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Effect of water activity on headspace volatile compounds produced in whey protein concentrate and other spray dried dairy products during accelerated storage

Lee, Yang Bong, Ph.D.
The Ohio State University, 1993
EFFECT OF WATER ACTIVITY ON HEADSPACE VOLATILE COMPOUNDS PRODUCED IN WHEY PROTEIN CONCENTRATE AND OTHER SPRAY DRIED DAIRY PRODUCTS DURING ACCELERATED STORAGE

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of The Ohio State University

By
Yang Bong Lee, B.S., M.S.

The Ohio State University

1993

Dissertation Committee:
Dr. Charles V. Morr
Dr. John B. Lindamood
Dr. Michael E. Mangino
Dr. David B. Min

Approved by

Advisor

Department of Food Science and Nutrition
Dedicated

To My Grandfather, Parents and Sister
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VITA

1960......... Born - Chunchon, Republic of Korea.

1979-1983.... B.S., Department of Food Science and Technology, Seoul National University, Sewon, Republic of Korea.

1983-1985.... M.S., Department of Food Science and Technology, Seoul National University, Sewon, Republic of Korea.

1986-1990.... Graduate research associate, Department of Food Science, Louisiana State University, Baton Rouge, LA.

1990-1993.... Graduate research associate, Department of Food Science and Technology, The Ohio State University, Columbus, OH.

FIELD OF STUDY

Major Field: Food Science and Nutrition
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I. INTRODUCTION

The production of spray-dried whey protein concentrate (WPC) in the U.S. is estimated at over 170 million lbs per year (Anonymous, 1991). However, off-flavors that develop in WPC during processing and storage limit its use in infant formula, health foods and drinks and other food products (Holsinger et al., 1974; Hugunin, 1987)

One important mechanism of off-flavor formation during processing and storage of WPC is non-enzymatic browning (Morr & Richter, 1988; Morr & Ha, 1991). Non-enzymatic browning reactions, of which the most important are the Maillard reactions, result in the formation of brown pigments and related off-flavors. These reactions require amines, amino acids, peptides or proteins with available amino acid residues, plus reducing sugars as reactants. WPC and other dried dairy products contain an abundance of these compounds. Maillard reactions yield several volatile compound classes such as aldehydes, reductones, and furfural. The rate of formation of these off-flavor compounds is dependent on food composition, temperature, time, and water activity ($a_w$).

Another important source of off-flavor formation is lipid oxidation. Even though whey is processed to remove most of the
residual milkfat prior to WPC manufacture, most of its phospholipoprotein complex and smallest milkfat globules are retained in the whey and WPC. WPC contains 5-6% residual lipids which are capable of autoxidation during spray drying and storage to produce volatile compounds that impart a typical stale whey powder flavor.

Volatile compounds can be measured by several conventional extraction methods such as solvent extraction (Ferretti & Flanagan, 1971a), vacuum extraction (Tanchotikul and Hsieh, 1990), and simultaneous distillation and extraction techniques (Tanchotikul and Hsieh, 1991). However, dynamic headspace analysis (DHA) techniques have recently been applied to cheese (Lee et al., 1992), WPC (Mills, 1986), and other food products. This latter technique requires much less sample preparation than by conventional flavor compound recovery procedures, thus reducing the likelihood of artefact formation. Several improvements have been made in this technique, i.e., remote, off-line sample device for analyzing WPC powder, a cryogenically focused capillary interface between the purge and trap concentrator and the gas chromatograph, and automatic injection of focussed volatiles onto the gas chromatographic column.

Volatile compounds in WPC and other dried dairy products will be separated and identified by DHA as a function of factors that are known to affect Maillard reaction and lipid oxidation, i.e., water activity and storage temperature.
II. LITERATURE REVIEW

1. Off-flavor formation in whey protein concentrate

Studies of flavors and off-flavors in dairy products have been done by many scientists (Shipe et al., 1978; Forss, 1979; Badings & Neeter, 1980; Thomas, 1981; Adda et al., 1982; Heath, 1983; Manning and Nursten, 1985; and Hammond, 1989). However, research on off-flavors of whey products has been relatively limited (Ferretti and Flanagan, 1971 a,b; and McGugan et al., 1979).

Aged, stale off-flavor is the single most important factor limiting the use of dried whey and WPC products as food ingredients (Holsinger, 1974; and Hugunin, 1987). The sources of these off-flavor compounds have been classified under four main headings: 1. those that have their origins in the milk; 2. those that are produced by indigenous enzymes, microbial enzymes, or by chemical reactions during cheese making; 3. Maillard browning type compounds generated by heat treatment during drying; and 4. those that are formed during storage of the dried whey or WPC.

Since WPC produced from high quality whey lacks noticeable off-flavor defects, the latter two mechanisms, i.e., Maillard and lipid oxidation, appear to be most
important to this study (Ramshaw and Dunstone, 1969; Ferretti and Flanagan, 1972; Min et al., 1990). It is also likely that indigenous compounds from the milk and those compounds formed prior to drying may act as catalysts for these latter two reactions. One additional factor may be the formation of off-flavors from riboflavin degradation, however, this reaction usually requires exposure of the product to light.

1.1. Maillard reaction mechanism

The Maillard reaction has received much attention as the source of off-flavor compounds in dehydrated foods. The Maillard reaction mechanism is extremely complex and many of the compounds that are formed by it have not yet been identified. Model systems have frequently been used to study the Maillard reaction mechanism for off-flavor formation in dried food products. The well known mechanisms are the pathways via Amadori and Heynes compounds. Amadori and Heynes reactions yield several volatile compounds such as aldehydes, reductones, and furfurals.

1.1.1. Maillard browning reaction in dehydrated dairy products

Non-enzymatic browning reactions, of which the most important are the Maillard reactions, result in the formation of brown pigments and related off-flavors. These reactions require amines, amino acids, peptides or proteins with
available amino acid residues, plus reducing sugars as reactants. The Maillard browning reaction, which involves interaction of protein and lactose as its initial step, is important in the formation of stale off-flavors in dried milk and whey products (Morr & Richter, 1988; Morr and Ha, 1991). WPC contains an abundance of these compounds, so WPC is also sensitive to this reaction.

Lactose, the major carbohydrate, and its hydrolysis products, glucose and galactose, are precursors of numerous nonenzymatically formed off-flavor compounds in dairy products. Two separate types of reactions occur. One reaction involves lactose fragmentation during heating and the other reaction involves its interaction with amino acids and other nitrogenous compounds as during the Maillard reaction. A great number of off-flavor compounds are formed by the heating of single sugars, ranging from formic, lactic, pyruvic and other aliphatic acids to phenols, benzaldehyde and other benzenoid compounds, and furans.

Dairy products are sensitive to heat treatment during processing and storage because they usually contain lactose. The nutritionally essential amino acid lysine reacts with lactose on heating and is extensively destroyed during the roller-drying of milk powders and also during the production of evaporated milk (Mauron, 1964). Maillard reactions of this type also occur during storage causing off-flavors and flavor deterioration which are one of the main obstacles to consumer
acceptance (Ferretti and Flanagan, 1972).

1.1.2. Volatile compounds formed by the Maillard reaction

Fors (1983) reviewed the literature pertaining to nearly 450 products arising from this reaction. Nursten (1981) classified hundreds of volatile products of the Maillard reaction into three groups: 1. Simple sugar dehydration or fragmentation products, i.e., furans, pyrones, cyclopentenes, carbonyls and acids; 2. Simple amino acid degradation products, i.e., aldehydes and sulphur compounds; and 3. Volatile compounds produced by further interaction of these compounds, i.e., pyrroles, pyridines, pyrazines, imidazoles, oxazoles and thiazoles.

Shibamoto (1989) studied a number of heterocyclic compounds reported in the Maillard reaction mixtures. This qualitative information on the volatile compounds in the Maillard reaction mixtures helps to understand their possible sources in food products. The major off-flavor compounds are nitrogen-containing and sulfur-containing heterocyclic compounds. For example, nitrogen-containing pyrazines contribute a characteristic roasted or toasted flavor to cooked foods. Sulfur-containing thiophenes and thiazoles give a characteristic cooked meat flavor. A striking property of these compounds is their extremely low odor thresholds for detection by sensory methods.
Ferretti and Flanagan (1971a and b, 1972) investigated the volatile compounds from the Maillard browning reaction using model lactose-casein and whey systems. They demonstrated that several different compounds were responsible for the stale off-flavor in dried milk and WPC.

1.1.2.1. Aldehyde and carbonyl volatile compounds

Aldehydes, which are produced by Strecker degradation of amino acids during the Maillard browning reaction and undergo an aldol condensation to form a wide range of flavor compounds. The Strecker degradation reaction results in amino acid deamination and decarboxylation in the presence of pyruvaldehyde or other dicarbonyl compounds to form aldehydes. A series of further reactions occurs which converts them to furfurals, dehydration products and polymers that result in brown pigment and off-flavor production (Hodge et al., 1972; Hammond, 1989).

Parks (1967) isolated 40 compounds from dry and concentrated fluid milk. Of these, methylpropanal, 3-methylbutanal, furfural, diacetyl and maltol were reported to be Maillard products. Scanlan et al. (1968) reported that raw milk contained 5 ppb diacetyl, whereas heated milk contained 38 ppb diacetyl, which is above its flavor threshold in unheated milk. They suggested that diacetyl contributes a richer flavor to heated milk products.
1.1.2.2. Sulfur-containing volatile compounds

Sulfur-containing amino acids such as methionine and cysteine are the sources of sulfur-containing volatile compounds from Strecker degradation. Strecker degradation products are condensed to form other compounds such as dimethyl disulfide and thiazoles. Cysteine may be broken to pyruvic acid, acetaldehyde, hydrogen sulphide and ammonia, and these compounds are condensed to sulfur-containing compounds such as trithiolane, dithiane and thiazoline. Strecker degradation of methionine yields methional.

The reaction of hydrogen sulfide, methyl mercaptans and other simple sulfur compounds with unsaturated carbonyl compounds is the origin of many dairy flavors. The further reaction of sulfur compounds and amino acids with milk lipid oxidation products can also occur. The reaction of methyl mercaptan with 2-hexenal in light-activated milk results in formation of a heated flavor, whereas reaction of 2-butanal and methyl mercaptan provides a cheesy flavor. Such reactions can actually eliminate oxidized flavors and generate more desirable food flavors. Malty flavors in milk and dairy products may arise from the reaction of amino acids and α-dicarbonyl compounds. Such reactions may take place during the nonenzymatic, Maillard browning during processing, drying and storage of dairy products.

The cooked flavor of heated milk is due to a large extent to the release of hydrogen sulfide from the sulfhydryl groups.
of the whey proteins. These compounds, which impart a typical cooked flavor, tend to mask the other undesirable flavors produced during storage. Free protein sulfhydryl groups are reported to inhibit non enzymatic browning of milk products (Jenness and Patton, 1959). Antioxidants also inhibit the nonenzymatic browning reaction (Pokorny, 1981). Dissolved oxygen plays a definite role in the oxidation of some sulfur compounds and thereby in reducing cooked flavor in UHT milk (Jeon, et al., 1978)

1.1.2.3. Nitrogen containing volatile compounds

Maillard reaction compounds result from high temperature heat treatments that cause caramelization of sugars and also from reactions that occur at ambient temperatures. For example, alkyl pyrazines, which have a nutty aroma, have been recovered from spray dried nonfat dry milk (Maga and Sizer, 1973).

Pyrazine formation mechanisms are complicated. Many steps may be involved in their formation, but one pathway involves self-condensation of amino ketones that originated from the Strecker degradation of amino acids (Newell et al., 1967).

Ferretti and Flanagan (1972) isolated 44 compounds from whey powder that had been stored two years and had acquired a stale off-flavor. The pyrazine volatile compounds isolated from whey powder were 2-methyl-pyrazine; 2,3-dimethyl pyrazine; 2,5-dimethyl-pyrazine; 2,6-dimethyl-pyrazine; 2,3,5-
trimethyl-pyrazine; 2-ethyl-5-methyl-pyrazine; 2-ethyl-6-methyl-pyrazine; 2-methyl-5-vinyl-pyrazine and 2-methyl-6-vinyl-pyrazine (Ferretti and Flanagan, 1971a). They concluded that on the basis of threshold values and approximate compound concentrations that 12 of the compounds could be responsible for the stale off-flavor in stored whey powder. These compounds included 2,3,5-trimethylpyrazine, methylethylpyrazine and N-ethyl-2-formylpyrrole. Also, Ferretti and Flanagan (1972) identified seven pyrazine compounds from stale nonfat dry milk. A lactose and casein model system provided five pyrazine compounds and casein alone also produced five pyrazine compounds during storage that included pyrazine; 2-methyl-pyrazine and dimethyl pyrazine.

