Evaluation of Flavor Variation in Swiss Cheese from Five Factories
Using Selected Ion Flow Tube Mass Spectrometry (SIFT-MS),
Descriptive Sensory Analysis, and Consumer Testing

THESIS

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By

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Abstract

Variation in Swiss cheese flavor is a problem in the Swiss cheese industry. The objective of this study is to identify the compounds and flavor attributes causing variation in Swiss cheese flavor by using selected ion flow tube-mass spectrometry (SIFT-MS), descriptive sensory analysis, and consumer sensory testing. Three Swiss cheese samples were obtained from each of five different factories for a total of fifteen samples. These cheeses varied in manufacturing dates, as well as the vat and block location. The trained panelists in the descriptive analysis found significant differences between the cheeses from different factories. Although there are some similarities between factories, there are also attributes that distinguish samples and are unique to each factory. To determine consumer preferred flavors, this study utilized an untrained consumer panel of 100 people who consume Swiss cheese, to determine which cheeses were liked the most, met expectations, and had the highest liking rating. Overall, cheeses from one factory were liked the most and were the best balanced in all attributes. SIFT-MS showed that the cheeses contained varying concentrations and odor activity values (OAV) of high impact volatile organic compounds (VOCs). OAVs (concentration/threshold) were utilized to discriminate all factories using Soft Independent Modeling of Class Analogy (SIMCA) indicating unique flavor profiles. A variety of variables inherent to Swiss cheese
manufacture may contribute to the variation in flavors associated with each factory. This study provided end point flavor characteristics and compounds to be traced back through fermentation pathways to help determine the source of flavor variation.
Dedicated to my mom, Deborah Taylor, for continued support, love, and inspiration.
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Fields of Study

Major Field: Food Science and Nutrition
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Chapter 1: Literature Review

1.1 Swiss Cheese

1.1.1 Background and Characteristics

Emmental (Emmentaler) is the best-known Swiss-type cheese and is referred to as Swiss cheese in the United States (Thierry and others 2010). Swiss cheese is now a generic term for the hard cheeses that were originally made in the Emmen valley in Switzerland (Daly and others 2010). Swiss cheese ranges in color from ivory to yellow and is easily identifiable by its characteristic round eyes that can vary in size. The minimum fat content of Swiss cheese is 41% while the maximum moisture content is also 41% by weight (FDA 2012). The high water and fat content of Swiss cheese contributes to the soft elastic structure which helps maintain the integrity of the curd during eye formation (Daly and others 2010). Swiss cheese is widely produced in the United States and must be at least 60 days old before it can be sold. In 2011, approximately 329,000,000 pounds of Swiss cheese was produced in the United States (USDA 2012). Ohio is the number one producer of Swiss cheese in the United States, producing 44% of the Swiss cheese in the United States.
1.1.2 Starter Cultures

Swiss cheese is dependent upon the activity of three different starter cultures. The three types of bacteria that are used as starter cultures in the production of Swiss cheese are *Streptococcus thermophilus*, *Lactobacillus* (*helveticus* or *delbrueckii*), and *Propionibacterium freudenreichii* (Daly and others 2010). *Streptococcus thermophilus* grows best at 37°C or higher and produces lactic acid from lactose during the make procedure (Høier and others 2010). *Lactobacillus* is utilized to produce lactic acid and control pH during pressing (Thierry and others 2010). The addition of *Lactobacillus* continues to lower the pH of the cheese for proper coagulation (Høier and others 2010). *Propionibacterium* is the only starter culture that does not grow in the milk; however it begins to grow when the cheese is moved from precooling to the warm room (Thierry and others 2010). *Propionibacterium* ferments the lactic acid made by the other starter cultures and produces propionic acid, acetic acid, and carbon dioxide. The production of carbon dioxide by *Propionibacterium* is responsible for the eye formation in Swiss cheese (Daly and other 2010). Non-starter lactic acid bacteria (NSLAB), originating from either the milk or the environment in which the cheese is produced, is also important to the make of Swiss cheese. These organisms produce lactic acid during pressing, but can also form carbon dioxide leading to excessive eye production (Thierry and others 2010).

1.1.3 Process

Swiss cheese is primarily made from cow’s milk, although buffalo’s milk can also be used (Thierry and others 2010). Originally, Swiss cheese was made from raw milk, but
now in the United States it is made from pasteurized milk. Once the milk completely cools, the starter organisms are added and held to initiate growth. Rennet is added to the milk to form a firm curd that is then heated slowly to 50°C (Thierry and others 2010). The cheese is cooked until the whey is expressed and then goes to the press to remove all excess whey. The cheese is brined, precooled to about 10°C, and then placed in the warm room at 18-24°C (Thierry and others 2010). To get eye formation, the cheese is held in the warm room for 20-30 days, and then is removed to the cold room to age for at least 60 days before being packaged and sold (FDA 2012).

1.2 Swiss Cheese Flavor

1.2.1 Swiss Cheese Volatiles

Swiss cheese flavor is very complex with over 600 volatile compounds having been reported (Harper and others 2011). These volatile compounds found in Swiss cheese are a result of proteolysis, lipolysis, and lactose, lactate, and citrate metabolism by microorganisms during ripening (Figure 1.1). There are many other compounds that are also important to the flavor profile of Swiss cheese such as hydrocarbons, alcohols, sulfur-containing compounds, furanones, esters, ketones, and aldehydes (Thierry and others 1999, Thierry and others 2010). Some of these compounds include 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, ethyl butanoate, ethyl 3-methylbutanoate, ethyl hexanoate, 2,3-butanedione, 2-heptanone, 3-methylbutanal, and methional (Thierry and others 2010). Preininger and Grosch (1994) detected some of those compounds and more in Swiss cheese including 2,3-butanedione,
3-methylbutanal, ethyl butanoate, ethyl 3-methylbutanoate, 2-heptanone, methional, 1-octen-3-one, ethyl hexanoate, furanones, skatole, γ-decalactone, and 2-sec-butyl-3-methoxypyrazine.

1.2.2 *Propionic and Acetic Acid*

The typical Swiss cheese flavor is described as sweet and nutty (Thierry and others 2010). The described nutty flavor of Swiss cheese has been positively correlated
with high concentrations of propionic acid (Lawlor and others 2002). Propionic and acetic acid are two volatile compounds that are important to the flavor of Swiss cheese. Both of these compounds are formed in the warm room as a result of *Propionibacterium* activity. Bachmann and others (2011), Lawlor and others (2002), and Preininger and others (1996) found that acetic acid and propionic acid are the most significant volatile compounds for the typical Swiss cheese flavor.

1.2.3 Esters and Furanones

Ethyl butanoate, ethyl 3-methyl butanoate, and ethyl hexanoate give Swiss cheese a fruity note while the furanones are responsible for the sweet, caramel flavor in Swiss cheese (Bachmann and others 2011). These esters (ethyl butanoate, ethyl 3-methyl butanoate, and ethyl hexanoate) were found to play an important role in the overall flavor profile of Swiss cheese (Preininger and Grosch 1994, Langler and others 1967). Grosch (1994), Preininger and Grosch (1994), and Preininger and others (1996) all determined that furanones (4-hydroxy-2,5-dimethyl-3(2H)-furanone and 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone), just like the esters, have a high impact on the flavor of Swiss cheese due to extremely high odor activity values (OAVs).

1.2.4 Ketones (2,3-Butanedione) and Alcohols

Many ketones and alcohols have been identified in Swiss cheese. Yang and Min (1994) found that ketones and alcohols accounted for 88% of the total volatiles (27 total compounds detected) in a Swiss cheese sample. In another study, 12 different ketones
were detected in Swiss cheese including 2-propanone, 2,3-butandione, 2-butanone, and 3-methyl-2-butanone (Thierry and others 2004). Langler and others (1967) also found that 2,3-butanedione plays a significant role in the flavor of Swiss cheese. 2,3-Butanedione was found at levels greater than its threshold illustrating its importance to the overall flavor profile (Preininger and Grosch 1994). Ethanol has been quantified in Swiss cheese at very high concentrations (Yang and Min 1994). Although Swiss cheese contains alcohols, especially ethanol, these compounds have little effect on the flavor of Swiss cheese because of their high thresholds (Langler and others 1967). On the other hand, the alcohols may contribute indirectly by aiding in the formation of esters with fatty acids (Langler and others 1967).

1.2.5 Sulfur-Containing Compounds

The formation of volatile sulfur compounds in fermented foods, including Swiss cheese, is very important to the overall flavor. These sulfur-containing compounds are formed from common sulfur-bearing precursors, including methionine and cysteine. (Landaud and others 2008). Compounds such as methional (boiled potato odor), hydrogen sulfide (rotten egg odor), methyl mercaptan (eggy odor), dimethyl disulfide ( garlic odor), and dimethyl trisulfide (garlic odor) have all been detected in cheese (Landaud and others 2008, Yang and Min 1994, Harper and others 2011). Methyl mercaptan, dimethyl disulfide, and methional are considered to have the greatest impact of the sulfur-containing compounds on the flavor of Swiss cheese (Harper and others 2011). Grosch (1994), Preininger and Grosh (1994), and Preininger and others (1996) all
determined that methional has a high impact on the flavor of Swiss cheese due to extremely high odor activity values (OAVs). Methional tends to have high odor activity values because it has quite a low detection threshold, which allows methional to provide an additional vegetable note to cheese aroma (Landaud and others 2008). Langler and others (1967) also found that dimethyl sulfide is important specifically to Swiss cheese flavor.

1.3 Analytical Methods for Volatile Compound Analysis

Many different analytical methods have been utilized to study the aroma and flavor of dairy products. For many years, gas chromatography-mass spectrometry (GC-MS) has been the instrument of choice for the analysis of volatile compounds in dairy foods (Spanel and Smith 1999). However, the GC-MS instrument must be calibrated for each trace gas and extensive sample preparation is required (Smith and Spanel 2005). In order to analyze volatile compounds using a GC-MS instrument, the volatile compounds released from the food product must first be collected to detect low threshold values. The collection process can often change the volatile compounds following their release from the food product or even artificially form new compounds. Static headspace (S-HS), dynamic headspace purge and trap extraction (D-HS), solid-phase micro extraction (SPME), and solid phase dynamic extraction (SPDE) are some examples of possible extraction methods for collecting volatile compounds (Harper and others 2011). Coupling these methods with GC-MS leads to a time consuming quantification of volatile compounds.
Atmospheric pressure ionization mass spectrometry (API-MS) is an analytical method that can be used for volatile quantification. API-MS does not use GC separation which results in complex spectra making the quantification of volatiles very challenging (Spanel and Smith 1999). Proton transfer reaction mass spectrometry (PTR-MS) is based on the chemical ionization of gas phase analytes which is determined by current kinetic data (Smith and Spanel 2011). PTR-MS only uses one precursor ion, H$_3$O$^+$, which can cause the spectra to be very complicated and difficult to analyze. Because chemical analysis of flavor volatiles in food products is complex, every analytical technique has its restrictions.

1.4 Selected Ion Flow Tube-Mass Spectrometry

1.4.1 Principles of SIFT-MS

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a direct mass spectrometric technique based on chemical ionization of a gas sample using selected precursor reagent positive ions along a flow tube at a known velocity. This technique is commonly used to quantify volatile compounds in real time at concentrations of parts per trillion (ppt) and does not require sample preparation. The method of utilizing SIFT-MS begins with the chosen mass selected precursor ions. Precursor ions H$_3$O$^+$, NO$^+$, and O$_2^+$ are used, because they do not react with the major components of air (N$_2$, O$_2$, H$_2$O, Ar, and CO$_2$) but selectively ionize only the trace gases and volatile compounds present in the air sample being analyzed (Spanel and Smith 1999). A schematic diagram of the analytical process used in a SIFT-MS instrument is shown in Figure 1.2.
The precursor ions are generated by microwave discharge and are transferred into the flow tube with a helium carrier gas via a quadrupole mass filter. The sample is introduced downstream into the mixture of the carrier gas and the precursor ions. This then produces the desired product ions. From there count rates of the precursor and product ions are determined using another quadrupole mass spectrometer detection system. The concentrations of individual vapors in the sample can be determined from the ratios of the product to precursor ion count rates.

SIFT-MS has been utilized in many laboratories world-wide over the past thirty years to identify the kinetic data for ion-neutral reactions (Smith and Spanel 2011). The kinetic data obtained includes the rate coefficient (k), the product ions, and their relative
amounts. The SIFT-MS analytical software, which stores all this kinetic data, can then be instantly utilized to calculate the concentrations of individual vapors \([\text{M}]\) in a gas mixture from the ratios of the product to precursor ion count rates according to equation (1).

\[
[M] = \frac{I_p}{ikt}
\]  

The reaction time \((t)\) can be simply defined by the known flow velocity of the helium carrier gas and the length of the flow tube. Based on all this kinetic data, SIFT-MS has quickly extended to the studies of ionic reactions relevant to other media (Smith and Spanel 2005).

SIFT-MS provides reliable, real-time identification and quantification of volatiles in a wide range of sampling methods. The soft chemical ionization used in SIFT-MS results in a smaller range of product ions than is common in gas chromatography-mass spectrometry (GC-MS). This allows SIFT-MS to circumvent the chromatographic separation leading to instantaneous quantification of volatiles (Harper and others 2011). In addition, the use of multiple reagent ions \((\text{H}_3\text{O}^+, \text{O}_2^+, \text{and NO}^+)\) reduces interferences and increases the specificity of SIFT-MS compared to other analytical methods, such as PTR-MS, that utilize only one ion. The sensitivity of this method is dependent on the time allowed for collecting data. A compound can be identified in a few seconds if it is present at greater than 10 ppb but a level as low as 0.1 ppb takes closer to 10 seconds (Spanel and Smith 1999).
2.4.2 Types of Scans

SIFT-MS can operate in two different modes, full scan (FS) mode and multiple ion monitoring (MIM) mode. The FS mode is a complete mass spectrum of both precursor and product ions and aids in identification of unknown compounds (Harper and others 2011). This spectrum is obtained by sweeping over a selected mass-to-charge ratio (m/z) range for a chosen time while a sample is introduced into the flow tube by a carrier gas (Smith and Spanel 2011). The second operating mode, MIM mode, only counts rates of the precursor ions and those of selected characteristic product ions. In this mode the downstream mass spectrometer rapidly switches between the masses of the precursor ions and the chosen product ions and dwelling on each mass for a predetermined short time interval. MIM mode provides better limits of quantification and better precision than FS mode because it provides a longer counting time than is possible with a FS (Harper and others 2011). The use of SIFT-MS for real time monitoring is possible because the response time is approximately 20 ms (Smith and Spanel 2011).

