ADHESION OF FOOD POWDERS DURING COATING AND THE EFFECTS OF ALKALIZATION AND ROASTING CONDITIONS ON COCOA VOLATILE COMPOUNDS

DISSERTATION

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ABSTRACT

The effects of powder resistivity, coating voltage, relative humidity and coating density on adhesion were investigated in both nonelectrostatic and electrostatic coating. Cocoa powder with a high resistivity (1.15 \times 10^{13} \text{\textOmega m}) showed a stronger electrostatic adhesion than starch powder with a medium resistivity (2.56 \times 10^{10} \text{\textOmega m}) and NaCl powder with a low resistivity (7.31 \times 10^5 \text{\textOmega m}). The adhesion of starch and cocoa powders coated at 0, 40 and 95 kV increased with increasing voltage. The adhesion at 0 kV was in the range of the theoretically calculated van der Waals force, and the measured adhesion forces at 40 kV and 95 kV were in the range of the theoretically calculated electrostatic image force at 40 kV and 95 kV respectively. For nonelectrostatic coating (0 kV), there was no significant change in adhesion when relative humidity (RH) increased from 30% to 60%, while adhesion increased when RH increased from 60% to 80%. For electrostatic coating, the adhesion decreased when RH increased from 30% to 60%, but the adhesion at 80% RH, close to the theoretically calculated capillary force value, was larger than the adhesion at 30% and 60% RH. For both nonelectrostatic and electrostatic coating, the adhesion force decreased as coating density increased to 1.0 mg/cm², but there was no significant change from 1.0 mg/cm² to 2.0 mg/cm².
Cocoa beans were alkalized before or after roasting and made into cocoa liquor before analyzing by selected ion flow tube-mass spectrometry (SIFT-MS). In both alkalized-before-roasting and alkalized-after-roasting samples, there were significantly higher concentrations of alkylpyrazines for the samples with pH above 7.0 than pH below 7.0. At pH 8, the concentrations of 2,3-, 2,5- and 2,6-dimethylpyrazine (DMP), 2,3,5-trimethylpyrazine (TrMP), 2,3,5,6-tetramethylpyrazine (TMP) and 2,3-diethyl-5-methylpyrazine (EMP) in the samples alkalized-before-roasting were higher than those in the samples alkalized-after-roasting. Volatiles increased under conditions that promoted the Maillard reaction. The partition coefficient was not significantly affected by pH from 5.2 to 8.0. The ratios of TrMP/DMP and DMP/TMP increased while the ratio of TMP/TrMP decreased as the pH increased. The concentrations of Strecker aldehydes and other volatiles followed a similar pattern as that of the alkylpyrazines. High pH favors the production of alkylpyrazines and Strecker aldehydes.

Alkalized and unalkalized Don Homero cocoa beans were roasted at 120, 150 and 170 °C in a rotary roaster. The real-time and end-of-roasting concentrations of cocoa volatiles in the headspace of the roaster were analyzed by SIFT-MS. The concentrations of total alcohol, acids, aldehydes, esters, ketones and alkylpyrazines reached peak concentrations within the first 15 min roasting. The concentrations of alkylpyrazines and Strecker aldehydes increased as the roasting temperature increased from 120 to 170 °C. For most of the volatile compounds compared, there was no significant difference between Arriba and Don Homero beans, but Arriba beans showed higher concentrations of 2-heptanone, acetone, ethyl acetate, methylbutanal, phenylacetaldehyde and
trimethylpyrazine than Don Homero beans. For unalkalized Don Homero beans (pH 5.7),
the time to peak concentration decreased from 13.5 to 7.4 min for pyrazines, and from
12.7 to 7.4 min for aldehydes as the roasting temperature increased from 120 to 170 °C.
Also, at 150 °C roasting, the time to peak concentration was shortened from 9.0 to 5.1
min for pyrazines, and from 9.1 to 5.0 min for aldehydes as the pH increased from 5.7 to
8.7.
This document is dedicated to my family.
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Chapter 1 : LITERATURE REVIEW

1.1 Powder adhesion

1.1.1 Introduction

Adhesion is the physical phenomena by which two materials stick together. Adhesion is important in numerous industries, and it can have either positive or negative effects. For example, the adhesion force between toner particles and carrier, photoreceptor and paper strongly influence the image quality in electrophotography. Adhesion is essential to ensure that the powder deposited on a workpiece will be kept on its surface throughout the process during powder painting (Takeuchi 2006). In dry powder inhalers (DPIs), the adhesion resulting from the interactions between the active substance and the excipient facilitates the delivery of the drugs (Bérard and others 2002). However, in the semiconductor industry, fine particles adhere to electronic components and cause contamination. In the food industry, adhesion of food to packaging surfaces may result in product loss, poor product appearance, and increase package recycling costs (Michalski and others 1997). On the other hand, in the food powder coating process, improvement in adhesion improves coating efficiency, minimizes powder waste and reduces dust.

Snack foods are an important sector of the food industry, which is a huge market with annual sales of more than $25 billion (Enggalhardjo and Narsimahan 2005). Powders such as seasonings or flavorings are often coated onto snack foods to enhance
flavor and taste after they are fried or baked. However, insufficient adhesion between seasonings and the surface of snacks results in the seasoning falling off the snacks, which makes a poor distribution of flavor on the product. Thus it is common for snack manufacturer to put 30% and, in some cases, as much as 50% more seasonings than actually needed into the coating process to ensure sufficient coating (Anon 1992). This powder waste increases the cost because the seasonings are normally expensive (Anon 1992). Moreover, more powder means more dust. There is a risk of explosion to operate under the air with high dust concentration, and dust damages the health of operators. Therefore, improvement of adhesion plays an important role in snack foods coating.

The most important forces involved in the adhesion between powders and food surfaces are capillary forces, van der Waals forces and electrostatic forces. Many factors, including operating conditions, properties of particles and properties of the food surface affect adhesion by influencing these forces. Relative humidity, particle size, particle charge, surface oil content and surface roughness are the main factors which need to be considered when studying food powder adhesion.

The purpose of this review is to introduce the forces and their mechanism of adhesion during food powder coating, to summarize which factors affect adhesion of food powders and why they are important.

1.1.2 Forces involved in adhesion

Bowling (1988) reviewed the forces of adhesion for small particles on surfaces and classified the interactions into three groups: long-range attractive interactions, interfacial reactions and very short range interactions. The long-range attractive interactions include
van der Waals forces and electrostatic forces, which attract the particles to the surface of
the substrate and establish the adhesion contact area. The interfacial reactions include
diffusion, condensation and mutual dissolution. This category also includes capillary
force caused by the establishment of liquid bridges. The very short range interactions
involve the forming of chemical bonds due to chemical reactions and intermediate bonds
such as hydrogen bonds (Bowling 1988). In food materials, protein in milk, for instance,
can be absorbed on porous glass surfaces when enough contact time is available. This
kind of interaction has special interest when considering food product storage (Michalski,
Desobry and Hardy 1997).

In the normal process of food powder coating, adhesion occurs between solid
particles and dry or moist solid surfaces. The long-range attractive interactions (for dry
surfaces) and capillary forces (for moist surfaces) are the main contributors to adhesion
as physical interactions are the primary forces, and very short range interactions are less
likely happened in such processes. Also it is difficult to quantify the very short range
interactions because they are very complex and specific to each case. These interactions
vary greatly depending upon particle and surface materials. However, the most important
forces, capillary forces, van der Waals forces and electrostatic forces can be
quantitatively measured (Bowling 1988).

1.1.2.1 Capillary forces

Condensation of water takes place in the gap between the particle and surface. The
resulting capillary forces can make a large contribution to the total adhesion. For particles
on smooth surfaces, the capillary forces are a function of the particle radius \( r \) and liquid surface tension \( \gamma \) (Bowling 1988).

\[
F(\text{cap.}) = 4\pi r \gamma \cos \theta
\]  

(1)

Based on Eq.1, theoretically, capillary forces are proportional to the particle size and the surface tension. Capillary forces have their greatest effect on a hydrophilic surface, where \( \theta \) is close to 0\(^\circ\), because this condition can ensure a total wetting of the surface. Capillary forces have their least effect on a hydrophobic surface where \( \theta \) is close to 90\(^\circ\), and the surface can barely be wetted (Zimon 1969).

In practice, all surfaces are rough and there is no absolutely smooth surface. Thus allowing for the effect of the roughness of the substrate, the capillary forces can be expressed as:

\[
F(\text{cap.}) = 4\pi r \gamma \left( \cos \theta_{\text{app}} / \alpha \right)
\]  

(2)

and \( \cos \theta_{\text{app}} = \alpha \cos \theta \)

Where, \( \theta_{\text{app}} \) is the apparent wetting angle, and \( \alpha \) is the roughness coefficient of the surface (Zimon 1969). Thus, capillary forces increase as the decrease of roughness coefficient.

Capillary forces can be explained by the theory of wetting and thermodynamic adsorption. A good wettability means that the liquid and solid have a strong affinity and are likely to adhere well (Michalski, Desobry and Hardy 1997). The theory of thermodynamical adsorption is based on the Young forces equation and the Dupré energy equation. Solid and liquid surface tensions (\( \gamma_S \) and \( \gamma_L \)), interfacial tension (\( \gamma_{SL} \)), and the liquid contact angle are related through the Young equation:

\[
\gamma_S = \gamma_{SL} + \gamma_L \cos \theta
\]  

(3)
While adhesive surface tensions and the work of adhesion are related to the Dupré energy equation:

\[ W = \gamma_s + \gamma_L + \gamma_{SL} \] (4)

Capillary forces are directly related to the relative humidity (RH), moisture and oil content. Capillary force dominates adhesion where sufficient moisture or oil is present and a liquid bridge is formed (Schubert 1987). Capillary condensation takes place over time, so that the effects of capillary forces are not felt immediately after the particle comes into contact with the solid surface (Zimon 1969). Therefore, during powder coating, in order to enhance the adhesion between seasonings and food products by adjusting RH, enough contact time should be allowed.

### 1.1.2.2 Van der Waals forces

Van der Waals forces are molecular forces, which include forces between molecules possessing dipoles and quadrapoles caused by the polarization of the atoms and molecules in the material. These also include nonpolar attractive forces known as London-van der Waals forces (Bowling 1988). Van der Waals forces are always present, and they are more important for smaller particles than for bigger particles (Bowling 1988). Van der Waals forces can be calculated with the equation:

\[ F_{(vdw)} = \frac{A r}{6 h^2} \] (5)

Where, \( A \) is the Hamaker constant, which depends on the surface properties such as roughness of the material and has a value of the order of \( 10^{-19} \)Nm (Rennie and others 1998), and \( h \) is the distance between the particle and the substrate. Van der Waals forces
are maximum for particles in contact with each other (Schubert 1987). As the separation distance increases, the van der Waals forces decrease sharply.

1.1.2.3 Electrostatic forces

Electrostatic forces include two types of forces, the electrostatic image force and electrical double layer forces. The first is due to bulk excess charges by corona charging or tribo charging on the particles. These net charges induce equal and opposite charges on the surface, which produce coulombic attraction and can be expressed as:

\[ F(\text{img}) = \frac{q^2}{(4\pi \varepsilon_0 \varepsilon_r r h)} \]  

(6)

For conductive powders, the excess charges are balanced by contact charge flow thus electrostatic image force is small; for insulators, such as polymers or powders with high resistivity, electrostatic attraction is significant (Bowling 1988).

The electrical double layer forces are induced by electrostatic contact potential. When two different materials are in contact, electrons are transferred from one solid to another until an equilibrium is reached where the current flow in both directions is equal. The final potential difference is called a contact potential difference \( U \) (Bowling 1988).

For a particle on a surface, this electrostatic double layer force is given as:

\[ F(\text{dl}) = 4dU^2 \]  

(7)

Where, \( F(\text{dl}) \) is the electrostatic double layer force, \( d \) is the particle diameter in \( \mu m \), and \( U \) is the contact potential difference in volts. The electrostatic double layer force is important for very small particles. When a very fine powder is used in food coating, the double layer force contributes greatly to the total adhesion.
1.1.2.4 Chemical adhesion

There is one more important force for food powders: chemical adhesion. Chemical adhesion is caused by chemical reactions. Chemical adhesion is highly dependent on the contact time between materials, as enough time must be available before a reaction occurs. This type of adhesion may be found during food storage (Michalski, Desobry and Hardy 1997).

1.1.2.5 Comparison of capillary forces, van der Waals forces, electrostatic forces and total adhesion forces

Capillary forces are present in a wet system, and the presence of liquid shields van der Waals and electrostatic forces, thus weakening these adhesion forces. In a dry system, however, capillary forces disappear, but electrostatic image forces act to bring particles to a surface and then van der Waals forces and electrical double layer forces will hold the particles on the surface (Bowling 1988). Van der Waals forces predominate for smaller particles (less than 50 µm), whereas electrostatic forces are important for larger or charged particles (Bowling 1988). For dry uncharged particles on a dry uncharged surface, only van der Waals forces and electrical double layer forces are significant forces (Bowling 1988). The dominant adhesion forces under different systems are summarized in Table 1.1. In an uncharged system, particle adhesion forces decrease in the order: capillary forces > van der Waals forces > electrostatic forces. However, for highly charged particles, electrostatic image forces can dominate in small particles (Schubert 1987).
Enggalhardjo and Narsimhan (2005) designed wind tunnel experiments to measure the capillary force, van der Waals force, electrostatic image force and total adhesion between seasonings and tortilla chips. The forces were tested at surface oil content 24%-32%, and particle size 32 to 180 µm. Based on the magnitude comparisons between these forces and total adhesion, only capillary forces significantly contribute to the total adhesion forces (Figure 1.1).

1.1.3 Measurement of adhesion

There are several methods used to measure adhesion, including bulk detachment methods and single particle detachment methods, and the test results can be in either absolute values, expressed in the force unit Newton (N), or relative values, expressed in percentage. However, no universal test method is available, thus the correct method depends on the case. Centrifuge and weighing methods are bulk detachment methods, while atomic force microscopy (AFM) method is a single particle detachment method.

1.1.3.1 Centrifuge method

The centrifuge method is a common adhesion measurement. It is a bulk detachment method and also one of the most reliable techniques, by which quantitative results can be obtained (Takeuchi 2006). The centrifuge method is based on the detachment of particles from a substrate surface due to centrifugal force. The centrifugal force to remove a certain percentage of the powders can be calculated as:

\[ F_c = m \omega^2 r \]  

(8)

Where, \( F_c \) is the centrifugal force, \( m \) is the mass of the particles attached, and \( \omega \) is the angular rotating velocity. In the case of pure adhesion measurement, where no shear
forces exist, the force necessary to detach a particle from the substrate surface almost equals to the adhesion force (Podczeck and others 1995). The average and median adhesion forces were measured to be $8.9 \times 10^{-8}$ N and $8.6 \times 10^{-8}$ N, respectively between a toner and polymer particles by applying an ultracentrifuge method (Takeuchi 2006).

1.1.3.2 Weighing method

The weighing method is also a bulk detachment method. Its main operation is to weigh the powders remaining on the contact surface after removal of the free powders by mechanical operations such as inversion, vibration, shaking and blowing. The test results give relative values and are normally expressed as a percentage, which can be calculated by dividing the weight loss by the initial weight, and then using 100% to deduct this ratio.

1.1.3.3 Atomic force microscopy

The atomic force microscopy (AFM) method is single particle detachment method (Louey and others 2001), by which direct measurement of adhesion is available. It is one of the most reliable and accurate methods due to its microscopy-aided measurement. AFM, combined with scanning electron microscopy (SEM) is frequently used to study the adhesion between the active substance and the excipient in pharmaceutics such as in the manufacturing of dry powder inhalers.

Bérard and others (2002) studied the adhesion between the zanamivir particles and lactose monohydrate surfaces. The direct measurement in controlled atmosphere by AFM of the forces and the interaction ranges between the probe and the substrate demonstrated that the average value of the adhesion force was $233.35 \times 10^{-9}$ N with a standard deviation
of $37.44 \times 10^{-9}$N. Although the application of AFM in pharmaceutics and other fields is increasing, its application in the study of food powder adhesion has not been reported.

