

THESIS

EFFECTS OF TIME AND TEMPERATURE DURING MELANGING ON THE VOLATILE
PROFILE OF DARK CHOCOLATE

Submitted by

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In partial fulfillment for the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2019

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ABSTRACT

EFFECTS OF TIME AND TEMPERATURE DURING MELANGING ON THE VOLATILE PROFILE OF DARK CHOCOLATE

Small-batch chocolate-makers use distinct equipment and process steps from that of large-scale industry leaders. Only one of these steps is unique to small-batch chocolate-making and is known as melanging—a refining process that takes place in a stone wet-grinder. Despite being essential to flavor and overall quality development, it has never been studied, and all data on its application to chocolate are anecdotal.

This research evaluated the melanging step at three different temperatures in order to determine, via Head Space Solid Phase Micro Extraction (SPME) coupled with GC-MS, which volatile flavor-active compounds are formed or lost during the melanging process and how the time and temperature affect the volatile composition of the chocolate mass. Univariate statistical analysis on the 36 samples (3 temperatures, 4 time points, and 3 technical replicates) was performed using analysis of variance (ANOVA) and multivariate statistical analysis was performed using principal component analysis (PCA) and orthogonal partial least squares (OPLS). These analyses found that time, rather than temperature, accounted for most of the variation among samples. Some trends among chemical classes were identified, and certain indicator compounds were highlighted for their particular relevance to the system. Pyrazines displayed the strongest pattern of any annotated chemical class. As a rule, these compounds started with moderate to high abundance at 0 hours and decreased to extremely low levels by 24 hours. However, most classes

of chemical compounds analyzed in this study did not follow strong patterns of behavior predicted by chemical class.

Particle size and pH were also measured for each sample. Majority particle size was found to be below 25 microns generally at all time points beyond 8 hours, and pH for all samples had a mean of 5.25 with a standard deviation of 0.177. Analysis showed significant temperature-dependent *p*-values for several compounds, but significant time-dependent *p*-values were apparent for a greater number of compounds. For compounds which showed dependency on both time and temperature, the time-dependent *p*-value was much smaller in most cases. Both PCA and OPLS analyses suggested the same trends.

It was determined first, that melanging is a viable method to carry out successful chocolate refining. The final flavor of chocolate made in a melanger is far more dependent on time spent in the melanger than on the temperature of the chocolate system. In addition, classes of chemical compounds did not behave according to unified patterns of behavior in melanging systems (with the exception of nitrogen heterocycles, which decreased in relative abundance). Finally, literature previously published on conching may be successfully extrapolated to melanging.

ACKNOWLEDGMENTS

Throughout this process I have received support and assistance from a number of parties. I am grateful to my advisor, Dr. Martha Stone, for reading numerous drafts and for keeping me on a productive path during my time at CSU, and to my other committee members, Dr. Jeffrey Miller and Dr. Joseph DiVerdi, for always being available to answer questions and provide guidance.

In addition, I would like to acknowledge Harmonie Bettanhausen, without whose advice and generosity none of this would have happened.

I would also like to recognize my parents, Doug and Bonnie Clark, and my husband, Pedro Fraguas Sainz, for their support, love, and most of all, patience.

Finally, I would like to thank Toby and Alix Gadd of Nuance Chocolate in Fort Collins, Colorado, USA, for donation of raw materials and equipment for this study and for constant encouragement over the years.

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DEFINITION OF TERMS

Small-batch chocolate: Chocolate made in small quantities, often by mom-and-pop-style entrepreneurs that have started businesses since 2007, usually focusing on high-quality chocolate that fetches a high price per volume.

Bean-to-bar chocolate: Chocolate made directly from the raw cacao bean at a single location, usually with minimal processing such as pressing or alkalization.

Cacao pod: The woody, outer shell of the cacao fruit.

Cacao bean: The seed of the cacao fruit, which is fermented, and roasted and then used to make chocolate and other cacao products.

Cacao husk: The paper outer layer of the cacao bean, which is separated from the interior and discarded before continuing with the chocolate-making process.

Cacao nibs: The meaty pieces of roasted cacao bean that are left behind when the papery outer husks are removed and the brittle inner bean shatters.

To winnow: The process of separating the papery outer layer of cacao beans (husk) from the meaty interior (nibs)

To refine: Usually refers to the process of reducing the particle size of the ingredients of chocolate and cacao products. This use must be disambiguated from a second, also common, use of the same word, which refers to flavor improvement of the same products.

Conche/Conch: A stirring machine, used in chocolate-making since 1880, that mixes refined ingredients, homogenizes them, and aerates the mass, resulting in improved flavor and texture properties of the final chocolate. Typically used in large-scale operations.

Melanger: A grinding machine, used in chocolate-making since at least 2006, that simultaneously refines cacao ingredients and mixes, homogenizes, and aerates the chocolate mass. Typically used in small-scale operations.

CHAPTER 1: INTRODUCTION

Chocolate is a confection made from the seeds of *Theobroma cacao*, one of 22 species of the genus *Theobroma* (Sotelo & Alvarez, 1991). Several of these species are used in the preparation of food and beverages, but *T. cacao* is most commonly used, and it results—with some minimal contribution in certain locals by *T. bicolor* and *T. grandiflorum*—in the foods and drinks collectively known as chocolate (Pérez-Mora, Jorrin-Novio, & Melgarejo, 2018). Modern-day chocolate is prepared in an elaborate series of processes which could only exist in a post-industrial, globalized market. This is because proper chocolate production requires time spent in two dramatically different climates, and it is rarely consumed near the place where it is produced (Moss, 2009).

Chocolate Production:

The first step in the chocolate production process involves harvesting the cacao pods, which is best done by hand because they do not all ripen at once. The cacao tree continuously produces hundreds of tiny, odorless flowers which are pollinated by the random action of small insects and mosquitos crawling across them, because there is no smell to attract pollinators (De La Cruz, Vargas, & Del Angel, 2009). Cocoa pods ripen within five to six months of pollination (Tannenbaum, 2008). Because fertilization is continuous, ripening is also continuous throughout the year, although it does experience peak seasons especially in Western Africa (Hamdouche, 2016). Ripeness can be tested by lightly scratching the skin of the pod or by watching for a color change; the specific color change depends on the variety of cacao, but it usually involves a shift from green to yellow, red, or orange (De La Cruz, Vargas, & Del Angel, 2009). After harvest, the pods are opened by tapping sharply with a machete or other long, narrow tool (Hamdouche, 2016).



Figure 1: Interior and exterior of ripe cacao pods (Terenzi, 2016)

Then laborers remove the seeds, covered in a sticky pulp known as mucilage, from the pod and collect them in a box, pile, or other vessel (Papalexandratou, Vrancken, de Bruyne, Vandamme, & de Vuyst, 2011). Fermentation begins as soon as the pods are opened and exposed to the environment (Sulaiman & Yang, 2015) and typically continues for 2 to 8 days depending on conditions at the farm (Hamdouche, 2016). At this point, the fermented beans are dried either under the sun, over fire, or in a mechanized dryer (Sulaiman & Yang, 2015) until they reach approximately 8% moisture (Hamdouche, 2016). Sun-drying is generally considered preferable, as some off-flavors have been associated with the use of other drying processes, although they may offer greater control over the final moisture level (Yaw, 2014). Beans can then be safely

packed and transported elsewhere as long as moisture levels are kept low, as cacao beans are extremely hygroscopic (De La Cruz et al., 2009).

The process continues when the cacao beans reach the factory or kitchen of a chocolate-maker. Beans are roasted, usually in a rotary or drum roaster (Chin, Miller, Payne, Hurst, & Stuart, 2013) with the objective of reducing the moisture level to around 3% and developing characteristic cacao flavors, mostly through Maillard reactions (Gutiérrez, 2017). Then in a process known as “winnowing” the brittle, meaty interior parts, known as nibs, are separated from the papery protective layer known as the husk, which is not useful in chocolate-making (Gutiérrez, 2017; Mermelstein, 2012). The husks may be discarded or used as raw material for other purposes like vermicompost or brewing (Molina-Murguía, García-Calderón, & Martín, 2009; Sadasivuni, Bhat, & Pallem, 2015; Sugar, n.d.) At this point, processing differs depending on whether the chocolate is going into large-scale production or small-batch production. In *State-of-the-Art Chocolate Manufacture: A Review*, Gutiérrez (2017) offers an excellent review and discussion of large-scale industrial chocolate processing.

Small-batch chocolate processing follows the roasting, winnowing, and de-husking steps by refining the chocolate with sugar and occasionally other ingredients, usually in a type of stone grinder called a melanger (Vreeland, 2015). When the chocolate is smooth, it undergoes a series of controlled heating and cooling steps to crystalize the fats into the most desirable structure. This process is known as tempering. Finally, the tempered mass is cooled in molds or made into confections (Afoakwa, 2011).

CHAPTER II: REVIEW OF LITERATURE

The stage of small-batch chocolate-making of interest to this study is the phase during which both the particle size and the flavor of the chocolate are refined. In large-scale operations, this is typically achieved according to a process known as conching, after the Spanish *concha* or “shell”, which the original conching machine resembled (Aidoo, Clercq, Afoakwa, & Dewettinck, 2014; Beckett, 2009; Hartel, von Elbe, & Hofberger, 2018). Because it is used in industrial operations, conching is well-studied and well-understood.

Researchers are still divided regarding the purpose and effects of conching (Albak & Tekin, 2016). Gutiérrez indicated that the purpose of conching is to eliminate acidity, bitterness, and some moisture (Gutiérrez, 2017). While Beckett agreed that these aspects of flavor development occur, he described conching as principally a method of improving the fluid mechanics of chocolate, with flavor development as a secondary goal (Beckett, Fowler, & Ziegler, 2017). Torres-Moreno (Torres-Moreno, Tarrega, Costell, & Blanch, 2012) noted the effect of conching on viscosity and texture.

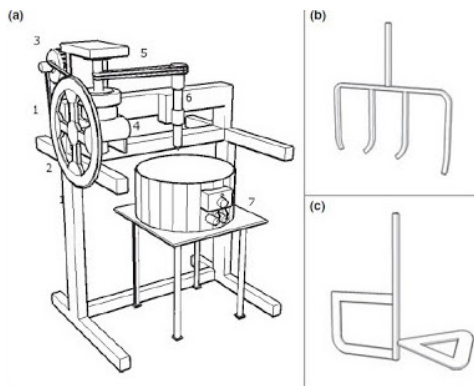


Figure 2: Diagram of a rotary conche. (A) General equipment scheme, (B) Yoke-type impeller, (C) Propeller-type impeller (Bordin Schumacher et al., 2009)

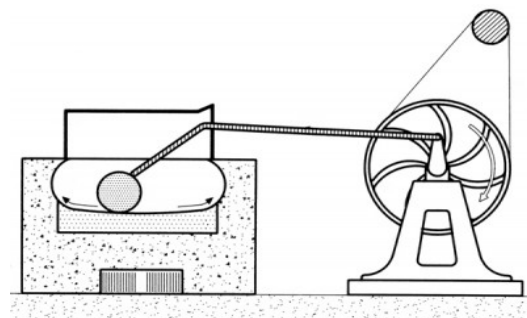


Figure 3: Diagram of a longitudinal conche (Domenichiello, 2019)

Conching Background:

Conching is essentially a flavor-development step, with some potential texture benefit as well, through homogenization of the fats, sugars, and cocoa solids by frictional heat and release of volatile compounds (Bordin Schumacher et al., 2009). However, it is not a particle-size refining step; when the chocolate mass enters the conche, its particle size has already been reduced to below the 25-micron limit by means of other refining equipment such as a roller refiner or ball mill (Rohm, Böhme, & Skorka, 2018). Later in process, fat will gradually coat all particles. Particles which have been ground too small in the refining stage will use up large amounts of fat as a coating, and the chocolate can thicken to the point of seizing if there are too many small particles; for this reason, it is advisable to keep an average particle size of around 20 microns and never allow any particle size to drop below 4 microns (Beckett, 2006; Hartel et al., 2018).