2.4.3 Ion chemistry

SIFT-MS is based on the known rate coefficients for the reaction of the reagent ions with the target compounds. Currently, the SYFT library has kinetic data for more than 500 volatiles that will aid in quantifying and identifying product ions. The SYFT library is continuously expanding in response to the demands of different SIFT-MS applications (Diskin and others 2002). Depending on the analytes and reagent ion, five types of reactions can occur in the SIFT-MS instrument (Harper and others 2011).
The first type of reaction that can occur in the flow tube is proton transfer (H$^+$) which is shown in equation (2).

$$H_3O^+ + M \rightarrow MH^+ + H_2O$$

(2)

The reagent ion, H$_3$O$^+$, typically reacts with most organic molecules (M) by rapid proton transfer producing water and MH$^+$ ions due to the greater proton affinity of the organic molecule than H$_2$O. Therefore the appearance of MH$^+$ ions is to be expected. For some alcohols, aldehydes, and carboxylic acids the MH$^+$ ions may partially or completely dissociate with the elimination of an H$_2$O molecule resulting in an [M-OH]$^+$ hydrocarbon ion (Spanel and Smith 1999).

The second type of reaction that can occur in the SIFT-MS instrument is charge transfer. The mechanism is shown in equations (3) and (4).

$$O_2^+ + M \rightarrow M^+ + O_2$$

(3)

$$NO^+ + M \rightarrow M^+ + NO$$

(4)

Both O$_2^+$ and NO$^+$ precursor ions undergo charge transfer reactions with organic molecules (M) producing parent M$^+$ ions and additional fragment ions. However these ions can only undergo charge transfer reactions with molecules that have ionization energies (IE) lower than the IE of O$_2^+$ (12.06) and NO$^+$ (9.26 eV) (Smith and Spanel 2005). Low molecular weight alkanes, aromatic hydrocarbons, and organosulfur
compounds typically undergo a charge transfer reaction with NO$^+$ due to their small IE (Spanel and Smith 1999).

The next reaction that occurs in the flow tube is dissociative charge transfer which is illustrated in equation (5).

\[ O_2^+ + M \rightarrow \text{Fragment}^+ + \text{Neutral fragments} + O_2 \]  

(5)

The IE of O$_2^+$ is greater than most organic molecules and therefore can react via dissociative charge transfer producing two or more fragment ions (Smith and Spanel 2011). Because several products can be formed, SIFT-MS spectra can be complex. This limits the usefulness of O$_2^+$ precursor ions in SIFT-MS analysis. O$_2^+$ precursor ions are utilized best for the detection and quantification of small molecules that do not react with either H$_3$O$^+$ or NO$^+$ ions (Smith and Spanel 2005).

The fourth reaction that can occur during this analytical process is association. The mechanisms for NO$^+$ and H$_3$O$^+$ association are shown in equations (6) and (7).

\[ \text{NO}^+ + \text{Analyte} + M \rightarrow \text{Analyte.NO}^+ + M \]  

(6)

\[ \text{H}_3\text{O}^+ + \text{Analyte} + M \rightarrow \text{Analyte.H}_3\text{O}^+ + M \]  

(7)

Ion-molecule association is common in the reactions of NO$^+$ with carboxylic acids, esters, and ketones (Smith and Spanel 2005). It becomes the most efficient when the IE (M) is not much different than IE (NO$^+$). Commonly, the helium carrier gas utilized in
SIFT-MS is the stabilizing agent for these association reactions. Association reactions can occur in parallel with some form of a charge transfer (Smith and Spanel 2005).

The final reaction that can occur in the flow tube of the SIFT-MS is hydride extraction which is displayed in equation (8).

\[ \text{NO}^+ + M \rightarrow [\text{M-H}]^+ + \text{HNO} \]  

(8)

Hydride extraction of NO\(^+\) results in a single product ion [M-H]\(^+\) and an HNO molecule. This reaction is a very dominant process that occurs in the reactions of NO\(^+\) with aldehydes resulting in a single product carboxy ion, which is convenient for SIFT-MS analysis (Spanel and Smith 1999). Hydride extraction is the only process that occurs in the reactions of NO\(^+\) with aldehydes and ethers (Smith and Spanel 2005).

1.4.4 Application Specifically in Food Science

SIFT-MS has been utilized in a variety of applications in fields including microbiology, health and medical sciences, environmental science, and food science. Some of these studies include breath analysis for medical diagnosis and therapeutic monitoring (Senthilmohan and others 2000), detection of volatiles produced by medically important fungi (Scotter and others 2005), and analysis of microbial volatiles for bacterial detection, identification, and determination of antibiotic susceptibility in a conventional blood culture system (Allardyce and others 2006).
SIFT-MS is also very practical and advantageous for studies in food science. An important application of SIFT-MS in the food industry is with fermented foods. In other studies SIFT-MS has been used to measure the volatile compounds that are produced during the processing of fermented sausages to study the effect of sausage fat content and processing on the volatile compounds (Spanel and Smith 2010) and to analyze in real-time high impact volatile sulfur compounds essential to the flavor profile of Swiss cheese (Harper and others 2011). Other foods including cut onion, crushed garlic and ripe banana (Spanel and Smith 1999), tomatoes and tomatillos (Xu and Barringer 2010), and cod fillets (Noseda and others 2010) have also been analyzed using SIFT-MS.

1.5 Determination of Volatile Organic Compound Importance in Food

1.5.1 Odor Thresholds

Many volatile organic compounds can be quantified in foods, but the importance of these compounds to the food can be assessed if their concentrations and odor thresholds are known (Van Gemert 2011). Odor thresholds can be defined as either detection thresholds or recognition thresholds. A detection threshold is the minimum concentration which can be detected without any requirements to identify or recognize the aroma while a recognition threshold is the minimum concentration at which an aroma or odor can be identified or recognized (Van Gemert 2011). Recognition thresholds, on average, are about three to five times the detection threshold (Hau and Connell 1998). In this study, recognition thresholds in air are utilized (when available) for volatile organic compounds as they best match the aroma experience of consuming food. These odor
thresholds are necessary to obtain a more accurate estimate of the contribution of each compound to the flavor of food by normalizing the compound concentrations using odor activity values (Qian and Reineccius 2003).

1.5.2 Odor Activity Values

Within the last 20 years, research has used odor activity values (OAV) as a means of focusing on compounds considered to be the most significant (high impact) flavor for a specific food. Although food products can contain hundreds of VOCs, not all of these compounds have a major effect on the flavor profile of the product (Grosch 1994). Only those compounds with OAVs greater than one significantly contribute to the flavor (Qian and Reineccius 2003). OAVs are defined as the concentration of the compound in the sample divided by the odor threshold value, in air, for that compound. When the OAV of a compound is greater than one, it is considered to have a high impact on flavor. OAVs less than one are considered to have little effect on flavor because they are below the detectable odor threshold.

This approach has been utilized to detect high impact compounds in the flavor profiles of many varieties of cheeses. Milo and Reineccius (1997) used the approach for Cheddar cheese and reported methional, homofuraneol, diacetyl, acetic acid, and butyric acid as odorants with high aroma impact. Volatile compounds in Parmigiano Reggiano cheese were also evaluated using OAVs. It was reported that 14 compounds, including some compounds found in Emmenthal cheese, had very high OAVs indicating those compounds are key components to Parmigiano Reggiano cheese flavor and aroma (Qian
and Reineccius 2003). Preininger and Grosch (1994) found the key odorants for Emmentaler cheese to be methional, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, and 5-ethyl-4-hydroxy-2,ethyl-3(2H)-furanone with a total of twelve compounds having OAVs greater than one in at least one cheese using this same approach. These twelve compounds are 2,3-butanedione, 3-methylbutanal, ethyl butanoate, ethyl 3-methylbutanoate, methional, ethyl hexanoate, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 5-ethyl-4-hydroxy-2,ethyl-3(2H)-furanone, 2-sec-butyl-3-methoxypyrazine, 1-octen-3-one, skatole, and γ-decalactone (Preininger and Grosch 1994).

1.6 Sensory Methods for Flavor Analysis

1.6.1 Types of Sensory Analysis Tests

Sensory perception is very important to all food products, especially dairy. The application of sensory science has actually been around for 60 years. Because of sensory science, a relationship can be established between sensory perception and instrumental tests (Drake 2007). Sensory analysis can be categorized into three groups of tests: traditional tests, analytical tests, and affective or consumer tests (Drake 2007).

Traditional tests include USDA grading and ADSA scorecard judging. In order to ensure product quality and consistency, these traditional sensory tests were created by the dairy industry. The overall goal of traditional tests is to assign a quality score or grade to each dairy product. Analytical sensory tests are more mainstream tests that are scientifically recognized. Some analytical sensory tests include triangle tests, alternative forced choice (AFC), degree of difference (DOD), threshold tests, and descriptive
sensory analysis (Drake 2007). The third group of sensory tests is affective or consumer tests. These tests typically measure preference and liking by untrained consumer panelists.

1.6.2 Descriptive Sensory Analysis

Descriptive sensory analysis is an analytical sensory test that is commonly used with dairy products. It consists of a group of trained individuals that can identify and quantify specifically trained characteristics of a food product. A panel typically contains six to twelve trained individuals which are sometimes trained for several hundred hours (Drake 2007). More training is necessary for more complex food characteristics. Although there is a time and financial commitment needed for descriptive sensory analysis, the panel and training are flexible to different studies and characteristics (Drake 2007).

Descriptive sensory analysis has been used in many food science applications. Some of these studies include the effect of grape seed extract on ground chicken during refrigerated storage (Brannan 2009), the flavor of natural and roasted Turkish hazelnuts (Alasalvar and other 2012), the effect of manufacturing company and the manufacturing season on the volatile composition of vanilla *torrone* (Speziale and others 2010), and sources of umami taste in Cheddar and Swiss cheese (Drake and others 2007). There have also been several studies using descriptive sensory analysis with Swiss cheese in particular. Kocaoglu-Vurma and others (2009) utilized descriptive sensory analysis to develop predictive models for the determination of sensory attributes of Swiss cheese.
based on infrared spectra. Descriptive sensory analysis has also been used to determine which flavor characteristics of Swiss cheese should be optimized and minimized to produce cheeses preferred by consumers (Liggett and others 2008).

1.6.3 Consumer Sensory Analysis

Consumer sensory analysis consists of a large array of tests that can measure food qualitatively and quantitatively (Drake 2007). The main commonality between the types of consumer sensory analysis is simply the testing with untrained consumers. Consumers provide an objective view point to the testing that can lead to the determination of the consumer likes and dislikes. A large number of consumers is highly recommended when conducting a consumer sensory test in order to consider all the variability amongst consumers. Therefore, demographic information is very important to consumer sensory tests in order to determine if these variables influence product liking (Drake 2007).

To measure food quantitatively, acceptance testing is often utilized. Acceptance testing, or degree of liking, presents consumers with products and they are asked to indicate the degree of liking on a suitable scale (Drake 2007). The most common scale that is used for acceptance testing is the 9-point hedonic scale where the anchors are dislike (zero) and like (nine). The just-about-right (JAR) scale is another scale that can be used for acceptance testing. This test is typically used in product development or optimization studies because the consumer will identify if specific product attributes (such as nutty flavor or dairy aroma) are “just about right” (Drake 2007).
Consumer sensory analysis has been used in many food science applications. Some of these studies include determining consumer opinions on the flavor and texture of fish (Sawyer and others 1988), the consumer preference of organically or conventionally grown vegetables (Zhao and others 2007), and the determination of liked and disliked Swiss cheese flavor characteristics by consumers (Liggett and others 2008).
Chapter 2: Materials and Methods

2.1 Swiss Cheese Samples

Fifteen cheeses were collected from November 2011 to April 2012 from member companies of the Swiss Cheese Consortium, where five factories contributed three cheeses each. The factories that make up the Swiss Cheese Consortium are located in Ohio, Illinois, Pennsylvania, and Iowa. These factories are designated as 148, 207, 374, 465, and 528. The Swiss cheeses varied in manufacturing dates, as well as the vat and block location (Table 2.1). Variation of cheeses within most factories was limited to different blocks from the same vat make. In addition, each of the five factories used different starter cultures in the processing of their Swiss cheese (Table 2.2).
Table 2.1 Basic information for all Swiss cheese samples analyzed based on factory.

<table>
<thead>
<tr>
<th>Factory</th>
<th>Manufacturing Date</th>
<th>Vat(s)</th>
<th>Block(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>148</td>
<td>1/3/2012</td>
<td>Different</td>
<td>Different</td>
</tr>
<tr>
<td>207</td>
<td>11/28/2011</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>374</td>
<td>4/20/2012</td>
<td>Same</td>
<td>Different</td>
</tr>
<tr>
<td>465</td>
<td>3/19/2012</td>
<td>Same</td>
<td>Different</td>
</tr>
<tr>
<td>528</td>
<td>12/26/2011</td>
<td>Same</td>
<td>Different</td>
</tr>
</tbody>
</table>

Table 2.2 Starter and adjunct organisms used by the different factories.

<table>
<thead>
<tr>
<th>Factory</th>
<th>Streptococcus</th>
<th>Primary Lactobacillus</th>
<th>Adjunct Lactobacillus</th>
<th>Propionibacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>207</td>
<td><em>S. thermophilus</em> B-9</td>
<td><em>L. helveticus</em> B-1</td>
<td><em>Lactobacillus</em> B-1</td>
<td><em>P. freudenreichii</em> B-1</td>
</tr>
<tr>
<td>148</td>
<td><em>S. thermophilus</em> C-1</td>
<td><em>L. delbruekii</em> C-1</td>
<td><em>L. casei</em> C-1</td>
<td><em>P. freudenreichii</em> C-1</td>
</tr>
<tr>
<td>465</td>
<td><em>S. thermophilus</em> D-1-8</td>
<td><em>L. helveticus</em> D-1</td>
<td>NONE</td>
<td><em>P. freudenreichii</em> D-1</td>
</tr>
<tr>
<td>528</td>
<td><em>S. thermophilus</em> E-1-7</td>
<td><em>L. delbruekii var lactis</em> E-A-1</td>
<td><em>L. casei</em> E-1</td>
<td><em>P. freudenreichii</em> E-1</td>
</tr>
</tbody>
</table>
2.2 Volatile Organic Compound (VOC) Quantification

2.2.1 Selected Ion Flow Tube-Mass Spectrometry

Each cheese was divided into smaller 500 g blocks, vacuum packed, and stored at -80°C. The cheeses were analyzed in random order in replicates of six over a period of two weeks. Data was reported in ppb. The cheese samples were shredded using a hand grater (OXO, New York, NY). An aliquot of 5 g was placed in a one liter bottle with a septa cap that could be punctured by a needle to introduce the head space into the SIFT-MS (SYFT Technologies Voice 200, Christchurch, New Zealand). Samples were heated to 40°C for 1 h prior to analysis.