**1.1.4 Factors affecting powder adhesion**

The adhesion between powders and a surface is influenced by many factors, especially operating conditions, properties of powder and properties of the surface. Relative humidity, particle size, particle charge, surface oil content and surface roughness are the main factors to consider. Different factors dominate adhesion under different circumstances.

**1.1.4.1 Relative humidity**

Relative humidity (RH) greatly influences adhesion by forming liquid bridges resulting in strong capillary forces, by modifying the surface topology and by increasing charge decay. Liquid bridges form from the condensation of atmospheric moisture at a RH above 65% (Williams 1990). Above 70% RH, capillary forces predominate over other forces in adhesion (Busnaina and Elsawy 2001). Hence, the effect of capillary forces must be considered for a relative air humidity of 65%-100% (Zimon 1969). As the RH increases, capillary forces increase greatly and become the strongest adhesion force.

An increase in RH of storage air from 5% to 55% had no influence on the adhesion force of particles to a compacted powder surface. However, a further increase in RH to 75% increased the adhesion force (Podczeck and others 1997).

The increase of environmental RH causes surface modification of the substrate which also changes the adhesion. Relative humidity greatly influences the amount of zanamivir fixed on a lactose monohydrate surface (Bérard and others 2002). As measured by AFM,
adhesion increases with relative humidity in the order: F_{0\%RH} < F_{32\%RH} < F_{85\%RH}. The increase of relative humidity progressively modifies the surface topology of the two components and increased the adhesion force.

The effect of RH on the resistivity of the particles is important. Water absorbs on the powder as the RH increases, decreasing of the resistivity and increasing charge decay to the environment, which reduces electrostatic image force, thus decreases adhesion (Grosvernor and Staniforth 1996). Cocoa powder’s resistivity decreases with increasing relative humidity, causing the charges to decay faster thus decreased the adhesion (Halim and Barringer 2007). Also, at high humidity, the electrostatic image force is greatly reduced because the liquid shields the charge (Bowling 1986).

1.1.4.2 Particle size

Adhesion varies with particle size in different ways. It may be directly proportional, or even inversely proportional, to the particle diameter, or alternatively, it may be independent of particle size. In the majority of investigations, the adhesion was inversely proportional to the size of the particles (Zimon 1969), which means smaller particles tend to have greater adhesion when coated on the surface.

The effect of particle size on adhesion can be opposite for electrostatic and nonelectrostatic coating. Nonelectrostatic adhesion between sucrose particles and crackers increased with increasing particle size (Mayr and Barringer 2006). Total adhesion force between seasoning particle and tortilla chip surface increased with seasoning particle size increase from 32 to 300µm (Enggalhardjo and Narsimhan 2005). For nonelectrostatic coating, adhesion increases with particle size because there are more
contact points between a larger particle and a substrate (Enggalhardjo and Narsimhan 2005).

Electrostatic adhesion between sucrose particles and crackers decreased with increasing particle size from 20 to 140µm (Mayr and Barringer 2006). In electrostatic coating, the smaller particles have a higher charge to mass ratio thus attain an improved adhesion to the substrate.

However, Halim and Barringer (2007) determined that adhesion for sugar coated on saltine crackers decreased with increasing particle size up to 200µm for both electrostatic and non-electrostatic coating. Above 200 µm, there was no significant electrostatic image force. The fact that electrostatic image force is significant for smaller particles but not larger particles is partially due to the fact that Coulomb force is proportional to the inverse of the separation distance squared, and the separation distance for larger particles tends to be longer than smaller particles (Halim and Barringer 2007).

1.1.4.3 Particle resistivity

The electrical resistivity has great influence on the electrostatic image force of powders. The higher the resistivity of the powder, the more significant the electrostatic image force is. To ensure effective electrostatic coating, the powder should be highly resistive so that it retains the charge for a long time. On the other hand, the substrate surface should be conductive to remove the induced charges so more powder can deposit onto the surface (Grosvenor and Staniforth 1996).

Three ranges of resistivity of powders were classified by Bailey (1998): greater than \(10^{13}\) Ωm, less than \(10^{10}\) Ωm, and in the intermediate range \(10^{11}-10^{13}\) Ωm. Particles with a
resistivity greater than $10^{13}$ Ωm are insulators, which have a charge relaxation time of minutes to hours and ensure the charged particles are retained on the substrate for a long time. Particles with resistivity less than $10^{10}$ Ωm act as conductors. They tend to charge more effectively than insulators in passing through a corona discharge. But when in contact with the substrate, they lose charges very quickly. Thus this type of particles is not an ideal particle for electrostatic coating. When the particles have a resistivity between $10^{11}$ Ωm to $10^{13}$ Ωm, it is difficult to predict powder coating performance. Charge decay is very fast, only a few seconds, and the electrostatic image force is poor.

Halim and Barringer (2007) compared nonelectrostatic and electrostatic adhesion by applying 11 types of food powders to coat on potato chips, saltine crackers, pork rinds, white bread and aluminum foil. Among these powders, salt and maltodextrin are conductive powders (resistivity < $10^{10}$ Ωm), so they can easily transfer charges between particles and to surfaces and do not have a good electrostatic image force during electrostatic coating. However, cellulose, 34% whey, soy flour, nonfat dry milk, 80% whey, sugar and cocoa powder have resistivities between $10^{11}$ Ωm and $10^{13}$ Ωm. They all showed significant electrostatic adhesion while cornstarch and sour cream powder did not.

The amount of charge on the particles covering a grounded surface is not constant over time. The charge decreases as the time spent by the particle on the surface increases. The reduction in the charge, in turn, leads to a decrease in Coulomb interaction (Zimon 1969). Too low a resistivity means the particles lose charge quickly upon contact with a grounded workpiece and may detach from the surface. Charge decay under these conditions is exponential with a time constant (Bailey 1998).
1.1.4.4 The ratio of charge to mass ($q/m$)

Increasing charge to mass ratio improves electrostatic image force because the particles are more strongly attracted to the grounded surface. Small particles can achieve a greater charge to mass than large particles because they have a greater surface area. The trajectory of small particles is also more affected by charge to mass ratio than large particles because they have low inertia and are less affected by gravity than large particles. Thus the effect of gravity on large powders is dominant over electrostatic forces.

There are also other powder properties affecting the transfer efficiency and adhesion during coating, including the particle shape (Miller and Barringer 2002, Buck and Barringer 2007), the cohesiveness and flowabilities of the particles (Mazumder and others 1997, Ricks and others 2002, Biehl and Barringer 2004, Sumawi and Barringer 2005).

1.1.4.5 Surface oil content

Surface oil content affects adhesion by allowing formation of liquid bridges between particles and substrates in a similar mechanism to relative humidity. Normally, adhesion increases with an increase in surface oil content. Increasing the oil content of a tortilla chip from 24% to 28% increased the adhesion force on dry seasonings. However, a further increase to 32% in the oil content did not result in a further significant change in the adhesion force (Enggalhardjo and Narsimhan 2005). Adhesiveness of sesame seeds increased from 0.02 to 0.59 when surface oil content increased from 0.43% to 0.77% (Takenaka and others 2006). High surface oil content causes the formation of an oil bridge between seeds.
Adhesion varies according to the surface oil content on the substrates. For potato chips with high surface oil content, increasing time between frying and coating did not change adhesion of salt because the chips were very oily (Buck and Barringer 2007). Even when they were stored one month, there was still enough oil remaining on the surface to form a liquid bridge, thus capillary forces dominate adhesion. For chips with low surface oil content, a significant decrease in adhesion was found in 1 day storage. This decrease in adhesion is caused by the decrease of surface oil content as surface oil is absorbed inside the chips. For chips with no surface oil, no significant difference in adhesion was found for both 1 day and 1 month old samples because van der Waals forces instead of capillary forces are the dominant forces in the dry system conditions.

1.1.4.6 Surface roughness

The relationship between roughness of surfaces and adhesion is complex. Adhesion may either decrease or increase as surface roughness increases (Packham 2005). Under conditions of good wettability, roughness improves adhesion because water or oil can fill the gaps to form liquid bridges; for poor wettability, roughness decreases adhesion because roughness increases the separation distance thus reduces van der Waals forces (Kendall and Stainton 2001). Surface roughness has a significant influence on adhesion when van der Waals forces and very small capillary forces dominate. Under this condition, adhesion is determined by the size and shape of the roughness peaks (Schubert 1987)

For substrates with poor wettability, the degree of roughness has a great impact on adhesion as it influences the true distance between the particle and the substrate. The
adhesion of polymer powders to aluminum substrates with various surface roughness initially decreases with an increase in surface roughness of the substrate (from 0.092 to 0.82 μm peak height), reaches a minimum, and then increases with surface roughness (from 0.82 to 1.97 μm) (Takeuchi 2006). The first decrease in the adhesion can be explained by the fact that van der Waals forces decrease with an increase of surface roughness due to less true contact area. The increase of adhesion after it reached a minimum is because as the roughness increases, the gaps on the substrate surface are large enough to hold the particles, thus improving the van der Waals forces (Takeuchi 2006). Model simulation predicts that the adhesion of particles smaller than or similar in size to the asperities depend mainly on the size and shape of the asperities and only weakly on the size of the particles (Katainer and others 2006). For particles larger than the asperities, the particle size has a significant effect on the adhesion.

Most food products have good wettability and are easily wetted by water or oil. The roughness influences the contact angle between the liquid and the surface of the food products.

1.1.5 Conclusion

Powder adhesion is complex and mainly involves 3 types of forces: capillary forces, van der Waals forces and electrostatic forces. Different forces dominate adhesion under different conditions. At high RH, moisture content, or surface oil content, a liquid bridge forms and capillary forces predominate over other forces. In dry charged system, electrostatic forces are the most important forces; for dry uncharged system, van der Waals forces are the most important forces. Relative humidity, particle size, particle
resistivity, charge to mass ratio, surface oil content and surface roughness are frequently important. The influence of these factors varies from case to case. Relative humidity and surface oil content can determine whether liquid bridge can form or not, thus influence capillary force; particle size has impact on both electrostatic image force and van der Waals forces; particle resistivity, charge to mass ratio mainly influence electrostatic image force, and surface roughness influences both capillary force and van der Waals forces.

1.1.6 References


Biehl HL, Barringer SA. 2003. Physical properties important to electrostatic and nonelectrostatic powder transfer efficiency in a tumble drum. J. Food Sci. 68: 2512-2515


1.1.7 List of symbols

A = Hamaker constant

d = particle diameter

$F_c$ = centrifugal force

$F_{(cap.)}$ = capillary force

$F_{(dl)}$ = electrostatic double layer forces

$F_{(img)}$ = electrostatic image force

$h$ = distance between the particle and the substrate

$m$ = mass of the particles attached

$q$ = charge of the particle or surface

$r$ = particle radius

$U$ = contact potential difference

$W$ = work to separate one surface unity of two adhered materials

$\alpha$ = roughness coefficient of surface

$\gamma$ = surface tension

$\gamma_L$ = liquid surface tension

$\gamma_S$ = solid surface tension

$\gamma_{SL}$ = solid-liquid interfacial tension

$\varepsilon_0$ = permittivity of vacuum ($8.85 \times 10^{-12}$ C$^2$/Nm)

$\varepsilon_r$ = relative permittivity of medium

$\theta$ = wetting angle
\( \theta_{\text{app}} = \) apparent wetting angle

\( \omega = \) angular rotating velocity
<table>
<thead>
<tr>
<th>Adhesion forces</th>
<th>Dry system</th>
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<tr>
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<td>Particle size ≥ 50 µm</td>
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<tr>
<td>Capillary forces</td>
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<td>NA</td>
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<td>Van der Waals forces</td>
<td>Dominant</td>
<td>NA</td>
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<td>Electrostatic forces</td>
<td>Electrostatic image forces (charged particles)</td>
<td>Dominant</td>
</tr>
<tr>
<td></td>
<td>Electrical double layer forces (uncharged particles)</td>
<td>NA</td>
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Table 1.1. Dominant adhesion forces under different systems (Adapted from Bowling 1998).
1.2 Volatile compounds in cocoa and chocolate

1.2.1 Cocoa beans

The fermented and roasted seeds of the cacao tree (*Theobroma cacao*) are enjoyed by consumers as the desirable key ingredient in beverage and chocolate confectionery due to its attractive aroma and typical taste (Stark and others 2005). There are several principle varieties of cocoa beans: Criollo, Forastero, Trinitario and Nacional. Criollo beans have a mild, nutty cocoa flavor, but are rarely grown due to its disease
susceptibility and low yields. Forastero bean is from the Amazonas region, which is the main type of cocoa with higher yield, resistance to pests and diseases but less emphasis on flavor. Trinitario is a hybrid of Forastero and Criollo, thus some Trinitario varieties produce special flavors. Nacional beans are only grown in Ecuador and have a full cocoa flavor with additional floral, spicy flavor called Arriba flavor. However, pure Nacional varieties are seldom grown, and the hybrids between Nacional and Trinitario, also have some of the Arriba flavor in Ecuador (Fowler 2009).

1.2.2 Major chocolate volatile compounds

To date, more than 600 volatile compounds have been identified, including pyrazines, aldehydes, hydrocarbons, alcohols, ketones, esters, sulphur compounds, acids, phenols, furans and so on (Ziegleder 2009). Among them, pyrazines and aldehydes are the most significant volatiles contributing to the chocolate flavor.

Pyrazines are nitrogenous heterocyclic compounds with two nitrogen atoms in a six-membered ring (Figure 1.2). There are many different substitutes in cocoa pyrazines such as methyl-, ethyl-, propyl-. These alkylpyrazines have a range of odor threshold values from 2 ng/kg to 10 mg/kg according to the substitution, and are important components to the flavor of roasted cocoa. 2,3-Dimethylpyrazine has the sensorial attribute of caramel and cocoa; 2,5-dimethylpyrazine and 2,3,5-trimethylpyrazine (TrMP) are cocoa and roasted nuts; 2,6-dimethylpyrazine is nutty, coffee, and green; 2,3,5,6-tetramethylpyrazine (TMP) is chocolate, cocoa, and coffee (Bonvehí and Coll 2002). Most pyrazines are generated during roasting, and only tetramethylpyrazine occurs in large amount in fermented unroasted cocoa. It reaches the maximum level soon at
medium roasting level and decreases because the consuming of its precursors. The concentrations of most pyrazines increased as roasting temperature or time.

Dimethylpyrazine (DMP), trimethylpyrazine (TrMP) and tetramethylpyrazine (TMP) are the primary alkylpyrazines in major volumes while other alkylpyrazines appear only at minor level. The concentration ratio between these methylpyrazines can be used to evaluate the roasting process. A concentration ratio of TMP/TrMP between about 1.5 and 2.5 was considered as a normal roasting, and below 1.0 showed over roasted flavor (Ziegleder 2009).

Aldehydes are another significant fraction of cocoa flavor, contributing the flowery, spicy, and pungent flavors to chocolate. Aldehydes from amino acids play important roles in chocolate flavor balance (Afoakwa and others 2008). They are mainly formed via Strecker degradation of amino acids and greatly generated during roasting, and their concentrations are far above the threshold values. The concentrations of 3-methylbutanal greatly increased during roasting (Ziegleder 1981). Aldehydes can also serve as intermediates and be converted to form complex compounds such as pyrazines and other heterocyclic compounds. Through comparison of the headspace volatiles during the roasting process, some methods for evaluating cocoa beans quality were suggested by utilizing some Strecker aldehydes, for example, 3-methylbutanal, 2-methylbutanal or 2-metylpronal as indicators (Keeney 1972; Ziegleder 1981).