1.1.2.4. Furan volatile compounds

Maga (1979) studied the formation and properties of key furans in foods. The basic formation of furan compounds is through carbohydrate thermal decomposition. Many furan compounds were identified in milk products. It can be concluded that the vast majority of furans in milk products are derived from further reaction of primary Maillard compounds. Carbohydrate degradation products have been proposed as the primary source of furans (Nursten, 1981). Thermal breakdown of terpenes has also been proposed as a mechanism for formation of furans (Vitzthum, et al. 1976). Normally, small amounts of furan compounds which have rather
low sensory thresholds.

2-furfural was identified in dry whole milk (Parks and Patton, 1966), whereas three additional furan compounds were identified in stale nonfat dry milk. Four furan compounds were identified from casein (Ramshaw and Dunstone, 1969), whereas twelve furan compounds were identified in stored sodium caseinate (Qvist, 1974). Twenty eight furan compounds were identified in whey powder (Ferretti and Flanagan, 1971a,b).

1.1.2.5. Other heterocyclic compounds

Other heterocyclic compounds formed by Maillard browning reactions include: unsaturated lactones, benzenoid compounds, and maltol. The lactones are isolated from stale dairy and heated products (Calve and Hoz, 1992). The importance of the other compounds in the flavor of dairy products has not been well described (Shipe et al., 1978).

1.1.2.6. Carbon dioxide

Carbon dioxide, which is a second major product of the Strecker degradation of amino acids, also contributes to the formation of brown pigment by the Maillard mechanism, and to the oxidative stability of the lipids in milk powder (Min et al., 1990)
1.2. Lipid oxidation

"Aged" or "stale" flavor, which is the single most important quality criticism in whey powder and WPC, is believed to be due largely to lipid oxidation. The relative importance of this latter mechanism must await further research to determine the importance of various chemical mechanisms for controlling flavor stability of WPC during storage.

1.2.1. Lipid oxidation mechanism

The exact nature of the initiation step for lipid oxidation is not fully understood although it can be promoted by suitable radicals, including those produced by a metal-catalyzed decomposition of preformed hydroperoxides (Gunstone, 1984). The reaction of unsaturated fatty acids with oxygen to form hydroperoxides is generally a free radical catalyzed process. The formation of free radicals in the initiation step of autoxidation is promoted by thermal or photodecomposition of peroxides or hydroperoxides, by metal catalysis and by ultraviolet irradiation. Propanal is the classical oxidation product of linolenic acid hydroperoxides, whereas, hexanal is one of the oxidation products of linoleic acid hydroperoxides. The role of singlet oxygen in the primary initiation reaction has been postulated (Rawls & van Santen, 1970). The reactivity of singlet oxygen with unsaturated fatty acids is about 1450 times greater than triplet oxygen (Rawls and van Santen, 1970
; Min and Lee, 1988). Lipid oxidation is accelerated by prooxidants, catalysts, and unsaturated lipids, and an oxygen-enriched headspace (Hall, 1985). Nitrous oxide also catalyzes the lipid oxidation reaction (Labuza, 1984). Bassette (1976) reported that 5 ppm of added copper resulted in the production of increased concentrations of acetaldehyde, propanal, n-pentanal, n-hexanal in pasteurized milk.

### 1.2.2. Volatile compounds in dehydrated dairy products

The chemical degradation of milk lipids has been studied extensively and substantial progress has been made (Day and Lillard, 1960; Lillard and Day, 1961; Sattar and deMan, 1975; Schwartz and Parks, 1978; Walstra and Jenness, 1984). The reaction of oxygen with unsaturated fatty acids in milkfat like other food lipids results in the formation of hydroperoxides which decompose to form a multitude of aldehyde and related chemical compounds (Frankel, 1985; Hammond, 1989). The possible formation of free radicals from proteins in dried dairy products by mechanical energy has been reported (Hansen et al., 1970). Milkfat oxidation products are reactive and are believed to also catalyze the Maillard browning reaction. Oxidized milkfat is commonly described as having an 'oxidized' or 'cardboard' flavor (Hammond, 1989), which is different from the stale flavor formed in dried whey and WPC. The fatty acids most susceptible to oxidation in dried milk and whey products are linoleic, linolenic, arachidonic and other polyunsaturated
fatty acids (Hammond, 1989). Stale flavor development in dried dairy products is related in part to the production of free fatty acids (Jeon, 1989). Dry whole milk, which contains 23.8-24.5% milkfat, is more susceptible to oxidative deterioration than low-fat milk powder (Labuza, 1982).

Lipid oxidation results in formation of a variety of volatile and non-volatile compounds that are responsible for undesirable flavors in dried milk and whey products. Non-volatile components include hydroperoxides, trihydroxy fatty acids and other oxygenated forms of fatty acids, oxidized lecithin and fatty acid dimers. Autoxidation of the unsaturated fatty acid components of phospholipids during processing generates off-flavors in milk and whey products. Phospholipids also possess an antioxidant activity due to their mineral ion-chelating ability or to their synergistic relation with other antioxidants. However, they themselves are rather easily oxidized, and may function as off-flavor precursors in finished food products (Porter, 1985; Min & Jung, 1989; Szuhaj & Sipos, 1989; Hamzawi, 1990). The phospholipid fraction of milk is oxidized more readily than the neutral triglyceride fraction and is therefore considered as an off-flavor precursor (Sattar & deMan, 1975). The relatively high concentration, i.e., 34-56%, of phospholipids in whey lipids, which are themselves rich in unsaturated fatty acids (Kurtz, 1974), makes WPC highly susceptible to the formation of off-flavors by lipid oxidation.
1.2.2.1. Aldehyde volatile compounds

Autoxidation of lipids proceeds at temperatures below 100°C through the addition of oxygen to a methylene group adjacent to a double bond. The resulting allylic hydroperoxides decompose to form straight-chain aldehydes such as for example hexanal in the case of the 13-hydroperoxide of linoleic acid. Other aldehydes may arise through isomerization or other secondary reactions. For example, 3-nonenal and cis-4-heptenal may arise from the isomerization of aldehydes produced by oxidation of 9, 15-isolinoleic acid, respectively.

Aldehydes resulting from autoxidation may react with other compounds to form important flavor compounds in dairy products. Nonanal and other saturated aldehydes oxidize to their acid forms which contribute to the off-flavor of dairy products (Jeon, 1989). The main volatile compounds which are identified from milkfat oxidation are n-alkanals, alk-2-enals, alk-2,4-dienals, and alkan-2-ones. Oct-1-en-3-one, 2,4-dienals, trienals and cis-4-heptenal have been implicated as being responsible for off-flavors in food products that contain oxidized milkfat.

1.2.2.2. Ketone volatile compounds

Saturated methyl ketones or 2-alkanones may result from either the enzymatic breakdown of β-keto acids arising from microbial oxidation of fatty acids such as caprylic (octanoic) or from the thermal decomposition of β-keto acids naturally
present in milk. Methyl ketones in dairy products contain an odd number of carbon atoms because they usually come from β-keto acids with an even number of carbon atoms. Chemically derived methyl ketones are responsible for stale off-flavors in liquid and dried milk products.

1.2.2.3. Aliphatic alcohols

Aliphatic alcohols have recently been discovered in dairy products. The formation of primary hydroperoxides by the decomposition of secondary hydroperoxides appears to occur at an early stage of oxidation. Primary hydroperoxides then form 1-alkanols and/or n-alkanals via the alkoxy radical. Thus, hexanal, pentanal, and 1-pentanol may be simultaneously derived from the 13-hydroperoxide of linoleate.

The flavor thresholds of aqueous C-7, C-9, and C-10 1-alkanol solutions are ≤ 1 ppm, i.e., the threshold of 1-pentanol is 4.5 ppm, and that of 1-octen-3-ol is 1 ppb.

1.2.2.4. Lactone volatile compounds

r-Lactones containing four carbon rings and δ-lactones containing five carbon rings have been classified as furanones and pyranones, respectively.
1.2.3. Prevention of lipid oxidation in dried dairy products

Milk fat oxidation may be inhibited by limiting oxygen content in the dry product. Nitrogen atmosphere, partial vacuum, and nitrogen atmosphere plus oxygen scavenging agents effectively inhibited off-flavor formation in dry whole milk during storage. Min et al. (1989) studied the effect of packaging conditions on the flavor stability of dry whole milk during storage. The milk powder was stored in air, nitrogen, and a 92% nitrogen and 8% hydrogen atmosphere. The product stored in nitrogen had a better flavor stability than that stored in air. The most stable storage conditions were with the 92% nitrogen and 8% hydrogen atmosphere due to the ability of the hydrogen to react with oxygen that had been adsorbed by the palladium catalyst in the packaging material.

Mixtures of Maillard reaction products function as antioxidants by varying the amino acid-sugar combinations (Lingnert and Eriksson, 1981).

2. Factors affecting the rate of off-flavor formation

The two main mechanisms of off-flavor formation of dried dairy products are Maillard reaction and lipid oxidation. Major factors that affect rate of off-flavor formation by these two mechanisms include $a_w$, temperature, oxygen, metallic ions, antioxidants, and light (O'Brien & Morrissey, 1989; Labuza & Saltmarch, 1981a,b).
2.1. Effect of moisture content on off-flavor formation

Water content is important for determining the rate of off-flavor formation in dried dairy products during storage by Maillard browning and lipid oxidation.

2.1.1. Relationship between water and food

Water exists in several forms in food products. The rate of chemical reactions such as Maillard browning and lipid oxidation are affected by the amount of water in each of the principal forms, i.e., active and bound or inactive (Rockland, 1969; Labuza, 1970, 1975).

Water activity is the term which is used to quantify the portion of total water that contributes to vapor pressure and is available to participate in chemical reactions. It is defined as:

\[
a_w = \frac{P_f}{P_o} \times \frac{RH(\%)}{100}
\]

where: \(a_w\) = water activity; \(P_f\) = water vapor pressure above food at temperature \(T\); \(P_o\) = vapor pressure of pure water at \(T\); and \% RH = equilibrium relative humidity at which food neither gains nor loses water.

The relationship between \(a_w\) and water or moisture content of the food is shown by moisture isotherms. Each food product exhibits a unique moisture isotherm which depends on its composition with respect to water binding compounds, i.e., mainly proteins, ions, and carbohydrates. To understand the
effect of $a_w$ on chemical reactions in food, total water must be considered as free or active and bound. At low $a_w$, water is tightly bound to surface polar sites and is generally unavailable for chemical reactions. The upper limit of this tightly bound water region has been named the BET monolayer moisture value, which occurs at $a_w 0.2-0.3$ for most foods.

The general equation for the BET isotherm model is:

$$\frac{a}{(1-a)m} = \frac{1}{m_0 c} + \frac{(C-1)}{m_0 c} a$$  \hspace{1cm} (2)

where: $m = \text{moisture content on a dry basis at water activity } a_w \text{ and temperature } T; \ c = \text{constant}; \text{ and } m_0 = \text{monolayer value.}$

The BET monolayer value corresponds to the most stable moisture content for most dehydrated foods (Salwin, 1959). At or above the monolayer, water is loosely held to varying degrees in multi-layers, in capillaries and possibly entrapped in various structural components of the food product. While the degree of freedom of this loosely bound water is somewhat limited, it is still available to act as a solvent and as a chemical reactant. With respect to the Maillard reaction, $a_w$ values above the monolayer value alter the reaction rate by controlling liquid phase viscosity and by mobilizing the reactant species (Duckworth, 1976).

There are two principal approaches to control the $a_w$ in the headspace over the food: 1. mechanical humidifiers; and 2. aqueous sulfuric acid, glycerol or saturated salt solutions.
Saturated salt solutions have been most commonly used to maintain a constant $a_w$ for food products to be tasted for off-flavor development. Several precautions are necessary when salt solutions are used for this purpose: 1. the salt and water must both be pure; 2. the saturated solutions must be prepared at temperatures above those at which the $a_w$ is to be controlled; 3. none of the salt crystals should protrude above the top of the solution, which should be no more than 1.5-2.0 mm in depth; 4. only nontoxic salts can be used for food applications where sensory evaluation may be required; and 5. vapors from certain volatile salts may alter the flavor of the food, the rate of chemical reactions in the food, and may also interfere with headspace analysis of the food sample. Sodium nitrite solutions release nitrous oxide vapors that are absorbed by the food and alter the reaction rate for lipid oxidation.