The head space of each 5 g sample was analyzed by the SIFT-MS instrument using three soft ionizing reagent ions (H$_3$O$^+$, NO$^+$, and O$_2^+$). The flow rate of the SIFT-MS was set at 0.26 torr L/sec. Identification and quantification of the VOCs were determined based on the known ion products and reaction rate coefficients for each compound in a method that contained 28 compounds including alcohols, aldehydes, ketones, esters, sulfur compounds, and pyrazines.

2.2.2 Odor Threshold Values

Odor threshold values were used to calculate OAVs in this investigation (Table 2.3). All odor threshold values referenced were recognition threshold values found in literature (Van Gemert 2011, Belitz and others 2009).
Table 2.3 Odor threshold values in air for volatile compounds found in Swiss cheese (Van Gemert 2011, Belitz and others 2009).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Odor Threshold (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-2-nonenal</td>
<td>0.19</td>
</tr>
<tr>
<td>2,3-butanedine</td>
<td>1.28</td>
</tr>
<tr>
<td>2-methylpropanal</td>
<td>139.03</td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td>0.28</td>
</tr>
<tr>
<td>3-methylbutanoic acid</td>
<td>191.63</td>
</tr>
<tr>
<td>3-methylindole</td>
<td>0.07</td>
</tr>
<tr>
<td>acetic acid</td>
<td>1723.21</td>
</tr>
<tr>
<td>ammonia</td>
<td>20558.62</td>
</tr>
<tr>
<td>butanal</td>
<td>25.71</td>
</tr>
<tr>
<td>butanoic acid</td>
<td>198.84</td>
</tr>
<tr>
<td>diethyl sulfide</td>
<td>5.69</td>
</tr>
<tr>
<td>dimethyl disulfide</td>
<td>4.93</td>
</tr>
<tr>
<td>dimethyl sulfide</td>
<td>6.26</td>
</tr>
<tr>
<td>dimethyl trisulfide</td>
<td>1.41</td>
</tr>
<tr>
<td>ethanol</td>
<td>431575.02</td>
</tr>
<tr>
<td>ethyl butanoate</td>
<td>17.37</td>
</tr>
<tr>
<td>ethyl hexanoate</td>
<td>1.07</td>
</tr>
<tr>
<td>ethyl methyl sulfide</td>
<td>39.81</td>
</tr>
<tr>
<td>furaneol</td>
<td>0.24</td>
</tr>
<tr>
<td>gamma-decalactone</td>
<td>0.19</td>
</tr>
<tr>
<td>homofuraneol</td>
<td>0.03</td>
</tr>
<tr>
<td>hydrogen sulfide</td>
<td>362.81</td>
</tr>
<tr>
<td>methional</td>
<td>0.01</td>
</tr>
<tr>
<td>methionol</td>
<td>5.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>methyl mercaptan</td>
<td>1.27</td>
</tr>
<tr>
<td>phenylacetaldehyde</td>
<td>0.35</td>
</tr>
<tr>
<td>propionic acid</td>
<td>562.13</td>
</tr>
<tr>
<td>tetramethylpyrazine</td>
<td>123.87</td>
</tr>
</tbody>
</table>

<sup>a</sup>Odor threshold determined in water.
2.2.3 Statistical analysis

VOC data was evaluated using one-way analysis of variance (ANOVA) with Tukey’s honestly significant difference (T-HSD) (Minitab v16, Minitab Inc, State College, PA, U.S.A.). Differentiation of Swiss cheeses based on OAVs was evaluated using soft independent modeling of class analogy (SIMCA) by Pirouette® software (version 3.11, Infometrix, Inc., Woodville, WA, U.S.A.).

2.3 Descriptive Sensory Analysis

2.3.1 Panelists

Eight panelists trained in the Spectrum method, each having at least 100 h of descriptive analysis experience with Swiss cheese flavor, evaluated all fifteen Swiss cheese samples.

2.3.2 Procedure

The descriptive analysis of Swiss cheese flavor was conducted at North Carolina State University. Panelists used a previously developed cheese flavor sensory language adapted to Swiss cheese flavor and typical sensory science techniques which included 21 attributes (Table 2.4). For assessment, panelists used a 15-point universal intensity scale in accordance with the Spectrum method. With the universal scale, panelists score intensities in the same manner across all attributes and all cheese samples.

Panelists learned to identify and scale flavor descriptors by using the same intensity scale through presentation and discussion of flavor definitions, references, and a
wide array of cheese types, including Swiss cheese. Cheeses were evaluated in duplicate in a randomized order on paper ballots in booths dedicated to sensory analysis and free from external aromas, noise, and distractions. Panelists were instructed to expectorate samples after evaluation, and spring water was available to each panelist for palate cleansing. Descriptive data was then evaluated by one-way analysis of variance (ANOVA) with Tukey’s HSD (honestly significant difference).
Table 2.4 Swiss cheese descriptive analysis lexicon (Liggett and others 2008).

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked/milky</td>
<td>Aromatics associated with cooked milk</td>
<td>Skim milk heated to 85°C for 30 min</td>
</tr>
<tr>
<td>Whey</td>
<td>Aromatics associated with Cheddar cheese whey</td>
<td>Fresh Cheddar whey</td>
</tr>
<tr>
<td>Diacetyl (buttery)</td>
<td>Aromatics associated with Diacetyl</td>
<td>Diacetyl</td>
</tr>
<tr>
<td>Milk fat</td>
<td>Aromatics associated with milk fat</td>
<td>Fresh coconut meat, heavy cream, γ-dodecylactone</td>
</tr>
<tr>
<td>Vinegar</td>
<td>Aromatics associated with vinegar</td>
<td>Distilled white vinegar, acetic acid</td>
</tr>
<tr>
<td>Dried fruit</td>
<td>Aromatics associated with dried fruits, specifically peaches and apricots</td>
<td>Dried apricot half</td>
</tr>
<tr>
<td>Fruity</td>
<td>Aromatics associated with different fruits</td>
<td>Fresh pineapple, ethyl hexanoate</td>
</tr>
<tr>
<td>Sulfur/eggy</td>
<td>Aromatics associated with cooked eggs</td>
<td>Hard-boiled egg (mashed)</td>
</tr>
<tr>
<td>Cheesy/butyric acid</td>
<td>Aromatics associated with butyric acid</td>
<td>Butyric acid</td>
</tr>
<tr>
<td>Brothy</td>
<td>Aromatics associated with boiled meat or vegetable stock</td>
<td>Canned potatoes, Wyler’s low-sodium beef broth cubes, methional</td>
</tr>
<tr>
<td>Nutty</td>
<td>The nutlike aromatic associated with different nuts</td>
<td>Lightly toasted unsalted nuts, unsalted cashew nuts, unsalted Wheat Thins</td>
</tr>
<tr>
<td>Sweaty</td>
<td>Aromatic associated with human sweat</td>
<td>Hexanoic acid</td>
</tr>
<tr>
<td>Cowy/phenolic</td>
<td>Aromas associated with barns and stock trailers, indicative of animal sweat and waste</td>
<td>Band-Aids, p-cresol, phenol</td>
</tr>
<tr>
<td>Sour</td>
<td>Fundamental taste sensation elicited by acids</td>
<td>Citric acid (0.08% in water)</td>
</tr>
<tr>
<td>Bitter</td>
<td>Fundamental taste sensation elicited by various compounds</td>
<td>Caffeine (0.08% in water)</td>
</tr>
<tr>
<td>Salty</td>
<td>Fundamental taste sensation elicited by salts</td>
<td>Sodium chloride (0.5% in water)</td>
</tr>
<tr>
<td>Sweet</td>
<td>Fundamental taste sensation elicited by sugars</td>
<td>Sucrose (5% in water)</td>
</tr>
<tr>
<td>Umami</td>
<td>Chemical feeling factor elicited by certain peptides and nucleotides</td>
<td>Monosodium glutamate (1% in water)</td>
</tr>
<tr>
<td>Prickle</td>
<td>Chemical feeling factor of which the sensation of carbonation on the tongue is typical</td>
<td>Soda water</td>
</tr>
<tr>
<td>Metallic</td>
<td>Chemical feeling factor elicited by metallic objects in the mouth</td>
<td>Aluminum foil</td>
</tr>
</tbody>
</table>

2.4 Consumer Sensory Testing

2.4.1 Panelists

A total of 100 untrained consumers (76 females and 24 males) were pre-recruited from the Food Industry Center database at the Parker Food Science and Technology
Building on the Columbus campus of The Ohio State University in the sensory testing area. Demographics indicated that over 75% of the panelists eat Swiss cheese regularly or at least twice every two weeks (Table 2.5).

Table 2.5 Demographics of how often panelists typically consume Swiss cheese.

<table>
<thead>
<tr>
<th>How often typically consume Swiss Cheese</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x Day</td>
<td>3%</td>
</tr>
<tr>
<td>2 x Week</td>
<td>24%</td>
</tr>
<tr>
<td>1 x week</td>
<td>18%</td>
</tr>
<tr>
<td>1 x 2 weeks</td>
<td>32%</td>
</tr>
<tr>
<td>1 x month</td>
<td>19%</td>
</tr>
<tr>
<td>1 x 3 Months</td>
<td>2%</td>
</tr>
<tr>
<td>1 x 6 months</td>
<td>3%</td>
</tr>
<tr>
<td>1 x Year</td>
<td>0%</td>
</tr>
<tr>
<td>Rarely</td>
<td>2%</td>
</tr>
<tr>
<td>Never</td>
<td>0%</td>
</tr>
</tbody>
</table>

2.4.2 Procedure

Cheeses (5 samples) were served at room temperature in 2 oz disposable plastic soufflé cups (GFS, Grand Rapids, M.I.) that had been labeled with three-digit codes. All samples were presented in randomized order to each testing booth to reduce potential first order bias. Testing was conducted under standard room conditions plus spot white lighting. Panelists evaluated samples individually in separate booths and entered their
own responses directly into a computer using Compusense five data collection and analysis software (Compusense® five, release 5.2, Compusense Inc., Guelph, ON, Canada). Panelists assessed visual characteristics before tasting samples, rating each sample independently for overall liking on a 9-point vertical hedonic category scale and Just About Right (JAR) scale. Panelists were allowed to swallow or expectorate samples, as desired; retasting was allowed; and each panelist proceeded at his or her own pace. Room temperature spring water was provided for rinsing between samples.

Demographics questions followed the sample evaluations (age, gender, Swiss cheese consumption, and race). Data was analyzed using one-way analysis of variance (ANOVA) with Tukey’s HSD (honestly significant difference).
Chapter 3: Determination of Flavor Variation in Swiss Cheese from Five Different Factories by Quantification of Volatile Organic Compounds (VOCs) Using Selected Ion Flow Tube- Mass Spectrometry (SIFT-MS)

3.1 Introduction

Swiss cheese flavor is very complex with over 600 volatile compounds having been reported (Harper and others 2011). Many studies have focused on identifying important volatile compounds found specifically in Swiss cheese. Preininger and Grosch (1994) detected thirteen compounds in Swiss cheese including 2,3-butanedione, 3-methylbutanal, ethyl butanoate, ethyl 3-methylbutanoate, 2-heptanone, methional, 1-octen-3-one, ethyl hexanoate, furanones, skatole, γ-decalactone, and 2-sec-butyl-3-methoxypyrazine. Acetic acid and propionic acid are the most important volatile compounds for the typical Swiss cheese flavor (Bachmann and others 2011, Lawlor and others 2002, Preininger and others 1996). Ethyl butanoate, ethyl 3-methyl butanoate, and ethyl hexanoate give Swiss cheese a fruity note and were found to play an important role in the overall flavor profile of Swiss cheese (Preininger and Grosch 1994, Langler and others 1967). Compounds such as methional (boiled potato odor), hydrogen sulfide (rotten egg odor), methyl mercaptan (eggy odor), dimethyl disulfide (garlic odor), and
dimethyl trisulfide (garlic odor) have also been detected in Swiss cheese (Landaud and others 2008, Yang and Min 1994, Harper and others 2011).

The most common method to identify and quantify these volatile compounds in food products has been gas chromatography and mass spectrometry (GC-MS) in combination with some method of sample concentration. However, this current method is not sensitive enough to measure VOCs directly in the head space of a food at levels as low as parts per billion-trillion by volume (ppb-ppt). Every analytical method used for volatile analysis has restrictions, but selected ion flow tube mass spectrometry (SIFT-MS) has the capability of measuring VOCs in real time, directly in the food head space at concentrations of ppb to ppt (Spanel and Smith 1999). This sensitivity is sufficient to determine concentrations of compounds without need for concentration, and the volatiles remain in the same proportion as they would be introduced to the consumer.

Although many studies have identified and quantified important volatile compounds in Swiss cheese, little research has focused on the flavor variability in Swiss cheese based on concentrations alone and linked the important compounds to biochemical pathways. A high quality, consistent product is necessary to attract and retain customers, who will not continue to purchase a product if it does not fulfill their flavor expectations (Drake 2004). Therefore this indicates that identifying flavor variability in Swiss cheese will not only benefit the factories producing the cheese but also the customers who purchase the cheese. In a previous study with different cheese samples, we determined that Swiss cheese from five factories had significantly different volatile compounds concentrations indicating flavor variation, but focused only on 21
high impact compounds determined by odor activity values (Taylor and others 2013). The objectives of this study were to utilize SIFT-MS to quantify VOCs in Swiss cheese and to identify variability in VOC profiles amongst five different factories to understand the cause of flavor variation in Swiss cheese through specific biochemical pathways.

3.2 Materials and Methods

3.2.1 Swiss Cheese Samples

Fifteen cheeses were collected from November 2011 to April 2012 from member companies of the Swiss Cheese Consortium, where five factories contributed three cheeses each. The factories that make up the Swiss Cheese Consortium are located in Ohio, Illinois, Pennsylvania, and Iowa. These factories are designated as 148, 207, 374, 465, and 528. The Swiss cheeses varied in manufacturing dates, as well as the vat and block location (Table 2.1). Variation of cheeses within most factories was limited to different blocks from the same vat make. In addition, each of the five factories used different starter cultures in the processing of their Swiss cheese (Table 2.2).

3.2.2 Volatile Organic Compound (VOC) Quantification by Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS)

Each cheese was divided into smaller 500 g blocks, vacuumed packed, and stored at -80°C. The cheeses were analyzed in random order in replicates of six over a period of two weeks. Data was reported in ppb. The cheese samples were shredded using a hand grater (OXO, New York, NY). An aliquot of 5 g was placed in a one liter bottle with a
septa cap that could be punctured by a needle to introduce the head space into the SIFT-MS (SYFT Technologies Voice 200, Christchurch, New Zealand). Samples were heated to 40°C for 1 h prior to analysis.

