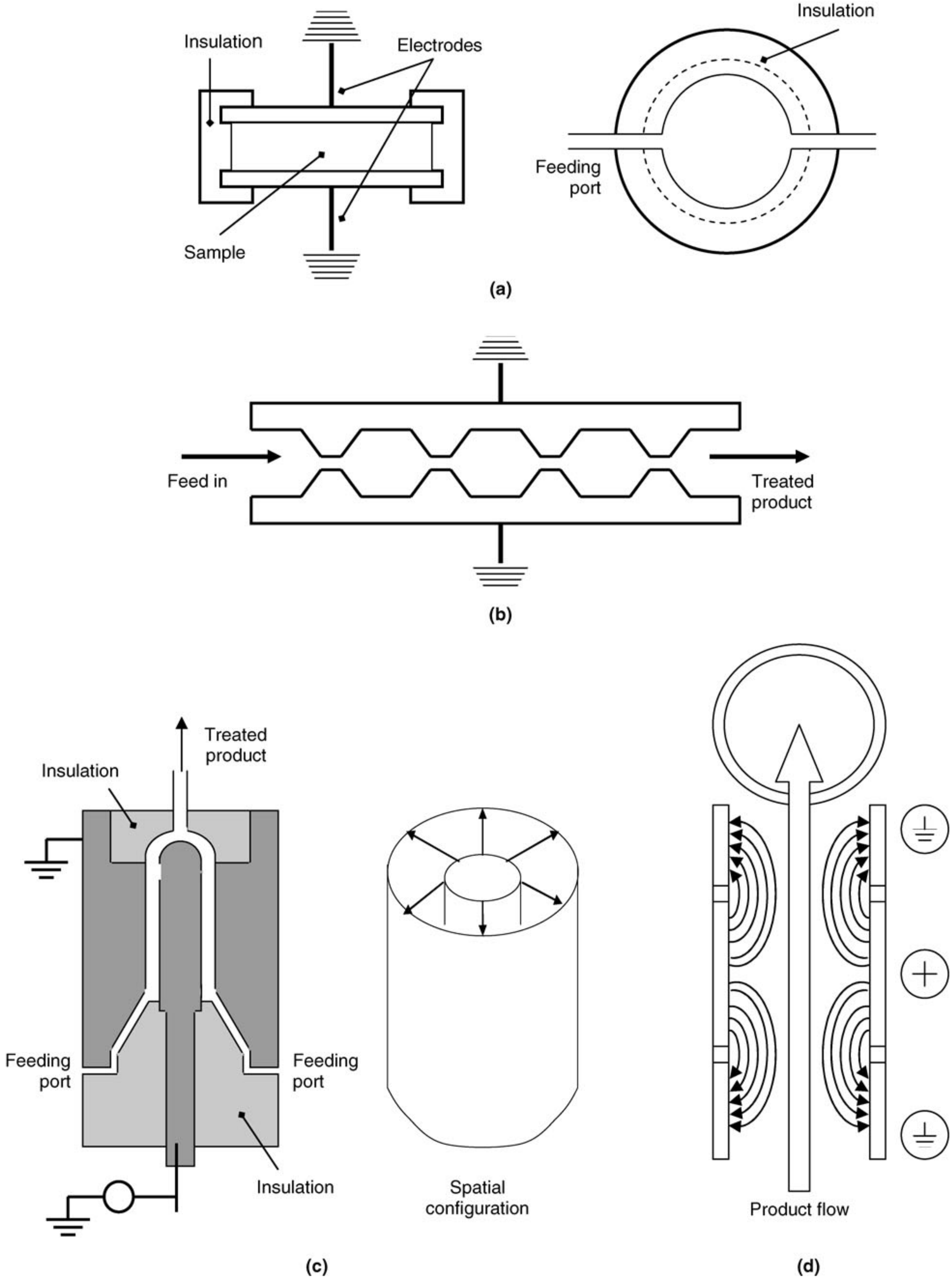

Figure 5 Designs of treatment chambers for pulsed electric fields equipment: (a) static chamber, (b) side view of a basic continuous design, (c) coaxial chamber, (d) colinear chamber.