In large commercial operations, conching usually consists of three distinct phases (Gutiérrez, 2017). First, in the dry phase, the refined cocoa mass enters the conche and most particles become covered in fat during mixing; 50°C is a typical temperature for this phase for the preparation of dark chocolate (Albak & Tekin, 2016). Moisture levels are already low when this phase starts—1-1.6% is typical (Beckett et al., 2017). The second phase, or pasty phase, completes moisture removal down to approximately 0.5% (Beckett et al., 2017; Bolenz, Kutschke, & Lipp, 2008) and begins flavor development (Gutiérrez, 2017). While dark chocolate may reach 80°C in this stage, milk chocolate need not rise above 55°C due to the larger proportion of unsaturated fatty acids in the milkfats, (Beckett et al., 2017) such as butyric, caproic, caprylic, and capric acids (Hartel et al., 2018). In the third or liquid phase, chocolate mass is fully liquefied, flavor development is complete, and a homogenous texture is generated while temperature decreases linearly to around 45°C for dark chocolate (Albak & Tekin, 2016) and 40°C for milk chocolate (Bolenz, Thiessenhusen, & Schäpe, 2003).

Although most industrial-scale chocolate-making processes carry out all three of these stages in a single conching machine, some producers—notably those in the French tradition—tend to move the chocolate mass between a series of increasingly specialized mixing and refining machinery, only coming to the conche in the very last stage (Gasparotto, 2018). This series of transfers between refining and aeration mechanisms is thought to create a silky texture and rounded flavor that cannot be accomplished by other means, and it also allows the chocolate maker to tailor the degree of processing to the purpose for which the chocolate product is intended (Gasparotto, 2018). A chocolate-maker may add additional cocoa butter—up to 5% of the final recipe—during the final conching stage (Beckett et al., 2017). Additional cocoa butter increases the melting rate of the chocolate, improving its smoothness and mouthfeel; it also decreases bitterness perception of chocolate by coating and “buffering” bitter cocoa solids (Prawira & Barringer, 2009). Time spent at each processing step is usually a closely-guarded industrial secret, but it is proportional to the volume of cacao mass in the conche and to the quality of the chocolate being produced (Rousseau, 2007; Tan & Balasubramanian, 2017). Gasparotto (2018) noted that “value-brand” chocolates may spend “only twelve hours” in the conche, but high-quality chocolates usually undergo the process for “many more hours”.

Gasparotto (2018) described two main types of conches: a longitudinal conche consisting of a long, relatively narrow trough in which a roller sweeps the cacao mass back and forth [the original conche pictured above (Fryer & Pinschower, 2000) was of this type] and a circular conche which employs either wheels or a pumping mechanism to create shear forces within a cylindrical drum, creating the agitation needed for this step. Gasparotto (2018) claims that the circular conche is the most widely used and this seems to have likely been true at least until the early 20th century, as a circular conche is advertised by Alfred Savy around this type as the only style of conche

available among his automated chocolate-making equipment (Frogerais, 2017). Beckett (2009) claims that the longitudinal conche has almost entirely fallen out of use.

Modern authors list a large number of alternative conche designs and mechanisms. Batch rotary conches (both horizontally- and vertically-stirred), small- and large-volume continuous conches, short dwell time continuous conches, cocoa liquor treatment conches, liquefiers, and in-line high-shear devices are just some of the modern conche styles described by Fryer and Pinschower (2000) and Hartel et al. (2018). They also list a number of combination grinder/conche systems, in which chocolate mass is recirculated between a conche and some type of mill (Fryer & Pinschower, 2000). Modern conches are both time-saving and able to produce acceptable flavor and texture results (Franke & Tscheuschner, 1991). They are able to reduce processing time by investing more energy input in the dry phase, which allows for more rapid evaporation of water and volatile compounds (Fryer & Pinschower, 2000). Anecdotally, this also satisfies consumer preferences, as studies have shown that consumers prefer chocolate made with a longer dry conche phase over that made with a longer liquid phase (Beckett, 2006).

What all these styles of conche have in common is their mechanism of action. In all cases, conches accomplish three specific objectives to improve the quality of the chocolate. First, they apply heat, either by friction or a temperature-controlled jacket to raise the temperature of the chocolate, causing the small remaining amount of water to evaporate (Fryer & Pinschower, 2000). Any steam-volatile compounds (especially volatile carboxylic acids) will volatilize with this water (Fryer & Pinschower, 2000; Hartel et al., 2018); it is for these reasons (evaporation of water and volatilization of compounds) that conches are all vented (Beckett, 2006). A number of chemical reactions important for chocolate flavor also take place at these higher temperatures, such as a reduction in alkaloids and polyphenols (Albak & Tekin, 2016), hydrolysis, and thermal

degradation (Hoskin, 1980). Secondly, to improve the quality of the chocolate, conches allow the chocolate mass to come into contact with oxygen, which may be accomplished by pumping, stirring, paddling, or several other mechanisms. Aeration of the chocolate further volatilizes unwanted flavor and aroma compounds and may allow other compounds to develop in oxygen-catalyzed reactions (Hartel et al., 2018; Hoskin, 1980). The final objective is to make use of shear forces within the conche to break up agglomerated particles in the chocolate mass and evenly coat all resulting fragments with fats (Fryer & Pinschower, 2000). As the liquid fats are distributed over all the particles, the chocolate will begin to thicken.

Conching allows development of the aroma and flavor of chocolate, reduces moisture and acidity, increases palatability, makes chocolate more workable and malleable, and gives chocolate a buttery, unctuous, creamy, texture (Gasparotto, 2018). It is the interactions that take place during this mixing stage that improve the quality of the chocolate beyond the contribution of the ingredients and the particle size alone.

Alternatives to Conching:

Smaller operations with less budget to invest in equipment may need to choose a refining option that requires less capital investment upfront. As of 2019, a small (for a 20kg batch) refiner-conche may be purchased for \$10,000-\$12,000 (VEC Refiner Conche, 2019) while a melanger that processes the same volume costs around \$2,400 (DCM Chocolate Melangeurs, 2019). The Stephan mixer and the ball mill have also been studied for chocolate-making with promising results (Aidoo et al., 2014; Alamprese, Datei, & Semeraro, 2007), but the most common choice for small-batch chocolate-makers is the melanger or wet-grinder (Vreeland, 2015). The modern melanger was developed in 1826 by Suchard (Moss, 2009), but stone grinders of various types have been in use for thousands of years (Berk, 2013; Vreeland, 2015). Stone grinding is also

thought to be the original refining technique applied to cacao in its native Central America (Fryer & Pinschower, 2000; Tannenbaum, 2008).

According to literature, French chocolate producers have been using a stone-grinder similar to the type now called a melanger (French, *malaxeur*) as part of the chocolate-making process since 1778 (Frogerais, 2017). However, its use in the French tradition has historically been different than how a similar machine is typically employed today by small-batch chocolate makers. Instead of using the *malaxeur* primarily for the entire refining step, producers treated this piece of equipment as only the first step in a series of refining and texture-development phases. In this production style, the cocoa mass was kept in the melanger only until the mixture was sufficiently homogenized that it could be passed to the next step, known as *broyage-affinage* (refining). At the end of *malaxage*, the cocoa mass was described as having a pleasant flavor, but with a particle size over 100 microns, still an unacceptably grainy texture (Gasparotto, 2018). The *broyage-affinage* that followed involved pulling a thin sheet of cocoa mass between rollers at 30 bars of pressure until particle size was reduced to 20 microns. Only then, and only for certain processes, was the “supplementary step” of conching recommended (Gasparotto, 2018).

The specific type of stone grinder used in small-batch chocolate production today has found more common application as a wet grinder for Indian dosa batter and has also found some limited application with soy on a laboratory scale (Bhattacharya & Bhat, 1997; Vishwanathan, Singh, & Subramanian, 2011). Similar machines have been used for nut-grinding for nut butters or nut-based confections (Nanci, 2016). Early records of melanger use in small-batch chocolate-making are poorly documented, but one record exists of some chocolate-makers using a modern melanger as early as 2006 (Spies, 2007). In 2005, John Nanci, a leader in the craft chocolate movement, first amended stone wet grinders for use as chocolate refining devices. At the time he was associated

with Santha, which later spun off a company devoted entirely to chocolate-making equipment, called Spectra (J. Nanci, personal communication, July 29, 2019). Availability of melangers further expanded when, in 2007, Inno Concepts LLC, an importer of wet grinders from India to the United States, found itself with an excess of grinders in the midst of an economic recession. Upon hearing that some of their customers used wet grinders for chocolate making, the owners experimented with modifications, such as adding a cooling mechanism, to repurpose their excess motorized stone wet grinders into chocolate-making devices, and the company was rebranded as Cocotown LLC (Williams, 2017). Cocotown marketed these updated grinders to the growing niche population of small-batch chocolate makers that were beginning to seek small-scale chocolate-making equipment at the time. Now, a number of types and brands of melangers are available to small-batch chocolate-makers.

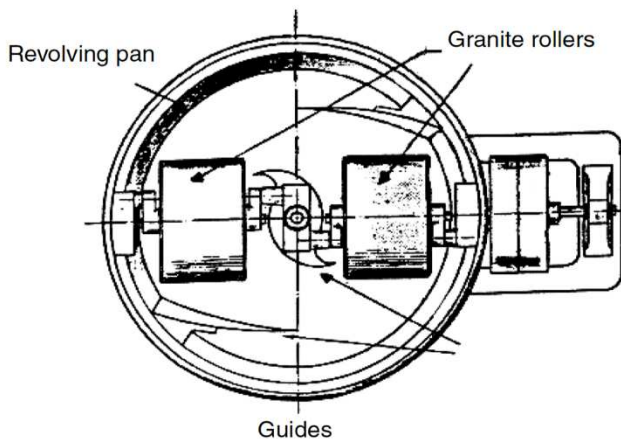


Figure 4: Diagram of the interior of a melanger (Beckett, 1980)



Figure 5: Image of a melanger in use (Terenzi, 2018)

Melanging:

While some researchers treat melanging as a type of conching because both techniques make use of crushing and shear forces to refine, mix, and aerate chocolate (Tan & Balasubramanian, 2017), there are some significant differences in the two processes. First, in a

conching process, the cocoa mass (cocoa butter, cocoa liquor, and sugar) is typically ground in a roller-refiner to its ultimate desired particle size before conching commences (Gutiérrez, 2017; Torres-Moreno et al., 2012). In modern-day conching it is thought that all particle size reduction has already taken place before the chocolate mass enters the conche, and no further reduction in particle size will occur during the conching step (Fryer & Pinschower, 2000). In melanging, however, particle size reduction takes place in conjunction with other processes, since the cocoa mass has not been fully refined when it enters the stone grinder (Owen, 2013; Tan & Balasubramanian, 2017). While in conching a chocolate producer may add cocoa butter towards the end of the process as described previously, in melanging all ingredients in their full proportions for the final recipe are added at the beginning of the process (Owen, 2013). Finally, while the precise times and temperatures of the two processes are trade secrets for the companies that use them, conching generally occurs for somewhere around 12 hours but can take as long as 72 hours (Bordin Schumacher et al., 2009); generally conching occurs at higher temperatures for shorter times than melanging (Stephen T. Beckett et al., 2017; Hinneh et al., 2019).

Although melanging is mechanically different than conching, it shares the same goals and works according to similar principles. Both methods make use of shear mixing (pressing particles between two surfaces) and elongational mixing (spreading particles along a surface) to reduce particle size (Berk, 2013). Any attempt to optimize a melanging profile would need to consider what is known about conching in terms of particle size and distribution, fat coverage, moisture evaporation, and volatile off-gassing. Together these processes contribute to the rheological and organoleptic properties of the final chocolate product (Albak & Tekin, 2016; Beckett et al., 2017; Hartel et al., 2018). Before this study can be undertaken, it is necessary to review what is already known about conching and discern how it may be relevant to refining with a melanger.

Determination of Successful Conching:

First we know that the effects of these processes that result in successful conching can be quantified according to the results of several tests. Yield stress and plastic viscosity may be measured to determine the rheological properties (characterized according to the Casson scale) of chocolate. An acceptable Casson yield stress for dark chocolate should be between 4-32 Pa, while Casson viscosity values should fall between 2.1 and 3.9 Pa (Aeschlimann & Beckett, 2000).