The head space of each 5 g sample was analyzed by the SIFT-MS instrument using three soft ionizing reagent ions (H$_3$O$^+$, NO$^+$, and O$_2^+$). The flow rate of the SIFT-MS was set at 0.26 torr L/sec. Identification and quantification of the VOCs were determined based on the known ion products and reaction rate coefficients for each compound in a method that contained 28 compounds including alcohols, aldehydes, ketones, esters, sulfur compounds, and pyrazines.

3.2.3 Statistical Analysis

VOC data was evaluated using one-way analysis of variance (ANOVA) with Tukey’s honestly significant difference (T-HSD) (Minitab v16, Minitab Inc, State College, PA, U.S.A.). Differentiation of Swiss cheeses based on VOC profiles was evaluated using soft independent modeling of class analogy (SIMCA) by Pirouette® software (version 3.11, Infometrix, Inc., Woodville, WA, U.S.A.).

3.3 Results and Discussion

3.3.1 Volatile Organic Compound (VOC) Concentrations

Differences were noted in the average VOC concentrations of 28 total compounds for the cheeses from the five factories (Table 3.1). These differences illustrate the complex flavor of Swiss cheese on a chemical basis. Ethanol had the highest
concentration in Factory 374 of 14,978 ppb while 3-methylindole had the lowest concentration also in Factory 374 of 1.01 ppb. Ethanol has been quantified in Swiss cheese at very high concentrations in other studies as well (Yang and Min 1994). Only one of the 28 compounds quantified, γ-decalactone, was not significantly different amongst the five Swiss cheese factories. The concentrations ranged from 3.55 ppb to 3.90 ppb for γ-decalactone. Preininger and Grosch (1994) also detected γ-decalactone in Swiss cheese at very low concentrations. All other compounds showed significant differences between at least two factories. Butyric acid was the only compound to be significantly different in the cheeses from all five factories, with P values < 0.05. On the other hand, there were six compounds that could be differentiated in four of the five factories. These compounds were 2-methylpropanal, butanal, furaneol, homofuraneol, methionol, and tetramethylpyrazine. Grosch (1994), Preininger and Grosch (1994), and Preininger and others (1996) all determined that furanones (furaneol and homofuraneol) are important compounds in Swiss cheese flavor.
Table 3.1 Average concentrations of volatile organic compounds in Swiss cheese from five different factories.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Average Concentration (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Factory 148</td>
</tr>
<tr>
<td>(E)-2-nonenal</td>
<td>1.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2,3-butanedione</td>
<td>92.26&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-methylpropanal</td>
<td>109.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td>65.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-methylbutanoic acid</td>
<td>64.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3-methylindole</td>
<td>1.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>acetic acid</td>
<td>1854.85&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ammonia</td>
<td>15.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>butanal</td>
<td>250.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>butyric acid</td>
<td>1045.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>diethyl sulfide</td>
<td>40.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>dimethyl disulfide</td>
<td>2.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>dimethyl sulfide</td>
<td>6.91&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>dimethyl trisulfide</td>
<td>6.05&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>ethanol</td>
<td>2008.65&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>ethyl butanoate</td>
<td>109.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ethyl hexanoate</td>
<td>14.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ethyl methyl sulfide</td>
<td>388.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>furaneol</td>
<td>7.94&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>gamma-decalactone</td>
<td>3.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>homofuraneol</td>
<td>5.59&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>hydrogen sulfide</td>
<td>1.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>methional</td>
<td>8.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>methionol</td>
<td>32.53&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>methyl mercaptan</td>
<td>17.80&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>phenylacetaldehyde</td>
<td>13.34&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>propionic acid</td>
<td>1019.99&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>tetramethylpyrazine</td>
<td>1.78&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abcd</sup>Different letters indicate significant differences amongst factories by compound.
3.3.2 Soft Independent Modeling of Class Analogy (SIMCA)

A soft independent modeling of class analogy (SIMCA) class projection plot, with all of the VOC concentrations, showed differentiation between the five factories (Figure 3.1). Factory 374 was completely isolated while there were overlaps between some of the other factories. Each factory can still have a unique VOC profile while also sharing flavor characteristics with another factory (Taylor and others 2013). For a measure of separation between classes in the SIMCA model, there are interclass distances (Table 3.2). The interclass distances between the five factories determined by the SIMCA model were all greater than 3 ($P < 0.05$), which is the value accepted to indicate statistically significant differentiation (Vogt and Knutsen 1985). The lowest value was 8.33 between factories 465 and 528, which were overlapping in the SIMCA class projection plot. Although these factories were shown to be similar in the SIMCA class projection plot, the interclass distance between the two factories illustrates that they are significantly different.
Figure 3.1 SIMCA classification plot showing discrimination of Swiss cheese from five factories based on concentrations of all VOCs analyzed.

Table 3.2 Interclass distances of Swiss cheese classified by factory based on the SIMCA model.

<table>
<thead>
<tr>
<th>Factory</th>
<th>528</th>
<th>465</th>
<th>374</th>
<th>207</th>
<th>148</th>
</tr>
</thead>
<tbody>
<tr>
<td>528</td>
<td>0</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>465</td>
<td>8.33</td>
<td>0</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>374</td>
<td>41.53</td>
<td>29.74</td>
<td>0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>207</td>
<td>30.25</td>
<td>23.33</td>
<td>42.05</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>148</td>
<td>31.37</td>
<td>19.25</td>
<td>22.19</td>
<td>22.84</td>
<td>0</td>
</tr>
</tbody>
</table>
The discriminating power based on the SIMCA model illustrates the importance that each compound contributes to the overall differentiation of the Swiss cheeses based on factory classification (Molfetta and others 2005). There were five compounds with discriminating powers greater than 1000 (Figure 3.2). These compounds were 2-methyl propanal, acetic acid, butyric acid, ethyl methyl sulfide, and propionic acid. Ethyl methyl sulfide had the highest discriminating power of 4575. In general, the formation of volatile sulfur compounds in Swiss cheese is very important to the overall flavor (Harper and others 2011). The formation of ethyl methyl sulfide in these Swiss cheeses was very important for discrimination amongst factories. These five compounds shown in the discriminating power plot are the most important compounds for distinguishing the cheeses from the five factories based on the SIMCA model.
Based on all the VOC concentrations quantified and the differentiation from the SIMCA model, there are several key compounds that specifically discriminated the VOC profiles of Swiss cheese from each of the five different factories (Table 3.3). All factories had acetic acid, propionic acid, and ethanol as common key compounds showing good differentiation between the different VOC profiles. Other studies also found that acetic acid and propionic acid are important volatile compounds for the typical Swiss cheese flavor (Bachmann and others 2011, Lawlor and others 2002, and Preininger and others 1996). Butyric acid was also significant to three of the five factories (Factory 374, 207, and 148). Factory 528 and Factory 465 had identical key compounds, which correlates with the overlapping in the SIMCA class projection plot. These factories clearly have
similar VOC profiles because they are discriminated using the same compounds. The key compounds for Factory 374 were determined to be acetic acid, propionic acid, ethanol, butyric acid, and 2,3-butanedione (Figure 3.3). Factory 374 has a distinctive VOC profile due to the unique key compound of 2,3-butanedione. Langler and others (1967) and Preininger and Grosch (1994) both also found that 2,3-butanedione plays a significant role in the flavor of Swiss cheese.

Table 3.3 Key compounds that specifically discriminate the VOC profiles of Swiss cheese from each of the five factories based on the SIMCA model.

<table>
<thead>
<tr>
<th>Factory</th>
<th>Key Discriminating Compound(s) (associated flavor description)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>528</td>
<td>Acetic acid (sour) Propionic acid (pungent) Ethanol (sweet)</td>
</tr>
<tr>
<td>465</td>
<td>Acetic acid (sour) Propionic acid (pungent) Ethanol (sweet)</td>
</tr>
<tr>
<td>374</td>
<td>Acetic acid (sour) Propionic acid (pungent) Ethanol (sweet) Butyric acid (sweaty, cheese) 2,3-butanedione (buttery)</td>
</tr>
<tr>
<td>207</td>
<td>Acetic acid (sour) Propionic acid (pungent) Ethanol (sweet) Butyric acid (sweaty, cheese)</td>
</tr>
<tr>
<td>148</td>
<td>Acetic acid (sour) Propionic acid (pungent) Ethanol (sweet) Butyric acid (sweaty, cheese)</td>
</tr>
</tbody>
</table>

*Additional information on aroma notes was obtained from Flavornet 2004.
3.3.3 *Links to Biochemical Pathways*

Based on the SIMCA model, seven compounds (propionic acid, acetic acid, ethanol, butyric acid, 2,3-butanedione, ethyl methyl sulfide, and 2-methylpropanal) were determined to be important to the discrimination of Swiss cheeses from five factories by VOC profile. Acetic acid, ethanol, propionic acid, 2,3-butanedione, and butyric acid are all formed during the secondary metabolism of lactic acid by non-starter lactic acid bacteria (NSLAB) and *Propionibacterium* (Law 2010). Butyric acid is specifically a result of the lipolytic activity of *Propionibacterium* (Fox and others 2004) while 2,3-butanedione is formed from the breakdown of citrate (Singh and others 2003). Sulfur compounds, like ethyl methyl sulfide, are formed from the breakdown of amino acids. 2-
Methylpropanal was also formed by the catabolism of amino acids, specifically valine (Singh and others 2003, Pripis-Nicolau and others 2000).

Often, the fermentation by NSLAB and the excessive fermentation by *Propionibacterium* can cause flavor defects such as excessive fruitiness and over acidification, which may be the cause of flavor variation in these cheeses (Law 2010). From this study, the breakdown of amino acids and secondary metabolism of lactic acid are the main biochemical pathways leading to flavor variation in Swiss cheese from five different factories.

### 3.4 Conclusion

Overall, the VOC concentrations were significantly different between the five factories. Ethanol was determined to have the highest concentrations while γ-decalactone had the lowest concentrations and was the only compound that was not significantly different amongst the five factories. Using the SIMCA model with the associated interclass distances, Swiss cheeses can be differentiated based on location (factory produced in) alone.

Based on this discrimination, Swiss cheese factories can begin to narrow down the cause of flavor variation in their Swiss cheese. SIFT-MS in combination with statistics led to the rapid identification of 2-methylpropanal, 2,3-butanedione, acetic acid, butyric acid, ethanol, ethyl methyl sulfide, and propionic acid as the key compounds from the breakdown of amino acids and secondary metabolism of lactic acid causing this variation. In the future, this information could be correlated with odor activity values and
sensory data to continue to identify the exact biochemical pathways causing flavor variation and to provide these factories a quality target for Swiss cheese therefore limiting flavor variation in Swiss cheese.

3.5 References


Chapter 4: Identification of High Impact Compounds in Swiss Cheese from Five Different Factories by Calculation of Odor Activity Values (OAVs)

4.1 Introduction

Although food products can contain hundreds of VOCs, research has shown that every compound does not have a major effect on the flavor profile of the product (Qian and Reineccius 2003, Milo and Reineccius 1997, Preininger and Grosch 1994). Only those compounds with odor activity values (OAV) greater than one significantly contribute to the flavor (Qian and Reineccius 2003). OAVs are defined as the concentration of the compound in the sample divided by the odor threshold value, in air, for that compound. OAVs less than one are considered to have little effect on flavor because they are below the detectable odor threshold, but those OAVs greater than one are considered to have a high impact on flavor.

Because comparing specific volatile concentrations does not illustrate the overall effect of each compound on the flavor, research has started to focus on the use of OAVs as a means of identifying the compounds considered to be high impact to the flavor of a specific food. Qian and Reineccius (2003) and Milo Reineccius (1997) used this OAV approach to evaluate volatile compounds in Parmigiano Reggiano cheese and Cheddar
Preininger and Grosch (1994) found the key odorants for Emmentaler cheese to be methional, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, and 5-ethyl-4-hydroxy-2-ethyl-3(2H)-furanone with a total of twelve compounds having OAVs greater than one in at least one cheese using this same approach. In a previous study, we also used the OAV approach to determine that methional, 2,3-butanedione, homofuraneol, furaneol, and 3-methylbutanoic acid are key odorants in Swiss cheese (Taylor and others 2013). We found that 20 compounds (total of 24 compounds) were high impact compounds (had OAVs greater than one) in at least one factory. Both studies showed that methional, homofuraneol, and furaneol are important to the overall flavor of Swiss cheese.

The objectives of this study were to utilize the OAV approach to determine the high impact compounds in Swiss cheese and utilize the high impact compounds to differentiate OAV profiles of Swiss cheese from five different factories. Although we used this same approach to discriminate cheeses from different locations (Taylor and others 2013), this study is confirming the OAV approach with the addition of new high impact compounds and links to biochemical pathways. In the future, the results of this study will be compared with sensory data for the same Swiss cheeses. Furthermore, a high quality, consistent product is necessary to retain customers, who will not continue to purchase a product if it does not meet their flavor expectations (Drake 2004), therefore this information can ultimately provide these five factories a quality target for Swiss cheese.
4.2 Materials and Methods

4.2.1 Swiss Cheese Samples

Fifteen cheeses were collected from November 2011 to April 2012 from member companies of the Swiss Cheese Consortium, where five factories contributed three cheeses each. The factories that make up the Swiss Cheese Consortium are located in Ohio, Illinois, Pennsylvania, and Iowa. These factories are designated as 148, 207, 374, 465, and 528. The Swiss cheeses varied in manufacturing dates, as well as the vat and block location (Table 2.1). Variation of cheeses within most factories was limited to different blocks from the same vat make. In addition, each of the five factories used different starter cultures in the processing of their Swiss cheese (Table 2.2).

4.2.2 Odor Activity Value (OAV) Calculations

Odor activity values (OAVs) were calculated by dividing the volatile organic compound (VOC) concentration by the odor threshold value of that specific compound in air. VOC concentrations were determined using selected ion flow tube-mass spectrometry (SIFT-MS) (SYFT Technologies Voice 200, Christchurch, New Zealand). Identification and quantification of the VOCs were determined based on the known ion products and reaction rate coefficients for each compound in a method that contained 28 compounds including alcohols, aldehydes, ketones, esters, sulfur compounds, and pyrazines. All concentrations were reported in ppb (Table 3.1). Odor threshold values were used to calculate OAVs in this investigation (Table 2.3). All odor threshold values
referenced were recognition threshold values in air (when feasible) found in literature (Van Gemert 2011, Belitz and others 2009).