studied (Jia et al., 1999, Yeom et al., 2000). It was found that 40% of decanal was lost by heat treatment at 90°C for 3 min, while no loss was observed by PEF treatment at 30 kV/cm, either at 240 or 480 μsec. Octanal showed a loss of 9.9% for the heat treatment and 0% for any of the two PEF treatments. Some compounds suffered losses for the PEF treatments, but always in less proportion than the heat-pasteurized juice. For example, 5.1% and 9.7% of ethyl butyrate were lost for the 240-μsec and 480-μsec treatments, respectively, but 22.4% was lost in the thermal process. The loss of these volatile compounds in orange juice treated by PEF was attributed to the vacuum degassing system of the PEF unit (Jia et al., 1999). The advantage of PEF compared with thermal processing was also observed in nutritive aspects. PEF-treated orange juice retained a significantly higher content of ascorbic acid than heat-pasteurized juice during storage at 4°C (Yeom et al., 2000). Although more research needs to be completed before considering PEF as the sole treatment to retain completely all flavor and color components of orange juice, it can be stated that PEF-pasteurized juice retains more flavor and shows less browning than conventionally pasteurized juice. Under certain conditions, PEF-treated orange juice retains ascorbic acid better than heat-treated juice. All these findings are important and may prove invaluable for the adaptation of PEF as a real alternative for orange juice pasteurization.

Flavor components in apple juice are numerous, so flavor identification may be considered more complex than the correspondent to orange juice due to the aromatic nature of apples. Eight odor-active volatiles have been, however, identified as the most important contributors for the aroma-flavor authenticity of apple juice (Cunningham et al., 1986). Zarate-Rodriguez et al. (2000) compared PEF and ultrafiltration (UF) for pasteurization of apple juice. No significant changes were observed in variables such as pH, °Brix, and acidity, expressed as malic acid, for the PEF-treated juice and the ultrafiltered one. Color was the quality attribute that did show change for membrane treatments. The observed trend was for the juice to become darker as a function of applied transmembrane pressure. Similarly to UF treatments, relative color changes were observed, but the registered effect was opposite, i.e., the treated juices became paler as a function of applied field strength. Color changes were independent of pulses number but dependent on field strength. The different color ratio perception in UF- and PEF-treated juices could be due to haze formation, which may be caused by tannins, proteins, and carbohydrate polysaccharides. Direct effects of PEF on volatiles of apple juice, and comparison with a conventional thermal treatment, have been also been investigated (Aguilar-Rosas et al., 2007). PEF and HTST were tested in order to determine decrease in concentration of eight odor-responsible volatiles. In general terms, PEF retained better most of the volatile compounds responsible for color and flavor of the treated apple juice. For example, as shown in Table 2, hexanal and hexyl acetate were only lost by 7% and 8.4%, respectively, when using PEF, while they were virtually lost by HTST. Also, important biochemical substances in apple juice, such as phenol compounds, were better retained by PEF than by HTST treatment.

Table 2 Percentage of volatiles losses, compared with untreated sample, in apple juice treated by two methods (differences by a Student *t*-test for independent samples: $p < 0.05$, $n = 3$)*

Compound	Loss percentage for PEF	Loss percentage for HTST
Acetic acid	39.792 ± 20.84	100
Hexanal	7.042 ± 9.32	62.348 ± 5.35
Butyl hexanoate	18.108 ± 7.72	36.273 ± 24.86
Ethyl acetate	77.458 ± 29.23	67.126 ± 39.33
Ethyl butyrate	60.190 ± 17.80	88.398 ± 12.46
Methyl butyrate	30.081 ± 31.37	51.200 ± 19.56
Hexyl acetate	8.408 ± 16.12	22.910 ± 21.99
1-hexanal	14.101 ± 7.65	69.307 ± 5.62

*Adapted from Aguilar-Rosas et al. (2007)

The effect of a PEF treatment applied in a continuous system, on physical and chemical properties of freshly squeezed citrus juices (grapefruit, lemon, orange, tangerine), was studied by Cserhalmi et al. (2006). The aim of the work was to investigate the effect of PEF technology on pH, °Brix, electric conductivity, viscosity, nonenzymic browning index (NEBI), hydroxymethylfurfurol (HMF), color, organic acid content, and volatile flavor compounds of the mentioned citrus juices. The juices were treated by 50 pulses at 28 kV/cm. The treatment temperature was below 34°C. The pH, °Brix, electric conductivity, viscosity, NEBI, and HMF of citrus juices practically did not change. Color also remained unchanged for all samples. In all cases, the absorbance spectra of treated and untreated samples were similar to each other. There was no significant change in organic acid content of juices. The volatile flavor compounds of treated juices were essentially equal to those present in unprocessed juice, as shown by SPME GC-MS results.