Other than pyrazines and aldehydes, volatile compounds such as acids, esters and alcohols which are not formed through Maillard reaction, may also contribute the chocolate with sour, fruity, and astringent flavor (Bonvehí 2005).
1.2.3 Chocolate flavor development

Chocolate flavor does not exist naturally in the bean, but it is generated by several processing steps after harvest (Martin 1987). The most important processes include the fermentation and drying of the cocoa seeds, the roasting of raw cocoa beans, and the conching of chocolate liquor (Ziegleder 2009). Hundreds of volatile compounds have been found in cocoa and chocolate, mainly pyrazines, aldehydes, ethers, thiazoles, phenols, ketones, alcohols, furans and esters (Keeney 1972; Dimick and Hoskin 1981; Schnermann and Schieberle 1997). But no unique compounds have been identified as exclusively belong to chocolate because most of these volatiles are also found in other foods.

1.2.3.1 Fermentation: the formation of flavor precursors

Fermentation is an important step for the development of flavor precursors. This process is two to eight days long depending on bean type and other factors (Martin 1987). During the fermentation process, the mucilaginous pulp surrounding the beans undergoes an ethanolic, acetic and lactic fermentation. During the first two days, an anaerobic break down of sugar starts in the pulp and ethanolic fermentation dominates over lactic acid fermentation. Ethanol and carbon dioxide are produced and displace air from the fermentation containers. As the fermentation proceeds, the pulp is drained off and more air is absorbed by the beans, promoting the oxidative formation of acetic acid (Ziegleder 2009). Ethanol and acetic acid can inhibit the germination of the bean. By the third day, the beans mass will be heated to about 45 °C, remaining at 45-50 °C until the
fermentation is complete. After four to five days, the production of acetic acid slows down and lactic acid is formed. Once the pulp has decomposed, the beans come in contact with the oxygen in the air and the fermentation undergoes an aerobic phase. Many oxygen-involved reactions occur, for example, the oxidation of polyphenols, which reduces the astringency of the bean by conversion of polyphenols into insoluble browning polymers (Ziegleder 2009).

After fermentation, the pH of the beans will decrease as with an increase in the acids formed in the beans during fermentation. The final pH of beans is crucial for optimum flavor formation (Biehl and Viogt 1999). Cocoa beans from Malaysia or Brazil have low pH (4.7-5.2), those from West Africa or Indonesia medium pH (5.2-5.5) and those from Ecuador, Venezuela or Guatemala high pH (5.5-5.8) (Jinap and Dimick 1990). Beans of higher pH (5.5-5.8) are considered unfermented and those of lower pH (4.75-5.19) well-fermented. Fermentation reduces the acid notes and maximizes the chocolate flavors due to the chemical changes and development of flavor precursors.

1.2.3.1.1 Chemical changes during fermentation

Sucrose and protein constituents are partially hydrolyzed, phenolic compounds oxidized, and glucose is converted into alcohols and oxidized to acetic and lactic acids during fermentation. Fresh cocoa beans contain 2-4% free sugars. The major sugar is sucrose in early fermentation, but it is soon hydrolyzed into glucose and fructose (Reineccius and others 1972). In Arriba or Sanchez cocoa, which are weakly fermented, sucrose was found up to 1% (Keeney 1972), while in well-fermented raw cocoas, sucrose drops to near zero but glucose and fructose increase to about 0.6%. Free amino acids and
peptides are the major nitrogen-containing precursors in the cocoa beans. Ripe cocoa beans contain 10-16% of protein but a low level of free amino acids. Proteins are degraded by enzymatic hydrolysis giving a rise to 1-2% of free amino acids (Ziegleder 2009). Hydrophobic amino acids, especially leucine, valine, alanine, isoleucine, phenylalanine and hydrophobic peptides are of major importance as precursors for the formation of cocoa flavor (Biehl and Ziegleder 2003). Polyphenols are a major group of cocoa components, making up about 10-20% of the cotyledon (Ziegleder 2009). The concentration of soluble polyphenols is greatly reduced by oxidation and polymerization due to enzymatic browning during fermentation thus the astringency of cocoa is reduced and the color changes from purple to brown. Fermentation improves the chocolate flavor and reduces the sourness, bitterness and astringency (Meyer and others 1989), and it also produces some important flavor-active components such as ethyl-2-methylbutanoate and tetramethylpyrazine (Afoakwa and others 2008). The drying process after fermentation stabilizes the cocoa beans for long-term storage and improves the flavor by further oxidizing and polymerizing polyphenols.

1.2.3.2 Roasting: the major flavor development process

Typical flavor of chocolate is dependent on the chemical precursors formed during fermentation coupled with the thermal reactions of compounds in the later stages of manufacturing cocoa (Bonvehí and Coll 2002). Roasting is an essential step to develop the chocolate flavor from the precursors formed during fermentation and drying. Prior to roasting, cocoa beans have bitter, acidic, astringent and musty flavor, After roasting, cocoa shows the typical intense aroma of cocoa and a reduced acidity due to the

28
diminished volatile acids such as acetic acid (Ramli and others 2006, Granvogl and others 2006). The degree of roasting is related to time and temperature applied, over periods of 5 to 120 min and in the range 120 to 150 °C (Afoakwa and others 2008). Roasting at temperature over 150 °C, or for too long a roasting time, results in cocoas with a significant bitter and burnt taste, which is described as over-roasted. The optimum roasting time used in industry depends mainly on the heat transfer characteristics of the roaster. Normally, a roaster with higher heat transfer efficiency needs shorter roasting time. Convective roasting is the most commonly used method of thermal processing of raw cocoa beans, in which cocoa beans are exposed to 130-150 °C for 15-45 min (Nebesny and Rutkowski 1998). The choice of roasting conditions depends on the type of beans, period of harvesting, the origin, postharvest treatment and the type of flavor desired (Ramli and others 2006).

1.2.3.2.1 Maillard Reaction during roasting

The flavor precursors interact in the roasting process to produce the desired cocoa flavor mainly via Maillard reaction. The free amino acids, peptides, and reducing sugars all participate in this important reaction (Rohan and Stewart 1967). Hydrophobic amino acids leucine, alanine, phenylalanine, and tyrosine released by proteinase activities in fermentation, are important contributors (Biehl and Ziegleder 2003), as well as the reducing sugars fructose and glucose derived from sucrose hydrolysis (Lopez and others 1978).

The Maillard reaction is generally divided into three stages: initial, intermediate and final. The initial stage involves the sugar-amine condensation and the Amadori
rearrangement. The intermediate stage involves sugar dehydration and fragmentation, and amino acid degradation via the Strecker reaction. The final stage includes the aldol condensation, the aldehyde-amine polymerization and the formation of heterocyclic nitrogen compounds. Browning occurs at final stage (Hodge 1953).

The Maillard reaction is initiated by a condensation reaction between carbonyl group of the aldose and the free amino group of an amino acid to give an N-subsstituted aldosylamine. It is basically an amine-assisted dehydration reaction of sugar. The condensation product loses water rapidly and is converted into Schiff base. This reaction is reversible. The Schiff base then cyclizes into the aldosylamine, followed by the Amadori rearrangement to form a ketosamine. The Amadori rearrangement is considered to be the key step in the formation of major intermediates for the browning reaction (Davies and Labuza 1997). Ketoses, such as fructose, react with amines to form aminoaldoses, this is called the Heynes reaction. The intermediates to this reaction are imines.

The intermediate stage is that the Amadori product degrades by different pathways depending on the conditions. The reaction pH influences the intermediates formed: acidic conditions favor 3-deoxyhexuloses (3-DH); basic or neutral pH favor formation of dehydroreductone intermediates 1-deoxyhexuloses (1-DH), which is critical to flavor formation. The 1-DH intermediates are dehydrated, fragmented, and degraded into smaller dicarbonyl molecules, or contribute to Strecker degradation depending on the temperature and the pH (Dimick and Hoskin 1999).
The Strecker degradation of amino acids involves their oxidative degradation by carbonyl compounds, which arise from the degradation of ketosamines. In this degradation reaction, amino acids first react to form Schiff bases and then undergo acid-catalyzed decarboxylation. The new Schiff base is easily hydrolyzed to form an amine and aldehyde. Strecker degradation is characterized by the production of aldehydes and CO₂. This degradation of amino acid is critical to the appropriate flavors for chocolate, and involves interactions of numerous compounds, leading to the structure derived from amino acids being split into three parts (Figure 1.3).

The intermediates are complex and their structure and exact nature of their formation is unknown, but the population of the intermediate compounds, quantitatively individually depend on reaction substrate and pH, polymerizes and determines the final chocolate flavor (Afoakwa and others 2008).

The final stage is characterized by the formation of roasted and toasted aromas and tastes, as well as brown pigments. The chemistry of these compounds is not well-known and their formation mechanism also remains obscure, but it is generally accepted that aldol condensation and cyclization lead to the formation of heterocyclic aroma volatiles such as pyrazines, while polymerization produces melanoidin pigments. One of the major pathways to form pyrazines during Maillard reaction is as follows: The amino acid is decarboxylated and deaminated via Strecker degradation, producing an aldehyde and carbon dioxide. The nitrogen-substituted 1,2-dioxos compound combine with a duplicate molecule. The two compounds react and are further oxidized to form pyrazines (Figure 1.4). The specific pyrazine structure can be dictated by side groups on dioxo compounds.
Pyruvaldehyde and valine, for example, form the end products of 2-methyl-propanal and 2, 5-dimethypyrazine (Dimick and Hoskin 1999).

The variety and quantity of the alkylpyrazine formation depend on the reactivity and type of amino acid used, pH, and the roasting conditions (Hwang and others 1995, Cremer and Eichner 2000). Chocolate is an abundant source of pyrazines, at least 80 of which have important roles in chocolate flavor (Counet and others 2002, Stark and Hofmann 2005).

1.2.3.2.2 The effect of roasting temperature on Maillard reaction

Roasting temperature and duration affect the intense of Maillard reaction thus influence the formation of volatile compounds. The Strecker degradation is favored by high temperature and different flavors are formed at different temperatures (Davies and Labuza 1997). An increase in temperature leads to an increase of the reactivity between the sugar and the amino group. The temperature dependence of a reaction rate constant \( k \) is often described by the Arrhenius equation:

\[
k = A \times \exp(-\frac{Ea}{RT})
\]

Where, \( k \) is the rate constant; \( A \) the frequency factor; \( Ea \) the activation energy; \( R \) the gas constant (8.3 Jmol\(^{-1}\)K\(^{-1}\)) (Martins and others 2001). Furthermore, temperature affects the activities of the reactants. High temperature favors the open chain configuration of the reducing sugar and this configuration is more reactive than that in the closed ring configuration (Figure 1.5) (Van Boekel 2001).
1.2.3.2.3 The effect of pH on Maillard reaction

As various steps in the Maillard reaction are acid-base catalyzed, the pH is a significant factor affecting the reaction. Generally, the rate of the reaction increases with increasing pH (Wolf from and Rooney 1953). The requirement of pH range is from 3 to 9, or other nonenzymatic interactions such as sugar-sugar and protein-protein will compete with the Maillard reaction (Davies and Labuza 1997). Similar to the high temperature, the high pH favors the open chain form of the reducing sugar. It also favors the unprotonated form of the amino acid group. Both are considered to be the reactive form (Figure 1.6) (Van Boekel 2001).

Moreover, the pH influences the degradation of the Amadori product in the Maillard reaction. It undergoes mainly 1,2-enolisation and forms furfural or hydroxymethylfurfural (HMF) at pH below 7, while at pH above 7, the degradation involves mainly 2,3-enolisation, yielding reductones and many fission products such as acetol, diacetyl and pyruvaldehyde (Martins and others 2001).

1.2.4 Alkalization of cocoa

Alkalization is the treatment of the unshelled beans, shelled beans (cocoa nibs), ground beans (liquor), or partially defatted liquor (cocoa powder) by adding a dry alkali or solution of alkali (Martin 1987). Alkalization is common for cocoa products such as hot chocolate drinks to enhance solubility. Alkalized cocoa is also frequently used in baking or coatings due to its darker colors than natural cocoa.

Alkalization before or during thermal treatments influences the flavor of chocolate because it increases the pH of the beans, thus affects the Maillard reaction pathway. It
also neutralizes the acids in the fermented beans and reduces the acidity. Sharif (1997) found that alkalizing Malaysian cocoa nibs to pH 6.0 did not significantly change flavor relative to a control but chocolates from nibs alkalized to pH of 7.2 and 8.1 were significantly different, and the chocolates from alkalized and thin-film processed cocoa liquor had better flavors than non-alkalized nib-roasted chocolate. However, the alkalized roasted cocoa has fewer volatile heterocyclic components than the natural roasted cocoa because pyrones and furaneol are destroyed (Ziegleder 1991). The natural roasting process increased the pyrazine concentration by almost 1.73 times the concentration detected in alkalized cocoa with same cocoa beans (Bonvehí and Coll 2002), and the formation of heterocycles by Maillard reactions depends strongly on the roasting temperature and time, amounts of alkali and the air injection during the roasting process.

1.2.5 Analysis of chocolate flavor

Varieties of volatile compounds detection methods have been applied in cocoa flavor studies. Gas chromatography / mass spectrometry (GC/MS) is the instrumental technique used most to identify the volatile compounds in cocoa aroma. The accuracy of the analysis depends on the extraction process, the aromatic intensity of the cocoa and the chromatographic procedure applied (Bonvehí 2005).

A total of sixteen compounds was identified by Bailey and others (1962) in the headspace volatiles of ground cocoa beans. A modified gas chromatograph was applied to quantitatively determine the levels of chocolate aroma from different commercial varieties of cocoa beans (Rohan 1965). Fifty-six compounds in the steam-volatile fraction
of roasted cocoa beans were identified by van Praag others (1968) and aldehydes, pyrazines, acetic acid, and isopentylacetate were suggested to contribute to cocoa aroma.

By using coupled steam distillation-microdistillation as the extraction method, the monomethyl-, 2,3-dimethyl-, 2,5-dimethyl-, 2,6-dimethyl-, trimethyl- and tetramethylpyrazines were detected in non-roasted cocoa beans, and their concentrations increased rapidly in roasted cocoa beans (Hashim and Chaveron 1994). The authors also suggested that tetramethylpyrazine was the principal volatile compound in fermented and roasted cocoa beans, and the ratios between the concentrations of alkylpyrazines could be used to evaluate the degree of cocoa bean roasting.

Ramli and others (2005) investigated the effect of roasting temperature and time on the concentrations of nineteen volatile compounds including nine pyrazines, five aldehydes, one methyl ketone, two alcohols and two esters in roasted commercial Malaysian cocoa beans through a combined steam distillation-extraction procedure and gas chromatography-mass spectrometry. In this study, tetramethylpyrazine was suggested to be the major pyrazine compound, and its concentration showed a linear correlation with roasting temperature, and the optimum condition of roasting was at 160 °C for 40 min. Trimethylpyrazine was believed to be formed during roasting since it was not found in unroasted cocoa beans. Other alkylpyrazines, e.g. 3-ethyl-2,5-dimethylpyrazine, 2-ethenyl-6-methylpyrazine, 2,5-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine were also detected as a sign of over-roasting. Four aldehydes including benzaldehyde, benzenacetaldehyde, α-ethyl benzenacetaldehyde and 5-methyl-2-hexenal were detected by the GC-MS system and the optimum conditions of roasting to generate the aldehyde
compounds are 150-160 °C for 20-40 min. Linalool and 2-heptanol were the two alcohols identified during roasting and their concentrations increased as a linear function of temperature until they reached the maximum, then remained constant and decreased at over-roasting conditions. Two esters, 4-ethylphenyl acetate and 2-phenylethyl acetate were detected in cocoa bean during roasting. Both concentrations increased as a higher temperature or a longer roasting time was applied, especially the cocoa beans were over roasted. The optimum conditions were 130-160 °C for 40 min. The authors drew conclusions that trimethylpyrazine and tetramethylpyrazine could be used as indicators of the roasting process, and 30-40 min roasting at 150 ºC was recommended as the optimum roasting conditions for Malaysian cocoa beans.