4.2.3 Statistical analysis

OAV data was evaluated using one-way analysis of variance (ANOVA) with Tukey’s honestly significant difference (T-HSD) (Minitab v16, Minitab Inc, State College, PA, U.S.A.). Differentiation of Swiss cheeses based on OAV profiles was evaluated using soft independent modeling of class analogy (SIMCA) and principle component analysis (PCA) by Pirouette® software (version 3.11, Infometrix, Inc., Woodville, WA, U.S.A.).

4.3 Results and Discussion

4.3.1 Odor Activity Values (OAVs)

Odor activity values (OAVs) were calculated to make a valid comparison between volatile compounds on the basis of their relative importance to the overall flavor to each Swiss cheese (Table 4.1). OAVs increase the weight of compounds that are more associated with flavor and aroma. This approach illustrates the flavor as a consumer would evaluate it instead of only looking at flavor on a chemical basis. Only six of the 28 compounds had an OAV less than one in all five factories. These compounds that provide little effect on the flavor of Swiss cheese were 2-methylpropanal, 3-methylbutanoic acid, ammonia, ethanol, hydrogen sulfide, and 2,3,4,6-tetramethylpyrazine. In a previous study, we also found that ammonia, ethanol, and 2,3,4,6-tetramethylpyrazine had an
OAV less than one in all five factories (Taylor and others 2013). Dimethyl disulfide had an OAV less than one in four of the five factories, but in Factory 374 it had an OAV of 2.20 illustrating its importance only in Swiss cheese from Factory 374.

All other compounds (22 compounds) had OAVs greater than one in all five factories indicating that these compounds are high impact and important to Swiss cheese flavor. In this study more high impact compounds were identified in Swiss cheese compared to Preininger and Grosch (1994) who found 12 high impact compounds and our previous study (Taylor and others 2013) which found 20 high impact compounds in Swiss cheese. Butyric acid was the only compound that was significantly different amongst all five factories with P values < 0.05, while γ-decalactone was the only compound that was not significantly different amongst any of the five factories. Previously in a study with different cheese samples from the same five factories, we only detected butyric acid in cheeses from two of the five factories and did not even quantify γ-decalactone (Taylor and others 2013). There were several compounds (butanal, furaneol, homofuraneol, and methionol) that could also be differentiated between four of the factories. The calculation of OAVs makes it apparent that there are many statistically significant differences for many different compounds between as few as two different factories.
Table 4.1 Average Odor Activity Values (OAV) for VOCs in Swiss cheese from five different factories.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Average Odor Activity Value (OAV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Factory</td>
</tr>
<tr>
<td></td>
<td>148</td>
</tr>
<tr>
<td>(E)-2-nonenal</td>
<td>6.66b</td>
</tr>
<tr>
<td>2,3-butanedione</td>
<td>72.19bc</td>
</tr>
<tr>
<td>2-methylpropenal</td>
<td>0.79a</td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td>235.95a</td>
</tr>
<tr>
<td>3-methylbutanoic acid</td>
<td>0.34c</td>
</tr>
<tr>
<td>3-methylindole</td>
<td>16.75c</td>
</tr>
<tr>
<td>acetic acid</td>
<td>1.08c</td>
</tr>
<tr>
<td>ammonia</td>
<td>0.001b</td>
</tr>
<tr>
<td>butanal</td>
<td>9.73a</td>
</tr>
<tr>
<td>butanoic acid</td>
<td>5.26c</td>
</tr>
<tr>
<td>diethyl sulfide</td>
<td>7.03b</td>
</tr>
<tr>
<td>dimethyl disulfide</td>
<td>0.47b</td>
</tr>
<tr>
<td>dimethyl sulfide</td>
<td>1.10c</td>
</tr>
<tr>
<td>dimethyl trisulfide</td>
<td>4.28bc</td>
</tr>
<tr>
<td>ethanol</td>
<td>0.005bc</td>
</tr>
<tr>
<td>ethyl butanoate</td>
<td>6.28c</td>
</tr>
<tr>
<td>ethyl hexanoate</td>
<td>13.68b</td>
</tr>
<tr>
<td>ethyl methyl sulfide</td>
<td>9.76b</td>
</tr>
<tr>
<td>furaneol</td>
<td>33.29bc</td>
</tr>
<tr>
<td>gamma-decalactone</td>
<td>20.53a</td>
</tr>
<tr>
<td>homofuraneol</td>
<td>216.50d</td>
</tr>
<tr>
<td>hydrogen sulfide</td>
<td>0.003b</td>
</tr>
<tr>
<td>methional</td>
<td>557.19c</td>
</tr>
<tr>
<td>methionol</td>
<td>6.51d</td>
</tr>
<tr>
<td>methyl mercaptan</td>
<td>14.01c</td>
</tr>
<tr>
<td>phenylacetaldehyde</td>
<td>38.55c</td>
</tr>
<tr>
<td>propionic acid</td>
<td>1.81c</td>
</tr>
<tr>
<td>tetramethylpyrazine</td>
<td>0.01cd</td>
</tr>
</tbody>
</table>

abcde Different letters indicate significant differences amongst factories.
4.3.2 Soft Independent Modeling of Class Analogy (SIMCA)

In order to classify the Swiss cheeses by factory, a soft independent modeling of class analogy (SIMCA) model was used. The SIMCA class projection plot helps to visualize the separation of the five factories based a 3-factor principal component analysis (Figure 4.1). There are some overlaps between three of the factories. Each factory can still have a unique OAV fingerprint while also sharing flavor characteristics with another factory (Taylor and others 2013). SIMCA models were also used to explore the relationship between the three cheeses within each factory (data not shown). Only two factories (Factory 374 and Factory 148) showed differentiation between all three cheeses based on SIMCA class projection plots and interclass distances. This discrimination within a factory obviously results in more flavor variation illustrating the significance of this study. The three cheeses from each of the other factories were determined not to be significantly different within their own factory.
Figure 4.1 SIMCA class projection plot showing discrimination of Swiss cheese from five factories based on OAVs.

Interclass distances between samples are a good measure of the separation between classes in the SIMCA class projection plot. The interclass distances were all greater than 3 (Table 4.2), which is the value accepted to indicate statistically significant differences between two classes (Vogt and Knutsen 1985). In a previous study with different cheese samples from the same five factories, we had one interclass distance that was less than 3 between Factory 374 and Factory 465 (Taylor and others 2013). In this study with different cheeses, all of the factories now had statistical differences based on the SIMCA model. The interclass distance between Factory 374 and 528 was the highest, with a value of 27.37. These factories had the most different OAV profiles. On the other hand, the lowest interclass distance value was 3.26 between Factories 528 and 465, which
is visualized in the SIMCA model. However, the overlapping on the SIMCA class projection plot does not completely illustrate the discrimination of factories based on OAV profiles.

The Cooman’s plot based on the SIMCA model is one of the best ways to visualize classification between different classes. In a Cooman’s plot, class membership is determined in terms of distance from the boundaries (95% confidence interval) of the categories in the classification model, in this case by factory. The axes are the distances of each sample from a specific class (factories) and the coordinates are calculated SIMCA distances (Pettersen and others 1998). The distances from Factory 465 and from Factory 528 have been plotted in a Cooman’s plot based on the SIMCA model (Figure 4.2). Factory 465 and 528 both are within their individual class indicating significant differences between their OAV profiles. As shown in the figure, none of the samples are associated as being part of both classes (lower left rectangle). The Cooman’s plot, by using five factors instead of just three factors used in the SIMCA class projection plot, shows distinct differentiation between all five factories.
Table 4.2  SIMCA model interclass distances of Swiss cheese classified by factory based on OAVs.

<table>
<thead>
<tr>
<th>Factory</th>
<th>528</th>
<th>465</th>
<th>374</th>
<th>207</th>
<th>148</th>
</tr>
</thead>
<tbody>
<tr>
<td>528</td>
<td>0</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>465</td>
<td>3.26</td>
<td>0</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>374</td>
<td>27.37</td>
<td>20.14</td>
<td>0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>207</td>
<td>5.39</td>
<td>3.33</td>
<td>14.31</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>148</td>
<td>4.98</td>
<td>5.66</td>
<td>23.30</td>
<td>11.54</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 4.2 Cooman's Plot from the SIMCA model for Factory 465 and Factory 528 indicates complete discrimination between these two factories.

The discriminating power that the SIMCA model provides illustrates the variable importance that each compound contributes to the overall discrimination of the five factories based on OAV profiles (Molfetta and others 2005). The five compounds that were found to be important to the overall discrimination based on the discriminating power are 2,3-butanedione, 3-methylbutanal, ethanol, methyl mercaptan, and ethyl methyl sulfide, which had the highest discriminating power of 885 despite having low OAVs (Figure 4.3). Ethyl methyl sulfide also had the highest discriminating power for the discrimination of Swiss cheeses from five different factories based on VOC profiles (Figure 3.2). The formation of volatile sulfur compounds, like ethyl methyl sulfide, in
Swiss cheese is very important to the overall flavor (Harper and others 2011). Despite having an OAV less than one in all five factories, ethanol was still important to the separation of OAV profiles between factories.

![Discriminating power plot showing compounds utilized to differentiate Swiss cheeses in the SIMCA model based on OAVs.](image)

Figure 4.3 Discriminating power plot showing compounds utilized to differentiate Swiss cheeses in the SIMCA model based on OAVs.

These compounds found to be important to the general differentiation of cheeses from all five factories based on OAV profiles were different than the compounds found important for separation based on VOC profiles (Figure 3.2). By calculating OAVs and normalizing concentrations, there was a shift in the compounds identified as important...
for discrimination. This change in discriminating powers illustrates the importance of calculating OAVs instead of utilizing only concentrations of volatile compounds. In addition, the compounds found in this study to be important to the discrimination of the cheeses by factory based on OAVs were not the same as in a previous study with different cheeses from the same five factories (Taylor and others 2013). 2,3-Butanedione was the only compound that both studies found to be important to differentiating the cheese by factory based on the SIMCA model, therefore illustrating the significance of the flavor variation in Swiss cheese as a growing problem.

There are also several key compounds specific to each factory that help discriminate the OAV profiles of their Swiss cheeses using both OAVs and the differentiation from the SIMCA model (Table 4.3). Only two compounds, homofuraneol and methional, were important to the individual discrimination of all five factories most likely due to the very high OAVs for both compounds in all five factories (Table 4.1). Previously, we determined these same two compounds were important to the discrimination of the cheeses from three of the five factories (Taylor and others 2013).

Also, Preininger and Grosch (1994) found that both methional and homofuraneol were overall key odorants for Swiss cheese. 3-Methylbutanal was found to be a key compound specifically to Factory 528, 465, and 148 while 2,3-butanedione was valuable to the discrimination of Factory 465 and Factory 374. 2,3-Butanedione has been found in many studies to be important to the overall flavor of Swiss cheese with high OAVs (Langler and others 1967, Preininger and Grosch 1994, Taylor and others 2013). Methyl
mercaptan was a unique compound only to the separation of the cheeses of Factory 207 from the other four factories.

Table 4.3 Key compounds specific to each factory that discriminate the OAV profiles of their Swiss cheese based on the SIMCA model.

<table>
<thead>
<tr>
<th>Factory</th>
<th>Key Discriminating Compound(s) (associated flavor description)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>528</td>
<td>Methional (cooked potato) Homofuraneol (caramel) 3-methylbutanal (sweat, acid)</td>
</tr>
<tr>
<td>465</td>
<td>Methional (cooked potato) Homofuraneol (caramel) 3-methylbutanal (sweat, acid) 2,3-butanedione (buttery)</td>
</tr>
<tr>
<td>374</td>
<td>Methional (cooked potato) Homofuraneol (caramel) 2,3-butanedione (buttery)</td>
</tr>
<tr>
<td>207</td>
<td>Methional (cooked potato) Homofuraneol (caramel, brothy) 2,3-butanedione (buttery) Methyl mercaptan (sulfur, garlic)</td>
</tr>
<tr>
<td>148</td>
<td>Methional (cooked potato) 3-methylbutanal (sweat, acid) Homofuraneol (caramel)</td>
</tr>
</tbody>
</table>

*Additional information on aroma notes was obtained from Flavornet 2004.

4.3.3 Principle Component Analysis (PCA)

Principle component analysis (PCA) explained the relationships between the compounds and the classification of Swiss cheeses based on factory. The first PCA plot
relates all 28 compounds by factory, where the first 2 principal components accounted for 61% of the total variability (Figure 4.4). PC1 accounted for 38% of the total variability while PC2 accounted for 23% of the total variability. The biplot associated specific compounds with each of the five factories. Factory 148 and Factory 374 had more compounds uniquely associated with their cheeses than the other three factories. Swiss cheese produced at Factory 148 (in gray) correlates mostly with 2-methylpropanal, 3-methylbutanal, and butanal while Factory 374 cheeses (in pink) were associated with sulfur compounds (hydrogen sulfide, methyl mercaptan, and dimethyl disulfide), ethanol, furaneol, and 2,3-butanedione. Factory 374 had the highest OAVs for 2,3-butanedione (Table 4.1), which was also a key compound that helped discriminate Factory 374 from the other four factories. Factory 207, 465, and 528 were all associated with 3-methylindole, homofuraneol, and propionic acid. Homofuraneol has been continually determined to be an important compound to Swiss cheese flavor due to its high OAVs (Preininger and Grosch 1994, Taylor and others 2013).
Figure 4.4 PCA biplot showing discrimination of OAV profiles for Swiss cheeses from five factories.

PCA was also used to explain the relationship between the key compounds identified by the SIMCA model and the five different factories (Figure 4.5). For the most part, this PCA plot agrees with Figure 4.4. Factory 374 continues to discriminate based on its high odor activity of 2,3-butanedione while Factory 148 separates because of its high odor activity of 3-methylbutanal. Factory 528 has a low OAV for methional, which gives it a unique OAV profile compared to the high OAVs of the other factories. Homofuraneol has the highest OAV in Factory 465 which is correlated in the discrimination of Factory 465 by homofuraneol in this PCA plot. Figure 4.5 does not
associate a unique volatile compounds with Factory 207 despite being significantly different based on the SIMCA model.