Another indicator for successful refining is thixotropy; this property is a response to shear forces characterized by a continuous decrease in apparent viscosity followed by a recovery of apparent viscosity when flow is interrupted. A plot of this trend would show a hysteresis loop, the area of which quantifies the degree of thixotropy. Chocolate with a thixotropic value less than one are considered to have been successfully conched (Aidoo et al., 2014).

It is also important to measure particle size as an indicator of successful conching. One of the principal objectives conching is to refine the diameter of the particles of sugar, cocoa solids, and fat micelles. Below 30 microns, the human tongue is unable to distinguish texture, so chocolate is made smooth by reducing particle size to below this diameter (Tan, n.d.) It is generally considered desirable to aim for a particle size of around 20 microns in finished chocolate, for a smooth and fine mouthfeel (Barel, 2001; Beckett, 2006).

Perhaps the most significant determiner of a successful conching step is flavor. Insufficiently conched chocolate is said to taste overly acidic, flavorless, and flat (Gasparotto, 2018), while fully conched chocolate is described as smooth-tasting and caramelized, with more noticeable chocolatey flavors (Hartel et al., 2018). Successful conching should also result in what is often called a “rounded” flavor (Ziegleder, Braun, Benz, Schreier, & Mikle, 2005); this results from a distribution of the flavor compounds from the fat phase to all phases of the system (Beckett et al., 2017). Eventually, flavor compounds will bind to the sugar, causing both fat and sugar to

become significant flavor carriers; this is the state described as “rounded” flavor (Beckett et al., 2017). Other observable organoleptic properties that are a direct consequence of proper conching are increased viscosity and a noticeably darker color (Gutiérrez, 2017)

One analytical method to determine degree of conching has been successfully developed; this technique uses GC-MS detection of tetramethyl pyrazine and benzaldehyde (Ziegleder et al., 2005). The team validated their methods to responses from 150 trained sensory panelists on a total of 14 different panels, and their results show that analytical methods can be used to more closely—if still imperfectly—determine conching success (Beckett, 2006). The same team has used analytical methods to probe rheological properties of conched chocolate, but these efforts did not result in a tool for determination of the degree of “doneness” from a texture or rheological perspective (Ziegleder, Balimann, Mikle, & Zaki, 2003).

Project Goal:

Melangers from Cocotown and other producers such as Cacao Cucina are now the most common flavor and particle size refining tool used by small-batch chocolate makers because of their scalability and low upfront capital investment (Vreeland, 2015). Despite this, there is little academic literature on the use of melangers for chocolate-making. Therefore, the goal of this study was to determine the effects of time and temperature on melanging outcome by measuring volatile profiles before, after, and at two stages during melanging under three different temperature treatments. Particle size and pH were also determined at each temperature and time point. By comparing these results to what is already known about conching, a further objective was to determine if melanging is an acceptable alternative to conching and to probe whether pre-existing data on conching might be extrapolated to melanging systems.

CHAPTER III: MATERIALS AND METHODS

Sample Preparation:

Sample preparation consisted first of gathering the ingredients to make the chocolate, then of making three batches of chocolate, each at a different temperature treatment, and finally of collecting samples at 8-hour time points within each temperature treatment.

Nuance Chocolate (Fort Collins, Colorado, USA) donated the raw materials and the bench-scale melanger for this study, as well as the facilities used to sort and roast the raw cacao beans. Each 0.5-kilogram batch of chocolate required 478 grams of raw cacao beans from Ghana, which were stored at 24°C until being used for this study in Nuance Chocolate's factory space. The 478.0 grams for each batch were individually weighed and hand-sorted to remove any stones, insects, or other non-cacao materials that could interfere with processing. The beans were roasted at a 163°C for 17 minutes, then removed from the oven and cooled. After being fully cooled, the roasted cacao beans were passed through a juicer (Champion, Lodi, CA, USA) connected to a Hoover L2310 vacuum (Hoover, Glenwillow, OH, USA) with a 10 Amp motor, for a process known as cracking and winnowing, which shatters the beans and removes the thin outer husk.

At this point, the roasted, cracked, and winnowed beans are known as cacao nibs. For each batch of chocolate, 350.0 grams of cacao nibs were placed into a Spectra 11 bench-size stone melanger (Spectra, Coimbatore, India) followed immediately by 25.0 grams liquified cocoa butter and 125.0 grams superfine cane sugar (both also donated by Nuance Chocolate). When all ingredients had combined into a visibly homogenous slurry (within five minutes of being mixed) the 0-hour time-point sample was taken for each batch. The melanger was then placed into a Caron 7000-25 series incubation chamber (Caron, Mariette, OH, USA) to control ambient temperature for each temperature treatment (16°C, 24°C, and 38°C). Further samples were taken at 8-hour, 16-

hour, and 24-hour time points. Each sample consisted of 2.0 grams of chocolate mass piped into a cylindrical glass SPME vial using a 1ml syringe, then further sealed inside a plastic container and incubated at 4°C, as per the recommendation of the Proteomics and Metabolomics Lab.

Metabolomics Analysis:

Now fully prepared, the samples were tested using headspace solid phase microextraction (HS/SPME) paired with GC-MS according to the procedure laid out by Rodriguez-Campos et al. (Rodriguez-Campos, Escalona-Buendía, Orozco-Avila, Lugo-Cervantes, & Jaramillo-Flores, 2011) with some modifications. Briefly, 2.0 grams of each sample was piped into the SPME flask using a 1ml syringe, then sealed in the flask and exposed to a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30m SPME fiber for 15 minutes at 60 °C to equilibrate, followed by a 30-minute exposure time at the same temperature. The needle was desorbed at 240 °C and injected onto a silica wax capillary column using hydrogen as a carrier gas. The GC temperature program was started at 40-60°C and was held for 5 minutes before being raised to 190-200°C at increments of 3°C/min and then being held again for 10 minutes. Quadrupole MS detection was done at 280°C at the source and an electronic ionization energy of -70eV with 1200V in the detector.

pH Measurements:

Meanwhile, parallel duplicates of each sample were tested for pH according to methods laid out by Jinap, Dimick, and Hollender (1995) with some modifications (Selamat Jinap & Dimick, 1990; Selamat Jinap, 1987). Briefly, 1.0 gram of sample was homogenized into 9.0 grams of boiling water. The mixture was then filtered, and the filtrate cooled to 24°C and its pH was recorded.

Particle Size:

Particle size measurements were measured using a stainless steel 0-50 micron grindometer (Boshi Electronic Instruments, Baiyun, Guangzhou, China) This is a device similar to that used by some small-batch chocolate-makers to determine when a batch of chocolate is ready to remove from the melanger (Gore, 2015). Results were expressed as a range of majority particle size, based on visual analysis of the grindometer. See Appendix for example images used for analysis.

Experimental Design / Statistical Analysis:

Volatile metabolite abundances were compared using two-way analysis of variance (ANOVA) via the *aov* function in the R statistical environment v. 3.5.1, and false discovery rate adjustment was performed on the ANOVA *p*-values using the Benjamini-Hochberg algorithm. Principal Components Analysis (PCA), and Orthogonal Partial Least Squares Analysis (OPLS) were conducted on 88 annotated metabolites with SIMCA software v. 15 (Sartorius Stedim Biotech, Umea, Sweden). Scores and loadings values from OPLS models were conducted in SIMCA software on z-transformed data for time and temperature (*y*) and annotated, unit variance scaled volatile metabolites (*x*). Predictive power (Q^2) was determined via cross-validation, by which the data was divided into seven parts and 1/7th of the data were removed, and the model was built on the remaining 6/7th of data remaining, and the removed 1/7th of data are predicted from the model.

CHAPTER IV: RESULTS

Particle Size Results:

In this study, all samples tested other than those at the zero time points showed a majority particle size below 30 microns. As expected, particle size continued to drop as melanger time was increased (Table 1). Owen (2013) explains that a particle size below 30 microns is desirable in chocolate for a smooth and finished texture because the tongue is unable to determine specific textures at this small diameter. Other authors concur, some claiming that 20 microns is the ideal particle size for finished chocolate (Barel, 2001; Cobigo, Lachenaud, & Barel, 2018). Tan (n.d.) states explicitly that a range of 15 to 25 microns is most desirable. By this definition, these results indicate that the chocolate samples could have been classified as “finished” with regard to particle size at any point beyond 8 hours, as all samples at 8 hours and beyond met the criteria of having a particle size below 30 microns.

Table 1: Chocolate particle size at each temperature and time point

Temperature (°C)	Time (Hours)	Particle Size (Microns)
16	0	50+
16	8	20-25, none below 15
16	16	15-20, none below 10
16	24	10-15, none below 5
24	0	50+
24	8	20-25, none below 15
24	16	15-20, none below 10
24	24	10-15, none below 5
38	0	50+
38	8	20-25, none below 15
38	16	15-20, none below 10
38	24	10-15, none below 5

pH Results:

Mean pH values were 5.25, with a standard deviation of 0.177. An IQR statistical test for outliers finds three; see highlighted values indicated on Table 2. These outliers were detected using the Interquartile Range times 1.5 added to or subtracted from the third and first quartile values, respectively. This test found that any values below 5.21 or above 5.3 would be classified as outliers. One measurement is notably aberrant: the first test of 38C and 24H (Figure 2). This outlier is unlikely to be a result of instrument error, as it was confirmed by a second pH meter. However, it may have been due to sampling contamination or human error.

Table 2: pH of chocolate mass at each temperature and time point

Temperature (°C)	Time (Hours)	pH
16	0	5.20
16	0	5.25
16	8	5.30
16	8	5.34
16	16	5.31
16	16	5.32
16	24	5.33
16	24	5.35
24	0	5.30
24	0	5.28
24	8	5.28
24	8	5.27
24	16	5.30
24	16	5.30
24	24	5.29
24	24	5.31
38	0	5.23
38	0	5.20
38	8	5.27
38	8	5.28
38	16	5.29
38	16	5.29
38	24	4.44
38	24	5.23
38	24	5.23

Metabolomics Results:

Samples from different time points had distinct profiles of volatile metabolites

To investigate if the samples varied in their volatile profiles, and if this variation was associated to time or temperature, a non-targeted metabolomics experiment was performed on 36 samples (4 time points, 3 temperatures, 3 technical replicates) using HS/SPME GC-MS. The MS detection and subsequent chemoinformatic methods resulted in the characterization of 88 compounds, which were annotated using spectral matching and classified according to ontological information from public chemical databases (Table 11, Appendix). Analysis of variance (ANOVA) on the 88 compounds characterized 86 volatile metabolites (98%) that varied among the samples for time and 59 volatile metabolites (67%) that varied for temperature (FDR adjusted $p < 0.05$, Table 11 Appendix). PCA was conducted on the 88 compounds which showed that variation was attributed to time and resulted in three principal components (Figure 6). PC1 (68.3% of the variation) was attributed to a distinct volatile profile of the samples at 0 hours. PC2 (14.5%) was associated with differences at 8 and 16 hours ($p < 0.05$), and PC3 (9.7%) demonstrated separation between 24 hours and the other time points ($p < 0.05$).

The influence of time

The metabolomics data demonstrate that time has much influence on variation seen among the sample profiles. Although analysis showed significant temperature-dependent p -values for several compounds, significant time-dependent p -values were apparent for a greater number of compounds. For compounds which show dependency on both time and temperature, the time-

dependent p -value was much smaller in most cases. Furthermore, both PCA and OPLS analyses suggest the same trends (Figures 6 and 7).

