AN ABSTRACT OF THE THESIS OF

Shilpa S. Mahajan for the degree of Master of Science in Food Science and Technology presented on June 28, 2004.
Title: Aroma Compounds in Sweet Dry Whey

Abstract approved:

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The objective of this study was to identify aroma volatiles in sweet whey powder. Volatiles were isolated by solvent extraction and solvent assisted flavor evaporation. Fractionation was followed to separate acidic volatiles from non-acidic volatiles. Gas chromatography/olfactometry and gas chromatography-mass spectrometry were used for the identification of aroma compounds. Osme methodology was applied to assess the relative importance of each aroma compound. Major free fatty acids detected were acetic, propanoic, butanoic, hexanoic, heptanoic, octanoic, decanoic, dodecanoic and 9-decenoic acids. Major non-acidic compounds detected were hexanal, heptanal, nonanal, phenylacetaldehyde, 1-octen-3-one, methional, 2,6-dimethylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine, furfuryl alcohol, p-cresol, 2-acetyl pyrrole, maltol, furaneol and several lactones. The aroma of

whey powder comprises mainly of curd fermentation products and compounds

formed during further chemical processes such as lipid oxidation and Maillard

reaction.

Aroma Compounds in Sweet Dry Whey

by

Shilpa S. Mahajan

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I understand that my thesis will become part of the permanent collection of Oregon

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Shilpa S. Mahajan, Author

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CONTRIBUTION OF AUTHORS

Dr. Michael Qian was involved in the experimental design and interpretation of data. Dr. Lisbeth Goddik directed with the structure and content of the manuscript.

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THIS THESIS IS DEDICATED TO MY PARENTS-

SHIVANAND V. MAHAJAN AND SHAILAJA S. MAHAJAN.

CHAPTER 1: LITERATURE REVIEW

WHEY

Whey is the liquid substance obtained by separating the coagulum from milk, cream, or skim milk in cheese making (ADPI, 2002). As a by-product of cheese production, whey was earlier discarded as a waste. However, worldwide shortages of protein have directed a considerable effort to the recovery of whey as a food source (Gillies, 1974).

DRY WHEY

Dry whey is the substance obtained by removal of water from whey, while leaving all other constituents in the same relative proportion as in liquid whey (ADPI, 2002). Figure 1 describes the preparation of dry whey from liquid whey.

Dry whey for human consumption complies with all provisions of the U.S. Federal Food, Drug, and Cosmetic Act (ADPI, 2002).

Whey flavor (applies to reconstituted form) is free from undesirable flavors, but may possess the following flavors to a slight degree: bitter, fermented, storage, and utensil; and the following to a definite degree: feed and weedy (ADPI, 2002). Flavor inconsistency and flavors that may carry through to the finished product can limit whey ingredient applications in dairy and nondairy foods. Though whey is used in several products, these products sometimes tend to be

heavily flavored, and off-flavors limit the use of whey in bland or delicately flavored foods (Whetstine et al., 2003).

Whey is generally classified as sweet, and acid (Table 1) depending on the acidity. This in turn depends on the type of cheese from which the whey is derived (Mahoney, 1985; ADPI, 2002). However, considerable variation in composition may occur depending on the milk supply and the nature of processing (Mahoney, 1985).
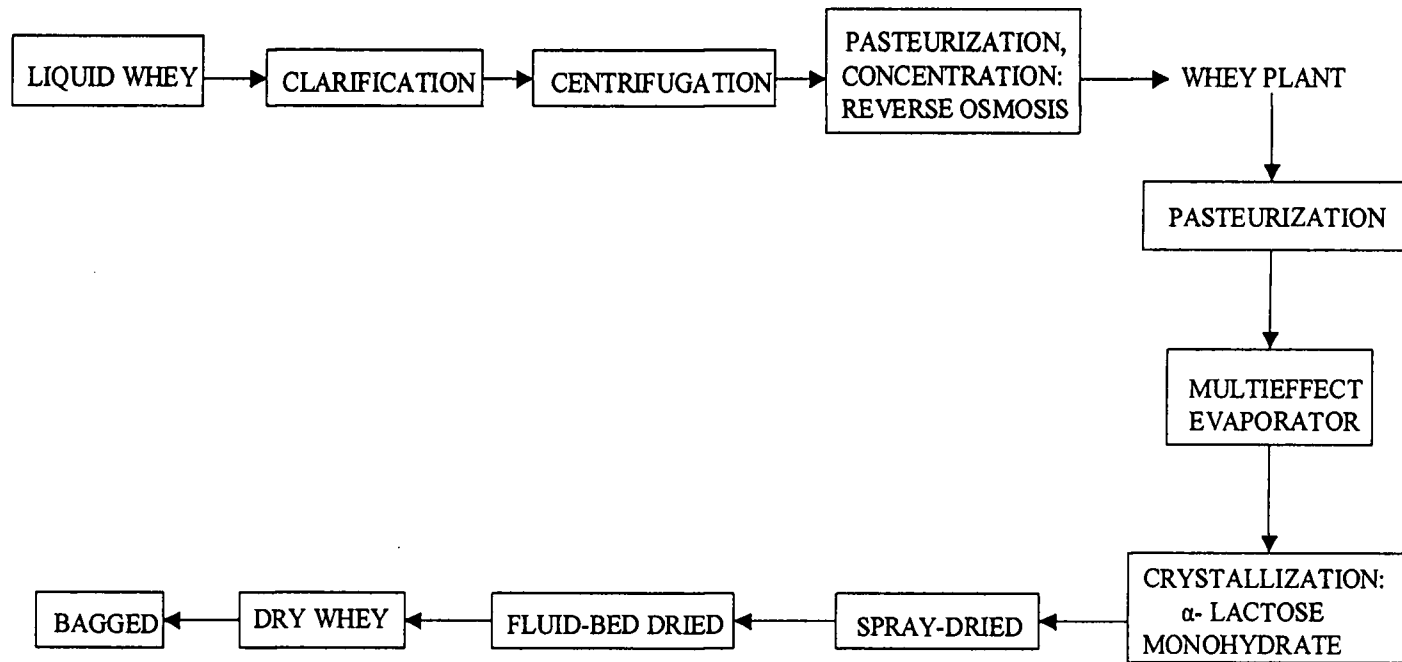
FIGURE 1.1 Preparation of dry whey from liquid whey

TABLE 1.1 Composition Of Dry Whey For Sweet And Acid Type (ADPI, 2002)

	Sweet-type	Acid-type
Constituent	Range	Range
Protein, N X 6.38 (%)	11.1 - 14.9	11.0 - 13.5
Non-protein Nitrogen (%)	0.41 - 0.57	0.52 - 0.64
Fat (%)	0.4 - 1.5	0.2 - 0.7
Lactose (%)	62.1 - 74.6	61.2 - 65.6
Total moisture (%)	3.4 - 6.9	4.3 - 7.3
Free Moisture (%)	1.1 - 4.9	1.6 - 5.0
Alkalinity of Ash (ml, 0.1N HCl/100g product)	54.4 - 195.9	277.0 - 393.0
Total ash	7.1 - 10.1	9.8 - 12.3
Titratable acidity (%)	0.07 - 0.20	0.35 - 0.44
pH	5.2 - 6.4	4.4 - 4.8

WHEY PRODUCTS

SWEET DRY WHEY/ SWEET WHEY POWDER

Dry sweet-type whey is obtained by drying fresh whey, derived from the manufacture of cheeses i.e. Cheddar, Swiss and similar cheeses which has been pasteurized and to which nothing has been added as a preservative. It is obtained from a procedure in which there is insignificant conversion of lactose to lactic acid (ADPI, 2002). It exhibits various applications and functionalities in products such as bakery products, process cheese products, frozen desserts, sauces, meat emulsions, salad dressings, beverages, confections, gravies, soups, meat products, snack foods.

SWEET WHEY POWDER FROM CHEDDAR CHEESE

Cheddar cheese is the most commonly used table cheese (USDEC, 2003) and is the most common natural cheese produced in the United States, accounting for more than 33% of total U.S. cheese production (NASS, 2003). It accounts for a large production as compared to many other cheeses produced in the U.S (NASS, 2003; USDEC, 2003). Hence sweet dry whey generated from Cheddar cheese occupies a large consumer market.

ACID DRY WHEY/ ACID WHEY POWDER

Dry acid-type whey is obtained by drying fresh whey, derived from the manufacture of cheeses like Cottage and Ricotta, which has been pasteurized and to which nothing has been added as a preservative. It is obtained from a procedure in which a significant amount of lactose is converted to lactic acid, or from the curd formation by direct acidification of milk (ADPI, 2002). It exhibits various applications and functionalities in products such as bakery products, prepared mixes, dry blends, salad dressings, snack foods, frozen desserts (sherbets).

REDUCED LACTOSE WHEY

Reduced lactose whey is obtained by the selective removal of lactose from whey. The lactose content of the dry product may not exceed 60%. Removal of lactose is accomplished by physical separation techniques such as precipitation, filtration or dialysis. The acidity of reduced lactose whey may be adjusted by the addition of safe and suitable pH adjusting ingredients (ADPI, 2002). It exhibits various applications and functionalities in products such as infant foods, confections, prepared dry mixes, bakery products, soups, sauces, gravies, dry seasoning blends, salad dressings, frozen foods, meat products.

REDUCED MINERALS WHEY

Reduced Minerals whey is obtained by the removal of a portion of the minerals from pasteurized whey. The dry product may not exceed 7% ash. Reduced minerals whey is produced by physical separation techniques such as precipitation, filtration or dialysis. The acidity of reduced minerals whey may be adjusted by the addition of safe and suitable pH-adjusted ingredients (ADPI, 2002). It exhibits various applications and functionalities in products such as infant foods, dairy products, dry blends, wet blends, confections, prepared dry mixes, bakery products, soups, sauces.

WHEY PROTEIN CONCENTRATE (WPC)

Whey protein concentrate is the substance obtained by the removal of sufficient nonprotein constituents from pasteurized whey so that the finished dry product contains $\geq 25\%$ protein. WPC is produced by physical separation techniques such as filtration or dialysis. The acidity of WPC may be adjusted by the addition of safe and suitable pH adjusting ingredients (ADPI, 2002). WPC34 and WPC80 are the most widely used in food industries. WPC exhibits various applications and functionalities such as water binding, emulsion and foaming in dairy products, dry blends, wet blends, prepared dry mixes, soft drinks/special dietary foods, infant foods, bakery products, confections, frozen desserts.

WHEY PROTEIN ISOLATE (WPI)

Whey protein isolate is obtained by the removal of sufficient nonprotein constituents from whey so that finished dry product contains not less than 90% protein. WPI is produced by physical separation techniques such as precipitation, membrane filtration and/or ion exchange. The acidity of WPI may be adjusted by the addition of safe and suitable pH adjusting ingredients (ADPI, 2002). WPI is known for its bioactive applications in nutraceuticals. It exhibits various applications and functionalities in products such as general protein supplement, protein functionality for gelation (yogurts, pudding), whipping (topping and filling), water-binding (meat, sausage), and emulsification (ice cream, margarine, mayonnaise).

FLAVOR

Flavor, color, shape, texture, and wholesomeness, determine food quality (Acree T., 1993). Of these, flavor plays a major role in the day-to-day and momentary regulation of food intake. Modification of the human diet for whatever reason is unlikely to succeed without taking flavor into account. Indeed, flavor could be a particularly useful medium through which to achieve recommended changes in eating patterns (Thomsan, 1986). Chemical compounds in food cause flavor and are hence important (Acree,1993). Flavor is defined as "The combination of taste and odor. It may be influenced by sensations of pain, heat and

cold, and by tactile sensations" (British Standards Institution, 1975). The challenge

for food science is to produce foods that are appealing, nutritional, profitable and

emotionally satisfying. Flavor is the key to success in this respect (Thomsan,

1986).

Foods are complex mixtures of organic and inorganic compounds, and the

flavor of food is mainly described by its volatile and non-volatile components

(Lawless and Lee, 1993). During the process of consumption, various physical and

chemical entities within a food may selectively stimulate our senses such as

somaaesthetic and kinaesthesis (Thomsan, 1986). The smell of food is composed

of "aroma", olfactory sensations from sniffed volatiles, and "odor", olfactory

sensations retro nasally obtained from volatiles released in the mouth (Acree,

1993; Lawless and Lee, 1993; Etievant et al., 1989).

HUMAN OLFACTORY SYSTEM

The olfactory system is extremely sensitive. If one compares the thresholds

of perception in substances that either smell or taste (i.e. the smallest quantity at

which they can be perceived), the sense of smell appears ten thousand times more

sensitive than that of taste, in that the former can detect certain volatile compounds

at concentrations as low as 10^{-18} molar. The human sense of smell is also

qualitatively more diverse than the sense of taste, and is characterized by an almost

infinite number of odor qualities. The peripheral receptors of the olfactory sense

are special neurons, which are localized in a relatively small area of the mucous-

covered inner surface of the nasal cavity, called the olfactory epithelium (Thomsan, 1986). The model of the olfactory system proposes that an odorant molecule may possess multiple combinations of numerous molecular features called "epitopes" (Firestein, 2001). Humans possess approximately 1000 proteinaceous olfactory receptors, and these receptors recognize different "epitopes" (Pickenhagen, 1989; Firestain, 2001). Most odor molecules are recognized by more than one receptor, and most receptors recognize several odors, "probably related by chemical property" (Firestain, 2001). Current experimental evidence suggests that the olfactory receptors have varied sensitivities to odorant molecular features such as chemical functional group and molecular length. Odor recognition (i.e. strength and quality) is then the function of which receptors are activated, and to what extent. This combinational strategy allows detection of the enormous collection of odors present (Pickenhagen, 1989; Firestein, 2001). However, this olfactory system shows large variation in acuity among the human population. Normal olfactory sensitivity can range up to 100-fold between the least and most sensitive observers (Amoore, 1971).

METHODLOGY

SAMPLE PREPARATION

A food product cannot be directly injected into a gas chromatography (GC) without sample preparation. The high temperature of the injection port will result in the degradation of nonvolatile constituents and create a number of false GC peaks corresponding to the volatile degradation products formed. In addition, very often the constituent of interest is required to be isolated from the food matrix simply to permit concentration such that it is above detectable limits for the GC (Reineccius, 1998). Sample preparation often involves grinding, homogenization, or otherwise reducing particle size. Many foods contain active enzyme systems that will alter the composition of the food product. This is very evident in the area of flavor work (Fleming et al., 1968; Kazeniak and Hall, 1970). Microbial growth or chemical reactions may also occur in the food during sample preparation. Chemical reactions will often result in the formation of volatiles that may give false peaks on the GC. Thus, the sample must be maintained under conditions such that degradation does not occur. Chemical preservation, thermal processing, drying and frozen storage often inhibit microorganisms. Thus sample preparation, component isolation, and concentration prior to GC analysis are important (Reineccius, 1998).

ISOLATION

The isolation procedure used will depend on the food matrix as well as the compounds to be analyzed. The primary considerations are to isolate the compounds of interest and to achieve reliable isolation of specific aroma and flavor compounds from non-volatile food constituents (e.g. carbohydrates, proteins, lipids, vitamins) or those that would interfere with GC (Teranishi and Kint, 1993). Methods for the isolation of volatile substances are headspace analysis, distillation and extraction methods, and the choice of isolation procedure is critical. An improper choice of method or poor technique at this step negates the best gas chromatographic analysis of the isolated solutes (Reineccius, 1998).