Frauendorfer and Schieberle (2008) applied a comparative aroma extraction dilution analysis on unroasted and roasted Criollo beans. In unroasted cocoa beans, twenty-two aroma compounds have been identified in concentrations above their odor thresholds including acetic acid, 3-methylbutanoic acid, ethyl-2-methylbutanoate, 3-methylbutanal, methylpropanoic acid, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, ethyl-2-methylpropanoate, 2-methylbutanoic acid, 2-phenylethanol and 2-phenylacetic acid, and these aroma compounds are likely contributors to the aroma of unroasted cocoa beans. Some pyrazines such as tetramethylpyrazine and 2-ethyl-3,5-diethylypyrazines were also detected in fermented but unroasted cocoa beans, while the concentration levels were low (Ziegleder 1991; Frauendorfer and Schieberle 2008). In roasted cocoa beans, twenty-seven compounds were found in concentrations exceeding their odor thresholds. Of the aroma compounds quantified, they demonstrated that only acetic acid was significantly
reduced, while 15 compounds including Strecker aldehydes such as 3-methylbutanal (malty) and phenylacetaldehyde (honey like), and alkylpyrazines such as 2,3,5-trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine, as well as 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like) increased in their concentrations.

1.2.6 Selected Ion Flow Tube Mass Spectrometry (SIFT-MS)

SIFT-MS is an analytical technique that applies judiciously-selected precursor positive ions for chemical ionization reactions coupled with mass spectrometric detection to rapidly quantify targeted volatile organic compounds (VOCs). The volatile compounds can be identified and quantified in real time based on the known rate coefficients for reaction of the reagent ions with the target compounds.

1.2.6.1 Principle of the SIFT-MS

The principle of the SIFT-MS can be explained in a schematic diagram of the analytical process shown in Figure 1.7. The analysis procedure can be divided into three steps: reactant ion selection, analyte ionization and analyte quantitation. In the first step, reagent ions are generated using a microwave discharge source and selected using the quadrupole mass filter. H$_3$O$^+$, NO$^+$ and O$_2$$^+$ are commonly selected as precursor ions because they do not react with bulk components of air (N$_2$, O$_2$, H$_2$O, CO$_2$ and Ar) but react with volatile compounds rapidly (Spanel and Smith 1999). The reagent ions are then passed into the flow tube with an inert carrier gas at a known precisely controlled velocity. In the second step, the sample is introduced into the flow tube to react with precursor ions under very well defined conditions. In the final step, the products of the
chemical ionization reactions, together with unreacted reagent ions, enter the downstream chamber and are filtered by a second quadrupole mass filter. A particle multiplier detects the ions at the selected mass and the count-rate is passed to the instrument computer for processing. The concentration of a volatile compound A can be calculated using the following equation based on the ion-chemical reaction:

$$[A] = \gamma \frac{[P^+]}{[R^+]kt}$$

Where, $[A]$ is the concentration of volatile compound A in the sample; $[P^+]$ product ion count rate; $[R^+]$ precursor ion count rate; $k$ reaction rate coefficient; $t$ reaction time.

The reaction rate coefficient, $k$, was obtained from a previous study of the ion chemistry of the precursor ions, H$_3$O$^+$, NO$^+$, and O$_2$$^+$ with a wide variety of volatile compounds, and was stored in the kinetic database of SIFT-MS. Reaction time $t$ is accurately defined by the known flow velocity of the helium carrier gas and the length of the flow tube (Spanel and Smith 1999).

There are three common reactions mechanisms existing in the flow tube, including:

Proton transfer (H$^+$)

$$H_3O^+ + \text{Analyte} \rightarrow \text{Analyte} \cdot H^+ + H_2O$$

Charge transfer (reagent removes a charge from analyte)

$$O_2^+ + \text{Analyte} \rightarrow \text{Analyte}^+ + O_2$$

Association (common for NO$^+$ and occasionally with H$_3$O$^+$)

$$NO^+ + \text{Analyte} \rightarrow \text{Analyte} \cdot NO^+$$

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

$$NO^+ + \text{Analyte} \rightarrow [\text{Analyte-OH}]^+ + HONO$$
SIFT-MS provides rapid identification and quantification of volatile compounds in a wide range of sample formats, including whole air and headspace. SIFT-MS uses multiple reagent ions (H$_3$O$^+$, O$_2^+$ and NO$^+$) to chemically ionize volatile compounds. Thus identification of some isomers without the need for chromatographic separation has become possible.

The calibration of SIFT-MS was established by analyzing several organic volatile compounds in dry air over a wide range of partial pressures by preparing standard atmospheres using the syringe injection technique, which includes ethanol, benzene, toluene, xylene, acetone, 2-butanone, 1-methoxy-2-propanol, and trichloroethylene (Smith and Spanel 2005), so conventional calibration of this instrument is unnecessary.

1.2.6.2 The choice of H$_3$O$^+$, O$_2^+$ and NO$^+$ reactions in SIFT-MS

SIFT-MS can be used as a sophisticated analytical instrument using mass selected precursor ions to ionize the trace gases in complex gas mixture. However, only H$_3$O$^+$, O$_2^+$ and NO$^+$ are suitable precursor ions species for SIFT-MS (Spanel and Smith 1995, Smith and Spanel 1996). The ion chemistry of H$_3$O$^+$, O$_2^+$ and NO$^+$ with series of volatile compounds had been investigated, which included alcohols, aldehydes, ketones, carboxylic acids, esters and ethers (Spanel and others 1997, Spanel and Smith 1997, Spanel and Smith 1998, Spanel and Smith 1999, Smith and Spanel 2005).

1.2.6.2.1 H$_3$O$^+$ reactions

The reaction of H$_3$O$^+$ with the large majority of organic compounds proceeds via proton transfer and the great advantage to SIFT-MS analyses of this proton transfer is that they frequently proceed with unit efficiency and usually result in only one or two product
ions (Smith and Spanel 2005). The product ion of the proton transfer reaction of H$_3$O$^+$ with molecule M is MH$^+$, whose mass is the mass of the reactant molecule plus 1. So the precursor ion H$_3$O$^+$ is not good to separate isomers because they produce the same mass spectra. When ions are introduced into humid air, clustering H$_3$O$^+$(H$_2$O)$_n$ may occur to the abundant water molecules (Smith and Spanel 1996). The presence of these cluster ions in the carrier gas of the SIFT-MS apparatus must be taken into account in the analysis. They can be used as an additional analytical tool due to their rapid reaction with many gas molecules, especially with polar molecules (Spanel and Smith 1995). Another important possible reaction is that the product ions such as MH$^+$, cluster with the abundant H$_2$O in wet air, producing a secondary reaction, which obviously influences the product ion signal distributions and intensities in SIFT-MS analyses. Both water clustering reactions produce masses MH$^+18$, MH$^+36$, etc. If M is an alcohol, aldehyde or a carboxylic acid, the dihydrate ions (MH$^+$(H$_2$O)$_2$) readily form. The increase of the number of final product ions generates more conflicts with other masses. Thus H$_3$O$^+$ reaction masses should be avoided for these compounds. But if M is a ketone, ester or ether, only the monohydrate ions (MH$^+H_2O$) readily form. Protonated phenols form mostly monohydrates and very small fractions of dihydrates (Wang and others 2004).

1.2.6.2.2 NO$^+$ reactions

The reactions of NO$^+$ with organic molecules are more varied than those of H$_3$O$^+$ but they usually result in one or two product ions. Several reaction processes occur in NO$^+$ reactions with organic molecules M, including charge transfer producing M$^+$ ions, hydride ion transfer producing MH$^+$, and ion-molecule association producing NO$^+M$ ions.
Charge transfer can only happen if the ionization energy (IE) of M is less than the IE of NO. Hydride ion transfer occurs in the reactions of NO\(^+\) with aldehydes and ethers (Spanel and others 1997, Spanel and Smith 1998). Ion-molecule association is common in the reactions of NO\(^+\) with some types of polar organic molecules at thermal energies (internal energy from the random movements of atoms and molecules), especially carboxylic acids and esters (Spanel and Smith 1998) and ketones (Spanel and others 1997). Although several reaction processes are represented in NO\(^+\) reactions, usually the reactions of NO\(^+\) with organic species proceed via only one of these processes. Therefore, NO\(^+\) is a very valuable precursor ion for SIFT-MS analyses. However, small compounds such as methanol react very slowly with NO\(^+\), which means those compounds have very high counts with H\(_3\)O\(^+\) but 20 to 50 times lower counts with NO\(^+\), so the NO\(^+\) is not suitable for their quantification.

**1.2.6.2.3 O\(_2\)\(^+\) reactions**

The reactions of O\(_2\)\(^+\) with most organic molecules are rapid, and they result in a single production ion or two or more product ions. The ionization energy of O\(_2\) molecules is greater than most organic molecules (Lias and others 1988), which ensures that their reactions with O\(_2\)\(^+\) ions proceed either via simple non-dissociative charge transfer or dissociative charge transfer reactions resulting in two or more fragment ions (Anicich 2003). O\(_2\)\(^+\) has higher energy than H\(_3\)O\(^+\) or NO\(^+\) so frequently splinters the compounds into the same fragments. The reactions of O\(_2\)\(^+\) with some aromatic organic compounds result in a single product ion, which is valuable for detection and quantification of these compounds. For instance, alkylpyrazines are best measured under O\(_2\)\(^+\) because they don’t
fragment but other compounds do. $O_2^+$ precursor ions are also valuable for the detection and quantification of small molecules such as NO, NO$_2$ and CS$_2$ that do not react with either H$_3$O$^+$ or NO$^+$ ions (McIntosh and others 1988, Hunter and Lias 1998). However, for $O_2^+$ reactions with polyatomic aliphatic hydrocarbons, several product ions can result thus the mass spectra are very complex. This limits the usefulness of $O_2^+$ precursor ions in SIFT-MS analyses.

1.2.6.3 Application of SIFT-MS in Food Science

SIFT-MS has been successfully used in a variety of areas, including monitoring air pollution in environmental science (Smith and Spanel 1996), analysis of breath volatiles for medical diagnosis and therapeutic monitoring in human physiology (Senthilmohan and others 2000; Smith and others 2003), and detection of bacterial metabolites in microbiology (Scotter and others 2005; Allardyce and others 2006). The applications of SIFT-MS for food emissions such as the volatile compounds emitted by ground Colombia coffee and crushed garlic cloves (Smith and Spanel 1999) have been reported. The volatile emission of volatiles from cut onions and ripe bananas were also measured using SIFT-MS (Smith and Spanel 1999), as well as the oxidation of olive oil (Davis and McEwan 2007). Recently, this technology has been used in the analysis of volatile compounds in tomatoes (Xu and Barringer 2009), cocoa (Huang and Barringer 2010), garlic (Hansanugrum and Barringer 2010), jalapeños (Azcarate and Barringer 2010). SIFT-MS has large potentials of trace gas analysis to the monitoring of food freshness, food preparation, and in the brewing industry (Smith and Spanel 2005)
1.2.7 References


Figure 1.2. Structures of methylpyrazines.
Figure 1.3. The formation of aldehydes from Strecker degradation of amino acids (Afoakwa 2008)
Figure 1.4. Major pathway for pyrazine formation via Strecker degradation (Mabrouk 1979).
Figure 1.5. Reducing sugar changing from the closed ring to open chain configuration at high roasting temperature.

\[
\text{Ring configuration} \quad \xrightarrow{\text{High } T} \quad \text{Open chain configuration}
\]

Figure 1.6. Free amino acids changing to deprotonation form at basic condition.

\[
\text{R-NH}_3^+ \quad \xleftrightarrow{\text{pH} > 7} \quad \text{R-NH}_2 + \text{H}^+
\]

deprotonation form

Figure 1.6. Free amino acids changing to deprotonation form at basic condition.
Figure 1.7. Principles of Selected Ion Flow Tube Mass Spectrometry (Syft Technologies)
Chapter 2: ADHESION OF FOOD POWDERS WITH NONELECTROSTATIC AND ELECTROSTATIC COATING

2.1 Abstract

This study investigated the effects of powder resistivity, coating voltage, relative humidity and coating density on adhesion. Cocoa powder with a high resistivity \((1.15 \times 10^{13} \, \Omega m)\) showed a stronger electrostatic adhesion than starch powder with a medium resistivity \((2.56 \times 10^{10} \, \Omega m)\) and NaCl powder with a low resistivity \((7.31 \times 10^5 \, \Omega m)\). The adhesion of starch and cocoa powders coated at 0, 40 and 95 kV increased with increasing voltage. The adhesion at 0 kV was in the range of the theoretically calculated van der Waals force, and the measured adhesion forces at 40 kV and 95 kV were in the range of the theoretically calculated electrostatic image force at 40 kV and 95 kV respectively. For nonelectrostatic coating (0 kV), there was no significant change in adhesion when RH increased from 30% to 60%, while adhesion increased when RH increased from 60% to 80%. For electrostatic coating, the adhesion decreased when RH increased from 30% to 60%, but the adhesion at 80% RH, close to the theoretically calculated capillary force value, was larger than the adhesion at 30% and 60% RH. For both nonelectrostatic and electrostatic coating, the adhesion force decreased as coating
density increased to 1.0 mg/cm², but there was no significant change from 1.0 mg/cm² to 2.0 mg/cm².

Keywords: adhesion, electrostatic coating, food powder

2.2 Introduction

Snack foods such as potato chips, pretzels, popcorn, and tortilla chips are often coated with seasonings or flavorings to enhance flavor and taste before they are packaged. Even distribution of seasoning toppings is important to the appearance and taste of snacks and it is essential to maintain a consistent quality of the product. However, insufficient adhesion between seasonings and the surface of snacks results in the seasonings falling off the snacks, leading to a poor distribution of flavor on the product. Thus it is common for traditional snack manufacturers to put 30% and, in some cases, as much as 50% more seasonings than actually needed into the coating process to ensure sufficient coating (Mitchell 1992). This powder waste increases product cost because the seasonings are expensive.

Electrostatic coating is one solution to the insufficient adhesion problem during food powder coating. Electrostatic powder coating applies charge onto food powders, and the charged particles repel each other because they all possess a similar charge, resulting in improved coating evenness. Once the charged particles deposit onto the grounded food product, they adhere to the product via electrostatic image forces (Bailey 1998). Based on Coulomb’s law, the electrostatic image force will be large since the distance is now small. Therefore the adhesion will be increased when charge is present (Hughes 1997). The
resistivity of the food powder determines how long the powder can retain the charge. Powders with higher resistivity can keep the charge longer than powders with lower resistivity, thus the adhesion between a powder with higher resistivity and the target is stronger than that between a powder with lower resistivity and the target.

Adhesion is the interaction of particles with a solid surface (Zimon 1969). It has been quantified as the work done in separating two surfaces that are in contact with each other (Michalski and others 1997). Adhesion is caused by a variety of forces. Forces between particles and a surface, bringing about adhesion, include long-range attractive interactions such as van der Waals forces and electrostatic forces (Bowling 1998). Electrostatic forces include both electrostatic image force and electrical double layer forces. Adhesion also occurs due to the establishment of liquid and solid bridges between particles and a surface, and the consequent capillary force (Bowling 1988). Under different conditions, different individual forces of adhesion will prevail. For dry uncharged particles on a dry uncharged surface, only van der Waals forces and electrical double layer forces are important (Bowling 1988). Charged particles add an additional electrostatic image force to the total adhesion. Different forces may dominate adhesion under different conditions. For instance, van der Waals force was the dominant force for uncharged toner particles at a relative humidity (RH) below 30%, while electrostatic force was the dominant force for corona-charged toner particles (Takeuchi 2006). Capillary forces appear in wet systems and can predominant over other forces for small particles (Bowling 1988). An increase of RH progressively modified the surface topology.
of zanamivir particles on lactose monohydrate and increased the adhesion force (Bérard and others 2002).

The objective of this study is to quantify the total adhesion force and individual forces between food powders and targets after nonelectrostatic and electrostatic coating, and investigate how the powder resistivity, coating voltage, RH and coating density affect the adhesion.