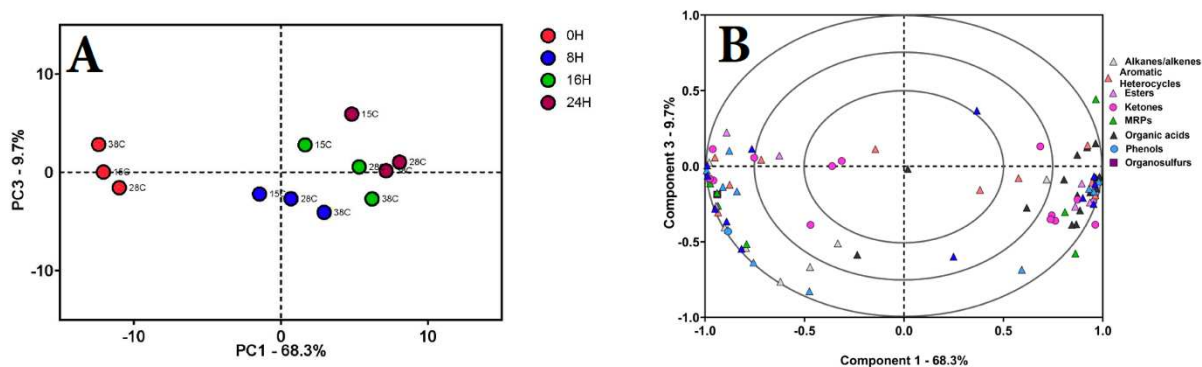


Figure 6: Scores plots from PCA analysis of 12 chocolate samples. (A) Samples, and (B) Loadings

OPLS characterized chocolate mass volatiles that influenced time

To investigate relationships between the times and temperatures of the samples and each of the 85 volatile metabolites, an orthogonal partial least squares (OPLS) model was developed with the data. OPLS is an extension of PLS and was used to model the relationship between the volatile metabolites (X matrix) and the time or temperature (Y matrix). OPLS was used to separate the systematic variation in X into two parts, one which explains and is related to Y (time or temperature) and one that is unrelated (orthogonal) to Y (Trygg & Lundstedt, 2007). The OPLS algorithm for time resulted in one predictive and two orthogonal components that explained 91.2% of the variation, with a predictive power of $Q^2 = 96\%$ to support that the model was not over-fit. The OPLS algorithm for temperature resulted in one predictive and four orthogonal components that explained 96.9% of the variation, with a predictive power of $Q^2 = 59.2\%$ to support that the model was not over-fit, but that temperature was not predictable. Analysis of the OPLS scores and loadings for time indicate that time can be linked to specific classes of volatile metabolites within the first two OPLS components, as indicated by correlation values of greater than $|0.50|$. A SIMCA

‘distance to model’ function was applied to characterize the metabolites with the largest contribution to explaining the variation in time (Figure 7). The data indicate associations with pyrazines, aldehydes, terpenes, alcohols, esters, and ketones, which are known classes of aroma compounds important in this system.

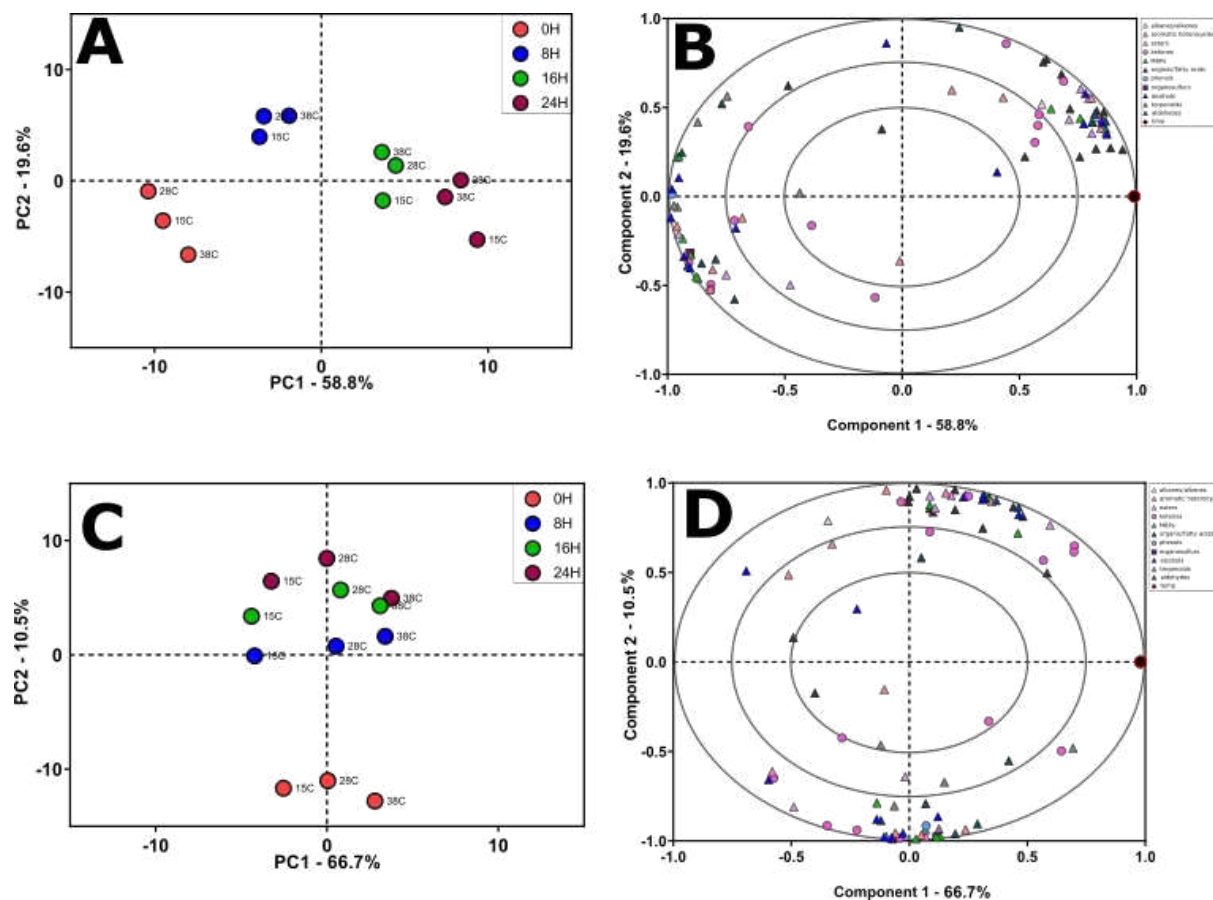


Figure 7: Scores plot of OPLS analysis of 12 chocolate samples. (A) Samples against time, (B) Loadings against time, (C) Samples against temperature, (D) Loadings against temperature

Prior to heat-mapping, volatile metabolite data were normalized within each variety via z-transformation (normalized peak area – mean peak area/standard deviation of total peak area of each metabolite). The resulting z-scores were converted into colors and grouped using hierarchical clustering on the Spearman’s rank correlation (r_s) between metabolite and sensory trait values (Zar,

1972). Correlation plots were generated in R using the `gplots`, `ggplots2`, `Reshape2`, and `stats` packages via the `heatmap.2`, `melt`, and `hclust` functions (Warnes et al., 2015).

Trends among chemical classes

As there are flavor and texture traits that may be driven by volatile chemistry, there were data taken and analyzed from four time points to evaluate the samples: 0-hour, 8-hour, 16-hour, and 24-hour. Data were evaluated to determine if trends of metabolite classes could distinguish the profiles of the chocolate masses at each time point, specifically, for lipids (to include fatty acid ester formation), Maillard reaction products (MRPs), organic acids (and their esters), aldehydes, ketones, and terpenes. Metabolite abundances were z-transformed to express the data as a profile within a variety. Therefore, a range in color denotes range in variation of a compound class within a variety, with very blue (high) or very red (low) indicative of the proportions of a metabolite's contribution to the profile.

Heatmaps organized by chemical class showed that most carboxylic acids increased in abundance. At 0 hours, of the 21 carboxylic acids found only 4 of them are high in abundance, but by 24 hours 15 are high in abundance. Of the 22 aldehydes and ketones analyzed, 7 increase in abundance, 12 decrease, and 3 remain relatively unchanged or undergo multiple shifts in abundance. For the four terpenes found, only one (*trans*-linalool) increases in relative abundance by 24 hours. In the case of the 10 alcohols, 5 decrease in abundance, 3 increase, and 2 remain mostly unchanged by 24 hours.

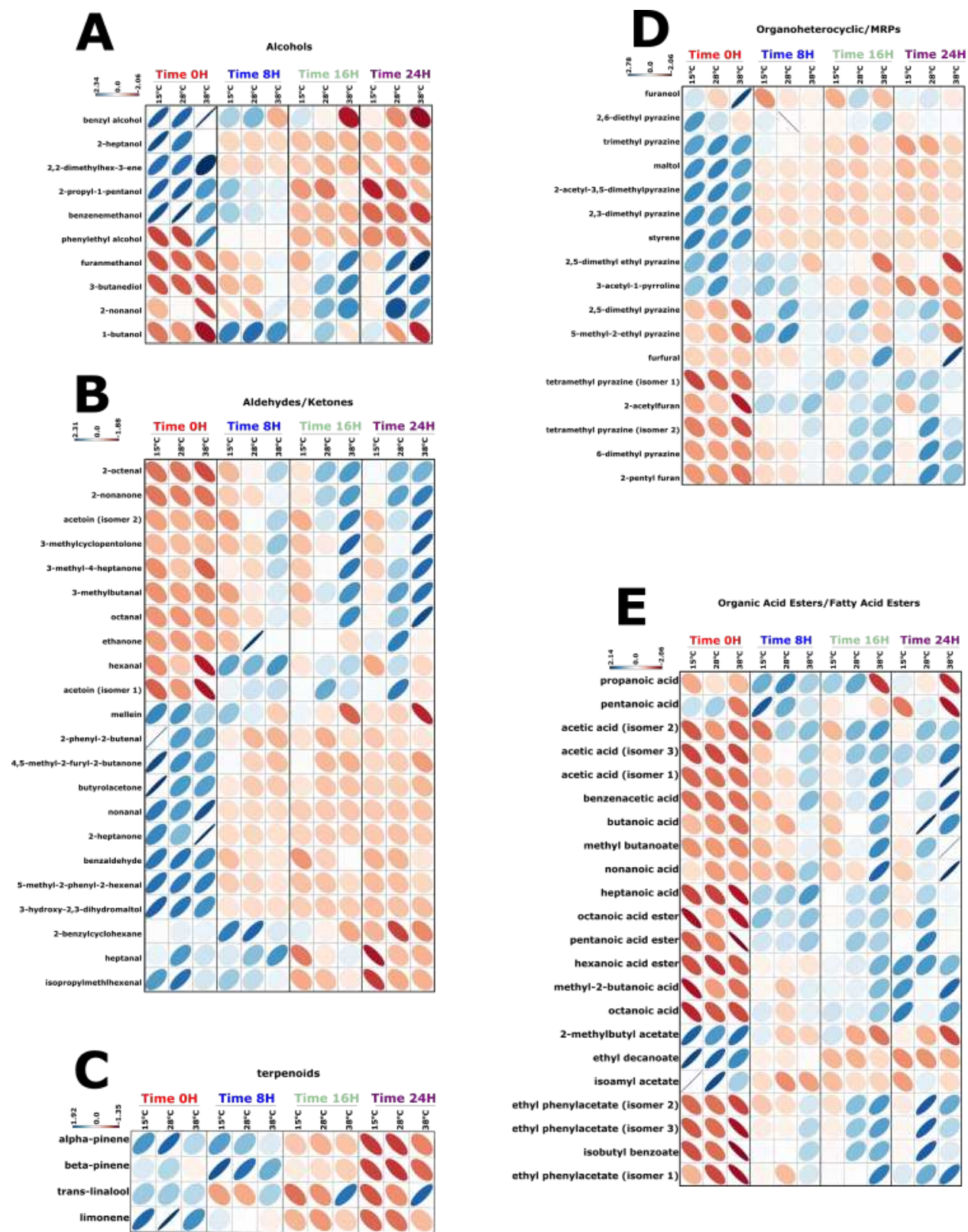


Figure 8: Heatmap plots of trends in relative abundance by chemical class. (A) organic alcohols, (B) aldehydes and ketones, (C) terpenes, (D) maillard reaction products, (E) organic and fatty acids

CHAPTER V: DISCUSSION

Summary

This study characterized chemical variation in chocolate mass samples that was attributed to differences in time and temperature. Specifically, the samples (i) displayed a majority particle size below 25 microns at all time points beyond 8 hours, (ii) had a mean pH of 5.25 with a standard deviation of 0.177, (iii) contained distinct flavor chemistry mediated by volatile metabolites, and (iv) associations between volatile metabolites and time/temperature could be determined. It is hypothesized that flavor chemistry will be principally temperature-dependent, and that data from these samples (made in a melanger) will show patterns similar to data collected on chocolate made in a refiner/conche system. Previous research has shown that flavor outcomes in refining/conching systems are strongly temperature dependent (Afoakwa, 2011; Beckett et al., 2017) and have laid out a list of outcomes that constitute successful conching. These include a reduction in particle size to between 4 and 25 microns (Barel, 2001; ST Beckett, 2006; Hartel et al., 2018), a decrease in overall number and concentration of volatile metabolites (Fischer, Abubaker, Hasselbarth, & Ullrich, 2008) and in an evolution/devolution of aroma compounds that can be estimated by examining the behavior of certain key indicator compounds, namely tetramethyl pyrazine, benzaldehyde, 2-methylpropanal, 2-methylheptadecane, and acetic acid (or carboxylic acids generally) (Albak & Tekin, 2016).