SOLVENT EXTRACTION

Solvent extraction is often the preferred method for the recovery of volatiles from foods. Recovery of volatiles will depend upon solvent choice and the solubility of the solutes being extracted. Solvent extractions utilize organic solvents to extract volatiles from food matrices (unless sugars, amino acids, or some other water-soluble components are of interest). Extraction with organic solvents limits the method to the isolation of volatiles from fat-free foods (e.g. wines, breads, fruit and berries, some vegetables, and alcoholic beverages), or an additional procedure must be employed to separate the extracted fat from the isolated volatiles (Reineccius, 1998). The choice of solvent is a function of

extraction time, and target volatiles characteristic (polarity, solubility, etc.). Many solvents are available for extractions, but the main choices are hydrocarbons, halogenated hydrocarbons, and ethers (Teranishi and Kint, 1993). Since many flavor compounds have limited water solubility, the use of apolar solvents rather than polar solvents allow more efficient extractions due to better interaction (Armstrong et al., 1989). Non-polar solvents such as pentane, hexane, or halogenated hydrocarbons are very effective in rejecting water and low boiling alcohols, while use of diethyl ether will extract more water, methanol, and ethanol (Teranishi and Knit, 1993). Solvent extractions may be carried out in quite elaborate equipment, such as supercritical CO_2 extraction, or can be as simple as a batch process in a separatory funnel. Extractions can be broadly classified as batch, continuous, or countercurrent processes. In batch extraction the solute is extracted from one solvent by shaking with a second, immiscible solvent. The solute partitions or distributes itself between two phases and when equilibrium is reached, the partition coefficient is a constant. The phases are allowed to separate, and the layer containing the desired component is removed (Rounds and Nielsen, 1998). Batch extractions can be quite efficient if multiple extractions and extensive shaking are used (Reineccius, et al., 1972). In Continuous extraction, solvent is recycled so that the sample is repeatedly extracted with fresh solvent. Different extractors are used depending on the density of the nonaqueous solvent (Rounds and Nielsen, 1998). In Concurrent extraction, two or more solutes with different partition coefficients are separated from each other by a series of partitions

between two immiscible liquid phases. Liquid-liquid chromatography is a direct extension of countercurrent extraction (Rounds and Nielsen, 1998). Solvent extractions may also pull many non-volatile components into the extract such as lipids that can foul GC systems or thermally degrade producing volatile artifacts (Takeoka and Full, 1997; Reineccius, 1998), and hence the extract may be required to be subjected for additional isolation steps.

DISTILLATION

Distillation processes are effective at isolating volatile compounds from foods for GC analysis. Product moisture or outside steam is used to heat and codistill the volatiles from a food product (Reineccius, 1998). The most commonly used distillation methods for volatile isolation is simultaneous steam distillation-extraction (SDE) (Teranishi and Knit, 1993; Zabetakis and Holden, 1997; Engel et al., 1999). This distillation-extraction may be run for hours with negligible solvent loss (Teranishi and Knit, 1993). However with this method there are concerns over elevated distillation temperatures that may create volatile artifacts, and alter the true sample volatile composition. (Engel et al., 1999).

To reduce distillation artifact formation, Weurman and others developed a high vacuum transfer (HVT) distillation technique suitable for distillation of food directly, or food solvent extracts (Engel et al., 1999). The method uses an extreme temperature differential between two connected vessels to evacuate the volatiles and leave non-volatiles behind. Modification to this technique led to the

development of the Solvent Assisted Flavor Evaporation (SAFE) distillation unit

(Engel et al., 1999).
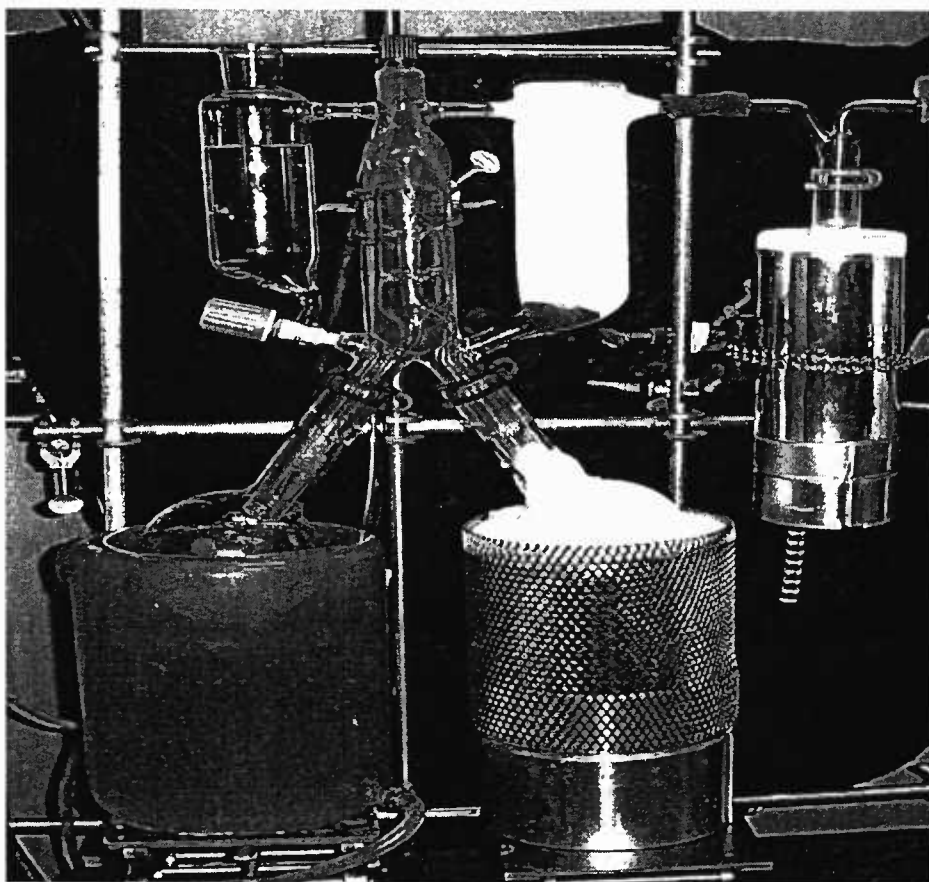
FIGURE 1.2 Solvent Assisted Flavor Evaporation (SAFE)

The apparatus consists of a sample-dropping funnel, a cooling trap, and a central distillation head bearing two "legs", to which is an attached ground glass jointed distillation and a recovery flask. The dropping funnel outlet feeds into the distillation "leg". The vapor inlets to the head and cooling trap are incorporated into the sides of the distillation and recovery "legs", respectively. Sample volatiles are extracted by first thermostatically heating the distillation head and "legs" to the same temperature as that set for distillation flask water bath. Then high vacuum is applied via the outlet in the cooling trap, and the dropping funnel stopcock is closed. The cooling trap and the Dewars flask surrounding the recovery flask are cooled with liquid nitrogen. The dropping funnel is filled with sample, and the sample is introduced into the distillation flask at a rate that will not collect liquid sample in the flask. The sample drops vaporize, and the vaporized volatiles and the solvent are evacuated into the distillation head, and then into the liquid nitrogen cooled recovery flask, where they condense. Non-volatiles remain in the distillation flask, and the extract can be dried and concentrated. SAFE produced higher yields of aromatic compounds from solvent extracts and fatty matrices (50% fat) compared to HVT. The method allows volatiles to be isolated from solvent extracts, aqueous foods such as milk, aqueous food suspensions such as fruit pulps, and even high oil content samples (Engel et al., 1999).

AROMA ANALYSIS

Once the sample has been isolated and fractionated to the desired degree, the next logical step is to determine the identity of one or more of its components (Mussinan, 1993).

GAS CHROMATOGRAPHY (GC) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GCMS)

Gas-chromatography (GC) is the preferred method for volatile compound analysis; in concert with compound separation and aroma characterization (Mussinan, 1993). It is regarded as the forerunner of modern instrumental analysis of volatile organic compounds. Some initial work was published in 1952 (James and Martin), and the method proved to be simple, fast, and appropriate for the separation of many volatile materials.

Gas-liquid chromatography (GLC, also described as simply GC) is the premier qualitative and quantitative analytical technique for flavor compounds (Teranishi and Kint, 1993). In this technique a vaporized sample is injected into the end of a heated fused-silica capillary column. A carrier gas (hydrogen, helium, or nitrogen) carries the volatiles to the column and onto the stationary liquid phase coating the interior; the liquid phase is cross-linked and covalently bonded to the interior surface of the capillary (Cserhati and Forgacs, 1999). The partitioning of

volatiles between the carrier gas phase and stationary liquid phase effects volatile separation (McNair and Miller, 1998; Cserhati and Forgacs, 1999).

Many different detectors and data analysis equipment have been developed to maximize their sensitivity, selectivity, and ability to quantify volatile sample components (Reineccius, 1998; Cserhati and Forgacs, 1999). The flame ionization detector (FID), is extensively used as it has high sensitivity to virtually all organic compounds, and wide linear detection range in response over a wide sample quantity range (10^{-3}-10^{-11} grams injected) (McNair and Miller, 1998). It responds to organics on a weight basis. FID is an excellent general-purpose detector and gives best response for compounds containing C-C or C-H bonds (Reineccius, 1998).

Mass spectrometer (MS) can provide qualitative and quantitative data on a wide range of volatile unknowns (McNair and Miller, 1998; Mussinan, 1993). "A mass spectrometer is an instrument that produces a beam of ions from a substance being investigated, sorts these ions into a spectrum according to their mass-to-charge ratios, and records the relative abundance of each species of ion present" (Busch and Kroha, 1985). Good separation is the key to successful identification. Mixed spectra are much harder to identify and can lead to erroneous conclusions (Mussinan, 1993).

Coupling of a gas chromatograph with a mass spectrometer (GC-MS) provides powerful identification capabilities by permitting mass spectrum on each component of a mixture to be obtained as that component elutes from the GC

(Mussinan, 1993; McNair and Miller, 1998). MS was first coupled to GC in 1959 (Gohlke, 1959), and by the late 1960's dedicated GC-MS systems were being designed to couple analysis speed and resolution of GC to the qualitative (compound structure, composition, molecular weight) and quantitative capabilities of MS (McNair and Miller, 1998). During the most common ionization process, the sample is bombarded with a stream of electrons that causes the chemical bonds to break apart. The resulting fragment ions are accelerated into a mass analyzer, most commonly either a magnetic or quadrupole mass analyzer. Here the ions are separated according to their abundance along a mass scale, and ultimately a bar-graph type "spectrum" is obtained (Silverstein et al., 1974). MS detection limits are 10^{-13} grams (Mussinan, 1993; Zabetakis and Holden, 1997; McNair and Miller, 1998). A mass spectrometer coupled to a GC allows the peaks to be identified or confirmed, and if an unknown is present, since compounds fragment in a set of pattern according to their chemical structure, the fragmentation pattern, or mass spectrum, of it can be identified using a computer-assisted search of a library known as MS spectra (Smith and Thakur, 1998). Library search routines compare each unknown spectrum to literally thousands of reference spectra providing a list of the 5 or 10 closest matches (McFadden, 1973; Silverstein et al., 1974; Aebi and Barnhard, 2002). A number of libraries are commercially available, such as the Wiley, National Institute of Standards and technology (NIST), EPA, NIH libraries (Aebi and Barnhard, 2002; Chem SW, 2004).

GAS CHROMATOGRAPHY-OLFACTOMETRY (GC-O)

Essences are separated into their constituents by using high-resolution GC and GCMS and, the odor is assessed by sniffing the volatiles as they elute from the GC columns. This technique is called gas-chromatography-olfactometry (GC-O) (Fuller et al., 1964). GC-O methods use human subjects to assess the odor quality (Van Ruth, 2001). Relating instrumental data with the sensory data is an essential step for acquiring a better understanding of the products flavor properties and stability. In odor research, the primary use of a sensory function is the assessment of odorant potency (Da Silvia et al., 1994). Once the volatile composition of the sample has been isolated, separated, and identified via GC analysis, the next step is to determine which of the potentially hundreds of identified volatiles actually contribute to the smell of the actual sample (Grosch et al., 1994). Individual volatile contributions to that smell are a function of volatiles potency (odor threshold), concentration, and its perception relative to other odor active volatiles (Takeoka and Full, 1997; Zabetakis and Holden, 1997). Mass spectrometry (MS) identifies the volatiles, while GC-Olfactometry (GC-O) techniques determine which of the volatiles possess odor activity (Van Ruth, 2001). Smell responds more to a change in stimulus than to the magnitude of the stimulus (Acree, T. E., 1993). It is reported that the human olfactory system has a theoretical odor detection limit of about 10^{-19} moles (Mistry et al., 1997), which is far more sensitive than any physical detector (Pollien et al., 1997). Moreover, combining the capability of a capillary column to separate compounds with high sensitivity

comparable to that of the human nose as a detector, GC-O turns out to be an optimal instrument to associate odor with eluting compounds (Boscaini E., et al., 2003). The choice of an olfactometric method depends mainly on the objective of the study, on the quality of the panel, and on the time scheduled for the analysis (Guen et al., 2000).

Food flavor analysis has generated three main olfactometric techniques that use the human nose as an "organic detector". These techniques provide qualitative and quantitative data on odor active volatiles in foods. They are generally described as intensity, dilution and detection frequency methods (Pollien et al., 1997; Guen et al., 2000; Van Ruth, 2001).

INTENSITY METHOD

OSME is the single and oldest intensity method developed in 1990 and is based on modern psychological concepts of odor perception (Miranda-Lopez et al., 1992; Da Silvia et al., 1994). McDaniel et al., (1990) and Miranda-Lopez et al., (1992) developed the *OSME* method. *OSME* is derived from a Greek word "οµ?" meaning smell. It is a quantitative bioassay method used to measure the perceived odor intensity of a compound eluting from a GC olfactometer. The subject rates the intensity of the compound odor by using an intensity device, thus providing an odor-peak. At the same time, verbal descriptions of the odor peak are recorded (Miranda-Lopez, et al., 1992). The *OSME* assessors sniff the GC effluent, and record odor descriptors of the detected volatile, the duration, and the perceived

odor intensity based on a 16-point scale, where 0 = not detected, 1= slight impact (just detected), and 15 = extreme impact (intensity) (Da Silva et al., 1994). By combining the information provided through Osme with the information provided by the gas chromatogram, it is possible to identify which volatile is important for each flavor attribute, and thus assess the significance of each volatile in the flavor system under study (Da Silva et al., 1993). This method is different from dilution analysis in that OSME is not based on odor detection thresholds but on odor intensity (Guen et al., 2000; Miranda-Lopez et al., 1992; Qian and Reineccius, 2002).

The Osme method has been used in several studies such as in wine (McDaniel et al., 1990; Miranda-Lopez, 1992), Parmigiano-Reggiano Cheese (Qian M., and Reineccius G., 2002), hop oils and beer (Sanchez et al., 1992), etc to identify significant odor-active compounds. In one study carried out by McDaniel et al. (1990) on Pinot Noir, the results of four assessors with four replicates were averaged to measure the perceived odor intensity of a compound eluting from a GC. In another study, odor active peak data was generated by first averaging individual assessor's responses (peak detection at least 50% of the time), then averaging those peak responses over all assessors (detection by at least 75% of assessors) (Miranda-Lopez et al., 1992, Sanchez et al., 1992). This method was modified for analysis done on corn snack, where consensus data was still obtained by averaging responses, but the criterion for peak detection was modified to those peaks detected at least once over all samples by at least two assessors. Missing

detections across the assessors were rated zero intensity in the averaging process (Da Silva et al., 1993). This form of data analysis is quite conservative for reporting the existence of an aroma peak; and possibly could lead the researcher to overlook minor odor active compounds. To exemplify this problem, it was considered that aroma peaks detected in all four replications by two of the four subjects, would not be present in the consensus Osmegram, and hence would be considered absent from the sample, despite the fact that it is quite unlikely that two of the four subjects would report a peak with similar odor quality in the same retention time range just by chance. In fact, it was more reasonable to assume that panelists who could not detect the given peak are less sensitive to that particular odorant. In this study, odor peaks detected at least once over all of the samples by at least two subjects, were computed as actual peaks; missing observations across four subjects were computed as 0 ratings in the averaging process (Da Silva et al., 1993).

Two variants of the intensity (*OSME*) method have been examined. One records intensity by movement of a computer mouse along a fixed scale (Guichard et al., 1995), while the other records intensity with a cross-modality method of intensity to finger span (Guichard et al., 1995; Etievant et al., 1999). Both variant methods used model odorant solutions; the finger span method used a trained panel, while the computer mouse method used untrained assessors.

Research by Da Silva et al (1994) indicated that subjects using the Osme method were sensitive, reliable and reproducible and reported odor intensity

change with physical stimulus change. Piggot (1990) reported that the Osme method (McDaniel et al., 1990) was a more effective approach than odor detection threshold techniques (Miranda-Lopez, et al., 1992). Osme combines the modern concepts of sensory descriptive analysis with techniques of computerized data collection. *OSME,* however, shows common characteristics of extensive and time-consuming training of panelists (Da Silva et al., 1994). The modified Osme data analysis is expected to provide the following advantages: it takes into account the concept that panelists show different sensitivities across compounds, it is less conservative in admitting the presence of an odorant in the flavor extract, it is less liberal in reporting differences among samples, it treats data similarly to other sensory techniques, thus allowing for statistical data analysis (Da Silva et al., 1993). It is assumed that OSME is more precise than the other GC-O techniques, but the repeatability and reproducibility of the method must be taken into account (Guen et al., 2000).