2.3 Materials and methods

2.3.1 Food powders

Cocoa powder, starch and NaCl were applied in this study. Powder particle size was measured using a Malvern Mastersizer X with a dry powder feeder (Malvern Instruments Ltd., Worcestershire, UK). The volume mean diameter $D_{4,3}$ of each powder was measured 3 times and the average was reported as the particle size for each powder. The cocoa powder (The Great American Spice Co., Fort Wayne, IN) had a particle size of 34 μm. The NaCl powder (Extra-Fine 325, Morton International Inc. Chicago, IL) had a particle size of 24 μm. The starch powder was modified starch (National Starch & Chemical (Thailand) Ltd., Amphur Muang, Thailand). The original particle size of the starch was 195 μm. It was ground in an ultracentrifugal mill (Glenmill Inc., Clifton, New Jersey, U.S.A.) at 18,000 rpm for 6 times to produce 59 μm particles.

Resistivity was measured using a resistivity cell (powder resistivity test cell, Electrostatic Solutions Ltd, Southampton, Hampshire, UK) connected to an electrometer.
(614 Electrometer, Keithley Instruments Inc, Cleveland, OH, USA) and voltage supply (DC supply, Kepco Inc, Flushing, NY, USA). Food powder (5 cm$^3$) was filled into the cell and tapped 30 s to remove air. A voltmeter (LCD Auto Range Digital Multimeter, Model 22-163, Radio Shack, Tandy Corporation, Fort Worth, TX) measured the voltage (175±5 volts) from the voltage supply and the current was read from the electrometer. The resistivity of the powder was calculated using the equation $\rho = \frac{KV}{I}$, where $\rho =$ resistivity (Ωm), $K =$ the cell constant (0.014), $V =$ the voltage applied (V) and $I =$ current (A).

The charge-to-mass ratio produced by tribocharging was determined using an aluminum foil covered piece of cardboard, which was first grounded then insulated from contact with the environment. The food powder was coated onto the aluminum foil by a corona charging system (Sure Coat Manual Powder Spray Gun, Part 302123D, Nordson Corporation, Amherst, OH). The coating voltage was set at 0 kV. An electrometer (Model 610c, Keithley Instruments, Inc., Cleveland, Ohio, U.S.A.) connected to the aluminum foil measured the charge captured by the aluminum foil. The mass of powder deposited on the aluminum foil was measured. The charge-to-mass ratio was determined from the charge of the powder dividing by the mass of the powder.

2.3.2 Coating system

All experiments were conducted in an environmentally-controlled room, where the relative humidity (RH) was kept constant at 35±5% using humidifiers (Environizer, Koz Inc., Southborough, MA; MoistAir, Emerson Electric, Hatfield, PA) or dehumidifiers (Maytag, Maytag Inc, Effingham, IL; Hampton bay, Fedders Corporation,
Liberty Corner, NJ) except for the study of effect of RH on adhesion. The temperature was controlled at 25±2°C. The corona charging system (Sure Coat Manual Powder Spray Gun, Part 302123D, Nordson Corporation, Amherst, OH) was set at 40 kV or 95 kV for electrostatic coating. The voltage was adjusted to 0 kV for nonelectrostatic coating. For both electrostatic and nonelectrostatic coating with the corona gun, an air compressor (5.0 hp, 15.0 gallon tank, Model WL650800AJ, Campbell Hausfeld, OH) was connected to the corona charging system to supply airflow. The flow rate air pressure was set at 6.9 × 10⁴ Pa (10.0 psi) and the atomizing air pressure was set at 3.5 × 10⁴ Pa (5.0 psi).

The powders were sprayed into a cardboard booth (47 × 38 × 40 cm) through an opening to contain the excess powder. The powder is charged as it exits the gun. The bottom of the booth was grounded foil. A Rigid Foam Insulation sheet (Owens Corning Foam Insulation, LCC, Toledo, OH) was cut to produce the coating targets (11.6 × 3.5 cm). A coating zone was drawn on the foil, which ensured the vertical position of the gun tip was 27 cm above the foil, and the horizontal distance of the gun tip to the coating zone was 15 cm. The targets were cleaned and dried at room temperature. Both the targets and the powders were put in the coating room at least 2 h before coating for tempering. The coating density was adjusted by controlling the amount of powder fed to the gun. The coating density of salt on commercial potato chips was determined to be 0.5 to 1.0 mg/cm². Thus all the tests were performed at this coating density range unless otherwise specified.
2.3.3 Adhesion force measurements

The adhesion was determined by applying detachment forces to remove the powder from the surface of the target. Centrifugal force and gravity were the two types of detachment forces employed. Targets (12 pieces) were put in 2 rows on the ground foil for coating. After coating with the corona gun, 3 targets with similar coating density were selected as replicates for each run. They were gently inverted 3 times after coating to remove loose powders by gravity. Then they were vertically placed into 250 ml centrifugal bottles (Fisher Scientific Company L.L.C.). The bottles were placed into the rotor of a Sorvall RC5C plus (Sorvall Company, Asheville, NC, U.S.A.), with the powder-covered surfaces of the targets facing outside the rotor. The targets were centrifuged at 100, 500, 1000, 1500 and 2000 rpm for 1 min, and the powder mass before and after applying each centrifugal force was recorded by weighing. The force due to gravity (\(F_g\)) was calculated with the equation:

\[ F_g = mg, \]

where \(m\) is the mass of a single particle, which equals the density of the powder (1491, 2200, and 1450 kg/m\(^3\) for starch, NaCl and cocoa powder, respectively) times the volume of a single particle. The volume of a single particle was calculated by assuming all particles were perfect spheres and had a diameter equal to the mean particle size measured. The gravitational acceleration (\(g\)) is 9.8 m/s\(^2\). The centrifugal force at each speed was calculated with the equation:

\[ F_c = m\omega^2x \]
where \( F_c \) is the centrifugal force, \( \omega \) is the angular velocity of the rotation, which equals to \( 2\pi n \), \( n \) is the spinning speed in revolutions per second, and \( x \) (8.63 cm) is the distance of the target surface from the axis of rotation. The detachment force was assumed to be the sum of gravity and centrifugal force at different speeds, and the adhesion force \( F_{50} \) was determined as the sum of gravity and centrifugal force required to remove 50% of the powders on the target.

### 2.3.4 Calculation of the individual forces

#### 2.3.4.1 Capillary force

The interaction between a sphere and plate surface associated with capillary force takes the following form:

\[
F_k = 4\pi\sigma r \cos\theta / \alpha
\]

where \( \sigma \) is the surface tension, (water at 25°C is 0.072 N/m), \( r \) is the radius of the particle, and \( \theta \) is the contact angle of water to the target surface, which is assumed to be 10°, and \( \alpha \) is the roughness coefficient of the surface, which was determined to be 118.61 for tortilla chips (Enggalhardjo and Narsimhan 2005).

#### 2.3.4.2 Electrostatic image force

The charged food particles induce equal and opposite charges on the target’s surface. According to columbic law, the electrostatic image force for a single particle \( (F_{im}) \) is theoretically calculated with the equation (Cross 1987)

\[
F_{im} = q^2 / \left[ 16\pi\varepsilon_0 \varepsilon_r (r+h)^2 \right]
\]

where \( \varepsilon_0 \) is the vacuum permittivity constant, \( 8.85 \times 10^{-12} \, \text{C}^2/\text{J*}\text{m} \), and \( \varepsilon_r \) is the relative permittivity constant. As the medium between the particle and substrate is air, the value
of relative permittivity is close to one, $h$ is the distance between the particle and target, which is assumed to be 0.05 μm, and $q$ is the charge on a single particle, which was estimated to be the saturation charge $q_{\text{max}}$ due to the Pauthenier limit. $q_{\text{max}}$ is given by (Cross 1987)

$$q_{\text{max}} = 4\pi\varepsilon_0 r^2 p E$$

where $p = 3 \varepsilon/(\varepsilon + 2)$, which varies between three for a conducting particle and one for an insulating particle, the permittivity of the particle is $\varepsilon$, and $E$ is the electric field in Vm$^{-1}$.

### 2.3.4.3 Van der Waals forces

The van der Waals force ($F_{\text{vd}}$) between a spherical food particle and a plate substrate at the distance of $h$ is given by

$$F_{\text{vd}} = A r / (6h^2)$$

where $A$ is the van der Waals constant, which is assumed to be $4.7 \times 10^{19}$ J based on sodium borate spheres (Zimon 1969).

### 2.3.5 Statistical Analysis

Two-way ANOVA with post hoc tests was performed to analyze the difference between different coating voltage, relative humidity, and particle size. Tukey HSD was used to determine the significant factors. A $p$ value less than 0.05 was used to indicate significant difference.
2.4 Results and discussion

2.4.1 Powder resistivity

Resistivity is a property of a material which indicates how strongly the material opposes the flow of electric charge. Powder resistivity determines the ability of a powder to hold charge and is critical to electrostatic adhesion. In terms of the magnitude of resistivity, particles with resistivity greater than $10^{13}$ Ωm are insulators, and less than $10^{10}$ Ωm are conductors (Bailey 1998). NaCl ($7.31 \times 10^5$ Ωm), starch ($2.56 \times 10^{10}$ Ωm) and cocoa ($1.15 \times 10^{13}$ Ωm) powders were chosen because they have low, medium and high resistivity respectively, and represent the range of resistivities present in food products. In order to minimize the size effect, powders with similar particle size were chosen to investigate the effect of resistivity on adhesion.

For nonelectrostatic coating, the percent of powder removed for different amounts of force for starch and NaCl powders were close, while cocoa powder showed a greater loss (lower adhesion) (Figure 2.1). Although no charge was intentionally added in nonelectrostatic coating, tribocharging always occurs due to friction during the handling of the powders. The net charge accumulated due to tribocharging can be large and tribocharging is greatest on powders and targets with high resistivity (Bailey 1998). The charge to mass ratio produced on cocoa powder (+62.0 nC/g) was significantly higher than that of NaCl (+48.8 nC/g) and starch (+30.0 nC/g). If the plastic foam targets were tribocharged positively during handling, then the powders would be repelled. NaCl and starch have little initial charge and their low resistivity means they lose their charge
quickly, while both cocoa powder and plastic foam have a high resistivity. Therefore the repulsion between cocoa powder and the targets may have been enough to lower the adhesion force. As capillary force is not significant at low relative humidity and electrostatic image force is not dominant for low charge to mass values, van der Waals force is the key force in nonelectrostatic coating. The measured adhesion force for cocoa powder at 0 kV, 30% RH ($F_{50} = 0.76$ nN) was of the same order of magnitude as the theoretically calculated van der Waals force for cocoa (0.53 nN). While the measured adhesion forces for NaCl ($F_{50} = 4.6$ nN) and starch ($F_{50} = 14.0$ nN) at 0 kV, 30% RH were larger than the theoretically calculated van der Waals force for NaCl (0.38 nN) and starch (0.92 nN). There are critical assumptions that must be made in calculating van der Waals force, including the distance between powder and the target, and the value of the van der Waals constant, which cannot be measured. Thus being of the same order of magnitude is as close as can be expected.

After electrostatic coating, on the other hand, cocoa powder showed the highest adhesion, followed by starch then NaCl powder (Figure 2.2). Particles with lower resistivity are charged more efficiently than those with higher resistivity when passing through a corona discharge, but when in contact with the target, they lose charge more quickly (Bailey 1998). Charge decay is exponential with a time constant. Cocoa powder is an insulating powder with a high resistivity, which has a slow charge decay rate and can retain charge longer, resulting in higher electrostatic adhesion. Starch powder has an intermediate resistivity and the electrostatic adhesion is between cocoa powder and NaCl powder. NaCl powder has a low resistivity and is a conductor. The charge applied on it
decays very fast and does not show significant electrostatic adhesion. Halim and Barringer (2007) also found that electrostatically coated cocoa powder had higher adhesion than NaCl.

### 2.4.2 Coating Voltage

Starch powder coated onto the targets at 95 kV showed the highest adhesion force, followed by 50 kV, then 0 kV (Figure 2.3). Because of its higher resistivity, the increase in adhesion force at high voltage for cocoa powder was even larger than for starch (Figure 2.4), while NaCl showed no difference (Figure 2.1, 2.2). In the remainder of the paper, the results for starch powder are presented because it has an intermediate resistivity and the majority of food powders have a resistivity in the intermediate range (Halim and Barringer 2007). Electrostatic coating has a greater effect on cocoa powder and less effect on NaCl, compared to starch. Electrostatic image force is an important contributor to adhesion force when the powders are charged. The electrostatic image force increases as the voltage increases because higher voltage creates higher charge density in the corona field, allowing the powders to pick up more net charge when passing through the corona area, thus achieving a higher electrostatic image force.

The measured adhesion force ($F_{50}=1.4 \text{ nN}$) for cocoa powder at 40 kV, 30% RH was similar to the theoretically calculated electrostatic image force ($2.6 \text{ nN}$), and larger than the calculated van der Waals force ($0.53 \text{ nN}$). At 95 kV, the measured adhesion force ($F_{50}=115 \text{ nN}$) for cocoa powder was higher than the calculated electrostatic image force ($10.6 \text{ nN}$). The theoretically calculated electrostatic image force was determined by assuming the food powders were saturated with charge when they passed through the
electrical field hence have the maximum charge levels. However, the actual charge the powder captures is frequently lower than the saturation level, thus lowering the actual electrostatic image force.

### 2.4.3 Relative humidity (RH)

For nonelectrostatic coating (0 kV), there was no significant change in adhesion between starch powder and the targets when RH increased from 30% to 60%, while adhesion increased when RH increased from 60% to 80% (Figure 2.5). There is little capillary force at 30% and 60% RH, while capillary force becomes significant at 80% RH. Relative humidity greatly influences adhesion by forming liquid bridges resulting in strong capillary forces. Capillary forces are the primary adhesion force at high relative humidity, which exceed all other adhesive components. Liquid bridges form from the condensation of atmospheric moisture at a relative humidity above 65% (Zimon 1969). Above 70% RH, capillary force predominates over other forces in adhesion (Busnaina and Elsawy 2001). The measured adhesion force at 80% RH ($F_{50}=277$ nN) was similar to the theoretically calculated capillary force (226 nN).

In electrostatic coating, the adhesion force at 30% RH, 95 kV was stronger than at 60% RH, 95 kV (Figure 2.6). As RH increases, powder absorbs water from the air, decreasing the powder’s resistivity and increasing the charge decay rate (Halim and Barringer 2007), therefore decreasing electrostatic image force. A further increase of RH to 80% leads to a lower resistivity and faster charge decay rate, which makes the electrostatic image force negligible. However, water bridges also form at high RH, thus capillary force appears and becomes the dominant force at 80% RH. Since capillary force
is much stronger than other individual forces, the adhesion force at 80% RH for electrostatic coating is larger than that at 30% and 60% RH.

2.4.4 Coating density

For both electrostatic and nonelectrostatic coating, as coating density increased to 1.0 mg/cm², the percentage of starch powder removed at 153 nN (1000 rpm) detachment force increased (Figure 2.7). However, when the coating density increased from 1.0 mg/cm² to 2.0 mg/cm², there was no significant change in the percentage removed. The fact that adhesion did not change indicated multilayers of powder were formed when coating density increased above 1.0 mg/cm². The theoretical monolayer value, based on the assumptions that all the particles are perfect spheres with the same size and there is no space between particles, is 2.9 mg/cm². This indicates the starch powders are not perfect spheres and/or multilayers form while there is still space between particles. The strength of multiple layers depends not only on their adhesion to the target surface but also on the autohesion between the particles (Zimon 1969). The adhesion force becomes smaller as layers increase because gravity dominates over autohesion, causing the particles to be removed from the targets more easily. Across the range of coating densities tested, the percentage of powder removed for 95 kV coating was lower than for 40 kV and 0 kV, indicating high voltage improves both monolayer and multilayer coating.