Models at Each Time Point

A regression model was created at each time point, taking into account data from all time points, to indicate which compounds had played the largest role in the development of the metabolic profile of the chocolate mass, indicated by their variation from zero on a coefficient plot

which plots each metabolite by the strength of its correlation with time (Figure 12, Appendix). At the 8-hour time point, beta-pinene and 2-benzylcyclohexane had the largest coefficients. Ketones, esters, and pyrazines, known to play specific roles in chocolate flavor development, were three dominant classes of compounds seen in this time range. At 16 hours, more organic acids had been incorporated into the model. The pyrazines as a category were not involved in the model at this time point. Hexanoic acid and octanoic acid were the two largest overall influencers of the model of flavor development up to 16 hours. At 24 hours, aldehydes and esters were major players in the model. The nitrogen heterocycle 2,6-diethyl pyrazine had the largest overall coefficient at 0.04 and isoamyl acetate was second at 0.03.

Data were also analyzed for major punctual contributors at each time point (independent from the model of the larger system). At 8 hours, 2-methyl-5-ethyl pyrazine and propanoic acid stand out as the two major contributors. However, what is most interesting about the 8-hour sample is that many compounds in a variety of categories act as important contributors (more than one, two, or even three standard deviations). Although these compounds represent a number of chemical groups, compounds with floral and fruity flavor associations were a dominant trend at this time point, as in 2-nonanol, propanoic acid, 1-butanol, 2-acetyl furan, methyl octanoate, and linalool (Figure 10b). At 16 hours, fewer of these floral and fruity compounds were relevant, and a greater number of compounds with buttery and dairy notes, such as acetoin and acetophenone, became important contributors. The contributors at 16 hours also included several carboxylic acids associated with rancid, sweaty, and unclean flavors. (Figure 10c). The 24-hour samples showed a more well-rounded flavor profile, with multiple contributions each from compounds previously identified in literature as having a floral aromas (ethyl phenyl acetate, 2-phenylethanol, octanal), fruity aromas (2-pentylfuran, isobutyl benzoate), roasted or caramel-like aromas (2,6-dimethyl

pyrazine, furfuryl alcohol, nonanone), dairy-like aromas (diacetyl, acetoin), and rancid-smelling aromas (butyric acid, valeric acid, isovaleric acid, hexanoic acid) (Table 3, Appendix). Another notable characteristic of the 24-hour samples is the relative absence or low importance of pyrazines; only one pyrazine appears on the contribution plot at 24 hours (Figure 10d).

What is perhaps most interesting about the emergence and devolution of compounds is that categories of compounds do not all follow consistent patterns. Few pyrazines appear even on the 8-hour contribution plot. Several carboxylic acids appear (more than on the contribution plots for the other time points) although they are different carboxylic acids than those that appear in later contribution plots. Aldehydes, esters, and ketones play a role on the 8-hour contribution plot as well. Fewer compounds overall are contributors at 16 hours. Ketones and carboxylic acids play a major role at this time point, although they are not the same ketones and carboxylic acids that were contributors at 8 hours. Pyrazines tended to be high on the contribution plot for 24 hours although only two of them appear. Esters and alcohols also appear high on the plot with contribution scores, while ketones and aldehydes have slightly lower scores and appear lower on the plot. No terpenes appear on the 24-hour contribution plot.

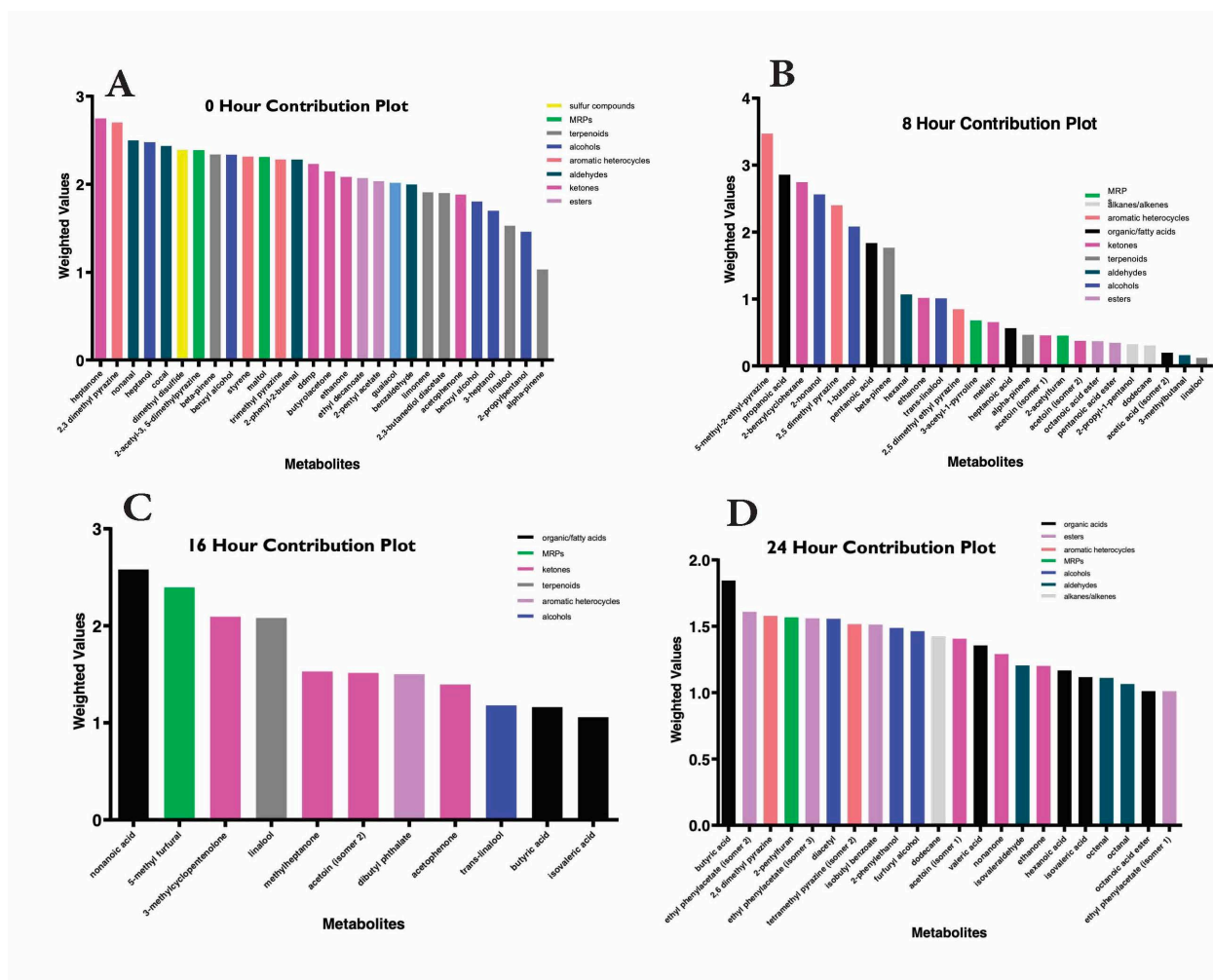


Figure 9: Contribution plots showing metabolites most significant influence on the flavor model, incorporating all temperature treatments at each time point. (A) 0 hours, (B) 8 hours, (C) 16 hours, (D) 24 hours

Specific Compounds of Interest

Albak and Tekin (2016) asserted that a decrease in tetramethyl pyrazine, benzaldehyde, and carboxylic acids and an increase in 2-methylpropanol and 2-methyl heptadecane are signs of successful conching. These data show that tetramethyl pyrazine levels decreased. Tetramethyl pyrazine could be viewed as a stand-in for pyrazines generally; since pyrazines as a group followed the same pattern of behavior, an observation of any nitrogen heterocycle should give an approximation of success. Benzaldehyde has long been recognized as an indicator of conching quality (Ziegler et al., 2005); further tests will show if it serves as an indicator of melanging

quality as well, but there seem to be many parallels between flavor development patterns in the two systems. In this system, its relative abundance decreases, accordance with Albak and Tekin's definition of successful conching.

The other indicator compounds they list not behave as Albak and Tekin predicted. The Strecker aldehyde 2-methylpropanal decreased over time; however, Counet et al. (2002) had previously shown that 2-methylpropanal does not always increase during a successful conche; it may drop slightly in actual or relative abundance. The nearly odorless 2-methylheptadecane was not annotated in this experiment, but it has not generally been considered a compound of interest in chocolate by other authors. Albak and Tekin's assertion that carboxylic acid levels should drop over time was true of some acids but not others in this system. This claim, too, has been disputed by other authors. Hoskin found that volatile fatty acid concentrations could not be predictably associated with conching time but only with rotation speed. They also showed that trained sensory panels could not detect a difference between chocolate samples with measurably distinct levels of acetic acid (Hoskin, 1980). While these data do not align perfectly with Albak and Tekin's work on variation of aroma content during conching (2016), a look at these compounds' behavior in view of previous work shows nothing unexpected.

Several volatile compounds were annotated that are worth mentioning because of their relevance in previous work. Of particular interest is dimethyl disulfide, which started quite high at zero hours but then disappeared nearly completely at all temperature points by 8 hours. Its disappearance is of note because it has been speculated (although not tested) that compounds of this class may be formed during conching (Afoakwa, Paterson, Fowler, & Ryan, 2008); these data indicated the opposite. The smoky off-flavor 2-methoxy phenol changed very little in relative abundance over the time measurements at all three temperatures; indeed, all three of its p-values

were very high compared to those of other compounds. Previous work has demonstrated that this compound is conferred in the roasting stage (Aprotosoaie, Luca, & Miron, 2016) but it can also be affected by storage conditions (Nightingale, Cadwallader, & Engeseth, 2012). These data showed that an attempt to minimize this compound in the refining stage will be futile; its temperature and time dependency is simply too low. These data also affirmed previous authors' assertions that pyrroles, such as the 3-acetyl-1H-pyrroline characteristic of dark chocolate, increased during refining and aeration (Liu et al., 2015). This compound started low in relative abundance and climbed throughout time point measurements to moderate or high (at 28°C) levels by 24 hours. Finally, dibutyl phthalate started low and remained low at all time measurements, although oddly it played an outsized role on the 16-hour contribution plot. This compound had not been previously identified in chocolate, and its presence can most likely be attributed to plastics in the environment or equipment used in the experiment. The bench-scale melanger used to collect these data included one small plastic part. It is possible that some small amount of plasticizer could have been volatilized over the course of the test, enough to play a role in some flavor models of the product.

Furaneol, shown in other works to be generated at high conching temperatures (Afoakwa et al., 2008; Counet et al., 2002), became an important contributor at the 16-hour time point and showed an even more important contribution at 24 hours. Previous authors have indicated that this essential flavor compound evolves during the conching step (Counet et al., 2002; Schnermann & Schieberle, 1997) while others claim it is produced during roasting (Aprotosoaie et al., 2016). These data support the latter view, as the compound appeared to be present in the system from the first measurements (Figure 11). Although its relative abundance was high early in the process at 8 hours, it did not become relevant to the flavor models until later sample times.

Maltol, which did not appear on any of the contribution plots, is nevertheless a compound worth mentioning. Data showed that it increased in relative abundance over time. It has been associated with nutty, caramel flavors and has been strongly correlated in sensory studies with the perception of chocolate aroma (Liu et al., 2015).