DILUTION METHOD

Dilution methods typically use one to three trained assessors to sniff a series of successively more dilute samples until no odors are detected in the GC effluent. Dilution techniques, Charm analysis (Acree et al., 1984; Pollien et al., 1997) and aroma extract dilution analysis (AEDA) (Ulrish and Grosch, 1987; Grosch, 1994) are suitable to screen impact odorants in food. Both methods are based on GC-O of an aroma extract that is diluted until no odor is detected at the

sniffing port. The principal difference between the two methods is that Charm analysis measures dilution value over the entire time the compounds elute, whereas AEDA simply determines the maximum dilution value (Grosch, 1994).

AROMA EXTRACT DILUTION ANALYSIS (AEDA)

AEDA was developed in 1987 and measures maximum relative intensity of odor active volatiles in a sample (Acree, 1993; Hanaoka et al., 2000). A solvent extracted volatile sample is dried and concentrated, and then serial dilutions of 1:2 (or 1:3) are made by addition of solvent, to produce a sequence of 7-10 samples where each member of the series is two (or three) times as concentrated as the next most dilute sample. As the separated volatiles exit the sniffing port, human subjects sniff the volatiles and record those detected by retention time and odor descriptors (Acree, 1993; Hanaoka et al., 2000). For each detected volatile, the greatest dilution (x) at which the odor is still detected is determined. The magnitude of the dilution $(2^x$, or $3^x)$ is called a dilution value (DV), or flavor dilution value (FD), and represents the odor intensity of that volatile in the sample (Ulrish and Grosch, 1987; Acree, 1993). The FD value is the ratio of odorant concentration in the initial extract to the odorant concentration in the greatest dilution at which the odorant is still detected (Grosch, 1994). It is assumed that volatiles with a "high" FD value contribute more to the smell of a food (Mistry et al., 1997; Hanaoka et al., 2000).

CHARM ANALYSIS

Charm analysis was developed in 1984, and is a "continuous AEDA" dilution method (Acree et al., 1984). Sample preparation is identical to that of AEDA, and the primary difference is that Charm analysis uses computer software to record FD values during the entire time the compounds are eluted, whereas AEDA generates only the maximum FD values for compounds (Acree, 1993; Mistry et al., 1997). AEDA output is a single maximum FD value, but the corresponding Charm output is a peak height (maximum FD value) and area ("Charm value", comparable to a chromatographic peak area) (Guichard et al., 1995; Hanaoka et al., 2000). The measure of odor activity in AEDA is the maximum FD value, in Charm analysis it is the Charm value (Takeoka and Full, 1997; Acree, 1993).

The major disadvantage of olfactometric dilution methods is that the relative measures of odor activity do not reflect a volatile's true odor contribution to a food (Van Ruth, 2001), and the odor response is linear to stimulus concentration, and that the linear relationship of all odor active compounds share the same slope (Guichard et al., 1995; Mistry et al., 1997). These suppositions are inconsistent with current sensory knowledge. The relationship of odor intensity versus concentration is a power function, a sigmoidal curve, with different shapes and slopes for different compounds (Acree, 1993; Guichard et al., 1995; Hanaoka et al., 2000). In addition, odorant losses during isolation are not taken into consideration (Buettner and Schieberle, 2001). The long time requirements also

make it difficult to do sample replicates, and check the reproducibility of assessor

results (Mistry et al., 1997). Dilution methods are described as "screening

methods" since their assumptions are not psychophysical, but based on odor

detection thresholds (Grosch, 1994; Guichard et al., 1995; Buettner and

Schieberle, 2001). AEDA requires alteast one judge with a very high sensitivity,

and is time-consuming (Guen et al., 2000).

DETECTION FREQUENCY METHOD

The detection frequency method was proposed by Linssen and others

(1993), and relates the odor intensity of a volatile to the number of experienced

assessors detecting it, either simultaneously in the same GC-O run (via multiple

sniffing ports), or from identical GC-O runs (Pollien et al., 1997; Van Ruth, 2001).

Dummy samples can be used to determine panel olfactory noise, the signal-to-

noise level of the assessors. (Van Ruth et al., 1996). A similar method by Pollien

and others reported satisfactory repeatability of results using untrained assessors

(Pollien et al., 1997). Van Ruth et al. (1995) developed a new technique, the

olfactometry global analysis, which is based on detection frequency method.

Numerous panel members sniffed the nondiluted extract, and the individual

aromagrams were summed. Peak heights were not linked to flavoring intensities

but to their detection frequencies (Guen et al., 2000). The panel used for detection

frequency method is required to have distinct sensitivities to be able to

differentiate enough odors. This method offers the advantage of smoothing

differences between or within individuals because each panelist participates in only 1/n of the final result, n being the number of panelist (Guen et al., 2000). The olfactometric global analysis allows one to obtain results in a short time with no specific panel (Guen et al., 2000). Disadvantages of using the detection frequency method is that it is not based on real intensities (Van Ruth, 2001). It is also not psychologically based, but overcomes limitations from the number of assessors used and the use of detection thresholds by correlating the number of assesssors detecting an odor to the odor's intensity.

SUMMARIZING GC-O METHODS

The development of GC-O for the analysis of food odor active volatiles is a logical extension of earlier GC separation and analysis of volatiles. It is well documented that the human olfactory system is a much more sensitive detector than currently available electronic detectors (Mistry et al., 1997; Pollien et al., 1997). The methods share some analytical concerns. Generally, analytical conditions and assessors qualities should be optimized for GC-O analysis. The effective odorant concentration delivered to the sniffing port is a function of the sample itself, supplemental air flow rate, and GC operating conditions, all of which affect sniffing port chromatographic peak shapes and heights (Van Ruth and O'Connor, 2001). Peak shape and height in turn affects odor perception, as human smell responds more to a stimulus change than stimulus magnitude (Acree, 1993). GC separation conditions (column type, oven temperature program) affect co-

elution, retention time, and resolution of volatiles. These separation characteristics directly affect an assessor's olfactory analysis by either overwhelming the ability to resolve and identify co-eluting or rapidly eluting volatiles, or by altering peak shape and height (Van Ruth, 2001). Although studies have recommended short (25 minutes or less) GC-O runs to avoid assessor fatigue (Acree, 1993; Pollien et al., 1997), others found no significant fatigue effects during 45-minute GC-O runs (Van Ruth and O'Conner, 2001). However, the use of trained panels is supported in the literature as a means to offset wide variation in assessors olfactory acuity, and to standardize odor descriptors (Mistry et al., 1997; Serot et al., 2001). All panels trained or not, display high variability of intensity measures within and between assessors (Pollien et al., 1997; Etievant et al., 1999). Regardless of olfactometric method used, cross-adaptation and compound contrast affects may alter the perceived intensity and odor character of volatiles (Mistry et al., 1997). Varying human sensitivity to odorants is a function of age, gender, genetic contribution, menstrual status, and life experiences (Doty et al., 1984; Stevens, 1991). In spite of infrequent use of *OSME* as designed, the literature implies this method is theoretically more acceptable because it is based on current concepts of psychophysics, and measures "real" intensities (Da Silva et al., 1994). Since all the GC-O methods discussed are significantly affected by the wide variability in sensitivity and reliability of the assessors used, all these methods can be considered to be no more than screening methods for the detection of potent odorant in food (Blank, 1997).

RETENTION INDEX

The retention index of a sample component is a number obtained by

interpolation using the adjusted retention time of the sample component to the

adjusted retention times of the two standards eluted before or after the peak of the

sample component (IUPAC compendium of chemical terminology, 1997).

Retention index determines the relative chromatographic retention for each odor-

active component detected during a sniff run (Acree, 1993). Under constant GC

conditions, the retention time of a compound remains constant. This retention time

is characteristic of the component, and therefore it can be used to identify the

component. Unfortunately, many compounds have the same retention time, and as

a result a positive identification is virtually impossible (Mussinan, 1993). In some

cases, the compound can be rechromatographed on a different stationary phase to

verify its identity. In many cases the absolute retention time is unreliable, and so

relative retention times are often calculated. Relative retention times are obtained

by relating the retention time of the unknown component to that of a standard

compound or a series of standard compounds. Selective detectors can provide

information about the chemical structure of an unknown component, and this

chemical structure information can be used in conjunction with other techniques

such as MS to determine the identity of the component (Mussinan, 1993). A

method of measuring column selectivity is by using retention indexing. Retention

index is the measure of the retentiveness of a compound relative to straight chain

hydrocarbons under a given set of chromatographic conditions. According to

Kovats modified method (IUPAC compendium of chemical terminology, 1997;

Van Den Dool, 1963)-

$$RI = 100Y + 100 \frac{[tr(X)-tr(Y)]}{[tr(Z)-tr(Y)]}$$

where,

$Y < X < Z$

tr= retention time

X= compound of interest

RI= Retention index of X

Y= alkane with Y carbon atoms eluting before compound X

Z= alkane with Z carbon atoms eluting after compound X

The table below summarizes the retention indices of various compounds on

Non-polar and Polar columns.

TABLE 1.2 Retention indices of aroma compounds on non-polar column

COMPOUND	AROMA	LITERATURE REFERENCES (See below)						
		1	2	3	4	5	6	7
		RETENTION INDEX						
Acetaldehyde	Sweet, solvent, ethanolic, pungent malty, green		< 500		<500			
Methanethiol	Putrid, rotten, sulphurous, sweet corn, onion				<500			
Trimethylamine	Fish house, crabby				<500			
Unknown	Rotten, fishy				<500			
Hydrogen sulfide	Rotten egg				<500			
Dimethyl sulfide	Marine, sulfury, canned corn, sulphurous, vegetable			724	<500			
C5 (RI = 500)								
2-Methylpropanal	Malty, dark chocolate, green		551		537			
Methylpropanal	Malty	572						
C6 (RI = 600)								
2,3-Butanedione (Diacetyl)	Buttery		584		606	621		
Acetic acid	Vinegar, sour			734				637
1-Pentene-3-one	Plastic,latex,ink,gasoline		680					
Pentanal	Butter,floral, malty, green, fruity		694	741				

TABLE 1.2 (Continued)

C7 (RI = 700)								
Ethanol				726				
Ethyl 2-methylpropanoate	Fruity	765						
Cyclopentanone				767				
Ethyl disulfide	Gasoline					768		
3-Methyl thiophene	Plastic					774		
1-Hexen-3-one	Plastic, water-bottle, rubbery				784	775		
Hexanal	Green, cut grass, garlic		795	771	808	794	799	
Ethyl butanoate	Bubble gum, fruity, ethereal, pineapple		798					803
C8 (RI = 800)								
Butanoic acid	Cheesy, soapy, faecal, rancid						804	825
Furfuryl alcohol	Vitamin, rubber					813		
2-Furanmethanol	Baked			808				
1-Hexanol				821				
2-Hexenal	Organic, fruity, vegetable		846					
Ethyl 2-methylbutanoate	Fruity, berries, pineapple	850						
Isovaleric acid	Swiss cheese, fecal, Sweaty, dried fruit						876	873
(Z)-4-Heptenal	Rancid, crabby, fishy, Sweet, biscuit-like				900	899	898	
C9 (RI = 900)								
Methional	Potato-like, boiled potato/fermented, roasted	906	897			899		

TABLE 1.2 (Continued)

3-(Methylthio)propanal	Baked potato				907		906	911
2-Heptanol	Mushroom/earthy, spicy, piney, floral, harsh, fruity		908					
Pentanoic acid	Sweaty, swiss cheese, cheesy						908	1041
2-Acetyl-1-pyrroline	Popcorn, roasty				927	916	921	
a-Pinene	Pine		933					
Camphene	Burnt rubber,unpleasant,mint		942					
Benzaldehyde	Musty,fruity,berry,raspberry		953	937				
2-Acetylpyrazine	Popcorn							965
Dimethyltrisulphide	Sulfurous, cabbage, rancid cheese, rotten, cooked cabbage, sewage, garlic	974	964		970	963	969	967
Phenol	Stale			973				
ß-Pinene	Gernium,woody,vegetative		975					
1-Octen-3-one	Mushroom, crayon, metallic		968		979	973	977	981
(Z)-1,5-Octadien-3-one	Metallic, mushroom				982	978		
Ethyl hexanoate	Fruity, fresh, floral, blackberry,sweet,citrus		992					1000
C10 (RI = 1000)								
a-Phellandrene	Tomato,grassy		1000					
Octanal	Fruity, soapy, sweet, wine-like, citrus, lemon				1005			
Hexyl acetate	Banana,floral,fruity		1005					

TABLE 1.2 (Continued)

D,L-Limonene				1017				
2-Acetylthiazole	Grilled hazel nut, popcorn					1018		
Hexanoic acid	Sweaty, vinegar, cheesy, rancid						1020	
Phenylacetaldehyde	Rosy, styrene, honey-like, floral, green	1043				1039		1045
(E)-2-Octenal	Nutty, raw peanut, stale				1045			
(E)-2-Octenal	Fatty, waxy					1060		
2-Octenal	Caramel, organic, coffee		1060					
Furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone)	Sweet, burnt sugar							1065
?-Terpinene	Spicy, floral		1065					
p-Cresol	Cowy, barny, medicine, sour	1079		1173		1070		1074
4-Methylphenol	Barnyard, medicine						1084	
2-Methoxyphenol	Smoky						1086	
2-Methoxyphenol	Smoky							
Maltol	Burnt sugar						1088	
Guaiacol	Spice, smoky							1089
a-Terpinolene	Earthy, chemical, musty		1090					
2-Isopropyl-3-methoxypyrazine	Earthy, soil							1094
Linalool	Sweet, floral, perfume, minty, citrus, plastic, moldy wine		1094	1098				1099
Furaneol	Burnt sugar						1096	
Nonanal	Fruity, soapy, floral		1098	1117				

TABLE 1.2 (Continued)

		C11 (RI = 1100)						
2-Acetyl-2-thiazoline	Popcorn					1103		
Octyl formate	Earthy, citrus, perfume		1110					
2-Phenylethanol	Flowery, rosy	1102				1114		1112
Sotolon (3-hydroxy-4,5-dimethyl-2-(5H)-furanone)	Curry, spicy, seasoning	1107					1108	
Allo-ocimene	Earthy, cooked fruit, green		1128					
(Z)-2-nonenal	Green, fatty, hay					1147		
(E,Z)-2,6-nonadienal	Cucumber-like,green, melon-like			994	1153	1148	1154	1151
2-Nonenal	Vegetative,green fruit,wood,fatty		1156					
(E)-2-Nonenal	Green, fatty, earthy, hay					1155		
2-Isobutyl-3-methoxypyrazine	Bell pepper-like							1174
4-Terpineol	Mold,rotten citrus		1181					
2-Methoxy-4-cresol	Sweet			1191				
Ethyl octanoate	Fruity							1196
		C12 (RI = 1200)						
(E,E)-2,4-nonadienal	Fatty, waxy, soapy					1211		
2,3-Dimethylnaphthalene	Nutty, coffee		1218					
Myrtenol	Bread,hot cereal,pungent		1218					
p-Anisaldehyde	Sweet, fragrance, floral	1244						
l-Carvone	Peppermint, liquorous, mint		1249					

TABLE 1.2 (Continued)

2-Phenylethylacetate	Orange,raspberry,sweet		1257					
Phenylacetic acid	Waxy/rosy, sweet	1263					1265	1262
Benzothiole	Rubbery					1269	1267	
Octanoic acid	Waxy, body odor, sweat, cheesy, goaty, fatty						1281	1281
Indole	Mothball-like						1290	
2-Undecanone	Pine,tea,floral,musty,soap		1292					
C13 (RI = 1300)								
o-Aminoacetophenone	Corn tortilla, grape, foxy					1308	1307	
(E,E)-2,4-Decadienal	Fatty, waxy, fried-fatty					1313		
(E)-2-Undecenal	Metallic					1360		
Eugenol	Benzene,wood,dirt		1360					
ß-Damascenone	Applesauce, cooked apple/grape like, floral, sweet,blackberry	1374	1401				1387	1391
3-Methylindole	Mothball-like, fecal						1396	
(E)-4,5-epoxy-(E)-2-Decenal	Fatty, unripe						1379	
Hexyl hexanoate	Plum,bellpepper,fruity		1384					
Decanoic acid	Soapy, waxy, sour, fatty						1387	
Skatole (3-Methylindole)	Fecal					1391		
Vanillin	Vanilla/candylike	1410					1399	
C14 (RI = 1400)								
Geosmin	Earthy, moistened soil							1417
Coumarin	Wild flower, herbaceous	1456						