2.1.2. Effect of water activity on Maillard reaction rate in dried dairy products

Interaction of water with various food components alters the physico-chemical state of the food system and affects the Maillard and other chemical reactions. In general, the non-enzymatic browning reaction rate is lowest below BET monolayer $a_w$ values and increases exponentially with increasing $a_w$ above the BET monolayer value, reaching a maximum rate at about $a_w$ 0.6 to 0.8. Above this latter $a_w$ range, however, additional
water dilutes the reacting species, thus decreasing the Maillard reaction rate (Loncin et al., 1968; Sharp, 1957; Eichner and Karel, 1972; Labuza, 1970), to decrease the reaction rate.

Non-enzymatic browning is a major deteriorative factor in the storage of dried dairy food products (Loncin et al. 1968). Coulter et al. (1949) reported higher rates of browning in dried milk powder containing 2-6% moisture on a wet basis. Henry et al. (1948) demonstrated that dried milk powder lost biological value due to a decrease in protein quality, especially at \( a_w = 0.55 \). Ben-Gera and Zimmerman (1972) observed greater losses of chemically available lysine in non-fat milk powder stored at 60% relative humidity \( (a_w = 0.6) \) than at 40% relative humidity \( (a_w) \) in the 20-40°C temperature range. Loncin et al. (1968) demonstrated a maximum rate of lysine loss and browning color development for dried milk stored at 37°C in the \( a_w = 0.6 - 0.7 \) range. Burvall et al. (1978) also demonstrated a browning reaction rate maximum at \( a_w = 0.6 - 0.7 \) in lactose-hydrolyzed milk powder. The greatest losses of lysine occurred in these latter powder samples stored at \( a_w = 0.62 \). The experiments of Henry et al. (1948) clearly demonstrated the importance of both moisture and temperature on the development of off-flavor in skim milk powder. In one study, these workers found that milk powders containing 7.3% moisture developed unpleasant 'gluey' flavors after about 10 days storage at 37°C, whereas powders containing 2.9% moisture required almost
500 days to reach the same degree of off-flavor development. At 20°C these changes occurred at a much slower rate, but powder containing 7.3% moisture deteriorated more quickly at 20°C than that containing 2.9% moisture stored at 37°C.

With respect to dried whey, Holsinger et al. (1973) reported that 8.18% moisture on a wet basis acid whey powder dried at temperatures ranging from 90 to 125°C exhibited greater lysine losses than those that contained 1.6% moisture. Kahrberg and Johnson (1975) demonstrated that the rate of browning in dried sweet cheese whey powder stored at room temperature also increased with increasing moisture contents in the range of 3.6 to 5.5% (wet basis). Labuza and Saltmarch (1981a) reported that the maximum browning reaction rate in whey powder is at \( a_w 0.44 \), which is considerably lower than the usual values of \( a_w 0.6-0.8 \) for most dried foods (Labuza & Saltmarch, 1981a,b).

2.1.3. Effect of lactose on Maillard reaction rate

Under certain conditions the maximum browning rate of whey powder can be shifted to \( a_w \) below 0.6-0.8 (Eichner and Karel, 1972; Warmbier et al. 1976 a,b.; Labuza, 1980). Hygroscopic whey powders have a maximum browning reaction rate at \( a_w 0.44 \) due to the moisture released during recrystallization of the hygroscopic lactose (Saltmarch and Labuza, 1980). The relative rate of lactose recrystallization increases with increased \( a_w \), whereas the relative rate of
diffusion of water from the matrix decreases with increased $a_w$. The browning reaction maximum rate should thus coincide with $a_w$ and temperature conditions that favor lactose recrystallization and enhance water diffusion rate (Saltmarch and Labuza, 1980).

Although a number of studies have examined the direct effect of water on the Maillard reaction, Huss (1970, 1974 a,b) related lactose crystallization to this reaction. Huss (1970) found that freshly dried skim milk powders contained lactose chiefly in the amorphous form. Upon exposure to increasing moisture levels, the lactose crystallized at the 40-42% RH ($a_w$ 0.4-0.42) range. Huss (1970) reported that available lysine loss was positively correlated with the extent of lactose crystallization in milk powders stored at 31 to 55% relative humidity ($a_w$ 0.31-0.55). Available lysine losses were negligible in powders stored at 4.9 to 6.8% moisture when lactose was in the amorphous form. Lysine losses increased dramatically in powders that underwent lactose crystallization at the 5.1-7.0% moisture content range. Later, Huss (1974a) reported that the greatest lysine losses occurred in skim milk powder stored at the 55% RH ($a_w$ 0.55) level which coincided with maximum lactose crystallization.

Huss (1974b) evaluated lysine losses in whey powders at different moisture contents and confirmed that the lactose state affected lysine losses. He also noted that the higher lactose content in whey powders (79%) contributed to greater
lysine losses at lower moisture content (1.5-4% wet basis) than in skim milk powder or in partially delactosed whey powder.

2.1.4. Effect of water activity on lipid oxidation

It has also been shown that $a_w$ affects the rate of lipid oxidation in food products. The rate of oxidation decreases as $a_w$ is decreased from high values to the monolayer $a_w$ value and then increases as the $a_w$ is lowered further. The fact that dried foods are most stable at or near the BET monolayer $a_w$ value has been attributed to changes in: 1. the activity ($E_a$) of trace metal catalysts; 2. availability and mobility of the trace metal catalysts which limit their movement to the lipid interface; 3. removal of peroxide intermediates by increased hydrogen bonding at the aqueous interface; and 4. rate of reaction of free radicals with proteins and other species in the aqueous phase (Hall, 1985).

2.2. Effect of storage temperature and time on off-flavor formation

2.2.1. Effect of storage temperature on off-flavor formation

By definition, the $a_w$ isotherm, which describes the relationship between water content and activity in food products must be determined at a single temperature. Because of the nature of the water binding phenomenon, foods that
exhibit the Type II isotherm bind less water at higher temperatures than at lower temperatures.

The effect of storage temperature follows the Clausius Clayperon equation:

$$\ln \frac{a_2}{a_1} = \frac{Q_s}{R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right)$$  \hspace{1cm} (3)

Where: $a_2$ = water activity at temperature $T_2$ °K, $a_1$ = water activity at temperature $T_1$ °K, $Q_s$ = heat of sorption in cal/mole which is a function of moisture content, and $R = 1.987$ cal/mole °K.

$Q_s$, which is the excess binding energy for removal of water, is the only unknown and, unfortunately, there are no standard tables of $Q_s$ for different foods. Therefore, the following procedure allows one to predict the $a_w$ of a food at various temperatures of interest: 1. determine moisture sorption isotherms for the product at several temperatures above and below the temperature of interest; 2. graph Log $a_w$ vs $1/T$ °K for the product at constant moisture content; and 3. determine the theoretical $a_w$ for the product at the given moisture content and temperature of interest from the straight line graph of $a_w$ vs $1/T$ °K.

In general, the effect of temperature on $a_w$ at constant moisture content is greatest for products with lower to intermediate $a_w$. $a_w$ increases as $T$ increases for a constant moisture content. $Q_s$ increases with decreasing moisture content, indicating a stronger interaction energy.
The effect of temperature on browning, which is the single most important factor, was first studied by Maillard (Maillard, 1916) by observing the rate of browning, the formation of carbon dioxide and the viscosity of glucose-glycine solutions at 34, 40, 100 and 150°C. He also reported that the rate of reaction increased with temperature.

Many workers have since confirmed this observation. In their classic study of the casein-glucose reaction, Lea and Hannan (1949) showed that the rate of reaction, as measured by the decrease in amino nitrogen content, increased uniformly with temperature over the range 0 - 90°C. The reaction rate was $9 \times 10^3$ times faster at 70°C than at 10°C and $4 \times 10^4$ times faster at 80°C than at 0°C.

Although the rate of the reaction at low temperature is quite low, it is the predominant reaction at temperatures above 35°C, especially in food systems with an intermediate moisture content (Labuza & Saltmarch, 1981a). The Maillard reaction has a relatively high temperature coefficient, e.g. the $Q_{10}$ for brown pigment formation is in the range 3-6 (Labuza & Saltmarch, 1981b).

### 2.2.2. Effect of storage time on off-flavor formation

Length of storage is also important. For example, Hurrell and Carpenter (1974) reported the loss of 76% and 85% of the available ε-amino lysine groups of an albumin-glucose mixture after 30 days storage at 37°C and after heating for 15 minutes
at 121°C, respectively. Although available lysine content may be severely reduced by Maillard reaction in foodstuffs stored at relatively low temperatures, such as 37°C, the resulting reaction products are colorless, non-volatile Amadori compounds. Browning occurs and off-flavor compounds are formed mainly at higher temperatures as a result of advanced Maillard reactions.

3. Headspace sampling techniques

Food is a complex material which consists of carbohydrates, lipids, proteins, minerals, water and numerous minor compounds, including volatile organic (flavor) compounds. The relative percentages of the volatile compounds vary widely for each food product and their chemical properties, i.e., polarities, solubilities and volatilities, also vary considerably.

A certain portion of the volatile compounds are partitioned between the food matrix and the headspace over the food. The composition of the volatiles in the headspace depends on the vapor pressures of the respective volatile compounds in their pure state at the temperature of the food and on the interaction of these compounds with the food matrix. At equilibrium, the headspace over the food contains a smaller amount of volatile aroma compounds than in the food matrix.

The isolation of volatile compounds from food may be
approached in two ways: 1. total volatiles analysis and 2. headspace analysis (Cronin, 1982). The 'total volatiles analysis' is done by isolating and concentrating the volatile components from the foodstuff which may potentially contribute to flavor (Weurman, 1969). The 'headspace analysis' involves the isolation and examination of the volatile compounds present in the vapor state above the food material.

Principal methods that have commonly been used for isolation and concentration of 'total volatiles' include solvent extraction, steam distillation and dialysis. These methods are generally time-consuming and require great care to avoid introducing artifacts by chemically altering the highly reactive components during isolation and concentration. However, these methods usually provide sufficient sample for a comprehensive chemical and sensory analysis.

On the other hand, headspace analysis is a much simpler procedure that can be performed on a small quantity of foodstuff with very little sample preparation, and with minimum likelihood of artifact formation. An additional advantage to this method is that the headspace vapor contains the aroma components in the relative proportions which one actually smells, i.e., it represents the effectual odor of the food product.

The main focus for developing headspace analytical techniques is to detect small quantities of important, higher-boiling volatile compounds and to remove water which is
commonly recovered along with flavor volatile compounds. Recent dynamic headspace analytical techniques, which collect and concentrate volatile vapors from large volumes of headspace purge gas by means of a cryogenic or chemical adsorbent trap, have to a great degree eliminated these problems.

Preparation of food aroma samples by these newer methods yields the most meaningful qualitative and quantitative patterns of aroma volatiles that contribute to flavor of a foodstuff.

3.1. Direct headspace injection

Headspace analysis generally provides the most accurate profile of the flavor compounds in the food system. Headspace analysis has been used extensively for monitoring changes in aroma volatiles, as for example, when food materials are processed or stored.

Direct analysis of the headspace vapors above a food product is the simplest analytical method. Direct injection of a small volume (5-10 ml) of the headspace vapor into the gas chromatograph is simple and rapid. This method has been used to analyze the volatiles of ripening banana (McCarthy et al. 1963), and to follow the development of oxidative rancidity and browning in dehydrated potato granules (Buttery and Teranishi, 1963).

The problem with direct headspace analysis is that too
little sample is available for instrumental analysis. Since direct headspace injections are generally limited to 10 ml or less, only those volatiles present at concentrations exceeding $10^{-7}$ g/L headspace are detected by the gas chromatograph (GC) and mass spectrometer (MS) (Schaefer, 1981). Since the concentration of volatiles above a food product ranges from $10^{-1}$ to $10^{4}$ g/L headspace (Weurman, 1974), only the most abundant volatiles above the food will be detected. Although this method cannot be used to evaluate trace volatiles (Dupuy et al., 1971), it may be the method of choice for those applications where trace volatile compound analysis is not necessary.

While the use of such splitless or on-column headspace sampling techniques substantially improves the sensitivity of the headspace methods, the technique is still not adequate for the analysis of trace volatiles in foods (Leahy and Reineccius, 1984). Additional problems with the direct sampling technique include: condensation of volatiles in the sampling syringe, absorption of volatiles by the septum of the sampling jar, irreproducibility of injection volume due to syringe leaks and vacuum in the sample bottle, and the inability to relate volatile compound concentrations in the headspace to actual concentrations in the food sample (Gregoire, 1985).