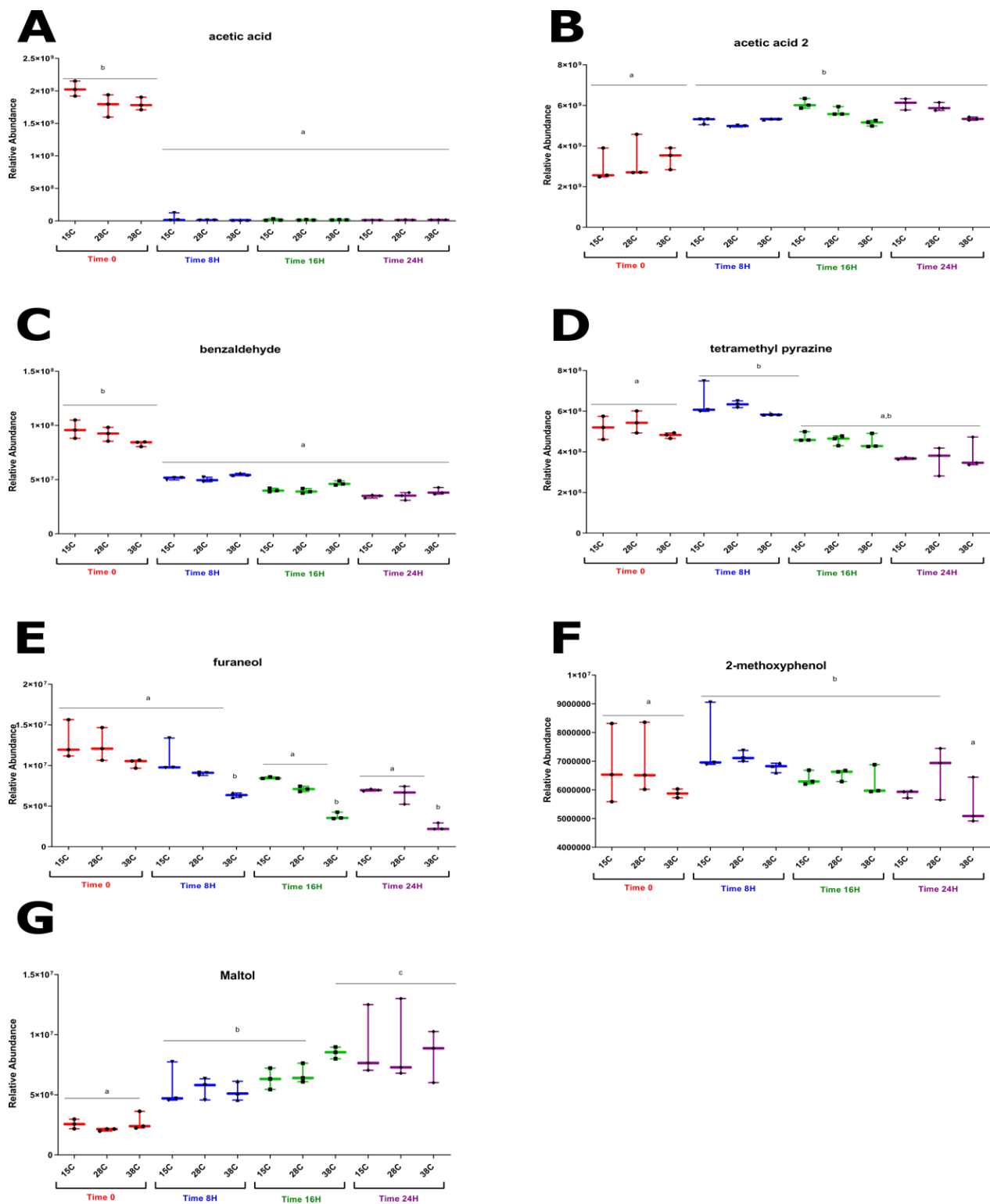


Figure 10: Box plots showing relative abundance over time of seven specific compounds of interest. (A) acetic acid isomer 1, (B) acetic acid isomer 2, (C) benzaldehyde, (D) tetramethyl pyrazine, (E) furaneol, (F) 2-methoxyphenol, (G) maltol

Trends by Chemical Class

If compounds are examined by chemical class, some trends appear. Relative abundance of the less volatile carboxylic acids was generally low at time zero but had climbed higher by 24 hours. It is possible that some aldehydes were oxidizing into their corresponding acids to cause this rise in relative abundance, but abundance of many aldehydes was also increasing so if this action occurred in the systems it must have been limited (see below). Those acids with greater volatility tended to decrease over the time of the experiment, but interestingly they were also generally present in greater abundance at time zero. Both detected isomers of acetic acid were very high at time zero, and while one isomer had disappeared completely by the 8-hour sample, the other isomer remained in surprising abundance throughout the experiment.

Many of the aldehydes in the chocolate system showed a trend of low (in some cases nearly zero) relative abundance increasing at each successive time point up to 24 hours. What makes these especially noteworthy is that they appeared to be more temperature-dependent than other annotated compounds; at higher ambient temperatures, their relative abundance increased more. Indeed, their temperature-dependent p-values were all in the bottom quartile of the annotated compounds. This is true of nonanal, octenal, octanal, hexanal, and heptanal. However, relative abundance of some other aldehydes decreased.

Terpenes followed less of a pattern than other chemical classes. Further testing would be needed to predictably model the behavior of terpenes in these systems. None of the terpenes appeared to be temperature-dependent, despite their volatility.

Pyrazines displayed the strongest pattern of any annotated chemical class. As a rule, these compounds started with moderate to high abundance at 0 hours and dropped off to extremely low levels by 24 hours. The only exception is 2,3,5-trimethyl 6-ethyl pyrazine, which demonstrated a

relatively unchanged abundance over time measurements, with slightly higher abundance at 8 hours at all temperature points.

Ketones displayed both increases and decreases in relative abundance. As was the case with other isomer groups, (i.e. acetic acid, linalool) the three isomers of ethanone seemed to behave independently of one another. The (3,5-dimethylpyrazinyl) ethanone started low and climbed to moderate, while 1-(2-furanyl) ethenone began very high but dropped to moderate by 8 hours and remained there. The third isomer of ethenone, 4-(5-methyl-2-furyl)-2-butanone, was high at 0 hours and climbed even higher by 24 hours, especially in higher-temperature systems.

Alcohols were also a divided class. Levels of 2-furanmethanol, 2-heptanol, and 1-pentanol all decreased in relative abundance, while all other alcohols increased in abundance or remained stable. This may warrant further investigation, as previous work has shown alcohols to decrease dramatically in abundance during conching (Albak & Tekin, 2016). It may be that one measurable difference between conching and melanging is the increased retention of alcohols during melanging due to lower temperatures achieved during the process.

Esters as a class did not all behave identically, but the behavior trends were determined by the acetate or non-acetate character of the ester. Perhaps unsurprisingly, all the acetate esters decreased in relative abundance while the less volatile esters show the opposite trend.

Possible Mechanisms

Previous work on conching has indicated that if melanging affects flavor in a way similar to conching, one would expect pyrazines, pyrroles, Strecker aldehydes, and acids to decrease (Albak & Tekin, 2016; Liu et al., 2015) while acid, alcohol, and hydrocarbon content is variable and may depend on the volatility of the specific compounds involved (Albak & Tekin, 2016; Owusu, Petersen, & Heimdal, 2012). It would also be reasonable to expect a decrease in overall

number and concentrations of aroma compounds (Albak & Tekin, 2016; Fischer et al., 2008; Owusu et al., 2012). Indeed, the melanging data showed similar outcomes. Over progressive time points, (independently of temperature) nitrogen heterocycles became less relevant (those that remain at the 24-hour time point are the less volatile polysubstituted rings) and Strecker aldehydes devolved into their decomposition products (although other aldehydes increase). Volatile terpenes and alcohols disappear or have smaller contribution scores in the model (Figure 10).

Many of the compounds annotated in this study fall into the category of Maillard precursors or products. As such, it is worth examining the role of Maillard reactions during melanging. Although the relative abundance of some notable Maillard products, like nitrogen heterocycles, decreased in all three temperature systems, it is theoretically possible that there were peaks in relative abundance between measured time points, or that Maillard products formed and degraded between measurements. However, it is unlikely that Maillard browning would occur in the melanging step. Early work by Rohan and later by Mohr showed that reducing sugars were the limiting factor in Maillard reaction progress in a cacao system; at the end of a typical cacao roast, reducing sugars had been entirely consumed while many free amino acids remained (Danehy, 1986). Although sugar is added to the melanging stage, it is typically in the form of sucrose, which is not a reducing sugar and would not contribute to Maillard reactions. Furthermore, even if sugar were added to the melanger in the form of glucose or fructose (or lactose, if milk were used), Maillard reactions would progress only sparingly. At pH values greater than 3, heat would be required to form Maillard products within the time frames described here (Afoakwa et al., 2008) Hoskin and Dimick (1980) previously confirmed that conching also does not reach the temperatures required for Maillard reactions. This was an indication that all the Maillard compounds in evidence here were likely formed during the roasting stage and had already achieved

their highest relative abundance at the beginning of the experiment. However, caramelization reactions can occur during chocolate refining, resulting in flavor compounds with similar properties to those of Maillard products (Afoakwa et al., 2008).

Two classes of compounds that appeared to evolve during the melanging time were aldehydes and fusel alcohols. It is not surprising to see evidence of an increase in aldehyde formation, as aldehydes can be formed through the oxidation of primary and secondary alcohols, and melanging introduces great amounts of oxygen into the chocolate system. The few aldehydes that seem to be disappearing could conceivably be condensing into Schiff bases, as this reaction happens easily at a pH similar to that of chocolate mass (Raj, Kumar, & Kumar, 2014) and the dehydration component of reactions like this would contribute to some of the viscosity changes that happen during chocolate refining. The behavior of the alcohols is more difficult to explain. They are unlikely to arise from ester, aldehyde, or ketone reduction because there is no reducing agent of sufficient strength present in the system. It is probable that those alcohols which increased in relative abundance did not actually grow in amount, but rather were stable enough to be non-volatile and so their abundance increased only relative to compounds that were volatilized.

CHAPTER VI: CONCLUSIONS AND RECOMMENDATIONS

Several conclusions can be drawn from these data. It was determined first, that melanging is a viable method to carry out successful chocolate refining; data show that both tools result in very similar flavor outcomes when used to refine chocolate. A melanger not only sufficiently reduces the particle size of the chocolate mass but also meets similar requirements specified by previous authors for successful conching from a flavor perspective. Second, the final flavor of chocolate made in a melanger is far more dependent on time spent in the melanger than on the temperature of the chocolate system. In addition, literature previously published on conching may be successfully extrapolated to melanging. Finally, classes of chemical compounds did not behave according to unified patterns of behavior in melanging systems (with the exception of nitrogen heterocycles, which decreased in relative abundance universally). Rather, within each class of compounds, behavior tended to show different trends for the more volatile compounds (often decreasing in relative abundance) than for the less volatile and poly-substituted compounds (often remaining stable or increasing in relative abundance). Subsequent work on this topic should pair targeted metabolomics analysis with sensory studies, perhaps in an effort to investigate the role of some of the less predictable classes of compounds discussed here.

Sensory work could also be performed to confirm the predictions of flavor models based on the melanging profiles presented in this work. From the data presented here, one might predict a flavor progression in the samples from time point to time point. Chocolate pulled from the melanger at 8 hours would be dominated by floral and fruity notes. Allowing melanging time to continue up to 16 hours would bring out a more roasted, toasty, and nutty flavor profile with rancid

or sweaty undertones. A melanging time of 24 hours would offer a well-rounded profile with compounds from a number of different chemical and flavor classes represented.