TABLE 1.2 (Continued)

Name	Odor	1	2	3	4	5	6	7
ß-Ionone	Hay					1482		
d-Decalactone	Peachy, coconut, sweet, fatty					1490	1494	1490
Elemicin	Floral,sweet spicy	> 1500						
C15 (RI = 1500)								
Unknown	Burnt protein					1547		
4-(4-Hydroxyphenyl)-2-butanone	Sweet, candylike	1555						
C16 (RI = 1600)								
d-Undecalactone	Green, cilantro					1653		
(Z)-6-Dodecenyl-d-lactone	Cheesy, soapy						1662	
?-Dodecalactone	Cheesy, soapy, green, sweet					1675	1680	
C17 (RI = 1700)								
d-Dodecalactone	Cheesy, coconut							1728
?-Decalactone	Peachy, coconut						1768	1467

RI= Retention index

C5, C6....C17= series of alkane containing hydrocarbons from 5, 6...17

#'s 1, 2...7= Literature references, giving the type of sample and the type of column used for its analysis, where-

1. Buckwheat honey, DB5 (Zhou et al., 2002)
2. Marion and Evergreen berries, DB5 (Klesk, K., and Qian, M., 2003)
3. Fermented bamboo shoots, DB5 (Fu et al., 2002)
4. American lobster, DB5 (Lee et al., 2001)

5. Nonfat dry milk, DB5 (Karagul-Yuceer et al., 2002)
6. Rennet casein, DB5 (Karagul-Yuceer et al., 2003)
7. British Farmhouse Cheddar cheese, Dimensions: 30 m X 0.32 mm i.d. X 0.25 μm (Suriyaphan O., et al., 2001)

TABLE 1.3 Retention indices of aroma compounds on polar columns

COMPOUND	AROMA	LITERATURE REFERENCES (See below)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
		RETENTION INDEX																
		C6 (RI = 600)																
Hydrogen sulfide	Rotten Egg		600															
Trimethylamine	Fish House, Crabby		609															
1,3-Pentanediene		624																
Methanethiol	Putrid, Rotten, Sulphurous, Sweet Corn, Onion	635	618	730	500	696	690											
Acetaldehyde	Sweet, Ethanolic, Pungent Malty, Green	645	655		701			677									727	

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TABLE 1.3 (Continued)

Compound	Description							
Formic acid, methyl ester		682						
Carbon disulfide				689				
Unknown	Rotten, Fishy		691					
C7 (RI = 700)								
Dimethyl sulfide	Marine, Sulfury, Canned Corn, Sulphurous, Vegetable	671	703		743			729	
Propanal		712							
2-Methyl propanal	Malty, Green	747							815
2-Propanone		753							
Unknown	Marine, Sulfury, Green							769	
Furan				781					
Acetic acid, methyl ester		782					909		
C8 (RI = 800)									

TABLE 1.3 (Continued)

Compound	Descriptor											
2-Methylpropanal	Malty, Dark Chocolate, Green		855		813							821	
3-Methyl furan		832		898									
Methylpropanal	Malty												839
Acetic acid, ethyl ester		850						921					
3-Methyl furan		858											
2-Butanone		866											
2-Methyl furan				870									
Propanoic acid, methyl ester		872											
Unknown	Roasty					887							
C9 (RI = 900)													
Butanone				900									
Unknown	Pungent					908							
2-Methylbutanal	Malty, Cocoa, Green	880		914	916					918			

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TABLE 1.3 (Continued)

Compound	Descriptor															
3-Methylbutanal	Plastic,Chemical,Yeasty	884		918						922						
3-Methylbutanal	Malty, Green											922				
Ethanol		913						937								
Dichloromethane				927				933								
2,5-Dimethylfuran		930														
3-Methylbutanal	Malty, Dark Chocolate, Sweet, Fruity, Mint, Green		932		921								906			921
Unknown	Fruity, Pyrogenous								931							
3- and 2-Methylbutanal	Malty										945					

TABLE 1.3 (Continued)

Compound	Odor																			
2-Ethyl furan				954																
Unknown	Popcorn								963											
Ethyl propanoate	Sweet, Fruity				955				975											
Ethyl 2-methylpropanoate	Fruity											967								
Pentanal	Butter,Floral, Malty, Green, Fruity			978	935			953	985											
2,3-Butanedione (diacetyl)	Buttery	962	981		978				985			985	970							982	
2-Butanol								988													
2-Pentanone														996							
2,3-Butandione	Buttery, Caramel								980		1000										
C10 (RI = 1000)																					
Methyl 2-methylbutanoate	Fruity, Floral,Sweet																			1014	
Thiophene		1021																			
a-Pinene	Piney																			1023	

TABLE 1.3 (Continued)

Compound	Descriptor																
2-Methyl-3-buten-2-ol													1035				
Trichloromethane				1023													
2-Butenal	Malty, Green				1023					1047							
Ethyl butanoate	Bubble Gum, Fruity, Ethereal, Pineapple				1037					1045							1041
Ethyl 2-methylbutanoate	Fruity, Berries, Pineapple							1047				1048					1023
S-Methyl ethanethioate				1048													
1-Propanol	Plastic								1052								
3-Hexanone		1053															
2,3-Pentandione	Buttery, Cheesy				1053						1054						
Ethyl 3-methylbutanoate	Fruity-Berrylike											1061					
2,3-Pentanedione	Buttery, Grassy	1058							1066								

TABLE 1.3 (Continued)

2-Vinylfuran		1075																
Dimethyl disulfide	Sulfury	1077		1067				904	1074									
Butyl acetate	Fruity, Floral															1075		
Hexanal	Green, Cut Grass, Garlic	1084	1083	1086	1097			1076	1089	1091	1082		1060		1077		1091	<1100
3-Methyl thiophene	Plastic											1078						
2-Methyl thiophene		1097																
Unknown	Grilled								1099									
C11 (RI = 1100)																		
2-Methyl-2-butenal		1102																
2-Methylpropanol	Waxy, Chemical, Mint	1103														1107		
2-Methyl-2-butenal	Green, Fruity									1108								
Unknown	Garlic								1114									
Unknown	Roasty				1116													
3 Penten-2one		1138																

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TABLE 1.3 (Continued)

3,4-Hexanedione		1143																
Ethyl pentanoate	Fruity								1146									
1-Hexen-3-one	Plastic, Water-Bottle, Rubbery		1096									1147						
m-Xylene								1148										
1-Methylpyrrole		1149																
Cyclopentanone							1154											
Unknown	Earthy								1158									
2-Vinylfuran-5-methylfuran		1160																
D,l-Limonene							1169											
ß-myrcene	Grassy, Metallic, Geranium, Pleasant																1169	
3-Penten-2-ol	Grilled							1177										
Unknown	Potato								1177									

TABLE 1.3 (Continued)

Compound	Descriptor																			
1-Ethyl-1H-Pyrrole		1194																		
2-Heptanone	Berry, Fruity, Sweet, Floral (Spicy,Banana), Blue Cheesy, Fruity			1177	1184					1193				1183						1188
Heptanal	Green			1182				1162		1195										
o-Xylene	Plastic Gas								1189											
Ethyl disulfide	Gasoline												1192							
C12 (RI = 1200)																				
Pyridine		1203																		
Unknown	Garlic							1204												
2-Pentylfuran								1215												
3-Methylbutanol	Malty, Fish, Moldy, Green, Wine	1220								1220	1200									1221

TABLE 1.3 (Continued)

Compound	Description														
Butyl 2-methyl-propanoate	Fruity, Sweet							1222							
2-Pentyl furan			1225												
2,5-Dimethylpyrrole		1225													
2-Hexenal	Organic, Fruity, Vegetable													1227	
Pyrazine		1231													
6-Methyl-2-heptanone			1233												
Ethyl hexanoate	Fruity, Fresh, Floral, Blackberry, Sweet, Citrus			1234				1245						1240	
(Z)-4-Heptenal	Sweet, Biscuit-Like								1238		1216				

TABLE 1.3 (Continued)

Compound	Descriptor													
2-Methylpyridine		1239												
(Z)-4-Heptenal	Rancid, Crabby, Fishy		1246									1231		
2-(Methoxymethyl)furan		1251												
(Z)-4-Heptenal	Boiled Potato							1255						
ß-Ocimene	Metal, Baked													1256
Unknown	Herbal, Sour						1257							
1-Pentanol				1259										
Methyl thiocyanate				1262										
3-Methyl-3-buten-1-ol		1264												
1,3-Thiazole		1270												
Unknown	Garlic, Sulfury							1280						
2-Octanone				1281										

TABLE 1.3 (Continued)

Compound	Odor														
2-Methyltetrahydrofuran-3-one		1283													
Octanal	Fruity, Soapy, Sweet, Wine-Like, Citrus, Lemon		1263	1286			1279		1280					1293	
2-Methyl pyrazine		1288													
1-Octen-3-one	Mushroom, Crayon, Metallic		1296						1296		1279		1298	1305	1310
C13 (RI = 1300)															
Cyclopentanol							1300								
Octanal	Citrus Fruit, Orange							1303							

TABLE 1.3 (Continued)

Compound	Descriptor	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13
4-Methylthiazole		1304												
(E)-2-Penten-1-ol	Mushroom						1318							
(Z)-2-Heptenal						1319								
2,3-Dimethylnaphthalene	Nutty, Coffee											1321		
1-Hydroxy-2-propanone		1323												
2-Acetyl-1-pyrroline	Popcorn, Roasty		1333						1330	1311	1327			1342
6-Methyl-5-hepten-2-one						1329								
2-Heptanol	Mushroom/Earthy, Spicy, Piney, Floral, Harsh, Fruity					1321		1332					1335	
3-Methyl-2-buten-1-ol		1337												

TABLE 1.3 (Continued)

Ethyl heptanoate	Fruity									1339								
2-Ethyl-5-methyl-pyrazine	Baked																1341	
(E)-2-Heptenal	Sulfury, Grassy								1342									
2,5-Dimethylpyrazine	Baked	1347															1303	
2,6-Dimethylpyrazine	Nutty, Chocolate	1353		1313						1344							1308	
1-Hexanol								1354										
2-Ethylpyrazine	Nutty	1359							1358									
Dimethyltrisulphide	Sulfurous, Cabbage, Rancid Cheese, Rotten, Cooked Cabbage, Sewage, Garlic		1381	1363	1398					1442	1372	1351	1360		1367		1377	

TABLE 1.3 (Continued)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2,3-Dimethyl pyrazine	Nutty, Coffee	1372							1363								
1-Ethyl-2,3-dimethylbenzene	Plastic							1374									
(Z)-1,5-Octadien-3-one	Geranium-Like									1377							
Allo-ocimene	Earthy, Cooked Fruit, Green																1377
Unknown	Citrus Fruit, Green							1380									
(Z)-1,5-Octadien-3-one	Metallic, Mushroom		1374									1384					
2-Nonanone	Green, Floral, Mint			1386									1389		1395		
Nonanal	Fruity, Soapy, Floral			1390			1386			1386			1389		1399		

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TABLE 1.3 (Continued)

(Z)-3-Hexen-1-ol + dimethyltrisulfide	Woody, Green, Marine								1391												
3-Octanol								1396													
Unknown	Savory							1397													
3-Hexenol	Green Piney, Grassy														1397						
3-Octen-2-one				1399																	1414
C14 (RI = 1400)																					
Cyclohexanol								1403													
2-Acetylthiazole	Grilled Hazel Nut, Popcorn								1667			1404									
2-Ethyl-6-methylpyrazine		1411																			
2-Butoxyethanol	Plastic								1413												
2-Ethylpyridine		1413																			

TABLE 1.3 (Continued)

Compound	Odor	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5-Ethyl-2-methyl-pyridine	Roasted, Baked							1415						1408	
2,4-Hexadienal	Green							1415							
(E)-2-Octenal	Fatty, Waxy								1419		1416				
2-Ethyl-5-methylpyrazine		1419													
2-Octanol							1421								
Trimethylpyrazine	Roasted, Baked							1422						1395	1407
2-Ethyl-3-methylpyrazine		1432													
2-Furfurylthiol	Roasty				1434	1435									
N-Propylpyrazine		1447													
(E)-2-Octenal	Toasted, Cucumber							1451							

TABLE 1.3 (Continued)

Compound	Odor	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17
3-Methylthiopropanal	Baked Potato		1456												1452			
Heptanol								1453										
Methional	Potato-Like, Boiled Potato/Fermented, Roasted				1458	1453	1448	1474	1477	1445	1448	1440	1427				1462	1463
3-Ethyl-2,5--dimethylpyrazine	Roasted, Baked									1455						1435		1449
5-Ethyl-2,3--dimethylpyrazine	Roasted, Baked									1460						1470		
Allyl disulfide								1465										
2-Vinyl pyrazine		1467																
2-Ethyl-3,5-dimethyl-pyrazine		1475																
Acetic acid	Vinegar, Sour	1480		1453		1454	1447	1433						1452		1460		

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TABLE 1.3 (Continued)

Compound	Descriptor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1-Hydroxy-2-propanone acetate		1484															
2-Furancarboxaldehyde	Roasted	1490		1457						1472							
2-Ethyl-1-hexanol		1509												1492			
1-Octen-3-ol	Mushroom/Plastic			1458				1429								1410	1461
(E)-2-Octenal	Nutty, Raw Peanut, Stale		1430														
Tetramethylpyrazine	Baked											1484					
Unknown	Earthy/Barny-Like							1490									
2-Ethyl-1-hexanol								1491									
2-Decanone														1496			
(Z)-2-Nonenal	Green, Fatty, Hay							1532			1496		1478				1508
Unknown	Boiled Potato, Grassy								1499								

TABLE 1.3 (Continued)

		C15 (RI = 1500)																	
Furfuryl formate		1519																	
Methylethenylpyrazine + (e,e)-2,4-heptadienal	Grassy, Marine								1520										
2-Methyl-6-vinyl-pyrazine		1521																	
2,3,5-Trimethyl-6-ethyl-pyrazine	Baked															1521			
6-Ethyl-2,3,5-trimethyl pyrazine	Nutty, Baked									1521									
4-Ethylbenzaldehyde								1521											
3-Mercapto-3-methylbutyl formate	Roasty					1523	1521												
Propanoic acid	Sour, Pungent,							1525										1525	

TABLE 1.3 (Continued)

Benzaldehyde	Musty,Fruity,Berry, Raspberry					1503					1531				
2-Nonanal	Plastic, Fruity						1533								
2-Methylpropanoic acid	Cheesy													1535	
2-Acetylfuran		1536													
Unknown	Bell Pepper/Spicy					1538									
Unknown	Plastic					1541									
1H-Pyrrole		1542													
Linalool	Sweet, Floral,Perfume, Minty,Citrus,Plastic,Moldy Wine					1552									1563
1-Acetyloxy-2-butanone		1554													
(E)-2-Nonenal	Green, Fatty,						1563	1527		1509	1557				

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TABLE 1.3 (Continued)

1-Octanol							1558											
Furfuryl acetate		1559																
Octanol	Organic, Citrus																1576	
Nonyl acetate	Cucumber, Rose, Fresh Grass																1587	
Unknown	Cucumber, Earthy							1587										
Butanoic acid	Cheesy, Soapy, Faecal, Rancid									1613				1588	1640			
(E,Z)-2,6-Nonadienal	Cucumber-Like, Green, Melon-Like		1593				1611		1577		1568		1578					
C16 (RI = 1600)																		
Unknown	Nutty							1604										
5-Methyl fufural		1605																

TABLE 1.3 (Continued)

Compound	Descriptor																			
2(3H)-Furanone, Dihydro								1608												
(E,E)-2,4-Octadienal	Cucumber, Green								1614											
2-Acetyl pyridine	Baked															1615				
1-Phenyl ethanol								1628												
Phenylacetaldehyde	Rosy, Styrene, Honey-Like, Floral, Green							1632		1661	1638	1623	1622			1609				
2-Furfurylfuran		1636																		
2-Furanmethanol	Baked			1644				1640		1676										
(E,Z)-2,4-Nonadienal	Green, Fatty										1647									
2-Furanmethanol	Baked														1655	1616				
Unknown	Nutty								1656											

TABLE 1.3 (Continued)

Compound	Descriptor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2-Formyl-1-methylpyrrole		1661																
Unknown	Dried Hay														1673			
?-Butyrolactone		1673																
3-Methylbutanoic acid	Sour, Sweaty Dry Fruit Like, Cheesy					1684	1676					1653				1630		
Furfuryl alcohol	Vitamin, Rubber	1686											1635					
(E,E)-2,4-=Nonadienal	Fatty, Waxy, Soapy										1698		1681					1709
Pentanoic acid	Sweaty, Swiss Cheese, Cheesy							1712								1698		
C17 (RI = 1700)																		
Unknown	Hay, Saffron												1710					
a-Terpineol	Floral, Over Ripe Fruit																1710	