Despite the many problems and limitations of direct headspace sampling, it has found substantial application in
flavor studies. Some applications of headspace sampling for flavor analysis may be found in the work of Crain and Tang (1975) on macadamia nuts; Williams et al., (1972) on dimethyl sulfide in processed foods; Seo and Joel, (1980) on a study of lipid oxidation; and Sullivan et al., (1974) on the flavor quality of dehydrated potatoes.

3.2. Concentration of headspace volatiles.

Detection and analysis of small quantities of food volatiles may be accomplished via headspace concentration or purge and trap techniques embodied in the dynamic headspace analysis. The dynamic or equilibrium headspace volatile vapors above a food may be removed by purging with an inert gas. The volatile compounds in the carrier gas are concentrated with cryogenic, porous polymer or on-column vapor traps. These newer techniques make it possible to analyze trace volatiles of food products by GC and MS.

The headspace volatile compounds of food have certain ratios with the volatiles which are bound to the food matrix. Therefore as purging time increases, the concentrations of volatile compounds in the headspace are decreased and bound compounds are volatilized to replenish those in the headspace according to Raults rule. Based on this theory, the isolation mechanism of headspace volatile compounds has been studied by two methods, i.e., 1. cryogenic trapping; and 2. activated carbon and porous polymer column trapping.
3.2.1. Cryogenic trapping

The simple method of cryogenic trapping of headspace volatiles was designed and used by Schreyen et al. (1976). A more advanced technique for concentrating headspace vapors is done by passing the purge gas through a series of cold traps. This purge and trap technique is the principle of dynamic headspace analysis (DHA). The volatiles over the food are swept by a stream of purified carrier gas and cryogenically condensed in a liquid nitrogen-cooled trap, from which they may be subsequently volatilized and injected directly onto the GC column. Several cryogenic trap designs have been developed for recovering organic volatile compounds from food and other products. These methods have improved our ability to analyze trace volatile compounds over what was possible by direct sampling.

Inclusion of water, which is a major volatile component of most food products, with the cryogenically trapped organic volatiles from food products is a major problem with dynamic headspace analysis (DHA). Since excessive amounts of water interfere with GC analysis of the volatile compounds, the headspace vapor sampling protocol must be controlled to limit the amount of water included in the sample. The use of various desiccant precolumns to selectively remove water from the purge stream during trapping has been investigated (Heatherbell et al., 1971). However, these devices adsorbed and thereby lowered the recoveries of oxygenated volatile
Although the procedures listed above are useful, they are limited in the degree of concentration which may be achieved. Also, while major volatile components of a food may be readily analyzed, headspace analysis frequently does not isolate the higher-boiling compounds of sensory importance in amounts sufficient for instrumental measurement. An additional step is generally necessary in these cases to separate the flavor constituents from the water before injection of the sample onto the GC column. The need for solvent extraction adds concerns for solvent impurities (artifacts), efficiency of extraction (uniformity) and flavor losses during extract concentration.

3.2.2. Activated carbon column trapping

Volatile flavors are often trapped on adsorbent materials to avoid co-condensation of water with the flavor volatiles. The trapping of volatile flavors on an adsorbent which has minimal affinity for water allows more extensive purging and eliminates the need for subsequent removal of water prior to injection onto the GC.

An approach was developed for trapping the aroma volatiles from large volumes of headspace vapors by activated charcoal which has a high adsorptive capacity, low affinity for water and reasonable chemical stability. As headspace vapors are passed over the activated carbon, the organic
flavor volatiles are preferentially adsorbed while water vapor passes through. Activated carbon is one of the earlier adsorbents used for the trapping of flavors from headspace vapors (Tang and Jennings, 1967; Jennings and Nursten, 1967).

Clark and Cronin (1975) showed that volatile compounds could be trapped effectively from a vapor stream on as little as 2 g of charcoal packed into a glass capillary tube. The volatiles were then desorbed rapidly from the trap by heating to 260°C and injected directly onto a capillary GC column for analysis.

Carbon adsorbs an amount of organic compounds approximately equal to its weight (Schaefer, 1981). Adsorbed flavor compounds may be desorbed from the charcoal by either thermal desorption (Paillard, 1965) or solvent extraction (Grob, 1973; Jennings and Nursten, 1967). However, after 10-100 L of headspace has been passed over the 1-10 mg carbon trap it becomes saturated and compounds of greater affinity displace those that are less strongly bound. Use of such overloaded adsorbent traps results in erroneous GC profiles.

A major advantage to the use of carbon adsorbent traps is their large adsorption capacity which far exceeds that of the synthetic polymers. However, concerns have appeared in the literature for artifact formation on impure carbon traps during thermal desorption (Baigrie et al., 1985; Palamand et al., 1968).
3.2.3. Porous polymer column trapping

An effective approach, which has greatly expanded the power of the headspace technique, and which is now becoming a well established practice in many laboratories, is the trapping of large volumes of headspace vapor on certain porous polymer adsorbents (Hollis, 1966). The outstanding property of these materials is their ability to adsorb and retain most volatile organic compounds at ambient temperatures, while displaying little affinity for water. Two series of such polymers are commercially available, namely the Porapak series (Waters Associates) and the Chromosorb Century series (Johns-Manville). The different members within each of these series possess somewhat different surface areas and polarities. The most widely used material among the Porapak series for trapping food flavor volatiles is Porapak Q, a non-polar copolymer of ethylvinylbenzene-divinylbenzene. In the study on the suitability of Chromosorb Century polymers for flavor work, the 'polyaromatic' Chromosorb 105 was most effective due to its low susceptibility to oxidative and thermal breakdown (Murray, 1977).

For headspace trapping a quantity of the polymer, usually in the range 100-500 mg, is packed into a glass tube and conditioned before use by purging with highly purified oxygen-free nitrogen at temperatures ranging from 180-225°C. The polymer trap is then attached to the sampling apparatus containing the foodstuff and the volatiles are swept through
the trap in a stream of purified air or nitrogen. Flow rates, while commonly around 50 ml/min can be as high as 300 ml/min. Volatiles may be collected from headspace volumes ranging from as little as one liter to 250 liters or more. After collection, the trap is dry-purged with purified nitrogen to remove most of the water. The volatiles, which are desorbed from the trap by heating and reversed carrier gas flow, are collected and concentrated in a short length of liquid nitrogen-cooled precolumn. They are then automatically injected onto the analytical GC column by rapidly heating the precolumn.

Schultz et al. (1977) present details of a relatively simple sample transfer procedure for this type of analysis, while a more sophisticated arrangement is described by Murray (1977). Recent applications have included the work of Toulemonde and Beauverd (1985) and Reineccius and Liardon (1985). Synthetic porous polymers have become the most commonly used adsorbent for trapping volatiles from purged headspace vapors (Charalambous, 1978). While many different polymers have been evaluated, Tenax GC has become the adsorbent of choice. While this choice is not obvious, Tenax GC permits superior recovery of adsorbed volatiles and exhibits relatively good thermal stability in comparison to the other synthetic polymers. These two advantages outweigh the poor adsorption capacity displayed by Tenax GC. Withycombe et al. (1978) compared Chromosorb 105, Porapak Q and Tenax GC
for the recovery of headspace volatiles from hydrolyzed vegetable protein and found that Tenax GC gave the most characteristic flavor isolate by aroma judgement. The limited adsorption capacity of Tenax is quite evident from the work of Buckholz et al. (1980) on peanut aroma. They used three traps in series to evaluate 'breakthrough' losses of peanut volatiles. The highly volatile components in peanut headspace vapors broke through the first two traps after only 15 min of purging at 40 ml/min.

Jennings and Filsoof (1977) have also done work on the effectiveness of porous polymers for headspace trapping. They found poor retention of ethanol, pentanol, hexanol and heptane on both Tenax and Porapak traps. Their data shows poor recovery of alcohols and higher boiling compounds. The higher boiling compounds show poor recovery due to their low volatility rather than 'breakthrough' as observed for the alcohols.

While there are problems with applying headspace adsorption techniques to flavor analysis, the method has become quite popular in the literature. The work of Simon et al. (1980); Ruen et al. (1982); Liardon et al. (1984) and Noble et al. (1979, 1980) has shown adsorption headspace methods to be exceptionally reproducible and accurate. This method has become the method of choice for quantitative flavor studies.
3.3. Extraction mechanism for headspace volatile compounds

There are two types of headspace analysis such as static and dynamic headspace analysis. The equilibrium time for sampling depends on the volatile compound composition and the vapor pressures of the major volatile compounds. Dynamic headspace analysis is more complicated than static headspace analysis.

3.3.1. Static headspace sampling techniques

The sample for headspace analysis is held at the desired temperature for a sufficiently long period of time to allow the headspace volatile compounds to equilibrate with those in the food matrix. The respective concentrations of each compound in the headspace \( (C_h) \) and food matrix \( (C_f) \) can be defined by the distribution coefficient \( (K) \) according to equation:

\[
K = \frac{C_h}{C_f}
\]  

The factors affecting \( K \) for each volatile compound depend on the relationship between food composition, volatile compounds in the food matrix, and temperature. In general, volatile compounds are present in higher concentration in the headspace with increasing temperatures. Therefore, the sampling temperature may be raised to increase headspace volatile compound concentrations and improve sampling.
sensitivity. However, sample decomposition or reaction and increased water concentration in the headspace are possible disadvantages to the use of high sampling temperatures.

After equilibrium is reached, the headspace volatile compounds are sampled with a gas syringe and injected directly into the gas chromatograph.

3.3.2. Dynamic headspace sampling techniques

A major disadvantage to the static headspace sampling technique is in detecting higher boiling compounds and compounds present in small concentrations. The dynamic headspace sampling technique was developed as a modification of the static headspace sampling technique. An inert carrier gas is allowed to flow over the food sample for a certain period of time to purge most of the volatile compounds. These volatile compounds are removed from the carrier gas by trapping them onto a precolumn trap that ideally will not strongly bind water vapor molecules.

Quantification of volatile compounds was attempted by Horwood (1989) from changes of their concentrations in the headspace as a function of purging time. Additional research is necessary to further define the methods needed to quantitate the volatile compounds present in the headspace of food products.
CHAPTER I

Identification of headspace volatile compounds from fresh whey protein concentrate

Flavor quality is one of the most important factors for determining consumer acceptance of dairy and other food products. Lipid oxidation and Maillard browning reactions that occur during processing of spray dried WPC result in the formation of volatile off-flavors that impair their flavor quality, thereby limiting their value as ingredients in formulated food products.

Analytical methodologies previously available for isolation, fractionation and identification of volatile compounds formed during processing of dried dairy products are time consuming, tedious and are sometimes subject to error due to artifact formation and incomplete compound recovery. The recently developed dynamic headspace analysis (DHA) technique is suitable for identifying and monitoring changes in concentrations of volatile compounds produced in dried dairy products during storage.

The major objective of this research was to identify headspace volatile compounds in commercial spray dried WPC by DHA. A secondary objective was to evaluate the reproducibility in analyzing headspace volatile compounds.

Introduction

Although solvent extraction is one of the most common techniques for flavor isolation, this is time consuming and may lead to artifacts from reaction the solvent with food components. The recently developed dynamic headspace analysis
(DHA) has several advantages such as good sensitivity and reproducibility. Also, this method has more convenient and less artifacts, compared to solvent extraction.

Lipid oxidation and Maillard reaction increase with high temperature during processing of spray drying for WPC. This off-flavor formation reduces its use as an ingredient in formulated food products. In order to overcome this problem, it is necessary to monitor the flavor changes during processing. However, there is only limited information available on flavors and off-flavors of WPC, due to technical difficulties in analyzing volatile compounds at such low concentrations (Ferretti & Flanagan, 1971a, b, 1972; Mills & Solms, 1984; Mills, 1986; Morr & Ha, 1991)

Procedure

Sampling of WPC: Commercial WPC was obtained from CALPRO (Corona, CA). Three grams of WPC were put in a 125 ml serum bottle (Cat. No. 223748, Wheaton 400, Millville, NJ) and sealed with a teflon-coated septum (Cat. No. 3-3200M, Supelco, Bellefonte, PA) and an aluminum cap (Cat. No. 3-3250M, Supelco) as shown in Fig. 1.