The influence of different PEF intensities and conventional HTST treatment on quality characteristics [pH, °Brix, total acidity, turbidity, hydroxymethylfurfural (HMF), color, microbial flora, PME activity, and sensory analysis] of blended orange and carrot juice were investigated (Rivas et al., 2006). HMF and color parameters did not vary with any of the treatments. Total acidity and turbidity were slightly higher after HTST treatment. Sensory characteristics of the PEF-treated juice were more similar to the untreated juice than to the HTST-pasteurized juice. Heat pasteurization was more efficient in inactivating microbial flora and PME and in preventing the growth of microbial flora and reactivation of PME at 2°C and 12°C for 10 weeks.

Cortés et al. (2008) evaluated the effect on color, browning, and HMF content of a pasteurized orange juice and the same orange juice treated by PEF, during 7 weeks, stored in refrigeration at 2°C and 10°C. Pasteurized orange juice presented greater yellow tendency and less red tendency than the untreated orange juice, while PEF-treated orange juice presented a color more similar to the untreated orange juice. Color variations during storage were greater in pasteurized orange juice than in PEF-treated orange juice. Nonthermal-treated orange juice has less nonenzymic browning than the pasteurized one. There was a significant increase in this parameter from the fourth week of storage in all the juices stored at 10°C, while in those stored at 2°C, the browning index values are maintained during more time. There were not significant variations in the HMF content of the juices pasteurized or treated by PEF with respect to the untreated orange juice. During refrigerated storage, HMF was always below the maximum values established.

Orange juice was processed by PEF at different processing conditions to test the effect on PME inhibition by Elez-Martínez et al. (2007). PME inactivation was greater when field strength, time, frequency, pulse width, and electric energy density were increased. PEF inactivation of orange juice PME with processing parameters was modeled. The first-order fractional conversion, and Fermi's and Hülsheger's models described with enough accuracy the orange juice PME inactivation by PEF as a function of the individual time and the combined effect of the field strength and the time, respectively. Residual orange juice PME activity was well related to frequency, pulse width, and electrical energy density, by a first-order fractional conversion model.

Mosqueda-Melgar et al. (2008) reported an investigation to determine effects of a PEF treatment in melon and watermelon juices, added with citric acid or cinnamon bark oil, on microbiological shelf-life and sensory attributes. Populations of different microorganisms were reduced by more than 5.0 log cycles in PEF-processed melon and watermelon juices containing 2.0% and 1.5% of citric acid, respectively, or 0.2% of cinnamon bark oil. The shelf-life of both juices stored at 5°C extended for more than 91 days. Hence, the microbiological quality and safety of these fruit juices by combining PEF and citric acid or cinnamon bark oil were ensured. However, the taste and odor of the PEF-treated melon and watermelon juices containing antimicrobials were significantly affected. Further studies are, therefore, needed to decrease the impact on the sensory attributes by using antimicrobials.

PEF effects on oxidative enzymes and color of fresh carrot juice were studied by Quitão-Teixeira et al. (2008). A response surface methodology (RSM) was used to evaluate the effect of pulse polarity (mono- or bipolar mode), pulse width (from 1 to 7 μsec), and pulse frequency (from 50 to 250 Hz) on color and POD inactivation of PEF-treated carrot juice. The total treatment time and the electric field strength were set at 1000 μsec and 35 kV/cm, respectively, at a temperature below 35°C. The physicochemical characteristics of carrot juice were measured. There was a linear relationship between electrical conductivity and temperature of the carrot juice. The results showed that PEF-treated carrot juice (35 kV/cm for 1000 μsec applying 6-μsec pulse width at 200 Hz in bipolar mode) achieved a 73.0% inactivation of POD. The color coordinates did not change significantly.