TABLE 1.3 (Continued)

Unknown	Baked												1710						
Ethylbenzaldehyde	Fruity, Anisic						1735												
Benzyl acetate	Sweet-Candy, Spicy, Liquorice													1740					
2-Acetyl-2-thiazoline	Popcorn										1743								
L-Carvone	Peppermint, Liquorous, Mint													1751					
(E,Z)-2,4-Decadienal	Green, Fatty								1752										1773
Naphthalene	Grilled Earthy						1764												
Unknown	Grilled, Fruity						1784												
(E,E)-2,4-Decadienal	Fatty, Waxy, Fried-Fatty								1804		1793								1818
p-Methyl acetophenone	Fruity,Sweet,Floral													1794					

TABLE 1.3 (Continued)

Compound	Description																
Hexanoic acid	Sweaty, Vinegar, Cheesy, Rancid							1814						1852		1797	
C18 (RI = 1800)																	
Unknown	Nutty							1800									
2-Methyl-1-decanol														1803			
N,N-Dibutylacetamide														1805			
2-Furfuryl methyl disulfide	Meaty	1516				1806	1813										
ß-Damascenone	Applesauce, Cooked Apple/Grape Like, Floral, Sweet,Blackberry									1806	1796			1821		1837	
2-Methoxyphenol	Smoky													1840			

TABLE 1.3 (Continued)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
N-Butylformide	Baked															1863	
2-Methylnaphthalene	Grilled, Earthy						1889										
C19 (RI = 1900)																	
Heptanoic acid	Cheesy, Goaty															1900	
2-Phenylethanol	Flowery, Rosy								1911	1893	1905						
2-Methoxy-4-cresol	Sweet					1915											
Unknown	Cucumber, Grassy						1927										
3-Methylbutaneamide	Baked															1931	
Unknown	Grassy, Boiled Potato						1948										
Maltol	Burnt Sugar									1953							
ß-Ionone	Hay										1954						
Benzothiole	Rubbery												1958				
Benzothiazole	Rubber		1964								1838	1968					

TABLE 1.3 (Continued)

d-Heptalactone										1976							
(E)-2-Undecenal	Metallic									1976							
Unknown	Popcorn											1980					
(E)-4,5-Epoxy-(E)-2-decenal	Fatty, Unripe										1997						
?-Octalactone										1999							
C20 (RI = 2000)																	
Phenol	Stale				1955					2006							
4,4-Epoxy-(E)-2-decenal	Metallic							2006									
p-Anisaldehyde	Sweet, Fragrance, Floral							2009									
2-Tetradecenal										2012							
2,5-Dimethyl-4-Hydroxy-3(2h)-Furanone (DMHF)	Caramel/ Burnt-Sugarlike							2018									

TABLE 1.3 (Continued)

2,6-Dimethylnaphthalene	Grilled, Boiled Potato							2038											
4-Hydroxy-2,5-Dimethyl-3(2H)-Furanone	Caramel-Like				2043	2040													
p-Cresol	Cowy, Barny, Medicine, Sour						2096				2073	2057							
Octanoic acid	Waxy, Body Odor, Sweat, Cheesy, Goaty, Fatty														2069		2008		
4-Methylphenol	Barnyard, Medicine												2077						
Unknown	Minty										2090								
Nonanoic acid	Fatty																		
C21 (RI = 2100)																			
Unknown	Animal										2153								

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TABLE 1.3 (Continued)

Compound	Description																				
?-Decalactone	Peachy, Coconut												2185	2136						
Sotolon (3-Hydroxy-4,5-Dimethyl-2-(5H)-Furanone	Curry, Spicy, Seasoning										2186									
d-Decalactone	Peachy, Coconut, Sweet, Fatty											2190	2229	2184						
2-Methoxy-4-vinyl-phenol	Clove-Like									2194										
C22 (RI = 2200)																				
o-Aminoacetophenone	Corn Tortilla, Grape, Foxy											2204		2202					2225	
3-Phenyl-pyridine	Baked														2216					
Unknown	Animal											2246								
Methylhexanoate	Fruity, Sweet, Pineapple												2251			1194				

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TABLE 1.3 (Continued)

Compound	Descriptor																
Decanoic acid	Soapy, Waxy, Sour, Fatty														2288		2184	
Unknown	Burnt Protein													2293				

C23 (RI = 2300)

Compound	Descriptor																
d-Undecalactone	Green, Cilantro													2356	2345			
10-Undecenoic acid														2358				
Unknown	Sour			2384														
?-Dodecalactone	Cheesy, Soapy, Green, Sweet													2384	2397	2376		
(Z)-6-Dodecenyl-?-lactone	Cheesy, Soapy															2390		

C24 (RI = 2400)

Compound	Descriptor																
?-Dodecenolactone															2427			

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TABLE 1.3 (Continued)

Compound	Description																					
Coumarin	Wild Flower, Herbaceous												2428									
Indole	Mothball-Like															2446						
d-Dodecalactone	Cheesy, Coconut														2466							
?-Tridecalactone															2488							
Skatole (3-Methylindole)	Mothball-Like, Fecal													2468		2489			2491			
C25 (RI = 2500)																						
Dodecanoic acid	Waxy, Soapy														2503		2538					
Phenylacetic acid	Waxy/Rosy, Sweet												2555									
Vanillin	Vanilla/Candylike												2563									
d-Tridecalactone															2565							

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TABLE 1.3 (Continued)

C26 (RI = 2600)																						
?-Tetradecalactone															2628							

C27 (RI = 2700)																						
d-Tetradecalactone															2701							
Tetradecanoic acid															2716							
Dibutyl phthalate															2726							
Tetradecenoic acid															2763							

C28 (RI = 2800)																						
Pentadecanoic acid														2819								
4-(4-Hydroxyphenyl)-2-butanone	Sweet, Candylike											2893										

C29 (RI = 2900)																						

TABLE 1.3 (Continued)

Hexadecanoic acid														2928			

RI= Retention index

C5, C6….C29= series of alkane containing hydrocarbons from 5, 6…29

#'s 1, 2…17= Literature references, giving the type of sample and the type of column used for its analysis, where-

1. Espresso coffee, HP-wax, (Maeztu et al., 2001)
2. American lobster, DB-wax (Lee et al., 2001)
3. Whey Protein concentrate, DB-wax (Lee et al., 1996)
4. Parmigiano Reggiano cheese, HP-FFAP (Qian, M., and Reineccus G. 2002)
5. Coffee drink, DB-wax (Kumazawa and Masuda, 2003)
6. Coffee drink, DB-wax (Kumazawa and Masuda, 2003)
7. Fermented bamboo shoots, DB-wax (Fu et al., 2002)
8. Cooked mussels, DB-wax (Guen et al., 2000)
9. Parmigiano Reggiano cheese, HP-FFAP (Qian, M., and Reineccus G., 2003)
10. Sour dough rye bread, DB-FFAP (Kirchhoff and Schieberle, 2001)
11. Buck wheat honey, DB-FFAP (Zhou et al., 2002)
12. Stored Nonfat dry milk, DB-FFAP (Karagul-Yuceer et al., 2002)
13. Sweetened condensed milk, DB-wax (Shimado, et al., 2001)
14. Rennet cheese, DB-FFAP (Karagil-Yuceer, et al., 2003)
15. Parmigiano Reggiano cheese, DB-FFAP (Qian, M., and Reineccus G.)
16. Marion and Evergreen blackberries, DB-wax (Klesk and Qian, 2003)
17. Corn-based snack, Supelco-wax (Da Silva et al., 1993)

REACTIONS

POSSIBLE SOURCES OF AROMA COMPOUNDS IN SWEET WHEY POWDER

This review describes the various aroma compounds generated in sweet whey powder during the current study and the possible sources for the formation of these compounds. Major compounds determined were short chain fatty acids, aldehydes, ketones, phenols, sulfur-containing compounds, lactones, furans, pyrrones, pyrroles, thiazoles, indoles, and pyrazines. These volatiles are believed to have originated from the breakdown of the major components of milk, volatile chemicals or their secondary reactions, or transfer from the forage.

The primary components present in milk are triacyglycerols, proteins, lactose, and a broad range of vitamins and minerals. Degradation of these components may occur by a wide range of microorganisms that leads to the development of many aroma compounds (White and White, 1999). During the manufacture of cheese, proteins, carbohydrates, lipids undergo an array of chemical changes due to fermentation, proteolysis and lipolysis (Eskin, 1990; Boscaini et al., 2003). Carbohydrates, proteins, and fats are also the major precursors of key flavors that develop on heating foods. The minor ingredients such as vitamins and minerals can also be involved, but their contributions are usually less profound (Scarpellino and Soukup. 1993). However, some of the

water-soluble vitamins, mainly riboflavin, and minerals from milk are lost in whey during cheddar cheese production (Eskin, 1990). In addition, studies on interactions between flavor compounds and macromolecules in model systems have indicated that carbohydrates and proteins can bind, adsorb, entrap, complex, or encapsulate flavor compounds and may also undergo chemical reactions with them (Chevance et al., 2000). Sources for some of the important reactions that may be involved in the formation of aroma compounds in sweet whey powder are described below.

LIPID OXIDATION

The potential for oxidation of milk fat in whey is greater than that of milk fat in whole milk, since the phospholipids, which are rich in unsaturated fatty acids, are at a higher concentration in whey fat compared to their concentration in whole milk (Hammond, 1989). Autoxidation is the reaction with molecular oxygen via a self-catalytic mechanism, and is the main reaction in oxidative deterioration of lipids (Nawar W. F., 1996). Autooxidation is complex and is promoted by heat, light, certain metals and enzymes known as lipoxygenases (Vaclavik V. A., and Christian E. W., 1998). The fatty acids of major importance to oxidation of fats in dried milk and whey products include phospholipids and polyunsaturated fatty acids such as linoleic, linolenic and oleic acid that are susceptible to oxidation (Hammond, 1989). Oxidized flavors result from the oxidation of unsaturated fatty acids by molecular oxygen to form hydroperoxides and is generally a free radical

process (Shipe et al., 1978). The reaction can be mainly separated into three

stages: initiation, propagation and termination. Formation of one free radical leads

to the oxidation of many unsaturated fatty acids. Hydroperoxides are very unstable

and decompose into compounds with short carbon chains, such as volatile fatty

acids, aldehydes and ketones. These are responsible for the characteristic odor of

rancid fats and oils. The termination stage of the reaction involves the reaction of

free radicals to form nonradical products. Elimination of all free radicals is the

only way to halt the oxidation reaction (Vaclavik and Christian, 1998). The initial

products of this oxidation, lipid hydroperoxides are quite bland but degrade rapidly

to hydrocarbons, acids, alcohols, aldehydes, and ketones which elicit undesirable

flavors (Shipe et al., 1978). Korycka-Dahl and Richardson (1980) and Richardson

and Korycka-Dahl (1983) proposed a number of alternative ways for generating

singlet oxygen, including: 1) chemical reaction between any residual hypochlorite

and hydroperoxide in milk; 2) Chemical or enzymatic catalyzed reactions

involving metalloproteins, for example, peroxidase; 3) photochemical oxidation in

the presence of a sensitizer, for example, riboflavin. The formation of free radicals

in the initiation step of autoxidation can also take place by thermal or

photodecomposition of peroxides or hydroperoxides, by metal catalysis and by

ultraviolet irradiation. The exact nature of the initiation step is not fully

understood, although it is known that initiation can be encouraged by suitable

radicals, including those produced by a metal-catalyzed decomposition of

preformed hydroperoxides (Gunstone, 1984). The possible formation of free

radicals in dry milk proteins by mechanical energy has also been reported (Hansen et al., 1970). Through autoxidation, phospholipids have the potential to participate in the generation of off-flavors in milk and whey products. It has been reported that phospholipids have an antioxidant activity due to their mineral ion chelating ability or to their synergistic relation with other antioxidants. At the same time, phospholipids are rather easily oxidized during heating or storage, and may become precursors of off-flavors in the finished products (Eskin, 1990).

DECOMPOSITION OF RIBOFLAVIN

Decomposition of riboflavin by photolysis has been reported to contribute to the undesirable flavors in milk (Sattar and Deman, 1975; Allen and Joseph, 1985) and would presumably be expected to occur in dried whey products as well. They stated that riboflavin is the primary factor responsible for light induced 'oxidized' flavor in fluid milk. Toyasaki et al., (1984) reported the acceleration of riboflavin photolysis in the presence of α-lactalbumin and β-lactoglobulin. They also reported that hydroxyl radicals as well as active oxygen were involved in the decomposition of riboflavin.

LIPID HYDROLYSIS

Bovine milk contains lipolytic activity of which the ß-type esterases are predominant. These include glycerol tricarboxyl esterases, aliphatic esterases,

diesterases and lipases (Downey and Andrews, 1969). Hydrolysis of ester bonds in lipids is catalyzed by enzymes (mainly lipase) or by heat and moisture, resulting in the liberation of free fatty acids. The release of short-chain fatty acids by hydrolysis is responsible for the development of hydrolytic rancidity (Coupland et al., 1996). Lipase activity has been detected in lactic acid bacteria and adventitious bacteria isolated from Cheddar cheese. Psychrotrophic bacteria that possess heat stable lipases are potential contributors to free fatty acid production during cheese ripening (Aston and Dulley, 1982).

FERMENTATION

A "starter" culture of lactic acid bacterium, Lactococcus lactis, is added to milk that ferments the carbohydrates via the hexose diphosphate pathway to pyruvate, which is then reduced to lactic acid and small quantities of acetic acid, (Eskin, M., 1990). Fermentation in whey occurs either by starter or contaminant bacteria. Polar carbonyls like dicarbonyls, a-keto acids, glyoxals and furfurals are formed during fermentation (Wadodkar et al., 2002).

PROTEOLYIS OF PROTEINS

The development of cheese flavor is due to the production of a wide range of compounds formed during the ripening period. Of these, amino acids are reported to have a major contribution. Proteolysis of milk proteins occurs by the

proteolytic enzymes that degrade proteins during the ripening of cheese and releases a range of nitrogenous compounds such as proteoses, peptones, amino acids, and ammonia (Eskin, 1990). The production of free amino acids and smaller peptides is brought about by the starter bacteria (Thomas and Mills, 1981; Eskine, M., 1990). The starter bacteria provide exo- and endopeptidases, although primarily exopeptidases results in selective amino acid production. Low molecular weight peptides mainly contribute to bitter flavors in cheese (Harwalkar et al., 1993). A wide range of decarboxylases have been reported in starter organisms that bring about the decarboxylation of amino acids. Transaminases and deminases are widely distributed in microorganisms catalyzing deamination of amino acids to form aldehydes, which can be oxidized or reduced to the corresponding alcohols or acids. The degradation reaction includes the hydrolysis of amide groups and cleavage of indole or phenol groups from tryptophan or tyrosine by lyases (Eskin M., 1990). Microorganisms can also degrade methionine by cleaving C-S bond. This process, referred to as gamma elimination, involves deamination by a deaminating methionine-demethiolase producing methanethiol (Law and Sharpe, 1978) that is reported to be a precursor for a wide range of sulfur compounds (Dumont and Adda, 1978).

BROWNING REACTIONS

Based on the results obtained for non-fat dry milk, storage temperature would be expected to be one of the most important factors affecting the quality of

spray-dried products (Driscoll et al., 1985; Karagul-Yuceer, et al., 2002). Heat treatments results in flavor changes, such as the appearance of cooked flavors that may carry over into the final product. Browning reactions in food are widespread phenomena, which take place during processing and storage. These reactions affect the flavor, appearance and nutritive value of the food products involved. The factors affecting browning reaction rate in dried food products include temperature, water activity, pH and availability of reactant (Salmarch et al., 1981). The rate of the reaction at low temperature is quite low, however, it is the predominant reaction at temperatures of >35°C, especially in food systems with an intermediate moisture content (Salmarch et al., 1981). The thermal breakdown of sugars can proceed by several mechanisms. These mechanisms have in common the dehydration of hexoses and pentoses. In the production of key flavors from sugars, at least two, and sometimes three, dehydrations are involved. Dehydrations without catalysis require high temperatures. These temperatures can be reduced, and the rates of reaction can be greatly increased by the presence of nitrogen containing bases. Three nonezymatic browning reactions mainly involved in foods are Maillard, Caramelization and Ascorbic acid oxidation. Caramelization reaction can occur both under acidic and alkaline conditions and does not require an amino group. Direct caramelization, which has high activation energy, is not of major consequence in most milk products, although some of the carbon dioxide formed on sterilization of milk can be traced to caramelization of lactose. Caramelization reaction requires sugar but not an amino group. The ascorbic acid oxidation

reaction requires the presence of oxygen and a slightly acidic condition (Eskin M., 1990).