Electrostatic coating, especially high voltage (95 kV) coating, showed a lower coating density than nonelectrostatic coating when the same mass of powder was applied to the gun. The charged powder evenly spread across the target, ground and surrounding area due to the repelling force between the charged powder particles instead of unevenly
dropping where gravity directed it onto the target, which occurred during nonelectrostatic coating. To achieve similar coating density for both nonelectrostatic and electrostatic coating, the feeding mass for electrostatic coating has to be increased. A feeding mass of 1g for 0 kV, 3g for 40 kV, and 5g for 95 kV coating was needed to create a similar coating density range of 0.5~1.0 mg/cm².

2.5 Conclusion

Powders with higher resistivity can retain charge better thus have higher electrostatic adhesion force than powders with lower resistivity. Adhesion force becomes stronger with increasing coating voltage, especially from medium voltage to high voltage. Adhesion force increased greatly when relative humidity is above 60% due to the formation of liquid bridges resulting in strong capillary force. For electrostatic coating, water in the air at moderate relative humidity (60%) decreases the resistivity of powder and causes faster charge decay, decreasing the effectiveness of electrostatic coating compared to lower relative humidity. Adhesion force decreased as the coating density decreased for both nonelectrostatic and electrostatic coating. While adhesion force can be theoretically calculated, the number of assumptions that must be made keeps the calculations from being very accurate.

2.6 References


Figure 2.1. Adhesion of different powders at 30% RH, 0 kV.
Figure 2.2. Adhesion of different powders at 30% RH, 95 kV.
Figure 2.3. Effect of coating voltage on adhesion for starch powder at 30% RH.
Figure 2.4. Effect of coating voltage on adhesion for cocoa powder at 30% RH.
Figure 2.5. Effect of RH on adhesion for starch at 0 kV coating
Figure 2.6. Effect of RH on adhesion for starch at 95 kV.
Figure 2.7. Effect of coating density on adhesion for starch at 35% RH.
Chapter 3 : ALKYLPYRAZINES AND OTHER VOLATILES IN COCOA LIQUORS AT pH 5 to 8, BY SELECTED ION FLOW TUBE–MS SPECTROMETRY (SIFT-MS)

3.1 Abstract

Cocoa beans were alkalized before or after roasting and made into cocoa liquor before analyzing by SIFT-MS. In both alkalized-before-roasting and alkalized-after-roasting samples, there were significantly higher concentrations of alkylpyrazines for the samples with pH above 7.0 than pH below 7.0. At pH 8, the concentrations of 2,3-, 2,5- and 2,6-dimethylpyrazine (DMP), 2,3,5-trimethylpyrazine (TrMP), 2,3,5,6-tetramethylpyrazine (TMP) and 2,3-diethyl-5-methylpyrazine (EMP) in the samples alkalized-before-roasting were higher than those in the samples alkalized-after-roasting. Volatiles increased under conditions that promoted the Maillard reaction. The partition coefficient was not significantly affected by pH from 5.2 to 8.0. The ratios of TrMP/DMP and DMP/TMP increased while the ratio of TMP/TrMP decreased as the pH increased. The concentrations of Strecker aldehydes and other volatiles followed a similar pattern as that of the alkylpyrazines. High pH favors the production of alkylpyrazines and Strecker aldehydes.

Keywords: Cocoa, alkylpyrazine, volatile, Maillard reaction, alkalization.
3.2 Introduction

Chocolate is one of the most aromatic foods. The distinctive flavor characteristics of chocolate are related to the cocoa bean genotype and growing environment (Clapperton 1994). However, chocolate flavor does not exist naturally in the beans; it is generated by a series of procedures that begins with the postharvest fermentation of the beans and continues through roasting (Martin 1987). Fermentation is an important procedure to reduce off-notes such as sourness, bitterness and astringency in cocoa beans (Meyer and others 1989; Biehl and others 1990). It is also a key step in the formation of reducing sugars and amino acids, which are the precursors of the Maillard reaction during roasting. Roasting is an essential process in developing chocolate flavor from the precursors formed during fermentation. The flavors produced during roasting include alkylpyrazines, aldehydes, ethers, thiazoles, phenols, ketones, alcohols, furans and esters (Dimick and Hoskin 1981).

Amino acids and peptides are released from proteins during fermentation of the cocoa beans. Fructose and glucose, derived from sucrose hydrolysis (Lopez and others 1978), are the most abundant reducing sugars in cocoa beans (Bonvehi and Coll 2002). These key precursors of flavor compounds interact during roasting and develop volatile heterocyclic compounds. The formation of these heterocyclic compounds is not fully understood but it is generally accepted that aldol condensation and cyclization lead to the formation of alkylpyrazines (Afoakwa and others 2008). The actual structure of the
alkylpyrazines is dictated based on the side group of the dioxo compounds, one of the intermediates of the Maillard reaction, and amino acids (Dimick and Hoskin 1999).

Heterocyclic aroma compounds are important volatiles to cocoa aroma (Bonvehi 2005). Among these heterocyclic compounds, the most dominant volatiles are the alkylpyrazines, which contribute to the desirable chocolate aroma (Counet and others 2002, Stark and Hofmann 2005). 2,3-Dimethylpyrazine has the sensorial attribute of caramel and cocoa; 2,5-dimethylpyrazine and 2,3,5-trimethylpyrazine are cocoa and roasted nuts; 2,6-dimethylpyrazine is nutty, coffee and green; 2,3,5,6-tetramethylpyrazine is chocolate, cocoa and coffee (Bonvehi and Coll 2002).

Alkalization is a treatment of the cocoa nib or liquor with alkali solutions. The primary aim of alkalization is to enhance the solubility of cocoa powders, but it also influences the color and flavor of the cocoa because pH influences the formation of intermediates in the Maillard reaction: acidic conditions favor 3-deoxyhexuloses while basic or neutral pH favor the formation of dehydroreductone intermediates in cocoa (Afoakwa and others 2008). The dehydroreductone compounds are critical for flavor formation since they can produce smaller dicarbonyl molecules or contribute to Strecker degradation (Dimick and Hoskin 1999).

Numerous studies have measured cocoa and chocolate volatiles using gas chromatography-mass spectrometry, frequently with solid phase micro-extraction or tenax trapping to concentrate the volatiles. Selected ion flow tube mass spectrometry (SIFT-MS) allows highly sensitive, real-time analysis of complex mixtures of volatile compounds without trapping or preconcentration (Spanel and Smith 1999). Thus artifacts
or changes in relative proportions are not introduced by the preconcentration steps. SIFT-MS has been used in the analysis of volatiles in coffee, onion, garlic, banana (Smith and Spanel 2005), tomatoes (Xu and Barringer) and oxidation of olive oil (Davis and McEwan 2007).

The purpose of this study is to investigate the effect of alkalization of cocoa beans before or after roasting on the formation of alkylpyrazines and other volatiles in cocoa liquors with different pH values.

3.3 Materials and methods

3.3.1 Cocoa liquor samples

Fermented cocoa beans from the Dominican Republic (Hispaniola '07, Chocolate Alchemy Company) were used. The cocoa beans were weighed (1 kg) and roasted in a convection oven (Doyon model JA 14, Dayton, OH) at 150°C for 20 min. After cooling to room temperature, the cocoa beans were cracked with a crankandstein cocoa mill, winnowed by an air gun (Model HG 201 B, Master Appliance Corp., Racine, WI) and ground by a juicer (The Champion, Plastaket Mfg. Inc., Lodi, CA) at 1725 R.P.M. to cocoa liquor. The alkalized-before-roasting cocoa liquor was prepared by soaking the cocoa beans in 700 ml potassium carbonate (Sigma-Aldrich, Inc., St. Louis, MO) aqueous solution for 30 min, then removing the alkali solution by passing through a screen, and drying at 70°C for 2 h before roasting. The concentration of the alkali solution varied from 42g/L to 204g/L depending on the desired final pH. The cocoa liquors were sealed and stored in a -18 °C freezer before testing. The alkalized-after-
roasting cocoa liquor was prepared in the same way except the alkalization step occurred after the roasting step.

Commercial cocoa powders (ADM Company Cocoa Division, Milwaukee, WI) N-11-N (pH 5.74), D-11-A (pH 7.07), D-11-V (pH 7.39), D-11-S (pH 7.84) and D-11-R (pH 8.04) with different degrees of alkalization were also analyzed. N-11-N is natural cocoa without alkalization; D-11-A is lightly alkalized; D-11-V is brown with medium alkalization; D-11-S powder is deep red made by a red alkalization process; and D-11-R is dark red. All these cocoa powders are claimed to have a rich flavor and a fat percentage of 10-12%.

To determine the effect of pH on the partition coefficient, the unalkalized cocoa liquor (20 g) was alkalinized by addition of 50 ml potassium carbonate solution to create different pH values. The samples were warmed in a water bath at 40 °C or 70 °C for 2h before being tested.

The pH of the sample was measured by placing cocoa liquor (20 g) or cocoa powder (10 g) in a 150 ml beaker, slowly adding with stirring 90 ml boiling hot distilled water, filtering, cooling to 20-25 °C then measuring with a pH meter (Accumet model 10, Denver Instrument Company, Denver, CO). The cocoa liquor sample size was larger to keep the non-fat solids content the same for all samples.

3.3.2 Selected ion flow tube-mass spectrometry (SIFT-MS)

Each cocoa liquor sample (20 g) or cocoa powder sample (20 g) was weighed without melting, transferred into 500 ml sealed bottles (Pyrex 1395, Corning, NY), added 50 ml pure water and warmed at 70°C in a water bath (Circulating bath model 260,
Precision Inc., Winchester, VA) for 1 h. The sample bottle was shaken and immediately transferred into a temperature-protecting sleeve, where the cap of the bottle was changed to a polytetrafluoroethylene-faced silicone septa (Pyrex 1395-45TS, Corning, NY) so that the inlet needle of the Selected Ion Flow Tube Mass Spectrometer (SIFT-MS) (Voice 100, Syft Technologies Ltd., Christchurch, New Zealand) could pierce the cap and draw volatiles from the headspace. The SIFT-MS instrument was described by Smith and Spanel (2005). The inlet needle (18 gauge) had been passivated to minimize reactions with the surface. Another long needle (14 gauge) was put at the bottom of the sample to circulate air inside the sample bottle.

In the SIFT-MS, analysis was performed using selected ion mode (SIM) scans, and the concentrations of volatile compounds were quantified from their reactions with the precursor ions H$_3$O$^+$, O$_2^+$ or NO$^+$ based on known kinetic parameters (Table 2). Applying the predetermined reaction rate constant for the volatile with the precursor ion, and accounting for dilution of the sample gas into the carrier gas, the concentration of the volatile was calculated (Smith and Spanel 1996). Calibration is performed each morning with a known concentration of benzene, toluene, ethylbenzene, and xylene.

Different volatiles can produce the same mass/charge (m/z) value, which creates conflicts that must be removed otherwise the results must be reported as a mixture. Thus the m/z values produced by reaction with one of the three precursor ions were carefully chosen based on published data for each compound (Table 1). The m/z values in Table 1 were chosen because they were not produced by other compounds in the sample and, thus, uniquely measured the stated compound, with the exceptions of 2,3-, 2,5- and 2,6-
dimethylpyrazine; and 2- and 3-methylbutanal, which were reported as mixtures. Compounds without known reaction kinetics, and compounds that had conflicts, except the ones just mentioned, were not reported in this study.

The parameters of the SIFT-MS were set as: inlet flow rate 120 cm$^3$/min, scan time 60 sec, calculation delay time 5 sec, product sample period 100 msec, precursor sample period 20 msec, heated inlet temperature 120°C, carrier gas argon pressure 200 kPa, helium pressure 30 psi. The flow tube vacuum pressure was 0.038±0.003 Torr.

3.3.3 Statistical analysis

One way ANOVA with Tukey HSD was performed to analyze for statistical differences between samples with different pH values, and a $t$-test was used to compare the samples alkalized before roasting and alkalized after roasting but having similar pH values. Significance was defined as $p < 0.05$.

3.4 Results and discussion

Alkylpyrazines and aldehydes are among the main compounds formed through the Maillard reaction and Strecker degradation during roasting, and both are major contributors to chocolate flavor (Bonvehi and Coll 2002, Dimick and Hoskin 1999). Many of these volatile compounds have relatively low threshold levels (Table 2), have an important influence on chocolate flavor and can be used as aroma quality indicators for chocolate products.
3.4.1 Alkylpyrazines in cocoa liquors

When the pH was above 7.0, there was a significant increase in the concentration of all the alkylpyrazines for both alkalized-before-roasting and alkalized-after-roasting cocoa liquors, because alkali conditions favor the formation of alkylpyrazines (Figure 3.1-2). The Maillard reaction can be divided into three stages: sugar-amine condensation and Amadori rearrangement; sugar dehydration and fragmentation and amino acid degradation; and formation of heterocyclic nitrogen compounds (Davies and Labuza 1997). Reducing sugars in the open-chain configuration and amino groups in the -NH$_2$ form are active reactants for the first stage of the Maillard reaction (Martins and others 2001). Basic conditions were more effective than acid in promoting the open ring formation of the reducing sugar at 70 °C (Yaylayan and others 1993). Also, basic condition facilitates the deprotonation of the amino group on the protein into the more reactive -NH$_2$ form (Davies and Labuza 1997). Therefore, the increase of the reactants (open-chain reducing sugar and amine in -NH$_2$ form) under alkali conditions accelerates the first stage reaction.

The alkali pH also alters which intermediates are formed during the second stage. Basic pH favors the formation of dehydroreductone intermediates (1-deoxyhexuloses) and fission products including acetol, pyruvaldehyde and diacetyl (Martins and others 2001). These dicarbonyls react with amino acids to yield the Strecker aldehydes and aminoketones which are converted via dimerization to alkylpyrazines (Davies and Labuza 1997). Thus at high pH the first stage is accelerated and more alkylpyrazine
precursors are formed in the second stage so more alkylpyrazines are formed than at low pH.

In the basic pH range, both alkalized-before-roasting and alkalized-after-roasting samples have increased levels of volatiles, but there is a greater increase in the alkalized-before-roasting samples (Figure 3.1-2). Comparing the samples around pH 8, there are significantly higher levels of volatiles in the alkalized-before-roasting samples. Most alkylpyrazines form during the Maillard reaction, which is accelerated at temperatures above 100°C (Koehler and Odell 1970). An increase in temperature leads to an increase in the reactivity between the sugar and the amino group (Martins and others 2001), thus the roasting process at 150 °C promotes this reaction. During roasting, the pH of the alkalized-after-roasting sample was only 5.2, which is the natural pH of cocoa beans, thus the formation of alkylpyrazines is slow. However, in the alkalized-before-roasting samples, the pH is above 7.0 during roasting, and the high pH accelerates the first stage and favors the formation of alkylpyrazines in the second stage of the Maillard reaction (Martins and others 2001). Therefore higher concentrations of alkylpyrazines were detected in alkalized-before-roasting cocoa liquors than alkalized-after-roasting liquors.

The Maillard reaction occurs rapidly at the high temperature of roasting, but this reaction also occurs at relatively moderate temperatures when basic conditions are present, for example, during drying (70 °C for 2 h) after alkalization. Thus in the alkalized-after-roasting samples, the cocoa liquors with a pH higher than 7.0 have higher concentrations of alkylpyrazines than those with a pH lower than 7.0 (Figure 3.1-2). The Maillard reaction is known to be significant at moderate temperature and high pH. For
instance, the rate of formation of Amadori products at 80 °C increased as the pH increased above 7.0 (Ge and Lee 1997). At 75 °C, the rates of pyrazine formation and the number of types of alkylpyrazines increased as pH increased from 5.0 to 9.0 (Leahy and Reineccius 1989). At 72 °C, the level of the volatiles produced by the Maillard reaction increased from pH 6 to 8 (Blank and others 2003).