However, further testing is needed to confirm this prediction. Sensory tests may illuminate interactions between compounds that cannot be predicted by metabolomics data alone. Compounds may act to reinforce one another or to cancel out one another's effect. For example, it has been established that 3-methyl butanal and dimethyl disulfide (both of which appear in contribution plots for 8 and 16 hours) interact to form a robustly chocolatey aroma essential for dark chocolate, although neither compound is able to produce this aroma on its own (Counet et al., 2002). Also, some of the major contributing compounds may be below threshold levels of detectability, despite having large contribution scores. For example, dibutyl phthalate, despite having the highest contribution score at 24 hours, is described as having a faint and barely detectable taste and aroma by sensory panels (Garg et al., 2018). It is also possible that some compounds may play an outsized role in sensory perception because they are present far above their terminal threshold. For instance, the only two pyrazines that appear on the 24-hour contribution plot are tetramethyl pyrazine (perception threshold 10ppm in water) (Counet et al., 2002) and 2,6-dimethyl pyrazine (perception threshold as low as 1.6 ppm in water) (Pevsner, Trifiletti, Strittmatter, & Snyder, 2006). Without more information, it is impossible to know how these compounds might be perceived in a fat suspension and in interaction with each other or other compounds. Pyrazines in general have very low flavor thresholds (Pevsner et al., 2006); it is possible that a pyrazine at even extremely low abundance may act to support other flavors even if it is not directly perceived itself. Finally, a compound that is described as having a certain flavor or aroma in isolation does not always contribute that same flavor in a system. For example, some of the same sweaty or rancid-smelling carboxylic acids that were described in the 16- and 24-hour

contribution plots have been identified in roasted coffee (Evangelista et al., 2014) and honey (Manyi-Loh, Ndip, & Clarke, 2011). However, neither of these products are traditionally associated with sweaty or rancid flavors.

An additional recommendation that follows from this study is to determine the scalability of the conclusions drawn from these data. While these samples were taken from chocolate made on a laboratory-scale test melanger, the larger volumes of chocolate made in full-scale melangers would require correspondingly longer time points to achieve the desired results. Further testing is necessary to determine if the results shown here have a linear relationship with the volume of chocolate mass in the melanger drum, or if any mathematical relationship can be obtained.

In short, these data have illuminated the relationship between conching and melanging and shown that work previously carried out on conching can be used to inform melanging profiles. This test has been the initial investigation into melanging systems. Further research is needed to elucidate the specific role of individual compounds and to manipulate variables that were not tested here.

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APPENDIX

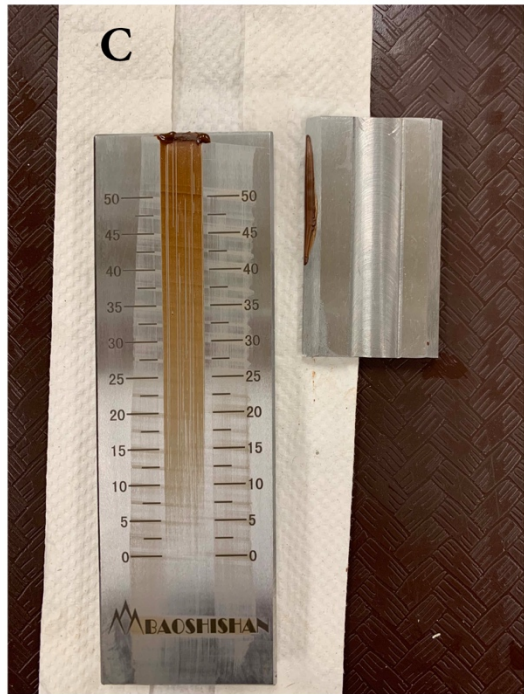


Figure 11: Grindometer readings showing an example of each result collected. (A) Tools before measuring; (B) 20-25 microns, none below 15; (C) 15-20 microns, none below 10; (D) 10-15 microns, none below 5

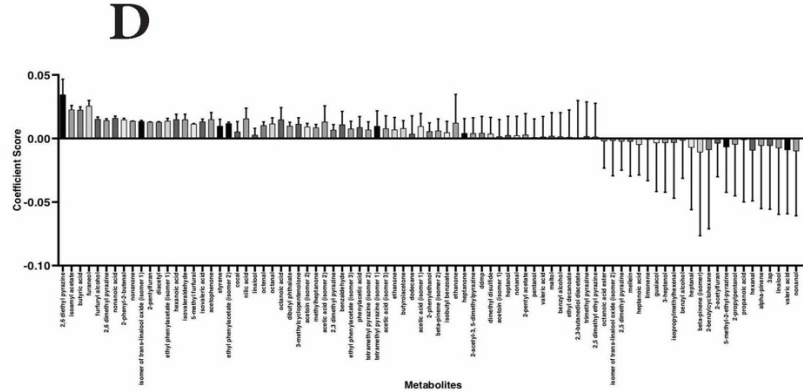
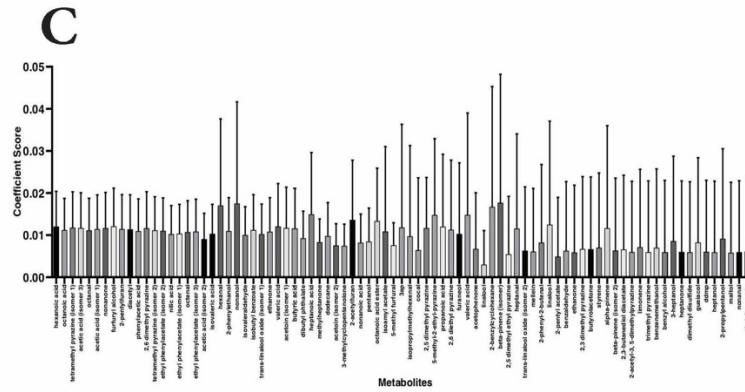
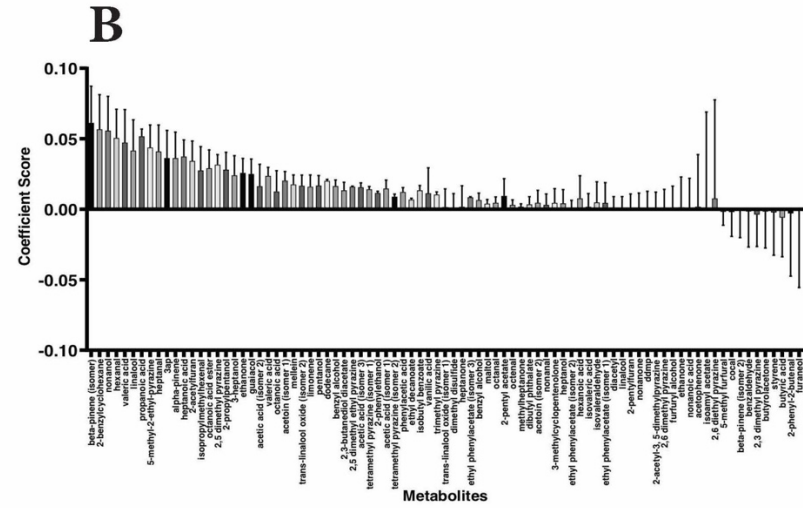
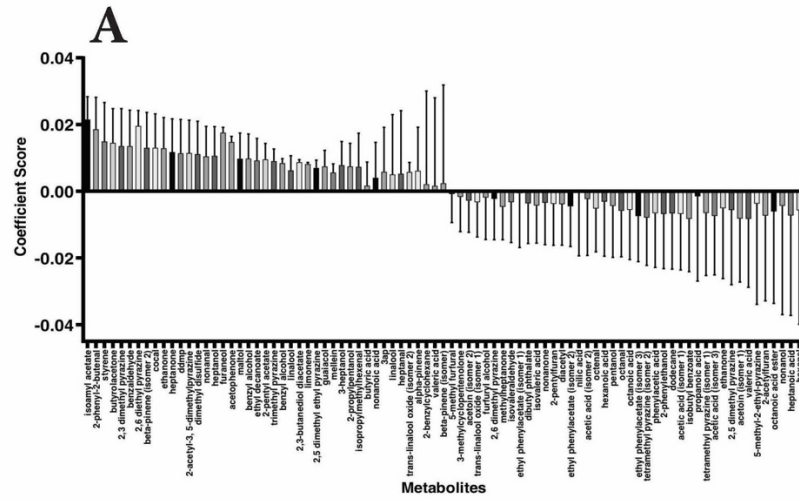


Figure 12: Coefficient plots at each time point expressed as the correlation of each metabolite to time. (A) 0 Hours, (B) 8 Hours, (C) 16 Hours, (D) 24 Hours

Table 3: Annotated compounds and their associated p-values and aromas

	Compound	Common Name	Temp p-value	Time p-value	Temp:Time p-value	Chemical Class	Aroma//Flavor	Database
1	(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	Linalool (Isomer 1)				Terpene	Floral, fresh, lavender	Flavor DB
2	Alpha-Pinene		0.002574	5.41E-17	0.399917	Terpene	Harsh, turpentine, minty, fresh, earthy, sweet, woody, terpene, pine, camphor	Flavor DB
3	Beta-Pinene (Isomer 1)		0.065505	5.89E-21	0.049975	Terpene	Resinous, resin, turpentine, green, hay, woody, pine, dry	FlavorDB
4	1-Butanol methyl ester		0.081432	2.35E-25	0.005418	Ester	Oil, alcoholic, burnt, whiskey, malt, banana, fusel, fruity	FlavorDB
5	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-	Mellein	0.000208	3.76E-09	0.026412	Ketone	None	The Good Scents Company
6	1-Pentanol		6.51E-05	7.23E-27	2.18E-06	Alcohol (1°)	Oil, balsamic, vanilla, fusel, sweet, balsam	FlavorDB
7	2,3,5-Trimethyl-6-ethylpyrazine		0.049962	0.001361	0.912315	MRP*	Roasted, baked potato, cooked	The Good Scents Company
8	2,3-Butanediol	Diacetyl	6.57E-15	3.47E-12	2.06E-09	Alcohol (2°)	Onion, fruit, creamy, buttery	FlavorDB
9	2,3-Butanediol, diacetate		0.2467	1.79E-13	0.002701	Ester	None	The Good Scents Company
10	2-Benzylcyclohexanone		2.17E-05	4.68E-10	0.003996	Ketone	Irritant	The Good Scents Company
11	2-Butanone, 1-(2-furanyl)-3-methyl-		1.27E-10	3.74E-13	2.67E-07	Ketone	Bitter, roasted	The Good Scents Company

12	2-Butenoic acid, 3-methyl-	Crotonic acid	2.62E-12	5.20E-14	0.002431	Carboxylic acid	Sour, sweat, acid, stinky, sweaty, animal, rancid, tropical, feet, cheese	FlavorDB
13	2-Furanmethanol		1.29E-05	7.32E-18	1.76E-05	Alcohol (1°)	Caramel, warm, cauliflower, vegetable, oily	FlavorDB
14	2-Heptanol		0.017628	2.65E-13	0.077559	Alcohol (1°)	Leafy, coconut, herbal, peony, strawberry, musty, sweet, woody, violet, green	FlavorDB
15	2-Heptanone		8.63E-05	5.75E-27	0.002492	Ketone	Coconut, Soap, Herbal, Sweet, Woody, Fruity, Spicy, Cinnamon	FlavorDB
16	2-Isopropyl-5-methylhex-2-enal	Isodihydroavandulyl aldehyde	0.000831	6.85E-07	0.477472	Aldehyde	Mint, fresh, peppermint, sweet, cool, minty, green	FlavorDB
17	2-Nonanol		0.44731	0.001551	0.673508	Alcohol (1°)	Fatty, dusty, rose, fat, floral, green, clean, wet, orange, fresh, bitter, oily	FlavorDB
18	2-Nonanone		0.317971	6.41E-09	0.026398	Ketone	Soap, herbal, hot milk, earthy, soapy, weedy	FlavorDB
19	2-Octenal, (E)-		1.44E-14	1.34E-16	6.74E-09	Aldehyde	Nut, fat, herbal, fresh, green, fatty, banana, waxy, leafy, cucumber	FlavorDB
20	2-Pentanol acetate		0.00112	4.16E-24	3.82E-05	Ester	Overripe fruit, banana, sweet, peanut, juicy	FlavorDB
21	2-Propyl-1-pentanol		1.85E-15	6.09E-18	4.21E-10	Alcohol (1°)	Found in feces	HMDB
22	3,4-Dihydro-6-methyl-2H-pyran-2-one	Ethanone (Isomer 1)	0.003632	1.26E-06	0.232933	Ketone	Nutty, roasted, cooked	FlavorDB
23	3-Acetyl-1H-pyrroline	3AP	0.031485	0.003478	0.347598	MRP*	Dry, sweet, woody, hawthorn,	Flavor DB