MAILLARD REACTION

Maillard reaction is the most predominant nonenzymatic browning reaction involved in the formation of various aroma compounds in sweet whey powder, involving lactose. This nonenzymatic "browning" reaction was first described by Maillard in 1912 (Maillard. and Hebd, 1912). The Maillard reaction essentially covers all those reactions involving compounds with amino groups and carbonyl groups present in foods. In food products, the nitrogen-containing bases are amino acids. Classically, the Maillard reaction occurs between an aldo or a keto sugar and the amino group of an amino acid. The overall reaction has been divided into three stages. These include amines, amino acids, and proteins interacting with sugars, aldehydes, and ketones, as well as products of lipid oxidation (Mauron, J. 1981). The amino acids have great chemical reactivity with respect to the carbonyl compounds, in particular sugars, according to the Maillard reaction. Reducing sugars is an essential component during the Maillard reaction, providing the carbonyl groups for interaction with the free amino groups of amino acids, peptides, and proteins (Eskin M., 1990). The carbonylamino reaction can develop in acidic or alkaline medium, although it is favored under alkaline conditions, where the amine groups of the amino acids, peptides, and proteins are in the basic form. Increasing the pH also ensures that more of the hexoses are in open chain or

reducing form (Maillard. and Hebd, 1912). Low moisture levels and moderate

storage temperatures are the critical factors affecting the interation between lactose

and protein. It was found that a linear range exists for the rate of reaction over a

temperature range of 0-80°C, with an increased rate with rise in temperature. This

reaction is favored at optimum moisture content (Danehy, 1986) with a maximum

water activity of 0.3-0.7. The maximum rate of browning in whey powder is

belived to occur at water activity of 0.44. The low water activity is related to

transition of lactose from amorphous to crystalline form (Eskin M., 1990). This

well-known reaction occurring in the food industries is generally described as a

dry favored reaction and at high temperature, leading to the intermediates of

Amadori and Heyns evolving by rearrangement and retroaldolization into a-

dicarbonyl compounds (Pripis-Nicolau, et al., 2000). Nucleophilic addition to the

carbonyl of a-dicarbonyl compounds during pasteurization and spray drying of

whey powder may take place because increased temperature favorable to the

reaction occur (Pripis-Nicolau, et al., 2000). The Maillard reaction involves three

distinct pathways. The early reaction involves the formation of the Amodori

compound, 1-amino-1-deoxy-2-ketose, which is uncolored (Finot. et al., 1981).

The second stage involves: dehydration of the Amadori compound by loss of three

molecules of water to form furfurals and reductones; fission, mainly by

dealdolization and Strecker degradation, i.e. the interaction of amino acids and

dicarbonyls. The final stage consists of the conversion of furfurals, fission

products, reductones and Strecker aldehydes into melanoidin pigments, with

further involvement of amines (Finot. et al., 1981; Salmarch et al., 1981; Moller, 1981; Mauron, 1981). The aldehydes formed during the Strecker degradation reaction contribute to flavor (Eskin, 1990).

AROMA COMPOUNDS IN SWEET WHEY POWDER

Attempts have been made to understand the contribution of the above reactions in the formation of aroma compounds in sweet whey powder.

FREE FATTY ACIDS

Any fat remaining in the whey is a source for the development of rancid and oxidized flavors. The free fatty acids in whey are believed to derive from three major sources: breakdown of the fat by lipolysis, lipid oxidation and metabolism of carbohydrates and amino acids by bacteria (Aston and Dulley, 1982; Wadodkar et al., 2002).

The starter culture of cheddar cheese basically consists of lactobacteria such as Lactococcus lactis subsp. lactis and/or L. lactis subsp. cremoris. These microorganisms, in addition to their essential role in milk acidification, are responsible for the production of peptides and amino acids, which subsequently convert to amino acids that play an important in the formation of aroma compounds. Both lactococci and nonstarter lactobacilli have the potential to produce aroma compounds from amino acids (Kieronczyk et al., 2003). The

lactobacillis produce high levels of keto acids and hydroxy acids, while L. lactis subsp. cremoris mainly produced carboxylic acids, which are potent aroma compounds. The carboxylic acids, such as isovaleric acid are very potent aroma compounds that contribute considerably to cheese flavor (Kieronczyk et al., 2003). Branched chain fatty acids are produced from deamination of amino acids i.e., propioni bacteria generate 2-methylpropionic acid from the breakdown of valine (Boscaini et al., 2003).

It was proposed that fermentation produces low molecular weight fatty acids from acetyl CoA. Thus acetic acid can be produced from citrate, lactose and amino acids (Aston and Dulley, 1982). Propionic acid is not normally associated with Cheddar cheese and is believed to originate during fermentation from non-starter Propionobacterium that has been identified in enzyme-modified Cheddar cheese (Kilcawley, 2001). Formic acid is also reported to be a product of fermentation (Varnam and Sutherland 1994b).

The bulk evidence indicates that lipolysis is the principal contributor of free fatty acids of chain length C4 or greater. (Aston and Dulley, 1982; Wadodkar et al., 2002; Kim et al., 2003). The short-chain fatty acids released by lipolysis of milk fat pointed to some fatty acid specificity for lipase. This is due to the physical property of the substrate, as ester groups with short chain fatty acids are less hydrophobic and more likely to be exposed at the oil-water interface compared to ester groups with longer chain fatty acids (Eskin, M., 1990). Octanoic acid was found to have originated from traces of residual fat in whey (Ferretti and

Flanagan, 1971). Decanoic and dodecanoic acids are reported to have soapy

flavors produced by bacterial enzyme activities (Reinnecius, 1991). Specifically

C10-C12 caused a soapy flavor in cheese (Woo and Lindsay, 1982). Hydrolysis of

plant acyglycerols and animal depot fats is the source of soapy-tasting fatty acids

(Lindsay, 1996).

9-decenoic acid is believed to arise from the enzymic oxidative cleavage of

polyunsaturated fatty acids (Salaun et al., 1988).

ALDEHYDES

Aldehydes in sweet whey powder are believed to be formed mainly by the

autooxidation of unsaturated fatty acids and Strecker degradation reaction.

Aldehydes are the major secondary products of autoxidation of unsaturated

fatty acids (Boscaini et al., 2003; Bendall, 2001; Wadodkar et al., 2002).

Aldehydes like n-alkanals, alka-2-enals, alka-2,4-dienals are known to be formed

by the autooxidation of unsaturated fatty acids of milk fat (Wadodkar et al., 2002).

Heptanal was reported in the thermally decomposed methyl oleate hydroperoxides

from photosensitized oxidation and autoxidation (Frankel, 1980). Nonanal, which

is the decomposition product of oleic acid, was detected in goat's milk cheese due

to the high contents of oleic acid (Kim et al., 2003). Hexanal is known to be a

prominent secondary oxidation product of linoleic acid (Ulberth and Roubicek

1995; Karagul-Yuceer et al., 2002). 2,4-dienals, trienals and cis-4-heptenal have

been implicated as being responsible for off-flavors in products that have

undergone milkfat oxidation. Grosch and co-workers (1994) reported (E)-2-nonenal and (E,E)-2,4-decadienal to be formed due to the effect of light exposure in butter oil.

Aldehydes are also produced by amino acid catabolism involving decarboxylation to amines, followed by oxidation via Strecker degradation to aldehydes (Boscaini et al., 2003). In this reaction, an amino acid reacts with a dicarbonyl compound to produce an aldehyde that has one carbon atom less than the original amino acid (Aston and Dulley, 1982). A variety of sources of dicarbonyl compounds in foods such as diacetyl and pentadione can participate in Strecker degradation. Other possible reactants are dehydroascorbic acid, 1-deoxyhexosone from the Amadori reaction, and the cyclic enolones (maltol, cyclotene, furaneol, etc.), which have diketo tautomeric forms. The reaction of oxygen with unsaturated fatty acids results in the formation of hydroperoxides which decompose to form a multitude of aldehyde and related chemical compounds (Frankel, 1980; Hammond, 1989) that are reactive and are believed to promote the Maillard browning reaction. Phenylacetaldehyde, which is a Strecker degradation product of amino acid phenylalanine, was found to be associated with floral aroma (Hoffman, 1989; Karagul-Yuceer et al., 2002).

KETONES

Diacetyl is considered to be a product of microbial action, involving lactic acid bacteria and citrate metabolism (Boscaini et al., 2003). It is known to be a

product of microbial action, involving *Lactococcus lactis* ssp. *lactis* biovar

diacetylactis and citrate metabolism (Bendall, 2001; Kaneko et al., 1987;

Kieronczyk et al., 2003). Metabolism of citrate by Streptococcus diactylactis also

leads to the production of diacetyl, which contributes to the flavor (Fox et al.,

1990). Both fermentation and autooxidation are reported to be involved in the

formation of 1-octen-3-one (Boscaini et al., 2003). Cadwallader and Howard

(1998) identified 1-octen-3-one as an aroma compound formed due to light

activation.

SULFUR-CONTAINING COMPOUNDS

Several sulfur-containing compounds have been identified as key odor

compounds that are formed by Maillard pathways involving cysteine, or possibly

from the thermal degradation of thiamine (Chevance and Farmer, 1999). When

methionine undergoes Strecker degradation, the main product is methional (Aston

and Dulley, 1982). Badings (1991) proposed that "sunlight" flavor in milk was due

to methional produced in light catalyzed reaction between methionine and

riboflavin. Methionine is also a well-known precursor of sulfides. Methional

formed is capable of further breakdown to methanethiol (Aston and Dulley, 1982),

which can further oxidze by radical degradation to form dimethyl disulfide and

dimethyl trisulfide (Counet et al., 2002; Boscaini et al., 2003; Bendall, 2001). It

has also been postulated that methanethiol is produced by non-enzymic chemical

reactions involving addition or substitution reactions between hydrogen sulfide

and casein or methionine. Starter microorganisms are believed to produce the reducing conditions that favor the production of methanethiol (Varnam and Sutherland, 1994a).

SIX-MEMBERED HETEROCYCLES

Maltol is known to be a product of Maillard reaction through sugar degradation and the amount of reducing sugar present can greatly influence the qualitative and quantitative presence of this compound. Glucose (a reducing sugar) is also formed during the enzymatic fermentation of lactose (Wadodkar et al., 2002). Maltol is formed by the heating of the hexose reductone produced in the transformations of Amadori products. The major product produced is 5-hydroxydihydromaltol, which can dehydrate to maltol. (Maillard, L. and Hebd C., 1912).

Pyrazines are heterocyclic nitrogen-containing compounds important to the flavor of many foods and have been recognized to be important flavor ingredients. They are of interest for the aroma chemistry because of their high odor potency and their characteristic sensory properties (Klocker et al., 2002). They are reported to be formed by Maillard reaction through Strecker degradation from nitrogen sources such as amino acids. They possess mostly burnt aroma notes and are found among the volatiles of most heated foods ((Alasalvar et al., 2003). Several researchers have proposed mechanisms for alkylpyrazines formation in various carbohydrate/amine systems. These pathways generally involve the formation of

aminocarbonyl fragments which condense, yielding dihydropyrazines or hydroxyl dihydropyrazines. These in turn yield pyrazines through oxidation (Buttery R. et al., 1977) or dehydration reactions (Eskin, 1990). Pyrazines show a broad spectrum of aroma impressions, whereby small differences in the structure of the four substituents and changes in their relative position at the heteroaromatic ring cause significant modifications of the aroma properties. The nutty-sweet smelling pyrazines contain only up to two carbon atoms at this substituent and can therefore be separated from other sensory properties by this descriptor. (Klocker et al., 2002). Alkylpyrazines are aroma active molecules that impart nutty, roasted, or earthy tonalities. In processed foods, the generation of alkylpyrazines is well-known to be associated with the Maillard reaction or pyrolysis reactions (e.g., roasted coffee). (Kurniadi, 2003). Free amino acids and monosaccharides are essential flavor precursors for the development of the unique flavors generated during roasting and give rise to pyrazines via Maillard sugaramine-type reactions. Another proposed mechanism for the formation of pyrazines during heating of foods involves the reaction of two α-amino carbonyl compounds, which arise either from an Amadori reaction or from Strecker degradation. Strecker degrdation of amino acids lead to the formation of very reactive a-amino ketones, which are the precursors of the formation of pyrazines by cyclic duplication (Laura Pripis-Nicolau, et al., 2000). The amino group of each molecule adds to the carbonyl group of the other to form a six-membered ring that contains two nitrogens and that has two hydroxyl groups attached. The loss of two molecules of water leaves a

ring with two nitrogens and two degrees of unsaturation. This compound oxidizes readily to produce a third degree of unsaturation, which results in a pyrazine. If both α-amino carbonyls are from diacetyl, the product is the parent compound, pyrazine. If one of the α-amino carbonyls is from diacetyl and the other from 2,3-pentadione, the product is methyl pyrazine. If both α-amino acids are from pentadione, the product is dimethylpyrazine. Formation of dimethylpyrazine is attributed to the condensation of two 3-carbon sugar fragments and nitrogen (Eskin, 1990). 2,5-dimethylpyrazine and 2,6-dimethylpyrazine are formed in the presence of methylglyoxal and cysteine (Laura Pripis-Nicolau, et al., 2000). The presence of an ethyl group in ethylpyrazine suggests a key role of alanine (Counet et al, 2002).

The non-enzymatic deamidation of proteins is a reaction commonly occurring during processing. It may take place by the direct hydrolysis of the amide bond in which an acid or a base acts as a catalyst to form an amino acid. The deamidation of an amide residue can release ammonia that can react with reducing sugars in much the same way as free amino acids (Ho et al., 1993). The formation of acetylpyrazine involves the addition of ammonia (resulting from protein breakdown) to a dicarbonyl compound. The resulting product reacts with C-methyltriose reductone from a sugar retro-aldol reaction to form an intermediate that loses two water molecules to form an acetylpyrazine. Many of the pyrazines have a nutlike aroma similar to that of peanuts. As a pyrazine becomes more complex and more alkyl-substituted, it loses its water solubility (Welty, 2001).

Pyrazines may be present in low concentrations but collectively may have a beneficial effect on cheddar flavor (Aston and Dulley, 1982). The pyrazines are the key contributors to nut flavors, and they play an important role in the flavor of heated foods, especially those that experience high temperatures, such as coffee, chocolate, cereals and meat. Pyrazines also contribute significantly to potato flavor (Ohloff., 1986).

FIVE-MEMBERED HETEROCYCLES

Reductones, dehydroreductones, and deoxyhexosones formed during Maillard reaction are important intermediates in the development of the key aroma compounds from carbohydrates. These intermediates also can play an important role in the catalysis of amino acid breakdown. The dehydroreductone from pentose can form a ring and dehydrate to form furfural (Anet E., 1964). Another important reaction in sugar breakdown is the retro-aldol reaction, which is the reverse of the aldol condensation. In the retro-aldol reaction, the unsaturated carbonyl group is hydrated at the double bond to form the α-hydroxy compound that can reverse to the two starting carbonyl compounds (Lane M. J. and Nursten H. J. 1983; Josephson, D. B. and Glinka J. 1989). In sugar breakdown, the retro-aldol reaction usually involves α-hydroxy carbonyls that revert to smaller carbonyl fragments. The retro-aldol condensations of deoxyhexosones to two- to four-carbon compounds are extremely important in the formation of heterocyclic flavor

compounds (Maillard, L. and Hebd C., 1912). Aldehydes produced by the Strecker degradation of the amino acids phase of the Maillard browning reaction undergo condensation and a series of further reactions to form furfurals and dehydration products (Hammond, 1989).

Furans arise from amino acids and sugars through Maillard and Strecker degradation reactions (Alasalvar, 2003). Sotolon could be formed by the aldolic condensation between acetaldehyde and 2-ketobutyric acid produced from threonine due to an enzymatic reaction. A strictly chemical degradation of threonine into 2-ketobutyric acid in acidic conditions has also been suggested (Takahashi et al., 1976). In addition, other studies have shown that 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) can be formed by the Maillard reaction as a result of condensation of molecules such as butane-2,3-dione (diacetyl) and hydroxyacetaldehyde which can arise from this mechanism (Hofmann and Schieberle, 1995).