![Figure 4.5 PCA plot showing discrimination of OAV profiles for Swiss cheeses from five factories based only on key compounds identified by the SIMCA model.](image)

4.3.4 Links to Biochemical Pathways

The analysis using the SIMCA model and PCA in combination with OAVs determined that only seven compounds were responsible for the discrimination of Swiss cheeses by factory. This is the same number of compounds found based on VOC profiles.
(Chapter 3, page 30), however the seven compounds are not identical. Based on OAV profiles, 2,3-butanedione, 3-methylbutanal, ethanol, homofuraneol, methional, methyl mercaptan, and ethyl methyl sulfide led to the discrimination of Swiss cheese by factory. Ethyl methyl sulfide, methional, methyl mercaptan, 3-methylbutanal, and homofuraneol are formed from the breakdown of amino acids. Methional and methyl mercaptan have been linked with the breakdown of methionine, and 3-methylbutanal has been associated with the Strecker degradation of leucine (Pripis-Nicolau and others 2000). Homofuraneol is a product of the Maillard reaction between reducing sugars and amino acids, such as glycine and glutamate, in cheese (Hayashida and others 1999, Ohata and others 2007, cited by Drake and others 2010). Milo and Reineccius (1997) suggests that production of homofuraneol in cheese can be effected by the differences in NSLAB. Ethanol and 2,3-butanedione are formed during the secondary metabolism of lactic acid by non-starter lactic acid bacteria (NSLAB) and Propionibacterium (Law 2010).

From this study, the breakdown of amino acids is the main biochemical pathway that led to the discrimination of Swiss cheese by factory. Frequently, the fermentation by NSLAB can lead to off flavors and result in variable Swiss cheese flavor (Law 2010). The secondary metabolism of lactic acid also aided in the differentiation of the Swiss cheeses. Although this analysis only gives insight into the general biochemical pathways, it will begin to help Swiss cheese factories narrow down the source of flavor variation in their Swiss cheese.
4.4 Conclusion

Swiss cheese from five different factories was determined to be significantly different based on OAV profiles. Twenty two high impact compounds (compounds with OAVs greater than 1 in at least one factory) were identified as significantly contributing to the flavor of Swiss cheese, which was more than previous Swiss cheese studies (Taylor and others 2013, Preininger and Grosch 1994). By using a SIMCA model and PCA, key compounds were identified as being important to the overall discrimination of Swiss cheese by factory. With only seven compounds (2,3-butanedione, 3-methylbutanal, ethanol, homofuraneol, methional, methyl mercaptan, and ethyl methyl sulfide), SIMCA and PCA in combination with OAVs led to rapid discrimination of Swiss cheese by factory primarily based on the breakdown of amino acids.

Overall from this study, Swiss cheese factories can begin to narrow down the volatile compounds causing flavor variation in the Swiss cheese from their specific factory. In the future, these OAVs and key high impact compounds can be correlated with descriptive and consumer sensory data to provide these factories a quality target for Swiss cheese. Identification of the relationship between volatile compounds and Swiss cheese characteristics will then allow for a deeper understanding of the biochemical pathways causing flavor variation in Swiss cheese.

4.5 References


Chapter 5: Evaluation of Flavor Variability in Swiss Cheese from Five Different Factories by Descriptive Sensory Analysis and Consumer Testing

5.1 Introduction

Cheese flavor is one of the most important criteria determining consumer choice and acceptance (Young and others 2004, Drake and others 2007). A consumer, whether it be at the grocery store or a product manufacturer, will not continue to purchase a product if it does not fulfill their flavor expectations (Drake 2004). Even though expectations can vary by consumer, it is very important to understand which flavor characteristics are desired by customers to produce a quality target for Swiss cheese factories. A high quality, consistent product is necessary to attract and retain consumers (Drake 2004). Because we determined there is currently variation in Swiss cheese flavor based on volatile compounds (Taylor and others 2013), there is also a need to identify the descriptive sensory characteristics causing the variability amongst cheeses from five different factories. While descriptive sensory analysis is used to identify and quantify information on the sensory aspects of products, effective consumer tests are also necessary to provide information on consumer liking (Meilgaard and others 1999, cited by Drake and Civille 2003). Being able to correlate the consumer preferences with the
descriptive sensory analysis will provide a more detailed understanding of the Swiss cheese variation problem from a consumer perspective.

Sensory perception has been used in many previous studies to analyze cheese flavor. Kocaoglu-Vurma and others (2009) utilized descriptive sensory analysis in combination with infrared spectra to develop predictive models for the determination of sensory attributes of Swiss cheese based on factory location. Descriptive sensory analysis and consumer liking testing has also been used to determine which flavor characteristics of Swiss cheese should be optimized and minimized to produce cheeses preferred by consumers (Liggett and others 2008). Liggett and others (2008) were able to identify that diacetyl, cabbage, cooked, whey, milk fat, umami, and vinegar were most important to the liking of Swiss cheese.

The objectives of this study were to utilize descriptive sensory analysis to evaluate flavor variation in Swiss cheeses from five different factories and to use consumer testing to determine the overall preferred Swiss cheese. This study also combined these two analyses to identify which flavor characteristics had the greatest impact on consumer liking. This study can also provide which attributes lead to the flavor variability problem in Swiss cheese from a consumer perspective. Ultimately, this analysis can be related to specific Swiss cheese volatile compounds to pinpoint the biochemical pathways and sources that are responsible for flavor variation in Swiss cheese.
5.2 Materials and Methods

5.2.1 Swiss Cheese Samples

Fifteen cheeses were collected from November 2011 to April 2012 from member companies of the Swiss Cheese Consortium, where five factories contributed three cheeses each. The factories that make up the Swiss Cheese Consortium are located in Ohio, Illinois, Pennsylvania, and Iowa. These factories are designated as 148, 207, 374, 465, and 528. The Swiss cheeses varied in manufacturing dates, as well as the vat and block location (Table 2.1). Variation of cheeses within most factories was limited to different blocks from the same vat make. In addition, each of the five factories used different starter cultures in the processing of their Swiss cheese (Table 2.2). Cheeses were cut into uniform 2.5 cm cubes, and any cube that contained large eyes was discarded. All three samples were randomly combined to represent that specific factory. For each factory, one cube was served at room temperature in a 2 oz translucent plastic soufflé cup (GFS, Grand Rapids, M.I.) labeled with a random three-digit factory code.

5.2.2 Descriptive Sensory Analysis

Eight panelists trained in the Spectrum method, each having at least 100 h of descriptive analysis experience with Swiss cheese flavor, evaluated all fifteen Swiss cheese samples. The descriptive analysis of Swiss cheese flavor was conducted at North Carolina State University. Panelists used a previously developed cheese flavor sensory language adapted to Swiss cheese flavor and typical sensory science techniques which included 21 attributes (Table 2.4). For assessment, panelists used a 15-point universal
intensity scale in accordance with the Spectrum method. With the universal scale, panelists score intensities in the same manner across all attributes and all cheese samples.

Panelists learned to identify and scale flavor descriptors by using the same intensity scale through presentation and discussion of flavor definitions, references, and a wide array of cheese types, including Swiss cheese. Cheeses were evaluated in duplicate in a randomized order on paper ballots in booths dedicated to sensory analysis and free from external aromas, noise, and distractions. Panelists were instructed to expectorate samples after evaluation, and spring water was available to each panelist for palate cleansing. Descriptive data was then evaluated by one-way analysis of variance (ANOVA) with Tukey’s HSD (Honestly Significant Difference). Correlation of descriptive data with consumer data was evaluated using partial least squares regression (PLSR) by Pirouette® software (version 3.11, Infometrix, Inc., Woodville, WA, U.S.A.) and principle component analysis (PCA) (SAS, version 8.2, Cary, N.C., U.S.A.).

5.2.3 Consumer Testing

A total of 100 untrained consumers (76 females and 24 males) were pre-recruited from the Food Industry Center database at the Parker Food Science and Technology Building on the Columbus campus of The Ohio State University in the sensory testing area. Demographics indicated that over 75% of the panelists eat Swiss cheese regularly or at least twice every two weeks (Table 2.5).

Cheeses (5 samples) were served at room temperature in 2 oz translucent plastic soufflé cup (GFS, Grand Rapids, M.I.) that had been labeled with three-digit codes. All
samples were presented in randomized order to each testing booth to reduce potential first order bias. Testing was conducted under standard room conditions plus spot white lighting. Panelists evaluated samples individually in separate booths and entered their own responses directly into a computer using Compusense five data collection and analysis software (Compusense® five, release 5.2, Compusense Inc., Guelph, ON, Canada). Panelists assessed visual characteristics before tasting samples, rating each sample independently for overall liking on a 9-point vertical hedonic category scale and Just About Right (JAR) scale. Panelists were allowed to swallow or expectorate samples, as desired; retasting was allowed; and each panelist proceeded at his or her own pace. Room temperature spring water was provided for rinsing between samples.

Demographics questions followed the sample evaluations (age, gender, Swiss cheese consumption, and race). Data was analyzed using one-way analysis of variance (ANOVA) with Tukey’s HSD (honestly significant difference). Correlation of descriptive data with consumer data was evaluated using partial least squares regression (PLSR) by Pirouette® software (version 3.11, Infometrix, Inc., Woodville, WA, U.S.A.).

5.3 Results and Discussion

5.3.1 Descriptive Sensory Analysis

The descriptive sensory analysis results indicated flavor differences among the Swiss cheeses (Table 5.1). Overall, the mean flavor attributes for all Swiss cheeses were low scoring between 0 and 4, which are typical intensities for cheeses (Liggett and others 2008). Brothy, cowy, and metallic attributes, commonly considered defects in cheese
flavor (Drake and others 2001), were not detected in any of the 15 Swiss cheese samples, which is similar to previous studies (Liggett and others 2008, Kocaoglu-Vurma and others 2009). Cooked cabbage, vinegar, milk fat, whey, cooked milk, sour, salty, sweet, and umami attributes were found in all 15 cheeses. Liggett and others (2008) also found cooked milk, milk fat, vinegar, and cabbage flavors to be present in all analyzed Swiss cheeses. However, there were some aroma attributes that were detected in only some of the samples. Dried fruit was found in 9 of the 15 samples, which is identical to the findings by Liggett and others (2008). Diacetyl was detected in 7 of the 15 cheese samples, and prickle attributes were identified in 6 out of the 15 Swiss cheese samples. However, prickle was found in all three cheese samples from Factory 465. Nutty had very low scores (highest score was 1.2) and was not detected in 10 of the Swiss cheese samples. Despite being considered an important attribute to Swiss cheese flavor (Lawlor and others 2003), the nutty aroma was almost non-existent in the cheeses from this study. Liggett and others (2008) also detected low intensities of nutty aroma and contribute these lower values to a better definition of nutty than in previous studies (Lawlor and others 2003).
Table 5.1 Mean values of descriptive analysis attributes for Swiss cheeses samples. Attributes were scored on a 15-point universal Spectrum scale, where 0 = absence of the attribute and 15 = very high intensity of the attribute. Not Detected (ND).

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</table>

Different letters indicate significant differences between factories.
Panelists identified significant differences in the intensities of the 21 attributes assessed between the five factories indicating flavor variation amongst the Swiss cheeses (Table 5.2). Butyric acid was only found in the cheese samples from Factory 465 while Factory 528 was the only factory that contained a sulfur-eggy aroma. In previous studies, butyric acid has not been detected in the majority of Swiss cheeses (Kocaoglu-Vurma and others 2004, Liggett and others 2008). Factory 207 was differentiated from all the other factories due to the presence of fresh fruit flavor in sample 207-1. On the other hand, Factory 148 had the highest levels of milk fat, nutty, and salty aromas which differentiated the cheeses from the other factories. Factory 374 was identified to have the highest intensities of diacetyl and the lowest intensities of sweet and dried fruit flavors. In addition, Factory 374 was the only factory to have all three samples contain diacetyl notes.
Table 5.2 Mean values of descriptive analysis attributes for Swiss cheese by factory.

Attributes were scored on a 15-point universal Spectrum scale, where 0 = absence of the attribute and 15 = very high intensity of the attribute. Not Detected (ND).

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>Factory 148</th>
<th>Factory 207</th>
<th>Factory 374</th>
<th>Factory 465</th>
<th>Factory 528</th>
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<td>2.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
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<td>Whey</td>
<td>2.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>0.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milk fat</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vinegar</td>
<td>1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dried Fruit</td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fresh Fruit</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eggy</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooked Cabbage</td>
<td>1.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butyric</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nutty</td>
<td>0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>0.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweaty</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sour</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bitter</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salty</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweet</td>
<td>2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Umami</td>
<td>2.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prickle</td>
<td>0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>Different letters indicate significant differences between factories.

In order to illustrate the differentiation amongst the five factories based on these 21 attributes, a principle component analysis (PCA) biplot of all flavor attributes of the Swiss cheese samples was made (Figure 5.1). It is clear that the Swiss cheese samples
vary in flavor profiles based on these measured attributes. The first 2 principal components are responsible for 55% of the total variability of the cheese samples. PC1 accounted for 40% of the total variability while PC2 accounted for 15% of the total variability. As previously stated, Factory 374 contained the highest intensities of diacetyl flavor which distinctly separates these samples from the other four factories. Although there are some similarities between Factories 148 and 207 as well as Factories 465 and 528, there are some attributes that can uniquely distinguish each factory from the others. Factory 148 was identified to contain the most desirable and important attributes for Swiss cheese flavor such as milk fat, cooked milk, and salty (Liggett and others 2008). Kocaoglu-Vurma and others (2009) also found that cooked milk and milk fat were correlated together based on PCA. High levels of milk fat in Factory 148 resulted in differentiation from Factory 207 which was the only factory containing fresh fruit notes. Attributes generally thought to be negative (bitter, butyric, cooked cabbage, and sulfur-egg) (Liggett and others 2008) were associated more with Factory 465 and Factory 528. The cheese samples from Factory 528 were the only ones to have intensities of sulfur-egg aromas slightly separating them from Factory 465 which contained butyric acid as its main differentiating attribute.
5.3.2 Consumer Testing

5.3.2.1 Overall Liking

Panelists evaluated all Swiss cheese samples (five total) for overall liking, nutty flavor, buttery or dairy flavor, and dairy aroma on a 9-point vertical hedonic category scale where 9 was extremely liked (Table 5.3). Factory 148 had the highest overall liking rating (7.13) however it was not significantly different from Factory 528 (Figure 5.1). Factory 207 was next in terms of overall liking, followed by Factory 465 and Factory 528. Factory 374 had the lowest overall liking rating (5.19).
Factory 374 and Factory 465 were both significantly different from Factory 148 and were evaluated as the least liked Swiss cheese samples in this sensory study.

Table 5.3 Liking attribute rating means for Swiss cheese samples by factory.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Factory 148</th>
<th>Factory 207</th>
<th>Factory 374</th>
<th>Factory 465</th>
<th>Factory 528</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Liking</td>
<td>7.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.62&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nutty Flavor</td>
<td>6.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.81&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.43&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Buttery (Dairy) Flavor</td>
<td>6.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.59&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dairy Aroma</td>
<td>6.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.95&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.49&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Meets Expectations*</td>
<td>4.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.95&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Measured on a 5-point hedonic scale. All other attributes were measured on a 9-point hedonic scale.

<sup>a,b,c</sup> Different letters indicate significant differences between factories.