Dynamic headspace analysis (DHA) of volatile compounds: The serum bottle with sample was attached to the dynamic headspace analyzer (DHA) as shown in Fig. 2.
Figure 1. Modified sampling device for dynamic headspace analysis.
Figure 2. Diagram of the dynamic headspace analyzer (DHA) with the 125 ml sealed serum bottle used as a control and external sample device.
Headspace volatile compounds were purged 10 min with ultrapure helium carrier gas at a flow rate of 40 ml/min and trapped onto a Tenax TA chemical column (Tekmar, Cincinnati, OH). Trapped volatiles were desorbed by heating the trap 4 minutes at 160°C and cryogenically focussed at a -150°C capillary interface that was cooled with liquid nitrogen. Focussed compounds were thermally injected onto the capillary gas chromatographic column by heating to 180°C within 1 minute. Compounds were separated with a 30 m x 0.25 mm x 0.25 μm film thickness DB-WAX column (Cat. No. 122-7032, J&W Scientific, Folsom, CA) in a gas chromatograph (Model 5890 series II, Hewlett Packard). The column was heated from an initial temperatures of 32°C to 160°C at a rate of 2°C/min. A total ion chromatograph (TIC) of eluted compounds was obtained with a mass-selective detector (Model 5971A, Hewlett Packard, Palo Alto, CA) and compounds were identified by comparison of their retention index values with those of reference compounds (Jenning & Shibamoto, 1980) and by computer-matching their mass spectra with that in reference NBS49K.L (Hewlett Packard).

Results and Discussion

Volatile compounds in fresh commercial WPC: Total ion chromatogram for volatile compounds obtained from fresh whey protein concentrate from CALPRO company was shown in Fig. 3 and headspace volatile compounds identified from fresh WPC were shown in Table 1.
Figure 3. Total ion chromatogram (TIC) of headspace volatile compounds from whey protein concentrate (WPC) before accelerated storage.
Table 1. GC-MS data for headspace volatile compounds recovered from fresh whey protein concentrate\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Compounds</th>
<th>Peak area (x 10\textsuperscript{6})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.66</td>
<td>pentane</td>
<td>4</td>
</tr>
<tr>
<td>2.06</td>
<td>heptane</td>
<td>2</td>
</tr>
<tr>
<td>3.63</td>
<td>propanal, 2-methyl-</td>
<td>1</td>
</tr>
<tr>
<td>4.16</td>
<td>methane, dichloro-</td>
<td>2</td>
</tr>
<tr>
<td>4.29</td>
<td>benzene</td>
<td>6</td>
</tr>
<tr>
<td>5.70</td>
<td>methane, isocyano-</td>
<td>5</td>
</tr>
<tr>
<td>6.23</td>
<td>ethene, tetrachloro-</td>
<td>6</td>
</tr>
<tr>
<td>6.34</td>
<td>methane, trichloro-</td>
<td>2</td>
</tr>
<tr>
<td>6.78</td>
<td>benzene, methyl-</td>
<td>11</td>
</tr>
<tr>
<td>8.33</td>
<td>hexanal</td>
<td>4</td>
</tr>
<tr>
<td>9.92</td>
<td>benzene, ethyl-</td>
<td>2</td>
</tr>
<tr>
<td>10.25</td>
<td>benzene, 1,4-dimethyl-</td>
<td>3</td>
</tr>
<tr>
<td>10.55</td>
<td>benzene, 1,3-dimethyl-</td>
<td>5</td>
</tr>
<tr>
<td>12.67</td>
<td>benzene, 1,2-dimethyl-</td>
<td>3</td>
</tr>
<tr>
<td>13.45</td>
<td>D-limonene</td>
<td>6</td>
</tr>
<tr>
<td>14.96</td>
<td>benzene, C3-alkyl-</td>
<td>2</td>
</tr>
<tr>
<td>18.08</td>
<td>benzene, C3-alkyl-</td>
<td>3</td>
</tr>
<tr>
<td>28.10</td>
<td>benzene, dichloro-</td>
<td>7</td>
</tr>
<tr>
<td>41.42</td>
<td>2-furanmethanol</td>
<td>1</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Based on data from a single injection of headspace volatile compounds from each of the three replicates.

Nine alkyl benzenes, 4 chloro-containing compounds, 2 aldehydes, 2 paraffins and limonene were identified. The origin of these alkyl benzenes were not clear even if these alkyl benzenes were found in many food system (Habu et al., 1985; Buttery et al., 1978; Vejaphan et al., 1988). Some may have formed by thermal degradation of sugar (Heyns et al., 1966). However, a sugar browning reaction model system did not produce significant amounts of alkyl benzenes (Umano & Shibamoto, 1987). Another research reported the formation of several alkyl benzenes from beef fats during heating (Watanabe & Sato, 1971).
Four chloro-containing compounds identified were dichloro-methane, trichloro-methane, tetrachloro-methane and dichloro benzene. The sources of these compounds are not clear like alkyl benzenes. Detergents used during the manufacturing of cheese and the pasteurization of whey and cleaning of membrane for WPC processing were believed as a possible source.

Two aldehydes such as 2-methyl propanal and hexanal were identified and believed to be lipid oxidation products. However, Maillard reaction products were not identified in fresh sample. It was believed that the purging temperature were room temperature and the Maillard reaction products were high boiling compounds.

**Evaluation of humidity control devices:** Headspace analysis techniques were developed to increase their sensitivity and reproducibility. The trap and purge method is the most common method to sample headspace volatile compounds for dynamic headspace analysis (DHA). The original sampling device supplied with the LSC 2000 Purge and Trap concentrator (Tekmar, Cincinnati, OH) was modified for this study. The U-shaped headspace sample holder, which was designed for sampling the headspace volatile compounds of environmental samples, was found to be inconvenient to use and difficult to clean. These problems were especially acute for analyzing powdered food samples, such as for the present study, where problems were encountered with particles being carried by the
carrier gas onto the Tenax trap (Morr and Ha, 1991). A special external sample holding device was designed for purging the headspace volatile compounds, consisting of a serum bottle that could be accessed by puncturing the septum used to seal it.

The common laboratory desiccator has most generally been used to maintain a constant humidity (a,) during storage of food samples. A new device, consisting of a septum-sealed serum bottle, was developed for this study. The volatile compounds formed during storage were concentrated into a smaller volume by this device, i.e., 150 ml for the serum bottle compared to ≥ 1 liter for the desiccator, and could be sampled directly without opening the chamber. The reproducibility of this new device was determined.

**Reproducibility of dynamic headspace analysis:**

Traditional flavor extraction methods are time-consuming and lack of reproducibility. Newly developed dynamic headspace analysis (DHA) techniques provide good reproducibility and sensitivity. The reproducibility of headspace volatile compounds recovery (Sensel and Griffiths, 1990) was calculated by the % relative standard deviation (% RSD):

\[
\text{%RSD} = \frac{\text{standard deviation}}{\text{mean}} \times 100
\]
Table 2. Reproducibility of recovery of the major headspace volatile compounds from whey protein concentrate stored 6 days at 60°C and RH 49.9%.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Compounds</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.78</td>
<td>dimethyl disulfide</td>
<td>4.8</td>
</tr>
<tr>
<td>8.33</td>
<td>hexanal</td>
<td>4.3</td>
</tr>
<tr>
<td>23.86</td>
<td>dimethyl trisulfide</td>
<td>14.9</td>
</tr>
<tr>
<td>25.60</td>
<td>nonanal</td>
<td>6.2</td>
</tr>
<tr>
<td>56.94</td>
<td>benzothiazole</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Based on data from a single injection of headspace volatile compounds from each of four sample replicates.

The recovery values for headspace volatile compounds by the Tenax TA purge and trap system ranged from 93.7% to 112% and their recovery reproducibility values ranged from 1.7% to 2.8% RSD (Sensel and Griffiths, 1990). The total ion chromatograms for the four sample replicates showed similar shapes and gave good reproducibility. Reproducibility values of five major headspace volatile compounds such as dimethyl disulfide, hexanal, dimethyl trisulfide, nonanal, and benzothiazole from stored WPC ranged from 4.3 to 14.9% RSD. These values were much less, so my method showed better reproducibility than those of the other research (Ha & Morr, 1992). The reason was believed that my method was used at room temperature for purging temperature and with prepurging.

Conclusion

The modified external sampling device showed good sensitivity and reproducibility as well as it was very convenient. The headspace volatile compounds isolated from
fresh commercial WPC were nine alkyl benzenes, 4 chloro-containing compounds, 2 aldehydes, 2 paraffins and limonene were identified.

Literature cited


CHAPTER II

Effect of water activity on the formation of headspace volatile compounds during accelerated storage of whey protein concentrate.

Water activity is one of the most important factors for controlling the off-flavor formation during storage of spray dried dairy and other food products. Lipid oxidation and Maillard browning reactions that occur during storage of spray dried dairy products are main reactions in the formation of volatile off-flavors that greatly impair their flavor quality.

Analytical methodologies previously available for controlling water activity were undesirable to analyze the delicate changes of headspace volatiles during storage of dried dairy products. The new miniaturized water activity ($a_w$) control chamber was designed for measuring the changes of headspace volatile compounds during accelerated storage studies on dried dairy products.

The major objective of this research was to study the changes of headspace volatile compounds produced by several water activities in commercial spray dried WPC by DHA. A secondary objective was to evaluate the newly designed miniaturized water activity ($a_w$) control chamber for conducting accelerated storage studies on dried dairy products, and that can be sampled for headspace volatile compounds.

Introduction

Although a lot of research have been done on functional properties of whey protein concentrate, relatively little attention has been given to research on off-flavors in WPC (Morr & Ha, 1991). Two major mechanisms involved in flavor
deterioration of WPC are lipid oxidation and Maillard browning reaction (Morr & Ha, 1991). Although processing conditions during spray drying are very important for preventing or inhibiting these chemical reactions, concentration and composition of whey protein concentrate is also important in this regard (Morr & Ha, 1991). Removal of lactose, lipid or minerals have been tried to improve whey protein functional properties as well as to improve flavor stability.

**Procedure**

**Sampling of WPC:** Commercial WPC (CALPRO 75) was obtained from CALPRO (Corona, CA). Three grams of WPC were put in a 125 ml serum bottle and sealed with a teflon-coated septum (Cat. No. 3-3200M, Supelco, Bellefonte, PA) and an aluminum cap (Cat. No. 3-3250M, Supelco).

The $a_w$ control chamber design is shown in Fig. 4. Saturated salt slurries of lithium chloride (LiCl), magnesium chloride (MgCl$_2$), sodium bromide (NaBr) or potassium bromide (KBr) were layered on the bottom of a 125 ml serum bottle (Cat. No. 223748, Wheaton 400, Millville, NJ) to a depth of 8 mm, respectively. This arrangement provided a theoretical $a_w$ of 0.11, 0.29, 0.50 and 0.79, respectively (Labuza, 1984). Six 0.5g aliquots of each dried dairy product were weighed into separate 10 x 75 mm, borosilicate glass, disposable culture tubes (Cat. No. 60825-538, VWR Scientific, Cleveland, OH) and placed in serum bottles. The serum bottles were then sealed
with a teflon-coated septum (Cat. No. 3-3200M, Supelco, Bellefonte, PA) and an aluminum cap (Cat. No. 3-3250M, Supelco).

Figure 4. Design of humidity (a_w) control device consisting of a saturated salt solution in a sealed 125 ml serum bottle.
Accelerated storage of WPC: The serum bottles were held 2 days in the dark at room temperature to equilibrate $a_w$ of the samples. They were subsequently exposed to accelerated storage for 6 days at 60°C in the dark and stored in a -20°C freezer until analyzed by DHA.

Dynamic headspace analysis (DHA) of volatile compounds: Serum bottles were held at room temperature, i.e., 20-24°C, for 30 min and attached to the dynamic headspace analyzer (DHA) as shown in Fig. 2. Headspace volatile compounds were purged 10 min with ultrapure helium carrier gas at a flow rate of 40 ml/min and trapped onto a Tenax TA chemical column (Tekmar, Cincinnati, OH). Trapped volatiles were desorbed by heating the trap 4 minutes at 160°C and cryogenically focussed at a -150°C capillary interface that was cooled with liquid nitrogen. Focussed compounds were thermally injected onto the capillary gas chromatographic column by heating to 180°C within 1 minute. Compounds were separated with a 30 m x 0.25 mm x 0.25 μm film thickness DB-WAX column (Cat. No. 122-7032, J&W Scientific, Folsom, CA) in a gas chromatograph (Model 5890 Series II, Hewlett Packard). The column was heated from an initial temperatures of 32°C to 160°C at a rate of 2°C/min. A total ion chromatograph (TIC) of eluted compounds was obtained with a mass-selective detector (Model 5971A, Hewlett Packard) and compounds were identified by comparison of their retention index values with those of reference compounds (Jennings & Shibamoto, 1980) and by computer-matching their mass spectra.
with that in reference NBS49K.L (Hewlett Packard).

**Results and Discussion**

Volatile compounds in stored commercial WPC: TIC of volatile compounds obtained from WPC stored 6 days at 60°C and at four different humidities are shown in Fig. 5.