A study of the effects PEF treatments on the bioactive components (polyphenols, catechins and free amino acids), color, and flavor of green tea infusions has been reported by Zhao et al. (2009). PEF efficiently retained polyphenols, catechins, and the original color of green tea infusions at electric field strength from 20 to 40 kV/cm for 200 μsec. PEF treatments also caused a significant increase in the total free amino acids of green tea infusions. The total free amino acids increased by 7.5% after PEF treatment at 40 kV/cm. The increase in total amino acids induced by PEF treatment, especially in theanine, is beneficial for the quality of commercial ready-to-drink green tea infusion products. There was no significant effect of PEF treatment at 20 or 30 kV/cm on flavor compounds of green tea infusions. However, PEF treatment caused losses of volatiles in green tea infusions to different extents when PEF dosage was higher than a critical level. The total concentration of volatiles lost was approximately 10% after PEF treatment at 40 kV/cm, for 200 μsec.

Sampedro et al. (2009) presented results of a project aimed at comparing thermal, PEF, and HPP processing on PME activity and volatile compounds concentration in an orange juice-milk beverage. Thermal treatment (85°C, 1 minute), PEF treatment (25 kV/cm, 65°C), or HPP treatment (650 MPa, 50°C) was needed to inactivate 90% of PME. Twelve volatile compounds were extracted by SPME and selected for quantification by GC-MS following the application of the different treatments. The average loss in concentration of volatile compounds was between 16.0% and 43.0% after thermal treatment. After PEF treatment, the average loss was between −13.7% and 8.3% at 25°C, 5.8% and 21.0% at 45°C, and 11.6% and 30.5% at 65°C. After HPP treatment, the average loss was between −14.2% and 7.5% at 30°C and 22.9% and 42.3% at 50°C. The results showed the potential of the nonthermal technologies in providing food with a higher standard of quality compared with thermal processing.

The degradation of ascorbic acid was determined in a ready-to-drink orange juice-milk beverage treated by PEF (Zulueta et al., 2010). The effects of PEF treatment were compared with those of heat pasteurization (90°C, 20 sec). Four electric field strengths (15, 25, 35, 40 kV/cm) and six treatment times for each field (from 40 μsec to 700 μsec) were studied. Ascorbic acid degradation was adjusted to an exponential model. For the shelf-life study, a 25 kV/cm at 280-μsec treatment was applied and the beverages were stored at 4°C and 10°C. The ascorbic acid degradation rate during storage was adjusted to zero-order kinetics, showing that beverages stored at 4°C had better ascorbic acid retention than beverages stored at 10°C. No significant differences were found between heat pasteurization and PEF treatments during storage.

CONCLUSIONS

Nonthermal food processing technologies hold several promises for modifying and improving quality and safety of foods. In particular, nonthermal treatments can be used to minimally process products and preserve their delicate sensory and nutritional qualities. Although impressive progress has been made in understanding the mechanism and influence of the different nonthermal processing technologies, much remains to be done. For example, even for some technologies that have been investigated for a long time, such as irradiation, there are pending issues. In this case, the development of off-flavors represents a challenge and research efforts are likely to continue on that line. Modeling processing variables need to be optimized for specific products to match equipment used in scale-up exercises. The use of nonthermal technologies to pasteurize a wide variety of foodstuffs and obtain high-quality, competitive products in world markets can be considered a plausible reality. Scientific validation of pasteurization efficiency for practically the most important technologies has been carried out, and they render results comparable with the conventional, thermal way of pasteurizing many food products. Safety is definitely preserved, while quality appears virtually unaltered by use of nonthermal ways of processing. As it appears, many of the nonthermal preservation technologies researched and developed in recent years are more suitable to treat liquid foods than solid foodstuffs. The beverage industry represents a huge market worldwide, with very strong companies distributing products around the globe. Most of these companies have a tradition of investing in research, and so, technology transfer based on research on nonthermal technologies is likely to grow considerably in the foreseeable future.

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