When the pH was below 7.0, no significant differences were found in the concentrations of alkylpyrazines in cocoa liquors alkalized-before-roasting and alkalized-after-roasting (Figure 3.1-2). Since the formation of alkylpyrazines is slow at acidic pH, there was no significant difference between the samples.

3.4.2 The effect of pH on partition coefficient

When cocoa liquor was alkalized and held at 40 °C, there was no significant change in the concentration of alkylpyrazines with pH from 5.2 to 8.0. Given that alkylpyrazines are basic, it would seem logical that volatility would decrease at low pH, but in this study, pH itself, without an increase in temperature to create the Maillard reaction products, did not produce a significant effect on volatile concentrations in the gas phase. When samples were held at 70 °C, TrMP, 2- and 3-methylbutanal and phenylacetaldehyde increased when pH increased above 7.0 (Figure 3.3), indicating that the Maillard reaction occurred rapidly in alkali conditions at this temperature. The other compounds did not change. However, the concentrations of the alkylpyrazines and Strecker aldehydes in the alkalized cocoa liquor samples were much lower than that in the alkalized cocoa bean samples, because the beans were heated much longer than the liquor, promoting the Maillard reaction.
3.4.3 The effect of pH on the ratios between alkylpyrazines

In the alkalized-before-roasting cocoa liquors, the ratio of TrMP to DMP increased from 0.79 to 2.29, and the ratio of DMP to TMP increased from 0.3 to 2.3 when the pH increased from 5.9 to 7.9 (Figure 3.4). The same trend was found in alkalized-after-roasting samples, where the ratio of TrMP to DMP and DMP to TMP increased when the pH increased from 6.0 to 8.4 (Figure 3.5). The change in the ratio of the different alkylpyrazines demonstrates that increasing pH favors the formation of TrMP and DMP, followed by TMP. Either the precursors or intermediates for forming TrMP and DMP must be more available than those for forming TMP as pH increases. Many of the amino acids have been shown to be precursors to TrMP and DMP, but not many are precursors for TMP. TrMP and DMP can be formed from alanine, valine, leucine, phenylalanine and threonine (Amrani-Hemaimi and others 1995, Arnoldi and others 1988). TrMP can also be formed from glycine and DMP from lysine, asparagine and glutamate. However, leucine is the only identified amino acid precursor for TMP (Arnoldi and others 1988). Bonvehi and Coll (2002) found the ratio of TMP to TrMP increased as the pH increased, opposite to our results. However, they used a different variety of cocoa beans, removed most of the cocoa butter, added water, and then roasted at a lower temperature for a longer time, thus the variety and/or exact conditions during roasting greatly affect the rate of formation of different alkylpyrazines.
Sensory evaluation has determined that the degree of roasting is normal if the ratio of TMP/TrMP is close to 1, in which case the cocoa liquors show a normal aromatic quality (Hashim and Chaveron 1994). If this ratio is greater than 1, the cocoa aromatic quality is poor because the concentration of TMP has not yet declined and not enough TrMP has developed (Hashim and Chaveron 1994). A ratio less than 1 indicates trimethylpyrazine is over-developed and a burnt flavor is present (Hashim and Chaveron 1994). No one has tested whether this ratio also works for alkalized cocoa, but if it does, the roasting conditions need to be adjusted for pH changes. For the samples with a pH below 6.5, a longer roasting time or higher temperature is necessary to increase the concentration of TrMP. For the samples with a pH above 7.0, the roasting time should be shorter or the temperature lower.

3.4.4 Alkylpyrazines in commercial cocoa powders

Commercial cocoa powder samples followed a similar trend of alkylpyrazines increasing with increasing pH (Figure 3.6). There was no significant change in the concentrations of alkylpyrazines between the samples at pH 5.74 and 7.07, while the alkylpyrazines increased for the samples at pH 7.39 and 7.84. But the alkylpyrazines at pH 8.04 were not higher than those with lower pH values (Figure 3.6). The commercial samples cannot be expected to match the laboratory results exactly since each commercial sample was made by a different, proprietary method unknown to these authors. However, it is known that the cocoa powder sample at pH 5.74 is unalkalized. The samples with pH 7.07, 7.39, 7.84 are probably made with 1-6% potassium carbonate at moderate temperatures for 30-60 min, while 4-6% sodium hydroxide with an oxidizer
and compressed air at a higher temperature for 1 h is probably used to produce the pH 8.04 sample (Ellis 1992). The pH 8.04 sample, a dark red cocoa powder, is intentionally made to have less flavor (Ellis 1992), which is shown by the lower level of alkylpyrazines.

3.4.5 Strecker aldehydes in cocoa liquors

Strecker aldehydes such as 2- and 3-methylbutanal, 2-methylpropanal, and phenylacetaldehyde, are products of roasting but are already present in the unroasted cocoa due to their biochemical formation during growth and fermentation (Granvogl and others 2006). The effect of pH during roasting on the formation of Strecker aldehydes showed a similar pattern to that of alkylpyrazines. In the samples with pH values below 7.0, there were no significant changes in the concentrations of 2- and 3-methylbutanal, 2-methylpropanal and phenylacetaldehyde in either alkalinized-before-roasting or alkalinized-after-roasting samples, while the concentrations of these aldehydes increased when the pH was above 7.0 (Table 4). Since alkali conditions favor the formation of dicarbonyl compounds during Strecker degradation, a significant increase in Strecker aldehydes was expected in cocoa liquors with a pH above 7.0.

Other volatiles such as furans, ketones, alcohols, nitrogen compounds, sulfur compounds, hydrocarbons and phenol also showed an increase in concentration with increasing pH for both alkalinized-before-roasting and alkalinized-after-roasting cocoa liquors (Table 4). Many of these volatile compounds are formed during the Maillard reaction and hence are promoted under alkali conditions.
3.5 Conclusions

The concentration of alkylpyrazines in cocoa liquor increased as the pH increased, and cocoa liquors with higher pH have a higher concentration of aroma compounds. Altering the pH alone did not affect the concentration of aroma compounds in the gas phase, except when samples were heated, which promoted the Maillard reaction. The ratios of TrMP/DMP and DMP/TMP increased while the ratio of TMP/TrMP decreased as the pH increased. The roasting conditions may be adjusted based on pH changes to produce optimum aroma.

3.6 References


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Table 3.1. Information of selected volatile compounds for SIFT-MS analysis.

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Precursor ion</th>
<th>k (10^-9 cm^3 s^-1)</th>
<th>m/z</th>
<th>Product ion</th>
<th>Reference</th>
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<td>3.3</td>
<td>$C_8H_8\text{N}^+$</td>
<td>Wang and others 2004b</td>
</tr>
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<td>$C_2H_6\text{S}_3^+$</td>
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<td>Toluene</td>
<td>$\text{NO}^+$</td>
<td>1.7</td>
<td>$C_7H_8^+$</td>
<td>Spanel, Smith 1998b</td>
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<td>$C_6H_6\text{O}^+$</td>
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Table 3.2. Odor thresholds in water of some alkylpyrazines and aldehydes.

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<th>Compounds</th>
<th>Odor description</th>
<th>Threshold level (ppb)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>2,3-dimethylpyrazine</td>
<td>Nutty</td>
<td>80</td>
<td>Mihara and Masuda 1988</td>
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<td>2,5-dimethylpyrazine</td>
<td>Strong nutty</td>
<td>38</td>
<td>Fors and Olofsson 1985</td>
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<td>2,6-dimethylpyrazine</td>
<td>Green nutty</td>
<td>57</td>
<td>Fors and Olofsson 1985</td>
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<td>2,3,5-trimethylpyrazine</td>
<td>Musty, nutty, caramel, sweet</td>
<td>38</td>
<td>Fors and Olofsson 1985</td>
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<td>2,3,5,6-tetramethylpyrazine</td>
<td>Walnuts, green</td>
<td>120</td>
<td>Fors and Olofsson 1985</td>
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<td>2,3-diethyl-5-methylpyrazine</td>
<td>Earthy, bitter, green, tallow</td>
<td>4</td>
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<td>2-methylbutanal</td>
<td>Malty</td>
<td>140</td>
<td>Rychlik and others 1998</td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td>Malty</td>
<td>13</td>
<td>Rychlik and others 1998</td>
</tr>
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<td>2-methylpropanal</td>
<td>Malty</td>
<td>0.1-2.3</td>
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<td>phenylacetaldehyde</td>
<td>Honey, sweet</td>
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Table 3.3. Concentrations (ppb) of aldehydes and other volatiles in cocoa liquors with different pH values.

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<th>7.87</th>
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<td>22302</td>
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<td>2- and 3-methylbutanal</td>
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<td>28227</td>
<td>23090</td>
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<td>5233</td>
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<td>(E,Z)-2,6-nonadienal</td>
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<td>157</td>
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<td>438</td>
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Figure 3.1. 2,3-, 2,5- and 2,6-dimethylpyrazines (DMP) and 2,3,5-trimethylpyrazine (TrMP) in cocoa liquors alkalized-before-roasting or alkalized-after-roasting to different pH values, with 95% confidence interval error bars.
Figure 3.2. 2,3,5,6-tetramethylpyrazine (TMP) and 2,3-diethyl-5-methylpyrazine (EMP) in cocoa liquors alkalized-before-roasting or alkalized-after-roasting to different pH values with 95% confidence interval error bars.
Figure 3.3. Alkylpyrazines and Strecker aldehydes in cocoa liquor alkalized to different pHs. 2- and 3-methylbutanal is plotted on the right hand axis with 95% confidence interval error bars.
Figure 3.4. The ratio between alkylpyrazines in cocoa liquors alkalized-before-roasting (DMP-dimethylpyrazines, TrMP-trimethylpyrazine, TMP-tetramethylpyrazine).
Figure 3.5. The ratio between alkylpyrazines in cocoa liquors alkalized-after-roasting (DMP-dimethylpyrazines, TrMP-trimethylpyrazine, TMP-tetramethylpyrazine).
Figure 3.6. 2,3-, 2,5- and 2,6-dimethylpyrazines (DMP), 2,3,5-trimethylpyrazine (TrMP), 2,3,5,6-tetramethylpyrazine (TMP) and 2,3-diethyl-5-methylpyrazine (EMP) in commercial cocoa powders with 95% confidence interval error bars.
Chapter 4: MONITORING OF COCOA VOLATILES PRODUCED DURING ROASTING BY SELECTED ION FLOW TUBE-MASS SPECTROMETRY (SIFT-MS)

4.1 Abstract

Alkalized and unalkalized Don Homero cocoa beans were roasted at 120, 150 and 170 °C in a rotary roaster. The real-time and end-of-roasting concentrations of cocoa volatiles in the headspace of the roaster were analyzed by selected ion flow tube-mass spectrometry (SIFT-MS). The concentrations of total alcohol, acids, aldehydes, esters, ketones and alkylpyrazines reached peak concentrations within the first 15 min roasting. The concentrations of alkylpyrazines and Strecker aldehydes increased as the roasting temperature increased from 120 to 170 °C. For most of the volatile compounds compared, there was no significant difference between Arriba and Don Homero beans, but Arriba beans showed higher concentrations of 2-heptanone, acetone, ethyl acetate, methylbutanal, phenylacetaldehyde and trimethylpyrazine than Don Homero beans. For unalkalized Don Homero beans (pH 5.7), the time to peak concentration decreased from 13.5 to 7.4 min for pyrazines, and from 12.7 to 7.4 min for aldehydes as the roasting temperature increased from 120 to 170 °C. Also, at 150 °C roasting, the time to peak concentration was shortened from 9.0 to 5.1 min for pyrazines, and from 9.1 to 5.0 min for aldehydes as the pH increased from 5.7 to 8.7.
Keywords: cocoa, Maillard reaction, roasting, volatiles

4.2 Introduction

After harvest, cocoa beans are fermented, dried and roasted. The fermentation process not only includes an external microbial fermentation which produces acetic acid and heat to prevent the germination of cocoa bean, but also activates the internal autolytic reaction, and promotes the formation of flavor precursors such as amino acids and reducing sugars (Martin 1987). The drying process stabilizes the cocoa beans for long-term storage and improves the flavor by oxidizing and polymerizing polyphenols. The most important step in the development of cocoa flavor is the roasting step because the cocoa aroma is formed during this process. There are two main purposes of roasting: the formation of typical roasted, sweet odorants of cocoa, and the removal of undesired compounds with low boiling points, such as acetic acid (Keeney 1972). The thermal treatment initiates the Maillard reaction between amino acids and reducing sugars in cocoa beans, which generates most of the aromatic volatiles contributing to the flavor of chocolate. To date, more than 600 volatile compounds have been identified in cocoa, including pyrazines, aldehydes, ethers, thiazoles, phenols, ketones, alcohols, furans and esters (Dimick and Hoskin 1981). Pyrazines and aldehydes are the major compounds formed during roasting (Heinzler and Eichner 1992), and they are the most important contributors to the desirable chocolate aroma (Counet and others 2002).

The concentrations of monomethyl-, 2,3-dimethyl-, 2,5-dimethyl-, 2,6-dimethyl-, trimethyl- and tetramethylpyrazine increase rapidly during roasting of cocoa beans (Hashim and Chaveron 1994). In roasted Criollo cocoa beans, twenty-seven compounds
were found in concentrations exceeding their odor thresholds. Of the aroma compounds quantified, only acetic acid was significantly reduced, while 15 compounds including Strecker aldehydes such as 3-methylbutanal and phenylacetaldehyde, and alkylpyrazines such as 2,3,5-trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine increased in their concentrations (Frauendorfer and Schieberle 2008).

The effect of roasting temperature and time on the concentrations of nineteen volatile compounds, including nine pyrazines, five aldehydes, one methyl ketone, two alcohols and two esters in roasted commercial Malaysian cocoa beans were investigated by Ramli and others (2005). They concluded that the quantities of trimethylpyrazine and tetramethylpyrazine could be used as indicators of the roasting process, and 30-40 min roasting at 150 °C was recommended as the optimum roasting conditions for Malaysian cocoa beans.

Alkalization is predominantly used to manufacture cocoa powder, which facilitates the solubility and influences the color of cocoa. The pH of the cocoa beans also affects the generation of volatile compounds during roasting because pH is an important factor affecting the Maillard reaction.

The concentrations of volatiles in roasted cocoa beans vary according to the types and origins of the beans. After roasting, Ghanaian beans have 698 µg/100g pyrazines, Ecuador (Arriba) beans have 238 µg/100g, while Mexico (Tabasco) beans contain as little as 142 µg/100g (Reineccius and others 1972). Caracas and Trinidad beans have high concentrations of isovaleraldehyde and isobutyraldehyde. The Arriba, Bahia, and Acca
beans have moderate, while Costa Rican, Sanchez and Tabasco beans have low concentrations of these aldehydes (Dimick and Hoskin 1999).

Roasting conditions affect cocoa aroma quality because the Maillard reaction is affected by temperature and the time retaining at this temperature. Despite the fact that roasting is the most important process in generating the key odorants of cocoa, the continuous change of these volatile compounds during roasting is unclear. Thus real-time monitoring of the concentrations of important volatiles during roasting becomes important, which provides direct and rapid results of the concentrations of important volatile compounds and aids in determining the preferred roasting temperature and time.

Many studies have measured cocoa and chocolate volatiles using gas chromatography-mass spectrometry. Selected ion flow tube mass spectrometry (SIFT-MS) allows highly sensitive, real-time analysis of complex mixtures of volatile compounds without trapping or preconcentration (Spanel and Smith 1999). Thus the preparation of sample is simplified and artifacts or changes in relative proportions are not introduced by the preconcentration steps. This technology has been used in the analysis of volatiles in coffee, onion, banana (Smith and Spanel 2005), tomatoes (Xu and Barringer 2009), garlic (Hansanugrum and Barringer 2010), jalapeños (Azcarate and Barringer 2010) and oxidation of olive oil (Davis and McEwan 2007).