The liking attribute rating means for nutty flavor, buttery flavor, and dairy aroma all followed the same pattern as overall liking (Table 5.3). Factory 374 and Factory 465 had the lowest ratings while Factory 148 had significantly higher ratings. Factory 207 had significantly similar ratings to Factory 528 for nutty flavor, buttery flavor, and dairy aroma, but both were slightly lower than Factory 148. For all characteristics evaluated with liking attribute ratings, Factory 148 had the highest ratings although Factory 528 and
Factory 148 were not determined to be significantly different from each other. The mean attribute liking ratings seem low in this study; however liking scores are generally very specific to the product being evaluated. Therefore high liking scores for a particular product are not necessarily found to be greater than 7 on a 9-point hedonic scale. Low mean attribute liking ratings have been determined in previous studies for both cheddar cheese (liking scores between 5.7 and 7.2) and Gouda cheese (liking scores between 5.5 and 6.5) (Young and others 2004, Yates and Drake 2007). This indicates that all Swiss cheese samples from the five factories analyzed in this study are considered “acceptable” Swiss cheeses with liking scores greater than 5.

This sensory study also analyzed whether or not the Swiss cheese samples met the expectations of the consumers using a 5-point hedonic scale with 5 being the highest rating of excellent (Table 5.3). Factory 148 had the highest rating for meeting expectations of Swiss cheese where the samples “definitely met expectations” for 59% of the panelists. Swiss cheese samples from Factory 528 had a similar rating for meeting expectations to the Factory 148 cheese samples. Factory 207 was the next Swiss cheese sample that met the expectations of the consumers with a rating of 3.58. Swiss cheese samples from Factory 374 and Factory 465 once again had the lowest ratings with Factory 374 having a liking attribute rating below 3.

5.3.2.2 Just About Right (JAR) Attributes

Panelists evaluated all Swiss cheese samples (five total) for dairy aroma, nutty flavor, and buttery flavor on a Just About Right (JAR) scale with the low end anchored
by “way too little” and the high end anchored by “way too much”. The middle category on the JAR scale is anchored by “just about right” denoting most acceptable (Gacula and others 2007). As with the liking attribute ratings, Factory 148 had the highest JAR frequency for amount of dairy aroma at 75% (Table 5.4). It was the most balanced Swiss cheese sample for dairy aroma. Factory 528 had a slightly lower JAR frequency of 70% however this cheese sample is also well balanced and not significantly different from Factory 148. All the other factories had too little dairy aroma according to the consumer panel with low JAR frequencies and high “slightly too little” frequencies.

Factory 148 had a slightly higher JAR frequency for amount of nutty flavor than Factory 528 (Table 5.4). The Swiss cheeses from these two factories were the most balanced for nutty flavor and buttery flavor. Factories 207, 374, and 465 all had significantly lower JAR frequencies for both buttery and nutty flavor. The consumers all evaluated these samples as having too little flavor.
Table 5.4 Just about right (JAR) frequencies (%) for dairy aroma, nutty flavor, and buttery flavor for Swiss cheeses by factory.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Factory</th>
<th>Much too Little</th>
<th>Slightly too Little</th>
<th>Just About Right (JAR)</th>
<th>Slightly too Much</th>
<th>Way too Much</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Aroma</td>
<td>148</td>
<td>2</td>
<td>16</td>
<td>75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>207</td>
<td>8</td>
<td>37</td>
<td>46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>374</td>
<td>15</td>
<td>23</td>
<td>56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>465</td>
<td>12</td>
<td>28</td>
<td>48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>528</td>
<td>7</td>
<td>19</td>
<td>70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Nutty Flavor</td>
<td>148</td>
<td>4</td>
<td>22</td>
<td>61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>207</td>
<td>9</td>
<td>31</td>
<td>38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>374</td>
<td>26</td>
<td>32</td>
<td>31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>465</td>
<td>9</td>
<td>24</td>
<td>40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>528</td>
<td>4</td>
<td>19</td>
<td>59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Butter Flavor</td>
<td>148</td>
<td>3</td>
<td>18</td>
<td>71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>207</td>
<td>5</td>
<td>22</td>
<td>61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>374</td>
<td>16</td>
<td>33</td>
<td>43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>465</td>
<td>8</td>
<td>39</td>
<td>47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>528</td>
<td>3</td>
<td>20</td>
<td>73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Different letters indicate significant differences between factories.

Overall, Factory 148 was liked the most in every category and best balanced in Just About Right (JAR) areas. The Swiss cheese samples from Factory 148 were determined to be the top rated Swiss cheeses in this consumer sensory study and met the expectations of 59% of the panelists. Factory 528 was similar (not significantly different) to Factory 148 in JAR frequencies and meeting expectations, but rated marginally lower for liking attributes. Factory 207 was the next in terms of liking however it scored low in
amount of dairy aroma, nutty flavor, and buttery flavor. Factory 374 and Factory 465 were rated similarly for liking with slight differences in JAR attributes. Factory 465 was generally slightly less flavorful. The panelists determined that nutty flavor, buttery flavor, and dairy aroma all contributed to the overall liking of Factory 148.

5.3.3 Correlation between Descriptive Sensory Analysis and Overall Liking

Principle Component Analysis (PCA) helped to correlate the descriptive sensory analysis with the overall liking of Swiss cheeses from five different factories. Factory 148, the most liked cheese by consumers, was correlated with milkfat, nutty, salty, and cooked milk (Figure 5.1). These descriptors had the highest scores in Factory 148 indicating that they are important positive attributes to consumers when determining likeness of Swiss cheese. Swiss cheeses from Factory 374 were the least liked cheeses overall and were associated with diacetyl based on PCA. Factory 374 was the only factory not to contain levels of dried fruit, had the highest levels of diacetyl, and even had the lowest intensities of sweet notes. Therefore high levels of diacetyl and low levels of sweet and dried fruit are important negative attributes consumers evaluate when deciding on a cheese they prefer. Liggett and others (2008) found that diacetyl, whey, milkfat, and umami were positive attributes while cabbage, cooked milk, and vinegar were negative attributes in cheese that are important to liking. This study correlated cooked milk positively and diacetyl negatively which conflicts with previous studies (Liggett and others 2008).
Descriptive sensory analysis descriptors were used to generate a partial least squares regression (PLSR) model by using overall liking from the consumer testing as a dependent variable. A total of 13 descriptors were used to build the PLSR model. The other 8 descriptors were generally not detected in the cheeses. PLSR analysis generated a prediction model with correlation coefficients of cross-validation (rVal) of 0.85 with a standard error of cross-validation (SECV) of 0.19. The first factor was responsible for 95% of the variance in liking while the second factor accounted for 0.75% of the total variance in liking. The descriptors that contributed the most to the overall liking of the Swiss cheeses (either positively or negatively) were umami, cooked milk, sweet, whey, milkfat, vinegar, salty, cooked cabbage, sweaty, and sour (Figure 5.2). Umami and cooked milk contributed the most to the overall liking.
Figure 5.2 Contribution of specific descriptors to overall liking of Swiss cheeses from five different factories based on PLSR model.

The descriptors that contributed the most to the overall liking based on Figure 5.2 can then be correlated to cheeses from specific factories and determined to be positively correlated or negatively correlated to overall liking in Figure 5.3. The first factor accounted for 95% of the variability in liking and was characterized by umami and cooked milk on the positive end and diacetyl, prickle, dried fruit, and nutty on the negative end. The second factor was responsible for only 0.75% of the total variability in liking. Factor 2 was characterized by diacetyl on the negative end and whey on the positive end. This analysis shows that whey and cooked milk were the characteristics
driving positive overall liking shown by cheeses from Factory 148 (the most liked factory). Factory 148 had the highest levels of cooked milk amongst all the other factories indicating its overall importance to flavor by consumers (Table 5.2). Other positive characteristics include milkfat, and umami which were two of the descriptors that contributed the most to overall liking (Figure 5.2). Liggett and others (2008) also found milkfat, umami, and whey to be important positive attributes to Swiss cheese flavor by consumers. Negative drivers were found to be diacetyl, nutty, dried fruit, and prickle in this study, which is illustrated by cheeses from Factory 374 (least liked factory by consumers). Swiss cheeses from Factory 374 had extremely high levels of diacetyl (significantly different from all other factories) and were the only cheeses not to have dried fruit, prickle, or nutty notes. These notes most likely led to a low overall liking score by consumers for Factory 374 (Table 5.1). Previous studies correlated the diacetyl attribute positively with overall liking (Liggett and others 2008), however in this study it was the most significant driving force for low overall liking scores in Factory 374.
Figure 5.3 Scores for Swiss cheeses from five different factories correlated with sensory descriptors and overall liking based on a PLSR model.

5.4 Conclusion

Panelists in both the descriptive sensory analysis and the consumer testing were able to differentiate between the Swiss cheeses by factory. In general, all the cheeses received low intensities and ratings. Characteristics perceived as defects in Swiss cheese (brothy, cowy, and metallic) were not detected in any of the cheeses in this study. Dried fruit, fresh fruit, diacetyl, butyric, and sulfur-eggy notes were the most important
attributes that led to discrimination amongst the Swiss cheeses by factory. Overall, Factory 148 was liked the most in every category and best balanced in Just About Right (JAR) areas because it contained attributes found to be positively correlated to overall liking (umami, cooked milk, and whey). Factory 374 was the least preferred cheese by consumers due to high levels of diacetyl and not containing prickle, dried fruit, and nutty notes which led to a negative correlation to overall liking scores.

All the differences assessed between factories could be due to a variety of variables. These differences amongst the factories could include differences in starter cultures, non-starter lactic acid bacteria (NSLAB), holding temperatures and times, and milk sources (Liggett and others 2008). In the future, this descriptive sensory data and the overall liking scores from the consumer testing can be correlated with odor activity values (OAVs) and key high impact compounds to provide these factories a quality target for Swiss cheese. Because of sensory science, a relationship can be established between sensory perception and instrumental tests, such as OAVs (Drake 2007). Having an increased understanding of the linkage between specific volatile compounds and flavor attributes is required to determine the source of flavor variation in Swiss cheese.

5.5 References


Chapter 6: Characterization of Flavor Variables in Swiss Cheese from Five Different Factories Using Odor Activity Values (OAVs) and Sensory Studies.

6.1 Introduction

There is currently variation in Swiss cheese flavor based on volatile compounds (Taylor and others 2013) and odor activity values (OAVs) (Table 4.1). OAVs are defined as the concentration of the compound in the sample divided by the odor threshold value, in air, for that compound. Our previous study and Preininger and Grosch (1994) used the OAV approach to determine the key odorants in Swiss cheese (Taylor and others 2013). Both studies showed that methional, homofuraneol, and furaneol are important to the overall flavor of Swiss cheese.

Descriptive sensory analysis and consumer testing showed flavor variation in Swiss cheese based on dried fruit, fresh fruit, diacetyl, butyric, and sulfur-eggy notes that were noticeable by consumers (Chapter 5, page 67). Descriptive sensory analysis and consumer liking testing has also been used to determine which flavor characteristics of Swiss cheese should be optimized and minimized to produce cheeses preferred by consumers (Liggett and others 2008). Liggett and others (2008) were able to identify that
diacetyl, cabbage, cooked, whey, milk fat, umami, and vinegar were most important to liking of Swiss cheese.

These studies on their own provided insight to the Swiss cheese industry about the variability in flavor and preference of the cheeses. In addition, correlation between OAVs and both sensory studies would help to characterize the flavor variables in Swiss cheese through prediction models. It is because of sensory science that a relationship can be established between sensory perception and instrumental tests (Drake 2007). Using prediction models based on instrumental testing to link specific flavors to volatile flavor compounds would help in the formation of a quality model for the Swiss cheese industry (Drake 2004). Kocaoglu-Vurma and others (2009) utilized descriptive sensory analysis in combination with infrared spectra to develop predictive models for the determination of sensory attributes of Swiss cheese based on factory location. They were able to build a tool that could be used for quality control of cheese flavor.

The objective of this study was to utilize a similar approach as Kocaoglu-Vurma and others (2009) with statistics to correlate odor activity values (OAVs) and sensory studies as a means of characterizing flavor variables in Swiss cheese. Maintaining a high quality, consistent cheese product is necessary to retain customers, who will not purchase cheese if it does not meet their flavor expectations (Drake 2004). This combination of OAVs and sensory data can provide prediction models which can ultimately provide these five factories a quality control tool for Swiss cheese. The factories can then improve manufacturing processes that will reduce undesirable flavor attributes and compounds and enhance desirable flavor compounds and attributes.
6.2 Materials and Methods

6.2.1 Swiss Cheese Samples

Fifteen cheeses were collected from November 2011 to April 2012 from member companies of the Swiss Cheese Consortium, where five factories contributed three cheeses each. The factories that make up the Swiss Cheese Consortium are located in Ohio, Illinois, Pennsylvania, and Iowa. These factories are designated as 148, 207, 374, 465, and 528. The Swiss cheeses varied in manufacturing dates, as well as the vat and block location (Table 2.1). Variation of cheeses within most factories was limited to different blocks from the same vat make. In addition, each of the five factories used different starter cultures in the processing of their Swiss cheese (Table 2.2).

6.2.2 Odor Activity Value (OAV) Calculations

Odor activity values (OAVs) were calculated by dividing the volatile organic compound (VOC) concentration by the odor threshold value of that specific compound in air (Table 4.1). VOC concentrations were determined using selected ion flow tube-mass spectrometry (SIFT-MS) (SYFT Technologies Voice 200, Christchurch, New Zealand). Identification and quantification of the VOCs were determined based on the known ion products and reaction rate coefficients for each compound in a method that contained 24 compounds including alcohols, aldehydes, ketones, esters, sulfur compounds, and pyrazines. All concentrations were reported in ppb (Table 3.1). Odor threshold values were used to calculate OAVs in this investigation (Table 2.3). All odor threshold values
referenced were recognition threshold values (when possible) found in literature (Van Gemert 2011, Belitz and others 2009).

6.2.3 Descriptive Sensory Analysis

Eight panelists trained in the Spectrum method, each having at least 100 h of descriptive analysis experience with Swiss cheese flavor, evaluated all fifteen Swiss cheese samples.

The descriptive analysis of Swiss cheese flavor was conducted at North Carolina State University. Panelists used a previously developed cheese flavor sensory language adapted to Swiss cheese flavor and typical sensory science techniques which included 21 attributes (Table 2.4). For assessment, panelists used a 15-point universal intensity scale in accordance with the Spectrum method. With the universal scale, panelists score intensities in the same manner across all attributes and all cheese samples.