![Total Ion Chromatogram (TIC) of headspace volatile compounds isolated from WPC after accelerated storage.](image)

Figure 5. Total ion chromatogram (TIC) of headspace volatile compounds isolated from WPC after accelerated storage.
Table 3. Effect of water activity on the concentrations of headspace volatile compounds isolated from WPC after 6 days accelerated storage at 60°C.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Relative Humidity (%RH)</th>
<th>Peak area (x 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11^c</td>
<td>22.3^c</td>
</tr>
<tr>
<td><strong>Aldehydes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butanal, 2-methyl-</td>
<td>914</td>
<td>21</td>
</tr>
<tr>
<td>Butanal, 3-methyl-</td>
<td>918</td>
<td>3</td>
</tr>
<tr>
<td>Pentanal</td>
<td>978</td>
<td>39</td>
</tr>
<tr>
<td>Hexanal</td>
<td>1086</td>
<td>81</td>
</tr>
<tr>
<td>Heptanal</td>
<td>1182</td>
<td>14</td>
</tr>
<tr>
<td>Octanal</td>
<td>1286</td>
<td>9</td>
</tr>
<tr>
<td>Nonanal</td>
<td>1390</td>
<td>12</td>
</tr>
<tr>
<td><strong>Ketones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Butanone</td>
<td>900</td>
<td>51</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>1177</td>
<td>7</td>
</tr>
<tr>
<td>2-Heptanone, 6-methyl-d</td>
<td>1233</td>
<td>ND</td>
</tr>
<tr>
<td>2-Octanone</td>
<td>1281</td>
<td>2</td>
</tr>
<tr>
<td>3-Octen-2-one</td>
<td>1399</td>
<td>2</td>
</tr>
<tr>
<td>2-Nonanone</td>
<td>1386</td>
<td>3</td>
</tr>
<tr>
<td><strong>Alcohols</strong></td>
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<td></td>
</tr>
<tr>
<td>1-Pentanol</td>
<td>1259</td>
<td>ND</td>
</tr>
<tr>
<td>1-Octen-3-ol</td>
<td>1458</td>
<td>ND</td>
</tr>
<tr>
<td>2-Furamethanol</td>
<td>1644</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Acids and esters</strong></td>
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</tr>
<tr>
<td>Acetic acid</td>
<td>1453</td>
<td>ND</td>
</tr>
<tr>
<td>Ethanoletic acid, S-methyl ester</td>
<td>1048</td>
<td>ND</td>
</tr>
<tr>
<td>Thiocyanic acid, methyl ester</td>
<td>1262</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Paraffins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentane</td>
<td>500</td>
<td>9</td>
</tr>
<tr>
<td>Hexane</td>
<td>600</td>
<td>9</td>
</tr>
<tr>
<td>Heptane</td>
<td>700</td>
<td>5</td>
</tr>
<tr>
<td>Decane</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Aromatic hydrocarbons</strong></td>
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<td></td>
</tr>
<tr>
<td>Furan</td>
<td>781</td>
<td>7</td>
</tr>
<tr>
<td>Furan, 2-methyl-</td>
<td>870</td>
<td>64</td>
</tr>
<tr>
<td>Furan, 3-methyl-</td>
<td>898</td>
<td>9</td>
</tr>
<tr>
<td>Furan, 2-ethyl-d</td>
<td>954</td>
<td>72</td>
</tr>
<tr>
<td>Furan, 2-pentyl-d</td>
<td>1225</td>
<td>34</td>
</tr>
<tr>
<td>2-Furan carboxaldehyde</td>
<td>1457</td>
<td>ND</td>
</tr>
<tr>
<td>Benzene</td>
<td>938</td>
<td>135</td>
</tr>
<tr>
<td>Benzene, methyl-</td>
<td>1037</td>
<td>20</td>
</tr>
<tr>
<td>Benzene, ethyl-</td>
<td>1120</td>
<td>2</td>
</tr>
<tr>
<td>Benzene, 1,4-dimethyl-</td>
<td>1128</td>
<td>3</td>
</tr>
<tr>
<td>Benzene, 1,3-dimethyl-</td>
<td>1134</td>
<td>5</td>
</tr>
<tr>
<td>Benzene, 1,2-dimethyl-</td>
<td>1195</td>
<td>3</td>
</tr>
<tr>
<td>Pyrazine, 2,6-dimethyl-</td>
<td>1313</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Sulfur containing compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon disulfide</td>
<td>689</td>
<td>5</td>
</tr>
<tr>
<td>Methane, thioind</td>
<td>730</td>
<td>4</td>
</tr>
<tr>
<td>Disulfide, dimethyl-</td>
<td>1067</td>
<td>180</td>
</tr>
<tr>
<td>Trisulfide, dimethyl</td>
<td>1363</td>
<td>8</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>1964</td>
<td>1</td>
</tr>
<tr>
<td><strong>Miscellaneous compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane, dichloro-</td>
<td>927</td>
<td>4</td>
</tr>
<tr>
<td>Methane, trichloro-</td>
<td>1023</td>
<td>265</td>
</tr>
</tbody>
</table>
(Table 3. continued)

a Average values of three replicates. ND means less than $1 \times 10^6$.
b Experimentally determined retention index.
c These values are for relative humidity controlled from saturated 
solutions of LiCl (11%), MgCl$_2$-6H$_2$O (29.3%), NaBr (49.9%) and KBr (79%).
d Tentatively identified by mass spectrometry.

Six aliphatic hydrocarbons, 7 furans, 7 sulfur containing 
compounds, 8 aldehydes, 7 ketones, 6 chloro-containing 
compounds, 10 alkyl benzenes, 4 alcohols, acetic acid, 
pyrazine and limonene were identified. Many of these compounds 
came from Maillard reaction and/or lipid oxidation. Maillard 
reaction products are believed to be 7 furans, 7 sulfur- 
containing compounds, acetic acid and 2,6-dimethylpyrazine 
(Nursten, 1981). The concentration of the volatile compounds 
increased at higher water activity. The largest peak was 
dimethyl disulfide. The concentration of many compounds 
increased sharply between 29.3% and 49.9% RH. The 
concentration of some aldehydes increased sharply between 
49.9% and 79.0% RH.

Sulfur-containing volatile compounds recovered from 
stored WPC were methane, thiobis; ethanethiol; ethanethioic 
acid, S-methyl ester; disulfide, dimethyl; thiocyanic acid, 
methyl ester; trisulfide, dimethyl; benzothiazole. In the case 
of dimethyl disulfide, the amount was increased sharply 
between 29.3% and 49.9% RH. The effect of $a_w$ on the 
concentration of these compounds during accelerated storage of 
6 days at 60°C is shown in Fig. 6. There are many volatile 
compounds which were believed to come from weed taints and
feed off-flavors. These latter compounds were dimethyl sulfide and dimethyl disulfide (Walker & Gray, 1970; Gordon & Morgan, 1972; Metha et al., 1974). Methanethiol was isolated from starter cultures (Badings & Neeter, 1980). Methanethiol is thought to be essential to Cheddar cheese aroma (Manning, 1974). Dimethyl disulfide is believed to be formed from the oxidation of methanethiol (Ferretti, 1973). This compound has been detected in several dairy products such as UHT milk, milk powder and sterilized concentrated milk (Jaddou et al., 1978; Hall & Anderson 1985; Arnold et al., 1966). These researches reported that the concentration of H$_2$S and methanethiol decreased as the intensity of the heat treatment in milk was increased. These results can be explained if these compounds which are formed on heating react with other molecules, especially with certain intermediates of the Maillard reaction, which increase in concentration at higher temperatures. The concentration of H$_2$S and methanethiol formed in milk during heat treatment decreased in concentration during storage (Jaddou et al., 1978). Also, they found that H$_2$S disappeared within the first week of storage and methanethiol within a month. This result can help to understand why H$_2$S and methanethiol were not found in the present study.

Furan volatile compounds isolated from stored WPC were furan; furan, 2-methyl; furan, 3-methyl; furan, 2-ethyl; furan, 2-pentyl; 2-furancarboxaldehyde and 2-furan methanol.
Figure 6. Effect of water activity on the concentration of sulfur-containing compounds and furan compounds in WPC after accelerated storage.
The effects of $a_w$ on the concentration of these latter compounds during accelerated storage are shown in Fig. 6. The most abundant compound among furan compounds isolated from WPC stored for 6 days at 60°C is 2-methyl furan. Also, Hall and Anderson (1985) isolated 2-methyl furan and 3-methyl furan from milk powder. These furan compounds are supposedly formed from the degradation and dehydration of lactose. 2-pentyl furan was identified as a component of the volatile decomposition products of autoxidized soybean and cottonseed oil. This compound is believed to be formed by autoxidation of linoleic acid (Krishnamurthy, et al., 1967).

The volatile compounds from lipid oxidation were believed to include aldehydes, ketones, esters, alcohols and lactones. Aldehydes that probably originate from lipid oxidation include hexanal, heptanal, octanal and nonanal. The most abundant aldehyde was hexanal. The next abundant compounds was nonanal. Volatile aldehydes isolated from stored WPC were butanal, 2-methyl; butanal, 3-methyl; hexanal; heptanal; octanal and nonanal. The effect of water activity on the formation of these compounds during accelerated storage is shown in Fig. 7. 3-methyl butanal which results from growth of *Streptococcus lactis*, var. *maltigenes* imparts a malty flavor to raw milk. This compound has also been found in pasteurized and sterilized milks (Badings et al., 1985). 3-methylbutanal is proposed to be formed from leucine during nonenzymatic browning (Ramshaw & Dunstone, 1969). 2-methylbutanal was also
Figure 7. Effect of water activity on the concentration of aldehydes and ketones in WPC after accelerated storage.
found in pasteurized and sterilized milks (Badings et al., 1985) and milk powder (Hall & Anderson, 1985). This compound was formed in aqueous solutions from the reaction of methylglyoxal with isoleucine via Strecker degradation (Griffith & Hammond, 1989). The amount of 4-cis-heptanal and hexanal was increased with oxidation flavor defects (Park et al., 1963; Bassette, 1976). The characteristic aroma of 4-cis-heptanal is described as creamy/putty (McGill et al., 1974).

Propanal is one of the dismutation products of linolenic acid hydroperoxide. Hexanal is one of the dismutation products of linoleic acid hydroperoxide.

Five ketone volatile compounds isolated from stored WPC were 2-butanone; 2-heptanone; 2-heptanone, 6-methyl; 2-nonanone and 3-octen-2-one. The effect of \( a_w \) on the concentrations of these compounds during accelerated storage are shown in Fig. 7. The concentration of methyl ketones is reported to be increased with more drastic heat treatments (van Duin, 1965). 1-octen-3-one was involved in oxidation flavor defects in milk. Methyl ketones is not believed to impart a typical Cheddar flavor (Walker & Keen, 1974; Wong et al., 1975).

Paraffin volatile compounds recovered from stored WPC were pentane, hexane, heptane and octane (Table 3). The peak area of paraffin compounds increased from less than \( 1 \times 10^7 \) to \( 5 \times 10^8 \) due to water activity. Paraffin volatile compounds also increased at \( a_w \) above 0.29.
Two chloro-containing volatile compounds isolated from stored WPC were dichloromethane and trichloromethane. The most abundant chloro-containing volatile compound was trichloromethane as shown in Table 3.

Seven benzene volatile compounds isolated from stored WPC were benzene; benzene, methyl-; benzene, ethyl-; benzene, 1,4-dimethyl; benzene, 1,3-dimethyl; benzene, 1,2-dimethyl and benzothiazole. The most abundant benzene volatile compound was benzene. Benzothiazole contributes a rubbery aroma (Parks et al., 1964) and was detected in UHT (Scanlan et al., 1968) and sterilized concentrated (Arnold et al., 1966) milks. Its origin is unknown.

Other volatile compounds isolated from stored WPC were acetic acid, 1-pentanol, 1-octen-3-ol and limonene. 1-octen-3-ol was characterized as mushroom flavor and detected in UHT milks (Scanlan et al., 1968) and in oxidized dairy products (Forss & Stark, 1963). The formation of 1-penten-3-ol was demonstrated in oxidized buttermilk (Stark et al., 1967).

Conclusion

The delicate changes in headspace volatile compounds from WPC during accelerated storage were determined as a function of water activity. Six aliphatic hydrocarbons, 7 furans, 7 sulfur containing compounds, 8 aldehydes, 7 ketones, 6 chloro-containing compounds, 10 alkyl benzenes, 4 alcohols, acetic acid, pyrazine and limonene were identified. In general, the
formation of headspace volatile compounds was greatest at higher water activity values during accelerated storage of WPC.