Furfuryl alcohol is believed to be a degradation product of furfural (Karagul-Yuceer, et al., 2002).

The compounds 2-acetyl-1-pyrroline and 2-propionyl-1-pyrroline were suggested as potent flavor compounds that exhibit roasty odors in popcorn. 2-acetyl-1-pyrroline and 2-propionyl-1-pyrroline possess a very low odor threshold and are frequently cited as compounds that directly contribute to roasted or smoky flavors (Da Silva, M. A. et al., 1993). 2-Acetyl-1-pyrroline is formed by the

interaction of pyruvaldehyde with 1-pyrroline, which is formed from amino acids. (Bendall, 2001) by Strecker degradation reaction (Eskin, 1990).

2,5-dimethyl-4-hydroxy-3(2H)-furanone (furoneol) is a product of sugar degradation/fragmentation in the Maillard reaction. Its odor has been described as caramel-like and tends to become fruity- and strawberry-like at low concentrations (Da Silva, M. A. et al., 1993).

2-acetylthiazole is often cited as the product of Maillard reaction formed in the presence of cysteine and methylglyoxal (Hoffmann and Schieberle, 1995; Meynier and Mottram, 1995).

Skatole is strongly associated with the unpleasant smell of cow's feces. They are produced from the amino acid L-tryptophan in the cow's rumen. Cows fed on a pasture diet receive more protein than cows fed on a supplement diet; however, as the pasture diet has less energy, much of the protein is broken down so that the gluconeogenic amino acids can be used as an energy source. Because L-tryptophan is not a gluconeogenic amino acid, the rumen of a pasture-fed cow will have to cope with far more free L-tryptophan than the rumen of a supplement-fed cow. More skatole will be produced and a small proportion of these powerful aroma compounds will enter the milk. Thus, the milk of pasture-fed cows was found to have higher concentrations of skatole than the milk of supplement-fed cows. (Bendall, 2001). Skatole is formed from indole acetic acid (IAA). Conversion of L-tryptophan to IAA occurs via three distinct route: 1) indole acetamide pathway catalyzed by Try 2-monoxygenase and indole acetamide

hydrolase 2) catalyzed by tryptophan side-chain oxidase and indole acetaldehyde

dehydrogenase 3) the indole pyruvic acid pathway which is initiated by Try

aminotransferase (Ummadi and Weimer, 2001).

Sweet whey powder showed some animal and cattle-like, odors. p-Cresol

and skatole may be contributors of these undesirable odors. Some compounds

released from the forage by the cow's metabolism may induce their development.

For example, certain types of weeds (Crucifera), lucerne, or *Brassica* sp, may

increase the concentration of indole, skatole, mercaptans, sulfides, nitriles and

thiocyanates (Forss, D. A. 1979). Loss of alanine moiety from tyrosine and

tryptophan causes the formation of phenol and indole respectively. Their

homologues, p-cresol is reported to contribute to the flavor of soft cheeses and

British Farmhouse Cheddar cheese and skatole is reported to contribute to the

flavor of soft cheeses (Suriyaphan et al., 2001).

PHENOLS

Phenolic compounds such as p-cresol, has long been known as the agent

responsible for the smell of cow urine imparting a barn-like/medicine-like flavor to

milk upon liberation from its precursors (Bendall, 2001; Kim et al., 2003). A

combination of p-cresol and acetic acid enhances the odor intensity of both

components in a synergistic manner that is more pronounced than the odors of

either component perceived individually. (Fu, 2002). p-Cresol is believed to be an

end-product of protein breakdown (Vanholder R., et al., 1999). It is one of the

metabolites of the amino acid tyrosine, and to a certain extent also phenylalanine

which by decarboxylation and putrefaction are converted to p-cresol (Curtius et al.,

1976; Vanholder et al., 1999).

LACTONES

Lactones are the products of lipid oxidation at elevated temperature

(Friedman, 1996). The C8 and C18 delta lactones and C12, C14 and C16 gamma

lactones have been identified in cheddar cheese extracts. Some correlation was

found between flavor and level of lactones and thus was concluded that lactones

influence cheese flavor directly and as modifying or blending agent on the overall

flavor. (Aston and Dulley, 1982). Lactones are produced during processing either

by hydrolysis of lactogenic glycerides to hydroxyl acids followed by ring closure

due to dehydration (lactonization) or by lipolysis of glycerides before ring closure

(Wadodkar et al., 2002). Esters are important aroma compounds that are formed by

many organisms (Winterhalter and Schreier, 1993). Hydroxy fatty acids occur as

natural components in many food materials (Friedman, 1996). In those cases

where a five- or six- membered ring can be formed, intramolecular esterification

occurs. Thus, a ?- or d- hydroxy acid loses water spontaneously to yield a cyclic

ester known as a lactone (Morrison and Boyd, 1996).

Based on the components present in milk and the various processes it

undergoes during the formation of whey powder, fermentation, proteolysis,

lipolysis, lipid oxidation and Maillard reactions are believed to be the principal

reactions responsible for the aroma compounds in sweet whey powder. Although the above mentioned reactions are considered to be the possible sources for the formation of these aroma compounds, appropriate experimental study is required to fully understand and validate its exact origin.

CHAPTER 2: AROMA COMPOUNDS IN SWEET WHEY POWDER

AROMA COMPOUNDS IN SWEET WHEY POWDER

S. S. Mahajan, L. Meunier-Goddik, M. Qian

ABSTRACT

The objective of this study was to identify aroma volatiles in sweet whey powder. Volatiles were isolated by solvent extraction and solvent assisted flavor evaporation. Fractionation was followed to separate acidic volatiles from non-acidic volatiles. Gas chromatography/olfactometry and Gas chromatography-mass spectrometry were used for the identification of aroma compounds. Osme methodology was applied to assess the relative importance of each aroma compound. Major free fatty acids detected were acetic, propanoic, butanoic, hexanoic, heptanoic, octanoic, decanoic, dodecanoic and 9-decenoic acids. Major non-acidic compounds detected were hexanal, heptanal, nonanal, phenylacetaldehyde, 1-octen-3-one, methional, 2,6-dimethylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine, furfuryl alcohol, p-cresol, 2-acetyl pyrrole, maltol, furaneol and several lactones. The aroma of whey powder comprises mainly of curd fermentation products and compounds formed during further chemical process such as lipid oxidation and Maillard reaction.

(**Key words:** sweet whey powder, aroma, Osme)

Abbreviation key: **GC** = gas chromatography, **GCMS** = gas chromatography mass spectrometry, **GCO** = gas chromatography olfactometry, **RI** = retention index, **SAFE** = solvent assisted flavor evaporation.

INTRODUCTION

Sweet whey powder is obtained by drying fresh fluid whey collected from the manufacture of Cheddar, Swiss, Mozzarella, Monterey Jack and similar cheeses. It typically contains about 70% lactose, 1.5% fat, 12% proteins, 4% moisture and 8.5% other solids (USDA, 2000). Whey has become an important ingredient in dairy and other food products (Whetstine et al., 2003) with a total production above 1.3 billion lbs in 2003 (USDA, 2004). In spite of its large use, whey is often implicated as having stale undesirable flavors that are unpleasant to consumers and limits its use in bland or delicately flavored foods (Whetstine et al., 2003).

Flavor quality in liquid whey has been evaluated recently. Whetstine et al. (2003) found that whey made from different types of cheese have different flavor, and flavor variability also exists within wheys from one cheese type. Tomaino et al. (2004) studied flavor variability of liquid whey produced from lab-scale Cheddar cheese using individual strains of *Lactococcus lactis* subsp. *lactis*. They demonstrated that there are differences in liquid whey produced in a noncommercial setting from different starter culture strains. It is possible that if flavor variability in liquid whey can be diminished, then a better quality of whey powder could be produced and the utilization of whey and whey ingredients could be increased.

Aroma compounds identified in liquid whey include 2,3-butanedione, 2-butanol, hexanal, 2-acetyl-1-pyrroline, methional, (E,E)-2,4-nonadienal, (E,E)-2,4-

decadienal, and various short-chain volatile acids (Karagul-Yuceer et al., 2003). Those compounds are believed to be very important to the flavor of liquid whey (Karagul-Yuceer et al., 2003).

Whey powder is expected to have different flavor profile than liquid whey because of the further steps involved in its process such as concentration and spray drying which may change flavor. Although Ferretti and Flanagan (1971) reported a wide variety of volatiles in whey powder, no direct information was provided on its flavor components. Livney and Bradley (1994) reported saltiness, brothiness, diacetyl, bitterness, acidity as some of the flavor characteristics in acid whey. They suggested that whey flavor may be due to components traced to original milk or developed during processing. Those compounds may carry over into the final product and limit its use in bland products. Whitfield (1992) hypothesized that Maillard reaction and lipid oxidation initiate flavor development in whey products but so far there is no experimental data to support this hypothesis.

To date, no information is available on the aroma compounds present in 'sweet whey powder' and hence this study is aimed at identification of flavor compounds in this dairy ingredient. In this research, flavor compounds in commercially produced sweet whey powder were analyzed using gas chromatography/Ofactometry and gas chromatography-mass spectrometry.

MATERIALS & METHODS

Sweet whey powder

Fresh sweet whey powder samples (Cheddar cheese whey, U.S. extra grade) obtained from the Midwest (Sample A) and the Pacific Northwest (Sample B) were used for analysis. The samples were stored frozen (-35°F) and used within three months.

Aroma extraction

One kg of sweet whey powder was mixed with 500ml of 2:1 freshly distilled pentane (Mallinckrodt baker, Phillipsburg, NJ) and diethyl ether (Honeywell International, Muskegon, MI) for four hours during each extraction, in a tightly capped bottle with intermittent shaking after every 15 minutes. Extractions were repeated three times. All extracts were combined and concentrated to 500ml under the hood. The extracts were distilled at 50°C by Solvent Assisted Flavor Evaporation (SAFE) under vacuum (28 Torr) to separate volatiles from non-volatiles. Liquid nitrogen was used for the condensation of volatiles. The distillate was further concentrated to 50ml and subsequently subjected to fractionation (Qian and Reineccius, 2002).

Fractionation

The aroma concentrate was separated into acidic and non-acidic fractions (neutral, basic). The concentrate (50ml) was washed twice with sodium bicarbonate solution (1M, 2 X 3ml) containing sodium chloride (0.05g, Sigma Chemicals, St.Louis, MO). The upper (ether) phase, containing the non-acidic volatiles, was dried over anhydrous sodium sulfate (EMP Chemicals, Gibbstown, NJ) and concentrated to 500µl for sample A and 50µl for sample B. The pooled aqueous phase (bottom layer) was acidified to pH 2.5 with hydrochloric acid (Reagent grade, USP, Intergra Chemical Company, Renton, WA). The acidic volatiles were further extracted with freshly distilled diethyl ether (3 X 0.5ml), which was then dried over anhydrous sodium sulfate and was concentrated to 500µl for sample A and 50µl for sample B.

Gas Chromatography (GC)

A Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector (FID) and an olfactometer was utilized. Samples were analyzed on DBwax column (30m X 0.25 mm id coated with crosslinked polyethylene glycol 20M, film thickness 0.5µm, J&W Scientific) and DB5 column (30m X 0.32 mm id coated with crosslinked 5% phenyl-methyl polysiloxane, film thickness 1µm, J&W Scientific, Folsom, Calif., U.S.A.). The column effluent was split 1:1 (by volume) into the FID and a heated sniffing port with fused silica outlet splitter

(Alltech Associates, Inc., Deerfield, IL). GC operating parameters were: injection port temperature= 250°C, detector temperature= 250°C, oven temperature from 40°C to 230°C at the rate of 5°C/min with initial and final hold times of 2 and 10 mins for DBwax, and 4 and 10 mins for DB5 column respectively. The nitrogen column flow rate was 2.0 ml/min measured at 25°C. 2μl of acidic fraction was injected on DBwax column and 2μl of non-acidic fraction was injected on both DBwax and DB5 columns at a split ratio of 1:1.

Gas Chromatography / Olfactometry (GCO)

Seven volunteers from Oregon State University performed GCO analysis on the whey samples. Panelists participated in four trial runs of each acidic and non-acidic fraction of the two samples to identify product attributes, clarify reference terminology, and verify or establish references for flavor and intensity values (Da-Silva et al., 1994). Each fraction was used at the same concentration as required for analysis. At the end of four sessions, four subjects were chosen based upon their ability to consistently perceive and describe odors as the aroma compounds eluted from the sniff port. During actual analysis, sniffing was performed twice on each fraction by each panelist using the Osme technique (McDaniel, 1990). A 16 point intensity scale with 0 as none, and 15 as extreme was utilized to assign intensity to aromas detected (Miranda-Lopez et al., 1992; Roberts et al., 2003).

Gas Chromatography-Mass Spectrometry (GCMS)

Gas chromatography-mass spectroscopy analysis was performed using an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector (MSD). System software control and data analysis were performed using enhanced ChemStation Software, G1707CA v. C.00.01.08 (Agilent Technologies, Inc., Wilmington, DE). Volatile separation was achieved with two fused silica capillary columns: DBwax (30m X 0.25 mm id coated with crosslinked polyethylene glycol 20M, film thickness 0.5μm, J&W Scientific) and DB5 (30m X 0.32 mm id coated with crosslinked 5% phenyl-methyl polysiloxane, film thickness 1μm, J&W Scientific, Folsom, Calif., U.S.A.). The oven temperature was programmed as for the GCO analysis. Injector, detector transfer line, and ion source temperatures were 250, 280 and 230°C respectively. Helium was used as the carrier gas with constant flow of 2 ml/min. A splitter was used at the end the column as described by Qian and Reineccius (2003). One ml of the flow was directed to the mass spectrometry while the rest of the flow was directed to an olfactometer. MSD conditions utilized were electron impact mass spectrometric data from m/z 35-300 collected at 5.27 scans/s and an ionization voltage of 70eV. 2μl of each fraction was injected in the splitless mode.

Retention Index (RI)

A sample containing series of hydrocarbons (C_5-C_{25}) was injected on both DB-Wax and DB-5 columns using the same conditions mentioned as above for the two samples. Retention indices for the aroma compounds were calculated using modified Kovats method (Van Den Dool, 1963).

Identification of odorants

Positive identifications were made by comparing calculated retention indices, panelist's aroma description and individual mass spectra (Wiley 138 mass spectral database). Tentative identifications were based on retention indices and odor descriptors.

RESULT AND DISCUSSION

Sweet whey powder from Midwest (Sample A) and Pacific Northwest (Sample B) were used in this study to identify the aroma compounds. Each fraction of sample A was concentrated to 500µl while that of sample B was concentrated ten more fold to 50µl.

The acidic fraction was analyzed by DB-Wax column. Several acids were identified (Table 1). But the most important acids found in whey powder were acetic, butanoic, 3-methylbutanoic, hexanoic and octanoic acids based on their high Osme value. Acetic acid with its sharp vinegar-like odor was one of the most

prominent among the identified acids. It showed a high Osme value indicating its important role in imparting the unique aroma to sweet whey powder. Butanoic, hexanoic and octanoic acids contributed to the cheesy and rancid odors. Of these, butanoic acid showed high aroma intensity. 3-methylbutanoic acid gave a sweaty odor with a relatively high Osme value. Pentanoic and dodecanoic acids gave pungent odors and octanoic acid gave an animal-like odor. Formic acid with its pungent odor showed a weak intensity in both samples. Propanonic, 2-methyl propanoic, decanoic, and dodecanoic acids showed a relatively low Osme value in the two samples. 9-decenoic acid, gave a fatty aroma with a weak Osme value in both the samples. From the 12 acids identified in the acidic fraction, all were present in sample A while 2-methyl propanoic acid and dodecanoic acids were absent in sample B. Short chain free fatty acids have been identified in cheese (Aston, 1982; Qian and Reineccius, 2002; Qian et al., 2002) as well as liquid whey (Karagul-Yuceer, 2003; Tomoino, 2004; Whetstine, 2003) Free fatty acids can be formed through the action of milk or bacterial lipases on triglycerides and phospholipids (Badings, 1991; Kim, 2003; Ferretti and Flanagan, 1971). Since the free fatty acids were isolated by their chemical properties and well separated and identified on the DB-Wax column, analysis was not necessary on an additional column.

The non-acidic fraction was analyzed by DB-Wax (Table 2) and DB-5 (Table 3) columns. The chemical classes of compounds identified on the two

columns consisted of aldehydes, ketones, lactones, sulfur containing compounds and many others.