							nutty, popcorn, phenolic	
24	3-Hexene, 2,2-dimethyl-		1.07E-09	1.48E-26	0.00495	Alcohol (2°)	Herbal, powerful, bitter, herb	FlavorDB
25	3-Methylcyclopentane-1,2-dione	Methylcyclopentenolone	7.24E-06	6.48E-06	0.790165	Ketone	Heated sugar, caramel, roasted, cooked, maple syrup	The Good Scents Company
26	4-Heptanone, 3-methyl-		0.00032	0.000321	0.313901	Ketone	Apple, banana, cheesy	The Good Scents Company
27	4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl-	DDMP	0.494169	0.353575	0.353095	Ketone, Alcohol	Sweet, bready, cookies, dried fruit	HMDB
28	5-Methyl-2-phenyl-2-hexenal	Cocal	1.27E-05	9.59E-10	0.003379	Aldehyde	Chocolate, bitter, butyric, cocoa, sweet, fruity, aldehydic, nut skin, green	FlavorDB
29	6,6-Dimethyl-2- vinylidenebicyclo[3.1.1]heptane	Beta-pinene (Isomer 2)	0.000358	0.000104	0.7675	Terpene	Resinous, fresh, green, hay, woody, pine, dry	Flavor DB
30	Acetic acid (Isomer 1)		0.043252	8.87E-26	0.043502	Carboxylic acid	Sour, pungent, sharp, vinegar	FlavorDB
31	Acetic acid, 2-phenylethyl ester	Phenylethyl acetate (Isomer 1)	0.023126	3.15E-07	0.092628	Ester	Bitter, rhubarb, gardenia, musty, floral, green, sweet, herbal, leafy, fruity	FlavorDB
32	Acetic acid (Isomer 2)		0.364977	1.32E-10	0.167655	Carboxylic acid	Sour, pungent, sharp, vinegar	FlavorDB
33	Acetoin (Isomer 1)		0.156661	2.15E-21	4.48E-06	Ketone, Alcohol (2°)	Butter, cream, milky, fatty, creamy, sweet, dairy	FlavorDB
34	Acetoin (Isomer 2)		4.87E-13	3.70E-20	6.84E-07	Ketone, Alcohol (2°)	Butter, cream, milky, fatty, creamy, sweet, dairy	FlavorDB
35	Acetophenone		2.96E-17	1.06E-16	5.89E-11	Ketone	Mimosa, hawthorn, sweet,	FlavorDB

							acacia, almond, pungent, flower, bitter, must	
36	Benzaldehyde		0.49303	3.67E-19	0.003219	Aldehyde	Cherry, almond, sweet, burnt sugar, sharp, strong, bitter	FlavorDB
37	Benzeneacetaldehyde		0.002102	3.30E-10	0.000182	Aldehyde	Cortex, radish, naricissus, sweet, beany, honey, cocoa, nutty	FlavorDB
38	Benzeneacetaldehyde, .alpha.-(2-methylpropylidene)-		0.000448	6.04E-08	0.015939	Aldehyde	Cortex, radish, naricissus, sweet, beany, honey, cocoa, nutty	FlavorDB
39	Benzeneacetaldehyde, .alpha.-ethylidene-		0.025975	1.29E-06	0.069616	Aldehyde	Cortex, radish, naricissus, sweet, beany, honey, cocoa, nutty	FlavorDB
40	Benzeneacetic acid		0.303721	8.55E-07	0.168876	Carboxylic acid	Honeysuckle, sour, waxy, floral, sweet, honey, civet, flower	FlavorDB
41	Benzeneacetic acid, ethyl ester	Ethyl phenylacetate	0.064238	2.20E-06	0.095382	Ester	Grapefruit, balsam, rose, anise, chocolate, honey, melon, sweet, floral, raspberry	FlavorDB
42	Benzenemethanol, .alpha.-methyl-, (R)-	Phenylethanol	0.188041	0.000569	0.258125	Alcohol (1°)	Gardenia, fresh, sweet, hyacinth	FlavorDB
43	Benzoic acid, 1-methylpropyl ester	Isobutyl benzoate	0.001358	2.91E-08	0.027771	Ester	Powdery, musty, sweet, fruity, balsam	FlavorDB
44	Benzyl alcohol		9.17E-16	1.44E-12	2.80E-12	Alcohol (1°)	Berry, balsamic, rose, floral, walnut, sweet, cherry, phenolic, flower, grapefruit	FlavorDB
45	Butanal, 3-methyl-		0.001036	3.14E-07	1.48E-08	Aldehyde	Peach, sour, chocolate, ethereal,	FlavorDB

							malt, fatty, aldehydic	
46	Butanoic acid		9.18E-06	0.000136	0.003384	Carboxylic acid	Butter, sweat, acetic, sharp, rancid, fruit, cheese	FlavorDB
47	Butanoic acid, 3-methyl-		2.86E-05	3.92E-14	1.73E-05	Carboxylic acid	Sour, sweat, acid, stinky, sweaty, animal, rancid, tropical, feet, cheese	FlavorDB
48	Butyrolactone		1.87E-09	7.19E-19	0.113993	Lactone	Caramel, oily, fatty, sweet, creamy	FlavorDB
49	Dibutyl phthalate		0.191277	0.183063	0.635292	Ester	Faint	FlavorDB
50	Disulfide, dimethyl		1.14E-06	5.26E-22	1.23E-07	Sulfur Compound	Fruity, herbal, vegetal, sulfurous, fatty, floral, nutty, meaty, spicy	FlavorDB
51	Dodecane		6.99E-06	7.44E-07	0.000321	Hydrocarbon	Alkane	FlavorDB
52	Dodecanoic acid, ethyl ester		1.75E-07	1.32E-12	0.00053	Ester	Apple, brandy, waxy, grape, oily, sweet, fruity, pear	FlavorDB
53	Ethanone, 1-(2-furanyl)-	Ethanone (Isomer 2)	0.000228	4.74E-27	0.000135	Ketone	Caramel, tobacco, balsamic, potato, coffee, cocoa, peanut, balsam, almond, beef	The Good Scents Company
54	Ethanone, 1-(3,5-dimethylpyrazinyl)-	Ethanone (Isomer 3)	0.008459	4.77E-06	0.248176	Ketone	Nutty, hazelnut, caramel, roasted, coffee, woodsmoke	FlavorDB
55	Furan, 2-acetyl		0.000228	4.74E-27	0.000135	MRP*	Caramel, tobacco, balsamic, potato, coffee, cocoa, peanut, balsam, almond, beef	FlavorDB
56	Furan, 2-pentyl-		1.40E-18	1.80E-16	2.41E-12	MRP*	Butter, green bean, vegetable, earthy,	FlavorDB

							beany, fruity, metallic, green	
57	Furaneol	Strawberry furone	9.90E-08	3.92E-10	0.635292	MRP*	Sweet, burnt, brown, caramellic, cotton candy	The Good Scents Company
58	Furfural		2.92E-09	1.80E-22	3.79E-05	MRP*	Sweet, brown, woody, bready, caramellic, with a slight phenolic nuance	The Good Scents Company
59	Furfural, 5-methyl		1.29E-10	3.74E-13	2.67E-07	MRP*	Caramellic, brown	The Good Scents Company
60	Heptanal		2.55E-26	4.11E-22	5.74E-20	Aldehyde	Citrus, ozone, fat, herbal, fresh, wine-lee, rancid, fatty, aldehydic, green	FlavorDB
61	Heptanoic acid		3.28E-08	2.52E-11	0.000378	Carboxylic acid	Sour, cheesy, sweat, rancid	FlavorDB
62	Hexanal		5.77E-19	7.82E-20	8.03E-12	Aldehyde	Leafy, grass, sweaty, tallow, fat, fresh, fruity, aldehydic, green	FlavorDB
63	Hexanoic acid		5.16E-06	4.65E-13	0.002848	Carboxylic acid	Fatty, sour, sweat, cheese	FlavorDB
64	Limonene		4.19E-06	2.08E-12	3.08E-08	Terpene	Mint, lemon, citrus, orange, fresh, sweet	FlavorDB
65	Linalool (Isomer 2)		0.122057	5.75E-10	0.248422	Terpene	Citrus, lemon, orange, floral, sweet, woody, blueberry, bois de rose, lavender, green	FlavorDB
66	Maltol		1.87E-09	3.28E-26	0.00219587	MRP*	Caramel, jam, bread, cotton, sweet, fruity, baked, candy	FlavorDB

67	Nonanal		1.26E-13	1.78E-15	5.03E-09	Aldehyde	Citrus, lime, orange peel, rose, fat, green, fishy, waxy, fresh, orris, grapefruit	FlavorDB
68	Nonanoic acid		0.311109	0.002302	0.766573	Carboxylic acid	Cultured dairy, fat, waxy, green, dirty, cheese	FlavorDB
69	Octanal		3.15E-09	2.25E-06	2.67E-05	Aldehyde	Lemon, citrus, soap, orange peel, fat, waxy, fatty, aldehydic, green	FlavorDB
70	Octanoic acid		0.355054	0.000115	0.830762	Carboxylic Acid	Cheesy, sweat, vegetable, waxy, fatty, rancid, oily	FlavorDB
71	Octanoic acid, ethyl ester		0.084083	2.30E-06	0.224517	Ester	Waxy, green, sweet, orange, aldehydic, vegetal, herbal	Good Scents Company
72	Pentanoic acid		4.63E-14	2.98E-16	9.75E-08	Carboxylic acid	Sweat, putrid, sweaty, acidic, animal, rancid, sickening	FlavorDB
73	Pentanoic acid, 4-methyl-		6.68E-07	1.18E-07	0.000373	Carboxylic acid	Cheesy, sweaty, acidic, sharp, animal, fruity, green	FlavorDB
74	Phenol, 2-methoxy-		0.09479	0.039384	0.798371	Phenol	Spice, vanilla, smoky, sweet, woody, phenolic, medicinal, smoke	FlavorDB
75	Phenylethyl Acetate (Isomer 2)		0.064238	2.20E-06	0.095382	Ester	Bitter, rhubarb, gardenia, musty, floral, green, sweet, herbal, leafy, fruity	FlavorDB
76	Phenylethyl Alcohol		0.139872	3.21E-05	0.368746	Alcohol (1°)	Lilac, rose, rosewater, honey, floral, spice, bitter, dried rose	FlavorDB

77	Propanoic acid, 2-methyl-		0.006571	1.91E-18	0.006265	Carboxylic acid	Rancid, butter, strawberry, cheese	FlavorDB
78	Pyrazine, 2,3-dimethyl-		0.229479	6.32E-25	0.000242	MRP*	Butter, caramel, nut, peanut butter, meat, leather, coffee, cocoa, almond, walnut	FlavorDB
79	Pyrazine, 2,5-dimethyl-		0.814056	7.31E-25	0.000735	MRP*	Roast beef, roasted nuts, grass, medical, cocoa, woody, medicinal	FlavorDB
80	Pyrazine, 2,6-diethyl-		0.088575	3.55E-09	0.18934	MRP*	Nutty, vegetable, meaty, baked	FlavorDB
81	Pyrazine, 2,6-dimethyl-		0.718412	3.69E-25	0.000555	MRP*	Roast beef, roasted nuts, coffee, cocoa, roasted nut	FlavorDB
82	Pyrazine, 2-ethyl-5-methyl-		0.774625	2.95E-18	3.84E-05	MRP*	Bean, fruit, sweet, coffee	FlavorDB
83	Pyrazine, 3-ethyl-2,5-dimethyl-		0.330785	5.32E-10	0.121184	MRP*	Roast, roasted, potato, cocoa, nutty	FlavorDB
84	Pyrazine, methyl-		8.48E-09	1.58E-30	5.49E-06	MRP*	Chocolate, roasted, cocoa, peanut, nutty, popcorn, green	FlavorDB
85	Pyrazine, tetramethyl-		0.359586	3.62E-09	0.661306	MRP*	Chocolate, burnt, lard, musty, coffee, cocoa, soybean, nutty	FlavorDB
86	Pyrazine, trimethyl-		0.976051	2.65E-17	0.000355	MRP*	Hazelnut, roasted, powdery, potato, musty, earthy, cocoa, peanut, nutty	FlavorDB
87	Styrene		8.76E-21	8.92E-14	9.83E-16	MRP*	Balsamic, gasoline, floral, sweet, plastic	FlavorDB
88	Trans-Linalool oxide (furanoid)		0.550188	9.23E-08	0.114751	Terpene	Floral, flowers	FlavorDB

*MRP = Maillard Reaction Product