Panelists learned to identify and scale flavor descriptors by using the same intensity scale through presentation and discussion of flavor definitions, references, and a wide array of cheese types, including Swiss cheese. Cheeses were evaluated in duplicate in a randomized order on paper ballots in booths dedicated to sensory analysis and free from external aromas, noise, and distractions. Panelists were instructed to expectorate samples after evaluation, and spring water was available to each panelist for palate cleansing.
6.2.4 Consumer Testing

A total of 100 untrained consumers (76 females and 24 males) were pre-recruited from the Food Industry Center database at the Parker Food Science and Technology Building on the Columbus campus of The Ohio State University in the sensory testing area. Demographics indicated that over 75% of the panelists eat Swiss cheese regularly or at least twice every two weeks (Table 2.5).

Cheeses (5 samples) were served at room temperature in 2 oz translucent plastic soufflé cup (GFS, Grand Rapids, M.I.) that had been labeled with three-digit codes. All samples were presented in randomized order to each testing booth to reduce potential first order bias. Testing was conducted under standard room conditions plus spot white lighting. Panelists evaluated samples individually in separate booths and entered their own responses directly into a computer using Compusense five data collection and analysis software (Compusense® five, release 5.2, Compusense Inc., Guelph, ON, Canada). Panelists assessed visual characteristics before tasting samples, rating each sample independently for overall liking on a 9-point vertical hedonic category scale. Panelists were allowed to swallow or expectorate samples, as desired; retasting was allowed; and each panelist proceeded at his or her own pace. Room temperature spring water was provided for rinsing between samples. Demographics questions followed the sample evaluations (age, gender, Swiss cheese consumption, and race).
6.2.5 Statistical Analysis

OAV data, descriptive data, and consumer testing data were all evaluated individually using one-way analysis of variance (ANOVA) with Tukey’s honestly significant difference (T-HSD) (Minitab v16, Minitab Inc, State College, PA, U.S.A.). Correlation of sensory data with OAV data was evaluated using partial least squares regression (PLSR) by Pirouette® software (version 3.11, Infometrix, Inc., Woodville, WA, U.S.A.).

6.3 Results and Discussion

6.3.1 Correlation between Odor Activity Values (OAVs) and Descriptive Sensory Analysis

A partial least squares regression (PLSR) model was generated from odor activity value (OAV) profiles obtained using selected ion flow tube-mass spectrometry (SIFT-MS) by using descriptive sensory scores as dependent variables. This model was built by only using 13 of the 21 descriptors because several of the descriptors were generally not detected in the cheeses. The PLSR analysis generated prediction models with correlation coefficients of cross-validation (rVal) between 0.36-0.98 and with standard errors of cross-validation (SECV) ranging from 0.10-0.45 (Table 6.1). SECV is an estimate of the expected error when using the models for unknown samples (Maurer and Rodriguez-Saona 2013). The number of factors used in the PLSR algorithm ranged from 2-10. PLSR selects the optimum number of factors that produces the lowest prediction residual error (Maurer and Rodriguez-Saona 2013).
Based on these results, it is not feasible to use SIFT-MS as a way to construct complete descriptive sensory models based on OAV profiles. In the future, if a prediction model is desired, a larger set of cheeses would need to be tested to continue to improve this PLSR model. It is necessary to have a wide range and even a different range of scores for each sensory descriptor to make a strong correlation model (Kocaoglu-Vurma and others 2009). Because there is so much variation in flavor amongst each cheese sample (even within a factory), it can be difficult to build a strong correlation model. Eventually with continued data collection this may be a practical way to predict descriptive sensory data based on OAV profiles.
Table 6.1 Partial least square regression (PLSR) model for correlation between odor activity values (OAVs) and sensory descriptors for Swiss cheese.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>SECV</th>
<th>r Val</th>
<th>SECV</th>
<th>r Cal</th>
<th>Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked/milky</td>
<td>0.37</td>
<td>0.48</td>
<td>0.22</td>
<td>0.84</td>
<td>6</td>
</tr>
<tr>
<td>Whey</td>
<td>0.24</td>
<td>0.69</td>
<td>0.08</td>
<td>0.99</td>
<td>10</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>0.15</td>
<td>0.85</td>
<td>0.10</td>
<td>0.96</td>
<td>3</td>
</tr>
<tr>
<td>Milkfat</td>
<td>0.20</td>
<td>0.83</td>
<td>0.06</td>
<td>0.99</td>
<td>6</td>
</tr>
<tr>
<td>Vinegar</td>
<td>0.10</td>
<td>0.98</td>
<td>0.03</td>
<td>0.99</td>
<td>10</td>
</tr>
<tr>
<td>Dried Fruit</td>
<td>0.42</td>
<td>0.58</td>
<td>0.41</td>
<td>0.66</td>
<td>2</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0.27</td>
<td>0.67</td>
<td>0.18</td>
<td>0.91</td>
<td>6</td>
</tr>
<tr>
<td>Nutty</td>
<td>0.45</td>
<td>0.36</td>
<td>0.42</td>
<td>0.64</td>
<td>4</td>
</tr>
<tr>
<td>Sweaty</td>
<td>0.41</td>
<td>0.64</td>
<td>0.35</td>
<td>0.76</td>
<td>2</td>
</tr>
<tr>
<td>Sour</td>
<td>0.18</td>
<td>0.70</td>
<td>0.10</td>
<td>0.93</td>
<td>6</td>
</tr>
<tr>
<td>Sweet</td>
<td>0.34</td>
<td>0.72</td>
<td>0.18</td>
<td>0.95</td>
<td>7</td>
</tr>
<tr>
<td>Umami</td>
<td>0.25</td>
<td>0.91</td>
<td>0.05</td>
<td>0.99</td>
<td>8</td>
</tr>
<tr>
<td>Prickle</td>
<td>0.29</td>
<td>0.81</td>
<td>0.10</td>
<td>0.99</td>
<td>10</td>
</tr>
</tbody>
</table>

SEC = standard error of calibration; rCal = correlation coefficient of calibration; SECV = standard error of cross-validation; rVal = correlation coefficient of cross-validation; Factors = number of factors that provide the optimal model for prediction.

However PLSR also provides specific correlations between OAV profiles and descriptive sensory analysis. Correlations between descriptive sensory analysis and OAV profiles can be seen by several descriptors including diacetyl, milkfat, vinegar, umami, and prickle (SECV ≤ 0.29 and rVal ≥ 0.81) (Table 6.1). As an example, the vinegar
descriptor made the strongest PLSR model for associating OAVs and descriptor scores (Figure 6.1). The vinegar descriptor generated a PLSR model with rVal greater than 0.91 and SECV less than 0.25 (Table 6.1). All predicted descriptive scores were closely associated with the actual measured vinegar scores in all 15 cheeses indicating a strong correlation between OAVs and descriptive scores. Also, the Swiss cheeses maintained differentiation by factory in this PLSR model.

Figure 6.1 PLSR model for the vinegar descriptor score used to correlated OAVs and descriptive sensory analysis of Swiss cheeses from five different factories.
The PLSR model for vinegar was able to characterize the descriptor by homofuraneol and methional on the positive end and 3-methylbutanal and 2,3-butanedione on the negative end (Figure 6.2). These compounds were found to be the most correlated with not only the vinegar attribute, but all of the attributes in the PLSR model. Liggett and others (2008) characterized 2,3-butanedione as a positive descriptor to overall liking, however in Chapter 5 (page 67) it is correlated negatively with overall liking and in this study with most of the eight descriptors (data not shown). This is most likely due to 2,3-butanedione having excessively high OAVs in Factory 374, which was determined to be the least preferred cheese by consumers in Chapter 5 (page 67). In a previous study, we and Preininger and Grosch (1994) both found that homofuraneol and methional are key odorants in Swiss cheese (Taylor and others 2013). Based on the PLSR model, homofuraneol and methional positively correlate with many descriptors for Swiss cheese (data not shown).

Acetic acid, which is the compound most associated with vinegar notes and is of great importance to Swiss cheese flavor (Bachmann and others 2011, Lawlor and others 2002, Preininger and others 1996), was not correlated with the vinegar descriptor in the PLSR model. On the other hand, there were many other volatile compounds in Swiss cheese that were associated with vinegar. This PLSR model suggests that descriptive attribute scores can be correlated with OAVs using a limited number of compounds by SIFT-MS.
Figure 6.2 Vinegar descriptor scores for Swiss cheeses from five different factories by descriptive sensory analysis correlated with volatile compounds by OAV profiles based on a PLSR model.

6.3.2 Correlation between Odor Activity Values (OAVs) and Overall Liking from Consumer Testing

A partial least squares regression (PLSR) model was generated from odor activity value (OAV) profiles obtained using selected ion flow tube-mass spectrometry (SIFT-MS) by using overall liking scores as the dependent variable. Based on the PLSR model for overall liking, SIFT-MS in combination with OAVs can be correlated to overall liking.
scores in Swiss cheese. This PLSR had a SECV of 0.35 and rVal of 0.8 after the removal of outliers (Figure 6.3).

Figure 6.3 PLSR model used to correlated OAVs and overall liking scores of Swiss cheeses from five different factories.

The PLSR model was able to indicate which volatile compounds based on OAVs contributed the most to overall liking of the Swiss cheeses (Figure 6.4). The first factor accounted for 98% of the variability in liking and was characterized by methional on the
positive end. The second factor was responsible for only 0.74% of the total variability in liking. Factor 2 was characterized by homofuraneol on the negative end and 3-methylbutanal on the positive end. This analysis shows that 3-methylbutanal and methional were the compounds driving positive overall liking. The most liked cheese by consumers was Factory 148, which had a statistically high OAV for 3-methylbutanal and a statistically low OAV for methional (Table 4.1). High odor activity of 3-methylbutanal and low odor activity of methional is therefore associated positively with overall liking scores in Swiss cheese. Factory 207, 374, and 465 all had significantly higher OAVs for methional than Factory 148. This suggests that consumers prefer a lower OAV for methional in their Swiss cheese. Homofuraneol is the only compound that is responsible for negatively driving overall liking; despite being an important compound to the overall liking score of Factory 528 which was the overall second most liked Swiss cheese (Table 5.3). This indicates that methional and 3-methylbutanal are driving the overall liking correlation more than homofuraneol. All three of these compounds that were correlated with overall liking scores were important to the discrimination of Swiss cheeses from different factories based on OAV profiles (Figure 4.4, Figure 4.5). A previous study also found that homofuraneol and methional are important to the flavor of Swiss cheese (Preininger and Grosch 1994), and this study suggests that consumers use these compounds as a means of determining overall likeness in Swiss cheeses.
Figure 6.4 Overall liking scores for Swiss cheeses from five different factories by consumer testing correlated with volatile compounds by OAV profiles based on a PLSR model.

2,3-Butanedione, or diacetyl, has been found in many studies to be important to the overall flavor of Swiss cheese with high OAVs (Langler and others 1967, Preininger and Grosch 1994, Taylor and others 2013). Swiss cheeses from Factory 374 had extremely high levels and high OAVs for 2,3-butanedione (significantly different from all other factories) which ultimately led to a low overall liking score by consumers (Table 5.1). However, the PLSR model does not find that 2,3-butandione is one of the
compounds that contributed the most to overall liking (Figure 6.4). A previous study correlated the diacetyl attribute from descriptive analysis, which is anchored by 2,3-butanedione in the Swiss cheese flavor lexicon (Table 2.4), positively with overall liking (Liggett and others 2008). In this study the diacetyl attribute was the most significant driving force for low overall liking scores in Factory 374 (Figure 5.3). Despite not being in the PLSR model correlating OAVs and overall liking scores, OAVs of 2,3-butanedione and scores of the diacetyl attribute were important for discrimination of Swiss cheeses from five different factories leading to the identification of flavor variation in Swiss cheese.

6.4 Conclusion

The PLSR model constructed using SIFT-MS as a way to understand descriptive sensory attributes based on the overall OAV profiles was successful. Using PLSR, the vinegar descriptor was characterized by homofuraneol and methional on the positive end and 3-methylbutanal and 2,3-butanedione on the negative end. Homofuraneol, 3-methylbutanal and methional were correlated with overall liking scores and were found to be important to the discrimination of Swiss cheeses from different factories based on OAV profiles.

This study showed that there is a relationship between sensory studies and instrumental testing. OAVs were shown to be able to determine important attributes preferred by consumers in Swiss cheese. This combination of OAVs and sensory data can continue to provide correlations which can ultimately offer these five factories a quality
control tool for Swiss cheese flavor and reduce flavor variability in a factory. The use of PLSR as a tool to analyze sensory data allows the industry to interpret instrumental results and pinpoint which volatile compounds are crucial for specific flavors (Drake 2007). Then, all factories can continuously work to improve manufacturing processes that will reduce undesirable flavor attributes and enhance desirable flavor compounds. In the future, this descriptive sensory data and the overall liking scores from the consumer testing can be correlated not only with odor activity values (OAVs) of volatile compounds but also with non-volatile compounds measured using FT-IR. Having an increased understanding of the linkage between specific volatile and non-volatile compounds and flavor attributes would continue to improve Swiss cheese flavor.

6.5 References


Chapter 7: Overall Conclusions

Variation in Swiss cheese flavor is a problem in the Swiss cheese industry, but current research has failed to focus on identifying the source of this variation. The variation in cheese flavor was identified and extensively analyzed for 15 Swiss cheese samples from five different factories by using SIFT-MS, descriptive sensory analysis, and consumer testing. Using only seven compounds (2,3-butanedione, 3-methylbutanal, ethanol, homofuraneol, methional, methyl mercaptan, and ethyl methyl sulfide), SIFT-MS in combination with OAVs provided rapid discrimination of Swiss cheese by factory. This was attributed primarily on the breakdown of amino acids. These differences in amino acid breakdown products amongst the five factories could be caused by differences in starter cultures, non-starter lactic acid bacteria (NSLAB), holding temperatures and times, and milk sources (Liggett and others 2008).

Besides identifying the source of flavor variation in Swiss cheese, a relationship between SIFT-MS in combination with OAVs and sensory studies was also established using a PLSR model. Ultimately, the combination of sensory studies and OAVs can offer the entire Swiss cheese industry a quality control tool for Swiss cheese flavor. Factories can continuously work to improve manufacturing processes based on results from the
PLSR model that will reduce undesirable flavor attributes and enhance desirable flavor compounds.

SIFT-MS was developed as a means of understanding the chemical basis of flavor, especially in food products. Although this study did not make a breakthrough in the understanding of the chemistry of Swiss cheese flavor, it has proven that SIFT-MS can be a powerful tool for flavor quality control. In combination with OAVs, SIFT-MS could provide the Swiss cheese industry with a quality target for the flavor of their cheese. Beyond Swiss cheese, this method of SIFT-MS in combination with OAVs and multivariate analysis could be used throughout the food industry to build quality targets for flavor for many different food products.
References