Many aldehydes were identified to have very intense aroma. Saturated aldehydes identified were hexanal, heptanal and nonanal, and they all had characteristic grassy, green, floral aromas. Most of the unsaturated aldehydes were tentatively identified by their retention indices and odor descriptive properties. The most prominent of these were (E)-2-octenal, (Z)-2-nonenal, (E,Z)-2,6-nonadienal, (E,E)-2,4-nonadienal, (E,E)-2,4-octadienal, (E,Z)-2,4-decadienal and (E,E)-2,4-decadienal. These aldehydes are known to be formed by the autoxidation of unsaturated fatty acids (Wadodkar et al., 2002). Phenylacetaldehyde, an important aroma compound with a floral note, was detected only in sample A. This compound is believed to have derived from Strecker degradation of phenylalanine (Counet et al., 2002).

Two straight chain ketones were identified in the whey powder. Diacetyl was identified as very important with its characteristic buttery note. It is known to be a product of microbial action, involving *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis* and citrate metabolism (Bendall, 2001; Kaneko et al., 1987; Kieronczyk et al., 2003). It is believed that diacetyl undergoes reduction to form acetoin, an aromatic hydroxy ketone (Hugenholtz et al., 2000). 1-octen-3-one was detected with a typical mushroom-like odor. Although the intensity for this compound was moderate, it showed unique earthy aroma characteristics to the odor profile of whey powder.

Several sulfur containing compounds were identified. Dimethyl sulfide, dimethyl trisulfide and methional have been identified in cheese (Aston and Dulley, 1982; Boscaini et al., 2003; Qian et al., 2002, Qian 2003) and liquid whey (Karagul-Yuceer, 2003; Tomaino, 2004; Whetstine, 2003). Both dimethyl disulfide and dimethyl trisulfide have an onion, cabbage note, while methional has a cooked potato aroma. Methional is an extremely potent flavor compound showing high Osme value in sample A. It was detected very close to the elution of 2,6-dimethylpyrazine, but was differentiated by the panelists with a cooked potato note for methional and a cooked meaty note for 2,6-dimethylpyrazine. Degradation of methionine can form dimethyl sulfide and methanethiol; and methanethiol can be further oxidizes to form dimethyl disulfide and dimethyl trisulfide (Bendall, 2001). Dimethyl sulfide can be oxidized to dimethyl sulfone (Livney and Bradley, 1994). Although a peak was detected for dimethyl sulfone by GCMS, no aroma was sensed. Methional is believed to have generated by Strecker degradation of methionine (Aston and Dulley, 1982; Boscaini, 2003; Da-Silva M.A, 1993; Fu et al., 2002).

Pyrazines are nitrogen-containing heterocyclic compounds which have been recognized for their characteristic baked and roasted, potato, nut and meat like aromas. The most intense of these were 2,6-dimethylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine. Liquid whey showed 2-methoxy-3-isopropyl and 2-isobutyl-3-methoxypyrazines (Karagul-Yuceer, 2003), but did not show any of the above pyrazines. However, they were

identified in Parmesan cheese as compounds responsible for the nutty aroma (Qian and Reineccius, 2002). Pyrazines are the products of Maillard reaction (Alasalvar et al., 2003; Friedman, 1996), possibly formed during concentration, spray drying, and other heat processes during the formation of whey powder.

2-Acetylpyrrole with its medicinal odor was seen only in sample A. 2-Acetyl thiazole, 2-acetyl-1-pyrroline and 2-propionyl-1-pyrroline were tentatively detected and were found to have roasted nuts and fried kind of aromas. 2-Acetyl-1-pyrroline is believed to be formed by the interaction of pyruvaldehyde with 1-pyrroline, which is formed from proline. (Bendall, 2001; Karagul-Yuceer, 2001). 2-Acetyl-1-pyrroline and 2-propionyl-1-pyrroline possess a very low odor threshold and are frequently cited as compounds that directly contribute to roasted or smoky flavors (Da-Silvia, 1993; Karagul-Yuceer et al., 2003).

Maltol and furoneol were identified in the whey powder that gave moderate to large intensity in both samples with a burnt sugar-like aroma for maltol and a sweet aroma for furaneol. Maltol and furaneol are believed to be products of sugar degradation in the Maillard reaction (Da-Silvia, 1993; Wadodkar et al., 2002). Sotolon, a five membered furan, gave a spicy note, with high intensity on both the samples. This compound is believed to be formed from a-ketobutyric acid and acetaldehyde (Takahashi et al., 1976). Maltol and sotolon have been identified in liquid whey (Karagul-Yuceer et al., 2003) while furaneol was found in stored nonfat dry milk (Karagul-Yuceer et al., 2001).

Most of the lactones were detected only by DB-wax column. Only a few compounds such as delta-decalactone and gamma-hexalactone were detected by both columns. Fruity, nutty and dairy aromas were identified by lactones in both the samples giving a wide range of intensity from very low to very high. Parliament et al., (1966) concluded that the precursors for lactones are triglycerides containing one esterified hydroxyl acid moiety.

p-Cresol, a phenolic compound with a typical cattle-like odor contributed sharp aroma intensity giving a high Osme value for sample A and a weak Osme value for sample B. It has long been known as the agent responsible for the smell of cow urine imparting a barn/medicine-like flavor to milk upon liberation from conjugate precursors (Bendall, 2001; Kim et al., 2003).

Skatole was also identified in the whey powder. It strongly associated with the unpleasant smell of cow feces. It is known to be produced from the amino acid L-tryptophan in the cow's rumen through their diet. Skatole has been found both in liquid and nonfat dry milk (Bendall, 2001; Karagul-Yuceer, 2001), and hence perhaps found in whey powder.

Although sample A and sample B showed some similar compounds, they cannot be compared based on their number or intensity. Sample A showed 45 aroma compounds on DB-wax and 21 on DB5. In addition, sample A also showed five unknowns by DB-wax and one by DB5. Sample B showed 39 aroma compounds on DB-wax and 15 on DB5. Three of the five unknowns analyzed by DB-wax and one by DB5 found in sample A were also present in sample B. Most

of the compounds were identified in both samples while the Osme values varied.

Some compounds, however, were only identified in one sample. For example,

ethylpyrazine was identified on DB-Wax column in sample B but was absent in

sample A, whereas 2-acetyl-1-pyrroline, 2,6-dimethylpyrazine, (Z)-2-nonenal,

phenyacetaldehyde and 2-acetyl pyrrole were identified in sample A but were

absent in sample B.

TABLE 2.1 Aroma volatiles in acidic fraction of sweet dry cheddar whey analyzed by DBwax column, showing retention indices and intensity for its characteristic aroma by *OSME* value

Compound	RI	Aroma	Osme value		Confirmed by
			A	B	
Acetic acid	1460	Vinegar-like	11	9	1,2,3
Formic acid	1510	Pungent	5	6	1,2,3
Propanoic acid	1550	Rancid	5	2	1,2,3
2-Methylpropanoic acid	1580	Buttery	6	0	1,2,3
Butanoic acid	1645	Cheesy	12	10	1,2,3
3-Methylbutanoic acid	1682	Sweaty	9	8	1,2,3
Pentanoic acid	1755	Pungent	6	4	1,2,3
Hexanoic acid	1858	Rancid	11	6	1,2,3
Heptanoic acid	1971	Cheesy	6	7	1,2,3
Octanoic acid	2072	Animal-like	9	6	1,2,3
Decanoic acid	2294	Soapy	6	4	1,2,3
9-decenoic acid	2348	Fatty	4	2	1,2,3
Dodecanoic acid	2505	Soapy	5	0	1,2,3

[1]Aroma descriptor
[2]Retention index (RI)
[3]Gas chromatography mass spectrometry
[4]A = Midwest with concentration 500μl, B = Pacific Northwest with concentration 50μl

TABLE 2.2 Aroma volatiles in non-acidic fraction of sweet dry cheddar whey analyzed by DBwax column, showing retention indices and intensity for its characteristic aroma by *OSME* value

Compound	RI	Aroma	Osme value		Confirmed by
			A	B	
Diacetyl	1005	Buttery	7	9	1,2
Dimethyl disulfide	1095	Cooked vegetable	4	6	1,2
Hexanal	1097	Grassy	5	4	1,2,3
Heptanal	1199	Grassy	12	10	1,2,3
(Z)-4-heptenal	1231	Sweet	8	8	1,2
1-octen-3-one	1307	Mushroom	6	3	1, 2, 3
Unknown	1323	Buttery	5	8	1
2-Acetyl-1-pyrroline	1331	Roasted nuts	7	0	1,2
2,6-Dimethyl pyrazine	1347	Cooked meat	15	0	1,2,3
2,5-Dimethyl pyrazine	1348	Cooked potato	12	10	1,2,3
2-Ethylpyrazine	1357	Roasted nuts	0	6	1,2
Dimethyl trisulfide	1367	Sulfury,cabbage	8	7	1, 2
2,3-Dimethyl pyrazine	1375	Nutty	10	7	1,2,3
2-Acetylthiazole	1397	Roasted nuts	9	10	1, 2
Nonanal	1406	Floral	10	7	1,2,3
(E)-2-octenal	1416	Roasted peanuts	10	4	1, 2
2-propionyl-1-pyrroline	1434	Fried	12	13	1,2
Methional	1464	Cooked potato	13	8	1,2,3
(Z)-2-nonenal	1493	Fatty	10	0	1,2
2,3,5-Trimethylpyrazine	1517	Roasted	9	5	1,2,3
Unknown	1522	Pungent	6	10	1
Unknown	1545	Sour	7	12	1
(E)-2-Nonenal	1558	Pungent	8	13	1,2
(E,Z)-2,6-Nonadienal	1604	Cucumber	8	13	1,2
Furfuryl alcohol	1613	Burnt rubber	7	5	1,2,3
(E,E)-2,4-Octadienal	1615	Cucumber	15	10	1,2

TABLE 2.2 (Continued)

Unknown	1619	Rancid	7	0	1
Phenylacetaldehyde	1632	Floral	8	0	1,2,3
(E,Z)-2,4-nonadienal	1646	Green	7	7	1,2
(E,E)-2,4-nonadienal	1681	Rancid	13	7	1,2
Gamma hexalactone	1736	Coconut	10	9	1,2,3
(E,Z)-2,4-decadienal	1752	Green	13	13	1,2
(E,E)-2,4-decadienal	1795	Baked	7	10	1,2
Delta octalactone	1988	Baked	5	5	1,2,3
2-Acetylpyrrole	2002	Herbal medicine	12	0	1,2,3
Maltol	2012	Caramel	8	12	1,2,3
Furaneol	2033	Sweet	8	8	1,2,3
Unknown	2056	Caramel	13	0	1
p-Cresol	2103	Cattle	11	6	1,2,3
Gamma decalactone	2143	Milk powder	9	10	1,2,3
Sotolon	2186	Spicy	10	15	1,2
Delta decalactone	2220	Fruity	10	9	1,2,3
Delta undecalactone	2345	Fruity	4	14	1, 3
Gamma dodecalactone	2384	Cheesy	8	5	1,2,3
Delta dodecalactone	2466	Buttery	3	4	1,2,3
Skatole	2486	Animal-like	6	8	1,2

[1]Aroma descriptor
[2]Retention index (RI)
[3]Gas chromatography mass spectrometry
[4]A = Midwest with concentration 500µl, B = Pacific Northwest with concentration 50µl

TABLE 2.3 Aroma volatiles in non-acidic fraction of sweet dry cheddar whey analyzed by DB5 column, showing retention indices and intensity for its characteristic aroma by *OSME* value

Compound	RI	Aroma	Osme value		Confirmed by
			A	B	
Diacetyl	606	Buttery	5	8	1,2
Hexanal	789	Grassy	5	5	1,2,3
Heptanal	899	Grassy	9	0	1,2,3
Methional	902	Cooked potato	9	3	1,2,3
2,6-dimethyl pyrazine	906	Cooked meat	11	0	1,2,3
2-acetyl-1-pyrroline	916	Roasted nuts	11	3	1,2
1-octen-3-one	974	Mushroom	6	8	1, 2, 3
Gamma hexalactone	1047	Coconut	10	8	1,2,3
2-acetyl pyrrole	1072	Herbal medicine	8	0	1,2,3
p-cresol	1074	Cattle	11	3	1,2,3
Maltol	1088	Burnt sugar	10	12	1,2,3
Nonanal	1101	Floral	6	0	1,2,3
Sotolon	1108	Spicy	11	0	1,2
(Z)-2-nonenal	1147	Fatty	8	6	1,2
(E,Z)-2,6-nonadienal	1153	Cucumber	8	12	1,2
(E)-2-nonenal	1155	Pungent	9	11	1,2
(E,E)-2,4-nonadienal	1211	Rancid	8	6	1,2
(E,E)-2,4-decadienal	1313	Baked	9	6	1,2
Skatole	1391	Animal-like	7	11	1,2
unknown	1482	Floral	10	11	1
Delta decalactone	1491	Fruity	12	0	1,2,3

[1]Aroma descriptor
[2]Retention index (RI)
[3]Gas chromatography mass spectrometry
[4]A = Midwest with concentration 500µl, B = Pacific Northwest with concentration 50µl

CONCLUSION

Important aroma volatiles analyzed in sweet whey powder were the short chain fatty acids, aldehydes and ketones, lactones, sulfur compounds, phenols, indoles, pyrazines, furans and pyrroles. Some of them are derived from milk or formed from cheese manufacturing, while others are formed during whey processing. While more sensory studies are required to validate the findings of each aroma compound, it is likely that the compounds identified in the current study are responsible for the characteristic aroma in sweet whey powder.

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CHAPTER 3: CONCLUSIONS

Aroma in sweet whey powder is due to both acidic and non-acidic compounds. Important aroma volatiles analyzed in sweet whey powder were the short chain fatty acids, aldehydes, ketones, lactones, sulfides, phenols, indoles, pyrazines, furans and pyrroles. Some of these aroma compounds are derived from milk or formed from cheese manufacturing, while others are formed during whey processing. Autooxidation of lipids, and Maillard browning reactions are believed to be the most important causes for the generation of these aroma compounds.

Short chain fatty acids like acetic, butanoic, 3-methylbutanoic, hexanoic, octanoic acids were mainly responsible for oxidized and rancid flavors that are possibly the products of fermentation, lipid hydrolysis and lipid oxidation.

Saturated aldehydes like hexanal, heptanal, nonanal and unsaturated aldehydes like (E)-2-octenal, (Z)-2-nonenal, (E)-2-nonenal, (E,Z)-2,6-nonadieanal, (E,E)-2,4-octadienal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal, (E,Z)-2,4-decadienal, phenylacetaldehyde were mainly responsible for green, grassy, cucumber, floral, fatty, rancid, baked, roasted, pungent odors that are believed to have generated due to Strecker degradation and lipid oxidation reactions.

Sulfur containing compounds like methional believed to be formed by Strecker degradation reaction gave a cooked potato aroma and dimethyl disulfide and dimethyl trisulfide gave cooked vegetable and cabbage aromas.

Phenols like p-cresol gave a cattle-like odor.

Ketones such as diacetyl and 1-octen-3-one, possible products of fermentaion and lipid oxidation gave buttery and mushroom aromas.

Several gamma and delta lactones were detected and gave a wide range of aromas from fruity, coconuty, to milk powdery, buttery, cheesy and baked. They are believed to be the products of mainly 4- or 5- hydroxy fatty acids.

Several five and six membered heterocylces were detected in and were supposed to be products of mainly Maillard reaction. Amongst the five-membered heterocylces, furans and thiazoles gave spicy, sweet, burnt rubbery and roasted aroma notes. Indoles like skatole were found to be responsible for animal-like odor. Pyrroles like 1-acetylpyrrole, 1-acetyl-1-pyrroline and 2-propionyl-1-pyrroline gave medicine, roasted, and fried aromas. Amongst the six-membered heterocycles, maltol, gave a caramel aroma and dimethylpyrazines, ethylpyrazine and trimethylpyrazine gave cooked, nutty and roasted aromas.

Aroma in sweet whey powder is thus believed to derive from milk and the various reactions that occur during its formation. The literature review prepared during the current study clearly states that fermentation, proteolysis, lipolysis, lipid oxidation and Maillard reactions are the primary reactions responsible for the aroma in sweet whey powder. Although these reactions are believed to be the possible sources for the formation of these aroma compounds, further experimental study is required to fully understand and validate the mechanism for its exact formation.

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