Literature cited


CHAPTER III

Effect of different salts on lipid oxidation in WPC during accelerated storage.

Lipid oxidation is one of the most important factors of off-flavor formation in dried dairy products during processing and storage. The headspace volatile compounds from lipid oxidation that occur during storage of spray dried WPC are difficult to measure due to simultaneously produced Maillard reaction products.

The recently developed sampling device for dynamic headspace analysis (DHA) with a miniaturized water activity (aw) control chamber for conducting accelerated storage of WPC was used to determine the headspace volatile compounds produced by oxidation of milk fat. This technique is suitable for identifying and monitoring changes in concentrations of volatile lipid oxidation compounds produced in WPC during storage.

The objective of this research was to study the effects of three saturated salt solutions, i.e., NaNO₂, Zn(NO₃)₂ and Mg(NO₃)₂ on the formation of headspace volatile compounds produced by oxidation of milk fat in WPC.

Introduction

Maillard reaction and lipid oxidation are the most common reactions responsible for off-flavor formation of dried dairy products during storage. Even if the lipid content of dried dairy products was decreased to produce lowfat or nonfat dry milks, the residual milkfat can still adversely affect their flavors. For example, WPC generally contain 4-7% lipid. Maillard reaction products may act as antioxidants to inhibit
off-flavor formation from lipid oxidation. However, both mechanisms involve different pathways and result in different products. It should be useful to devise an analytical methodology to distinguish lipid oxidation and Maillard reaction products in stored dairy products. Three conditions of water activity, temperature and nitrous oxide were employed to distinguish between lipid oxidation and Maillard reaction products by DHA.

Procedure

Whey protein concentrate: Commercial whey protein concentrate containing 75% protein and 7% milkfat was obtained from CALPRO (Corona, CA).

Miniaturized aw control chamber: The aw control chamber design is shown in Fig. 4. Saturated slurries of Zn(NO₃)₂, Mg(NO₃)₂ and NaN₃ crystals in water were layered on the bottom of separate 125 ml serum bottles (Cat. No. 223748, Wheaton 400, Millville, NJ) to a depth of 8 mm to control water activity, respectively. These saturated salt solutions provided 15%, 43% and 59% RH at 60°C, respectively. Six 0.5g aliquots of WPC were weighed into separate 10 x 75 mm, borosilicate glass, disposable culture tubes (Cat. No. 60825-538, VWR Scientific, Cleveland, OH). These were placed in each of the serum bottle, which were then sealed with a teflon-coated septum (Cat No. 3-3200M, Supelco, Bellefonte, PA) and an aluminum cap (Cat No. 3-3250M, Supelco).
Accelerated storage of whey protein concentrate: The serum bottles were held 2 days in the dark at room temperature to equilibrate the $a_w$ of the samples. They were subsequently exposed to accelerated storage for 6 days at 60°C in the dark and held in a -20°C freezer until analyzed by DHA.

Dynamic headspace analysis (DHA) of volatile compounds: Serum bottles were held at room temperature, i.e., 20-24°C, for 30 min and attached to the dynamic headspace analyzer (DHA). Headspace volatile compounds were purged 10 min with ultrapure helium carrier gas at a flow rate of 40 ml/min and trapped onto a Tenax TA chemical column (Tekmar, Cincinnati, OH). Trapped volatiles were desorbed by heating the trap for 4 minutes at 160°C and cryogenically focussed at a -150°C capillary interface that was cooled with liquid nitrogen. Focussed compounds were thermally injected onto the capillary gas chromatographic column by heating to 180°C within 1 minute. Compounds were separated with a 30 m x 0.25 mm x 0.25 μm film thickness DB-WAX column (Cat. No. 122-7032, J&W Scientific, Folsom, CA) in a gas chromatograph (Model 5890 Series II, Hewlett Packard). The column was heated from an initial temperature of 32°C to 160°C at a rate of 2°C/min. A total ion chromatograph (TIC) of eluted compounds was obtained with a mass-selective detector (Model 5971A, Hewlett Packard) and compounds were identified by comparison of their retention index values with those of reference compounds (Jenning & Shibamoto, 1980) and by computer-matching their mass spectra
with that in reference NBS49K.L (Hewlett Packard).

Results and Discussion

Total ion chromatograms for volatile compounds isolated from WPC that stored 6 days at 60°C over the three different saturated salt solutions are shown in Fig. 8. Table 4 shows the concentration of volatile compounds isolated from the WPC after accelerated storage.

Figure 8. Total ion chromatogram (TIC) of headspace volatile compounds from whey protein concentrate (WPC) stored over three different salt solutions.
Table 4. Total ion chromatogram peak areas for headspace volatile compounds isolated from WPC stored for 6 days at 60°C and at controlled humidity provided by different saturated salt solutions.

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<tr>
<th>Compounds</th>
<th>$R_l^b$</th>
<th>NaNO$_2$</th>
<th>Zn(NO$_3$)$_2$</th>
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(Table 4. continued)

Miscellaneous compounds

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<sup>a</sup> Average values of three replicates. ND means less than $1 \times 10^{-6}$.

<sup>b</sup> Experimentally determined retention index.

<sup>c</sup> Tentatively identified by mass spectrometry.

Volatile aldehydes isolated from stored WPC included propanal, 2-methyl; butanal, 2-methyl; butanal, 3-methyl; hexanal; heptanal; octanal and nonanal. Concentrations of aldehydes were greater for WPC equilibrated against NaNO₂ than for WPC equilibrated against the other salts (Fig. 9).

Volatile ketones isolated from this study are 2-butanone, 2-pentanone, 2-heptanone, 6-methyl-2-heptanone, 2-octanone, 2-nonanone and 3-octen-2-one. Fig. 10 shows the effect of the three different salt slurries on ketone production. The production of 2-ketones, which are known to be lipid oxidation products, shows the same patterns as the aldehydes. The concentration of 2-ketones decreased with longer chain lengths as shown Fig. 10.

Alcohols identified from accelerated storage of WPC are 1-pentanol and 1-octen-3-ol. Although acetic acid was isolated from stored WPC, its concentration was small. Two sulfur-containing esters were identified as ethanethioic acid, S-methyl ester and thiocyanic acid, methyl ester.
Figure 9. Effect of nitrous oxide and water activity on the concentrations of aldehydes in WPC after accelerated storage.
Figure 10. Effect of nitrous oxide and water activity on the concentrations of ketones in WPC after accelerated storage.
Volatile Maillard reaction and lipid oxidation products were formed at the highest concentrations when stored over NaNO₂. An interesting finding is the identification of pyrazine and 2,6-dimethyl-pyrazine in WPC stored over NaNO₂ solution.

Furan compounds identified from stored WPC are furan; furan, 2-methyl; furan, 3-methyl; furan, 2-ethyl; furan, 2-pentyl; 2-furanmethanol and 2-furancarboxaldehyde. Sulfur containing compounds which are known to be Maillard reaction products included methane, thiobis; dimethyl, disulfide; trisulfide, dimethyl- and benzothiazole.

Conclusions

Use of the different water activity control salt solutions allowed us to study the effects of accelerated storage on lipid oxidation and Maillard reaction separately. The water activity control device over NaNO₂ solutions catalyzed lipid oxidation due to volatile nitrous oxide (Labuza, 1984), so this method may allow us to separate the effects of lipid oxidation and Maillard reaction on flavor stability of dried dairy products.

Literature cited


CHAPTER IV

Changes of headspace volatile compounds due to oxidation of milkfat during accelerated storage of dried dairy products

Flavor quality is one of the most important factors for determining consumer acceptance of dairy and other food products. Lipid oxidation and Maillard browning reactions that occur during storage of spray dried dairy products result in the formation of volatile compounds that greatly impair their flavor quality, thereby limiting their value as ingredients in formulated food products.

Analytical methodologies previously available for isolation, fractionation and identification of volatile compounds formed during storage of dried dairy products are time consuming, tedious and are often subject to error due to artifact formation and incomplete compound recovery. The recently developed dynamic headspace analysis (DHA) technique is suitable for identifying and monitoring changes in concentrations of volatile compounds produced in dried dairy products during storage.

The major objective of this research was to study the changes of headspace volatile compounds produced by oxidation of milkfat in selected, commercial, spray dried dairy products by DHA. A secondary objective was to design a miniaturized water activity ($a_w$) control chamber for conducting accelerated storage studies on dried dairy products, and that can be sampled for headspace volatile compounds.
Introduction

Although considerable research has been done on the development of off-flavors in milk and fermented dairy products over the past 10-15 years, relatively little attention has been given to research on off-flavors in dried milk and dairy products (Ferretti & Flanagan, 1971a; 1971b; 1972; McGugan et al., 1979). Two major mechanisms are usually involved in flavor deterioration of dried milk and dairy products, i.e., lipid oxidation and Maillard browning reaction (McGugan, 1979; Ramshaw & Dunstone, 1969; Min, et al., 1990). Ferretti and Flanagan (1971b) identified 24 volatile compounds in the steam distillate from commercial spray dried whey powder that included alkylpyrazines, furans, pyroles, alkylpyrazines, α-methyl-γ-butyrolactone, isobutyramide, N-methyl-2-pyrrolidinone, 3-hydroxy-2-butanone, benzaldehyde, phenol, benzyl alcohol, maltol, dimethylsulfone, and several organic acids. No attempt was made to distinguish compounds produced by lipid oxidation from those produced by Maillard browning reaction. Although storage temperature is very important for preventing or inhibiting these chemical reactions (Driscoll et al., 1985), moisture and lipid concentrations of the dried products are also important in this regard (Morr & Ha, 1991). Atomization of milk or whey concentrates into ≥ 150°C air during spray drying likely promotes initiation of the lipid oxidation mechanism. Once initiated, reaction of unsaturated lipids with peroxides,
hydroperoxides and free radicals to form the undesirable volatile compounds responsible for the "oxidized" off-flavor would be accelerated.

Oxidized milkfat is commonly described as having an "oxidized" or "cardboard" flavor (Hammond, 1989), which differs from the "stale" flavor formed in dried whey and whey protein concentrate (WPC) products. Oxidation of milkfat results in the formation of undesirable volatile compounds in dried milk and whey products (Hill et al., 1969) and is one of the most important factors limiting the manufacture and utilization of these products. Linoleic, linolenic and several unsaturated fatty acids in milk are especially susceptible to oxidation (Hammond, 1989). Aldehydes and other lipid oxidation products from these unsaturated fatty acids are chemically reactive and are believed to promote the Maillard browning reaction (Hill et al., 1969). Phospholipids are more readily oxidized than the neutral glyceride lipid fraction and is therefore considered as one of the off-flavor precursors in milk (Sattar & deMan, 1975).

Procedure

Dried milk products: commercial dried dairy products obtained for this study included: whole milk powder (WMP) that contained 26% milkfat from Maple Island, Inc., Stillwater, MN; nonfat dry milk (NFMP) that contained 0.8% milkfat, potassium caseinate (PC) that contained ≤ 1% milkfat, and whey protein
concentrate (WPC) that contained 7% milkfat from DMV Ridgeview, La Crosse, WI; whey protein isolate (WPI) that contained < 1% milkfat from Le Sueur Isolates, Le Sueur, MN; and sweet whey powder (WP) that contained 1% milkfat from Holmes Cheese Company, Millersburg, OH.

Miniaturized a control chamber: The a control chamber design is shown in Fig. 4. A saturated slurry of sodium nitrite (NaNO₂) crystals in water was layered on the bottom of a 120 ml serum bottle (cat. no. 223748, Wheaton 400, Millville, NJ) to a depth of 8 mm. This arrangement provided a theoretical aᵢ of 0.59. Six 0.5g aliquots of each dried dairy product were weighed into separate 10 x 75 mm, borosilicate glass, disposable culture tubes (cat. no. 60825-538, VWR Scientific, Cleveland, OH) and sealed with a teflon-coated septum (cat no. 3-3200M, Supelco, Bellefonte, PA) and an aluminum cap (cat no. 3-3250M, Supelco).

Accelerated storage of dried dairy products: The serum bottle was held 2 days in the dark at room temperature to equilibrate aₑ of the samples to 0.59. It was subsequently exposed to accelerated storage for 6 days at 60°C in the dark and stored in a -20°C freezer until analyzed by DHA.

Dynamic headspace analysis (DHA) of volatile compounds: Serum bottles were held at room temperature, i.e., 20-24°C, for 30 min and attached to the dynamic headspace analyzer (DHA) as shown in Fig. 2. Headspace volatile compounds were purged 10 min with ultrapure helium carrier gas at a flow rate
of 40 ml/min and trapped onto a Tenax TA chemical column (Tekmar, Cincinnati, OH). Trapped volatiles were desorbed by heating the trap 4 minutes at 160°C and cryogenically focussed at a -150°C capillary interface that was cooled with liquid nitrogen. Focussed compounds were thermally injected onto the capillary gas chromatographic column by heating to 180°C within 1 minute. Compounds were separated with a 30 m x 0.25 mm x 0.25 μm film thickness DB-WAX column (cat. no. 122-7032, J&W Scientific, Folsom, CA) in a gas chromatograph (model 5890 series II, Hewlett Packard). The column was heated from an initial temperatures of 32°C to 160°C at a rate of 2°C/min. A total ion chromatograph (TIC) of eluted compounds was obtained with a mass-selective detector (model 5971A, Hewlett Packard) and compounds were identified by comparison of their retention index values with those of reference compounds (Jenning & Shibamoto, 1980) and by computer-matching their mass spectra with that in reference NBS49K.L (Hewlett Packard).

Results and Discussion

Volatile compounds in control, dried dairy products: TIC for the six spray dried dairy products prior to accelerated storage are shown in Fig. 11. These control products exhibited a highly acceptable and clean odor.
Figure 11. Total ion chromatogram (TIC) of headspace volatile compounds from whole milk powder (WMP), nonfat milk powder (NFMP), potassium caseinate (PC), whey powder (WP), whey protein concentrate (WPC), and whey protein isolate (WPI) before accelerated storage.
(Figure 11. continued)

Retention time (min)

**whey powder**

**whey protein concentrate**

**whey protein isolate**
The mass spectrum of the unidentified volatile compound exhibiting the largest TIC peak fractionated from all dried milk products except WPC prior to accelerated storage, and with a retention time of approximately 6 min, is presented in Fig. 12. The largest TIC peak eluted from the headspace of WPC with a retention time of 6.8 minutes, was identified as trichloromethane. None of these compounds are believed to be responsible for the off-flavors observed in stored, dried dairy products.

![Figure 12. Ion spectrum of major, unidentified volatile compound with retention time of 6 min isolated from all dried dairy products before accelerated storage by dynamic headspace analysis (DHA).]
Volatile compounds in stored, dried dairy products: TIC of the spray dried dairy products that have been stored for 6 days at 60°C and a_w 0.59 are presented in Fig. 13. Tentative identifications of these volatile compounds are provided in Table 5.

![Graphs showing TIC of volatile compounds in different dairy products](image)

Figure 13. Total ion chromatogram (TIC) of headspace volatile compounds from whole milk powder (WMP), nonfat milk powder (NFMP), potassium caseinate (PC), whey powder (WP), whey protein concentrate (WPC), and whey protein isolate (WPI) after accelerated storage.
(Figure 13. continued)

![Chromatograms of different types of whey proteins.](image)

**Whey Powder**

**Whey Protein Concentrate**

**Whey Protein Isolate**

Retention time (min)
Table 5. Total ion chromatogram peak areas for headspace volatile compounds isolated from spray dried dairy products stored 6 days at 60°C and 0.59 a_w

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<th>Compounds</th>
<th>Rt^b</th>
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<th>NFMP^c</th>
<th>WP^c</th>
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^a Average values of three replicate determinations. ND means less than 1 x 10^6.
^b Experimentally determined retention index.
^c Product identification: WMP = whole milk powder, NFMP = nonfat milk powder, WP = whey powder, and WPC = whey protein concentrate.
^d Tentatively identified by mass spectrometry.
Uniquely different TIC profiles were obtained for each spray dried dairy product after accelerated storage.

Contrary to expectations, NFMP exhibited a TIC profile corresponding to higher concentrations of most volatile compounds than from WMP. Since NFMP contains a much lower concentration of milkfat than WMP, this phenomenon may likely be due to differences in fatty acid composition of the two products. For example, NFMP lipids might be expected to contain predominantly smaller sized milkfat globules with higher proportions of phospholipids and lipoprotein-containing milkfat globule membranes and proportionately less neutral triglycerides than WMP lipids. Concentrations of 18:1, 18:2; and 18:3 fatty acids in bovine milk phospholipids are 46.7, 12.4, and 3.4 mole %, respectively (Morrison, 1970). Differences in phospholipid concentrations in milk and milk products are given by Kurtz (1974). Milkfat in whole milk and skim milk contains 0.87 and 17.29% phospholipid, respectively. These lipid compositional differences could well explain the greater production of volatile compounds in NFMP than in WMP. In addition, differences in the design and operation of the spray dryer as well as in compositional and processing-related factors, i.e., antioxidants, prooxidants, heat treatment, pH, and homogenization, affect the oxidation reaction in milk and dairy products (Weihrauch, 1988).

Major differences were observed in the TIC profiles for WP, WPI and WPC, which were more complicated than for WMP or
NFMP. The reasons for these differences are not known, but may be due to the major variations in processing technologies used to manufacture them. For example, the WP used in this study was made from Swiss cheese whey, a by-product of the cheese manufacturing industry. Milk is subjected to biochemical action of casein coagulating enzymes and lactic acid bacteria during cheese curd formation which may alter the susceptibility of the residual whey lipids to oxidation after spray drying. Membrane fractionation and other processing steps in the manufacture of WPC and WPI may also alter the susceptibility of residual whey lipids after drying. Differences in protein-milkfat ratios in the various whey and milk powders may also result in an altered microstructure of their particles such that the milkfat is more or less protected against oxidation. Differences in polar lipids, lipoproteins and other milkfat globule membrane components may also have a bearing on the formation of volatile compounds in dried dairy products during accelerated storage. The highly unsaturated phospholipid fraction associated with the milkfat globule membrane material is believed to be the site of oxidative deterioration in fluid milk and cream (Badings, 1970).

Potassium caseinate (PC) and WPI, the products with the lowest milkfat concentrations, exhibited considerably less production of headspace volatile compounds than the other products during accelerated storage. The best spray dried
dairy products from a flavor stability standpoint should be PC and WPI, which contain lowest lipid concentrations. However, additional and more costly processing steps, i.e., ion exchange adsorption for making WPI, are required for manufacturing these latter products (Morr, 1992).

Mechanisms for volatile compound formation in dried dairy products: The two main mechanisms for volatile compound formation in dried dairy products during storage are Maillard browning reaction and lipid oxidation (Ferretti & Flanagan, 1972; Ramshaw & Dunstone, 1969; Min, et al., 1990). The substantial concentration of protein and lactose in all of these dried dairy products makes it virtually impossible to rule out the Maillard browning reaction in these studies. In fact, it is possible that these two reactions may be synergistic in nature in dried dairy products. Intermediate and final products from one reaction may actually catalyze the other reaction. Thus, many of the volatile compounds identified in TIC from stored, dried dairy products undoubtedly result from both of these chemical reactions.

Volatile compounds expected to arise from milkfat oxidation, were recovered from the headspace of dried dairy products by DHA after being subjected to accelerated storage for 6 days at 60°C by DHA (Table 5). These included: aldehydes i.e., 2-methyl propanal, butanal, 2-methyl butanal, 3-methyl butanal, hexanal, octanal and nonanal; and ketones, i.e., 2-propanone, 2-butanone, 2-pentanone, 2-heptanone, 6-methyl-2-
heptanone, and 2-nonanone. Acids and esters that were identified included: acetic acid, ethyl ester; ethanethioic acid, S-methyl ester; and thiocyanic acid, methyl ester. Acids and lactones were not detected in the headspace of stored, dried dairy products, due likely to their low volatility at the temperature employed for purging.

Milkfat contains 30% oleic acid among fatty acid and approximately 3% linoleic acid (Dairy Handbook). Oleic acid reportedly breaks down to form octanal, nonanal, 2-undecenal and 2-decenal, and linoleic acid breaks down to form hexanal, 2-octenal and 2,4-decadienal (Kinsella, 1969). Aldehydes and 2-ketones, which possess a common ion fragment with a mass/charge (m/z) ratio of 44 (Silverstein, 1991), were among the most abundant compounds formed by lipid oxidation of dried dairy products in the present study. Chromatograms of all volatile compounds isolated from WMP, WP, and WPC that exhibited a m/z ratio of 44 are shown in Fig. 14. Comparison of results for these three spray dried dairy products indicates that the four most abundant aldehydes were in decreasing order hexanal (RT 8.3 min), heptanal (RT 12.8 min), nonanal (RT 25.2 min), and octanal (RT 18.7 min). These results would not be expected on the basis of the percentages of oleic and linoleic acids.
Figure 14. Forty four ion chromatogram of headspace volatile compounds from nonfat milk powder (NFMP), whey powder (WP), whey protein concentrate (WPC), and whey protein isolate (WPI) after accelerated storage.
WPC stored at 60°C over saturated NaNO₂ solution to maintain an \( a_w \) of 0.59 for 6 days exhibited the highest concentrations of the three aldehydes, hexanal, nonanal, and octanal, of all dried dairy products studied. WPC also showed the poorest flavor stability among the dried dairy products studied. The reasons for the high susceptibility of WPC to lipid oxidation is not known. WPC contained 7% milkfat and the powder was quite fluffy, thus allowing a more intimate contact of substrate with the residual oxygen from the headspace atmosphere in the serum bottle. It is also likely that WPC had a smaller particle size distribution than the other dried products. Information on the fatty acid composition of the residual lipids in WPC is lacking but it is possible that these lipids may contain a higher percentage of unsaturated fatty acids that are more susceptible to oxidation than the other products. It is for this reason that researchers are attempting to remove the residual lipids from cheese whey prior to manufacturing WPC (Morr, 1992).

Water activity is an important factor that affects the off-flavor formation of spray dried dairy products during storage. The rate of lipid oxidation of spray dried dairy products increased above an \( a_w \) of 0.22 (Roozen & Linssen, 1992). However, Maillard reaction products, which are simultaneously formed during storage of dried dairy products, functioned as antioxidants (Waller et al., 1983), thus making it difficult to evaluate the relationship between \( a_w \) and lipid
oxidation rate.

It was shown previously that the use of NaN0₂ for controlling \( a_w \) during accelerated storage resulted in a greater degree of lipid oxidation in dried dairy products than was obtained with other \( a_w \)-controlling chemicals (Labuza, 1984). The release of N₂O by NaN0₂ into the headspace is believed to catalyze lipid oxidation (Labuza, 1984).

**Conclusion**

Although all of the dried dairy products, i.e., whole milk powder, nonfat dry milk, potassium caseinate, whey protein concentrate, whey protein isolate and whey powder, exhibited a clean and bland flavor before accelerated storage, DHA results revealed different susceptibilities to lipid oxidation during 6 days of accelerated storage at 60°C and \( a_w \) of 0.59. DHA 44 ion chromatograms provided a reliable indicator of the degree of oxidation experienced by these dried dairy products during storage. The humidity (\( a_w \))-controlling device effectively controlled \( a_w \) during accelerated storage and also allowed direct sampling of headspace volatiles for DHA of the stored products. The order of increasing lipid oxidation rates for the dried dairy products was: PC, WPI, WMP, NFMP, WP and WPC.
Literature cited


IV. GENERAL CONCLUSIONS

1. A humidity controlling device in a serum bottle was designed to analyze headspace volatile compounds during storage. The humidity ($a_H$)-controlling device effectively controlled $a_w$ during accelerated storage and also allowed direct sampling of headspace volatiles for DHA of the stored products.

2. DHA provided good reproducibility and sensitivity for analyzing the volatile compounds formed during accelerated storage of WPC and other dried dairy products. Reproducibility values of headspace volatile compounds isolated from stored WPC ranged from 4.3 to 14.9% RSD.

3. Volatile Maillard reaction and lipid oxidation products were formed in highest concentrations in WPC stored over saturated NaNO$_2$ solution.

4. The concentrations of headspace volatile lipid oxidation products formed in dried dairy products during accelerated storage increased with temperature, water activity and the presence of N$_2$O.
5. Mass ion (44) chromatograms from commercial dried dairy products were useful for detecting lipid oxidation products formed in dried dairy products during accelerated storage.

6. Commercial spray dried dairy products, i.e., whole milk powder (WMP), nonfat milk powder (NFDM), potassium caseinate (PC), whey protein concentrate (WPC), whey protein isolate (WPI) and whey powder, exhibited a clean and bland flavor before accelerated storage.

7. The order of increasing lipid oxidation rates for the dried dairy products was: PC, WPI, WMP, NFMP, WP and WPC. These trends may be due to differences in the fatty acid composition of these products.
V. BIBLIOGRAPHY


Buckholz, L. I., Withycombe, D. A. and Daun, H. 1980. Application and characteristics of polymer adsorption method used to analyze flavor volatiles from peanuts.