The purpose of this study was to apply SIFT-MS for real-time monitoring of the important volatile compounds during roasting hence elucidating how roasting temperature and pH affect the formation of important cocoa volatile compounds.
4.3 Materials and methods

4.3.1 Cocoa bean samples

Two types of fermented and dried cocoa beans from Ecuador were used in this study. One was Don Homero (Chocolate Alchemy Co., Eugene, OR), and the other was Arriba (The Hershey Company, Hershey, PA). The cocoa beans were stored at -18 °C in the freezer. Prior to roasting, they were thawed at room temperature for 2 h. To compare the volatiles of cocoa beans with different pH values, the unalkalized cocoa beans (pH 5.7) were treated with alkali solution to make alkalized cocoa bean samples with pH values at 7.2 and 8.7. The alkalized samples were prepared by soaking the thawed cocoa beans in 1 L potassium carbonate (Sigma-Aldrich Inc., St, Louis, MO) aqueous solution for 3 h, then removing the alkali solution by passing through a screen, and drying at 70 °C for 3 h before roasting. The concentrations of the alkali solutions were 100 g/L and 250 g/L. The cocoa beans were weighed (300 g per batch), fed into a roaster (Model CBR-101 Gene Café, Genesis, Hwasung-Si, Kyungki-Do, South Korea) and roasted at 120, 150 and 170 °C separately. The roasting time varied from 15 to 60 min.

The pH values of the cocoa beans were determined by grinding the cocoa beans into cocoa liquor. The cocoa liquor (20 g) was then placed into a 150 mL beaker, adding 90 mL boiling hot distilled water with stirring, passing through filter paper, cooling to 20 °C, and measuring with a pH meter (Accumet model 10, Denver Instrument Co., Denver, Colo., U.S.A).
4.3.2 SIFT-MS analysis

For the cocoa bean samples, the sampling needle of the SIFT-MS was inserted into the headspace of an aluminum vent pipe which was connected to the outlet of the roaster so that the instrument could continuously detect the concentrations of the volatiles produced during roasting. Glass wool was placed in the roaster outlet to prevent chaff from being sucked into the sampling needle. The heating temperature for the capillary and the arm of the SIFT-MS inlet was adjusted to 180 °C so that the volatiles did not cool down when sampled into the SIFT-MS.

The total concentration of each volatile compound category was calculated by summing up all the individual concentration of each compound in the same category. For instance, Total concentration of pyrazines is the sum-up of the concentrations of 2-methylpyrazine, dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine and 2, 3-diethyl-5-methylpyrazine.

The in-jar cocoa bean sample was prepared by taking 50g roasted cocoa beans out of the roaster, putting them in a sealed jar, and equilibrating in a 50 °C waterbath for 90 min before tested by SIFT-MS.

A method for roasted cocoa volatiles was developed and imported into the SIFT-MS. There are 60 volatile compounds in this method, including 5 pyrazines, 13 aldehydes, 8 ketones, 8 alcohols, 5 acids, 4 furans, 3 esters, 5 nitrogen compounds, 2 phenols, 5 hydrocarbon and 2 sulfur compounds. The concentrations of volatile compounds were quantified from their reactions with the precursor ions $\text{H}_3\text{O}^+$, $\text{NO}^+$, or $\text{O}_2^+$ based on known kinetic parameters (Table 1). The m/z values produced by reaction with one of the
three precursor ions were carefully chosen to avoid conflicts (different volatiles produce the same \( m/z \) value) based on published data (Table 1). The \( m/z \) values in Table 1 were chosen because they were not produced by other compounds in the sample and, thus, uniquely measured the stated compound, with the exceptions of 2,3-, 2,5- and 2,6-dimethylpyrazine, which were reported as dimethylpyrazine, the mixture of the three dimethylpyrazines; as well as 2-methylbutanal and 3-methylbutanal, which were reported as methylbutanal, the mixture of the two methylbutanals. Also, compounds with irresolvable conflicts or concentrations below the detection threshold are not reported.

4.3.3 Parameter settings of SIFT-MS

The parameters of the SIFT-MS was set as: inlet flow rate 120 cm\(^3\)/min, scan time 3600 sec for 60 min roasting, 900 sec for 15 min roasting, calculation delay time 5 sec, product sample period 100 msec, precursor sample period 20 msec, heated inlet temperature 180\(^\circ\)C, carrier gas argon pressure 200 kPa, helium pressure 30 psi. The flow tube vacuum pressure was 0.038±0.003 Torr.

4.3.4 Statistical Analysis

Two factors analysis of variance (ANOVA) was performed to analyze for statistical differences between the two cocoa bean varieties. One factor is the roasting temperature, and the other is the type of the cocoa beans. Significance was defined as \( p < 0.05 \).
4.4 Results and discussion

4.4.1 Real-time concentration vs. roasting time

The concentration of total alkylpyrazines, aldehydes, alcohols, acids, esters and ketones in the headspace of the roaster increased as the roasting time increased, reached a peak concentration within the first 15 min then quickly decreased and leveled off (Figure 4.1). To validate that these results were not an artifact of the air flow in the roaster or the sampling method, cocoa beans were removed from the roaster every 3 min during roasting, placed in a jar, and the headspace analyzed. In both roaster and jar headspace, the volatiles followed the same pattern. Ramli and others (2005) also found a peak and fall during their roasting study.

Free amino acids and reducing sugars are the flavor precursors which interact during the roasting process to produce cocoa volatiles. These precursors are abundant in the fermented and dried cocoa beans at the very beginning of roasting, thus the generation rate of these volatiles is very fast. The volatiles accumulate in the headspace of the roaster and their concentrations continuously increase to a peak. As the roasting proceeds, both the free amino acids and the reducing sugars are consumed. More than 85% of the total free amino acid is consumed by roasting at 135 °C for 3 min (Bonvehí and Coll 2002), and about 70% of the glucose and fructose is consumed at 140 °C in 15 min (Rohan and Stewart 1966). The rate of generation of new volatiles slows down so the concentrations decrease until a balance between the generation rate and loss rate is established where the concentrations do not change greatly. The roaster used was a
convection roaster with highly efficient heat-transfer, thus most volatiles peaked in concentration within the first 15 min of roasting.

4.4.2 The effect of roasting temperature on the volatiles

Alkylpyrazines and Strecker aldehydes are important compounds in cocoa volatiles. Both categories are major contributors to chocolate flavor (Dimick and Hoskin 1999; Bonvehí and Coll 2002). The concentrations of these important volatiles, including dimethylpyrazine, trimethylpyrazine, acetaldehyde and methylbutanal, increased in the roaster as the roasting temperature increased from 120 °C to 170 °C (Figure 4.2–4.5).

Roasting below 120 °C is regarded as slightly roasted (Ziegleder 1982). Only a low level of alkylpyrazines (below 20 ppb) and a small amount of aldehydes (less than 100 ppb) were produced at this temperature. Roasting between 120 °C and 140 °C is defined as normally roasted, between 140 °C and 160 °C is strongly roasted and above 160 °C is over-roasted (Ziegleder 1982). At 150 °C, the peak concentration of dimethylpyrazine and trimethylpyrazine was increased to 60 ppb, and the acetaldehyde and methylbutanal to 250 ppb. At 170 °C, the peak concentrations of the alkylpyrazines further increased to 180 ppb, and the aldehydes to 350–450 ppb. This temperature effect occurs because of different pathways in the Maillard reaction. In stage two of the Maillard reaction, the Amadori product is degraded by one of three main pathways depending on the conditions (Figure 4.6). The pathways are through reductones, fission products, or Schiff base of hydroxymethylfurfural (HMF). High temperature favors the formation of fission products such as acetol, diacetyl and pyruvaldehyde. These dicarbonyls are very reactive and they react with amino acids to form Strecker aldehydes and aminoketones which are
converted via dimerization to yield pyrazines at higher temperatures (Davies and Labuza 1997). Other pathways may also form aldehydes and pyrazines but this is the major pathway. Therefore, high temperature favors the formation of alkylpyrazines and Strecker aldehydes. Hashim and Chaveron (1994) also demonstrated that the concentrations of all methylpyrazines, except for tetramethylpyrazine, increased linearly in relationship with the roasting temperature.

During roasting, dimethylpyrazine and trimethylpyrazine had similar peak concentrations (170-180 ppb) at 170 °C (Figure 4.2-4.3), and both were higher than tetramethylpyrazine, which was only 40 ppb at its peak concentration. Dimethylpyrazine has eight amino acid precursors, including alanine, valine, leucine, phenylalanine, threonine, lysine, asparagine and glutamate. Trimethylpyrazine has six, including alanine, valine, leucine, phenylalanine, threonine and glycine (Arnoldi and others 1988; Amrani-Hemaimi and others 1995), while only leucine is identified as the precursor for tetramethylpyrazine (Arnoldi and others 1988). Seventeen free amino acids, including all of these pyrazine precursors, are present in cocoa beans (Bonvehí and Coll 2002). Among them, the total content of the precursors for dimethylpyrazine and trimethylpyrazine were 283 and 191 mg/100g separately, but the leucine content was only 29 mg/100g (Bonvehí and Coll 2002). The precursor amino acids to form dimethylpyrazine and trimethylpyrazine are abundant while the precursor to form tetramethylpyrazine is limited. Thus, more dimethylpyrazine and trimethylpyrazine were formed during roasting than tetramethylpyrazine.
The peak concentrations of Strecker aldehydes at 170 °C, 400 ppb for acetaldehyde and 350 ppb for methylbutanal (Figure 4.4-4.5), were higher than that of alkylpyrazines (180 ppb), indicating the generation of these aldehydes is greater than the alkylpyrazines or the volatility of these aldehydes in roasted cocoa beans is higher than alkylpyrazines. However, alkylpyrazines have a higher Henry’s Law Constant than aldehydes, so the concentration of alkylpyrazines should be higher if both volatile compounds were generated in equal volume. Since the alkylpyrazine concentration in the headspace is lower than the aldehyde concentration, the generation of aldehydes must be larger than that of alkylpyrazines. Alkylpyrazines are the advanced Maillard reaction products, while Strecker aldehydes are intermediates. These intermediates are generated during roasting and only a portion of them continue reacting with amino acids to form the alkylpyrazines or aldimines.

4.4.3 Type of beans

Arriba cocoa is from the variety Nacional, which has a full cocoa aroma with additional floral, spicy and green flavors, and is a premium Ecuador cocoa often used in fine dark chocolate (Fowler 2009). Don Homero cocoa is a hybrid variety (CCN51) which isn’t classified as a fine cocoa and is said to have acidic and harsh flavors (Afoakwa and others 2008). During roasting of both types of beans, the concentration of all volatile compounds increased as temperature increased from 120 °C to 170 °C, and the time to peak concentration was quite similar. For most of the volatile compounds compared, there was no significant difference between the two types of beans, but Arriba beans did have significantly higher concentrations of 2-heptanone, acetone, ethyl acetate,
methylbutanal, phenylacetaldehyde and trimethylpyrazine than the Don Homero beans (Table 2). 2-Heptanone has a spicy aroma (Ansorena and others 2001), acetone has a faintly aromatic, sweetish aroma, ethyl acetate has a fruity and sweet aroma, methylbutanal has the aroma of chocolate, phenylacetaldehyde has a flowery and honey aroma, and trimethylpyrazine has the aroma of cocoa, roasted and green (Counet and others 2002). The flowery, spicy and green aromas represent the typical Arriba aroma. The higher concentrations of 2-heptanone, acetone, ethyl acetate, phenylacetaldehyde, methylbutanal and trimethylpyrazine in Arriba than that in Don Homero may suggest the flavor difference between the two varieties.

4.4.4 Time to peak concentration

Both temperature and pH affect the time to peak concentration during roasting. For the samples with the same pHs, the ones roasted at higher temperatures showed a shorter time to reach the peak concentration. The time to peak concentration of unalkalized Don Homero beans decreased from 13.5 to 7.4 min for pyrazines, and from 12.7 to 7.4 min for aldehydes as the roasting temperature increased from 120 to 170 °C. The effect of temperature is best defined by the temperature dependence of the rate constant in the Arrhenius equation (Davies and Labuza 1997), in which the relationship between the rate constant and the temperature is exponential. An elevated roasting temperature promotes the reactions between the amino acids and the reducing sugars. Also, under the same roasting temperature, the time to peak concentration was shortened as the pH increased. For example, at 150 °C roasting, the time to peak concentration decreased from 9.0 to 5.1
min for pyrazines, and from 9.1 to 5.0 min for aldehydes as the pH increased from 5.7 to 8.7 (Table 3).

The pH of cocoa beans is another important factor affecting the time to peak concentration during roasting because pH influences the reaction velocity. The cocoa beans at pH 8.7 showed the highest concentration of dimethylpyrazine, followed by cocoa beans at pH 7.2 and 5.7 (Figure 4.7), which matches the cocoa liquor results found in a previous study (Yang and Barringer 2010). Also the time to peak of dimethylpyrazine at pH 8.7 (4.8 min) occurred earlier than samples at pH 7.2 (8.0 min) and pH 5.7 (9.6 min) when roasted at 150 °C (Figure 4.7). Basic condition (pH > 7.0) favors the formation of reducing sugars in the open-chain configuration. It also facilitates the deprotonation of the amino group on the protein into the –NH₂ (Davies and Labuza 1997). Reducing sugars in the open-chain configuration and amino acids in the –NH₂ form are active reactants for the first stage of the Maillard reaction (Martins and others 2001). Since more active reactants are available at basic conditions than acidic conditions, the Maillard reaction occurred quickly and the reaction products, aldehydes and pyrazines, reach peak concentration quickly and the time to peak is shortened. Temperature and pH are crucial factors affecting the Maillard reaction. High temperature and basic conditions accelerate the Maillard reaction, thus these volatiles are accumulated in a shorter time.

4.5 Conclusions

Most volatiles, including pyrazines, aldehydes, alcohols, acids, esters and ketones were generated and reached maximum concentrations within the first 15 min of roasting. The real-time concentrations of alkylpyrazines and Strecker aldehydes increased as the
roasting temperature increased. Arriba cocoa showed higher concentrations of some important alkylpyrazines and aldehydes than Don Homero cocoa. The peak concentration occurs earlier as the roasting temperature or the pH of the cocoa increases.

4.6 References


Table 4.1. Information of selected volatile compounds for SIFT-MS analysis

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<th>Volatile compounds</th>
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<th>$m/z$</th>
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<td>Spanel and Smith 1998</td>
</tr>
<tr>
<td>acetic acid</td>
<td>( \text{NO}^+ )</td>
<td>9.0</td>
<td>90</td>
<td>( \text{CH}_3\text{COOH}\text{NO}^+ )</td>
<td>Spanel and Smith 1998</td>
</tr>
</tbody>
</table>
Table 4.2. Concentrations (ppb) of volatile compounds in the headspace of roasted Arriba and Don Homero Ecuadorian cocoa beans at the end of 15 min roasting.

<table>
<thead>
<tr>
<th>Bean type</th>
<th>Arriba</th>
<th></th>
<th></th>
<th>Don Homero</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Roasting temperature (C)</td>
<td>120</td>
<td>150</td>
<td>170</td>
<td>120</td>
<td>150</td>
<td>170</td>
</tr>
<tr>
<td>(E)-2-nonenal</td>
<td>3</td>
<td>12</td>
<td>15</td>
<td>4</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>(E)-2-octenal</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>(E,E)-2,4-decadienal</td>
<td>0</td>
<td>25</td>
<td>20</td>
<td>1</td>
<td>7</td>
<td>31</td>
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<tr>
<td>1-octen-3-one</td>
<td>2</td>
<td>16</td>
<td>26</td>
<td>0</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>2,3-butanedione</td>
<td>38</td>
<td>73</td>
<td>62</td>
<td>26</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>2,3-diethyl-5-methylpyrazine</td>
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<td>2</td>
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<td>0</td>
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<td>32</td>
<td>3</td>
<td>9</td>
<td>43</td>
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<td>107</td>
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<td>73</td>
<td>14</td>
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<tr>
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<td>29</td>
<td>6</td>
<td>17</td>
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<td>9</td>
<td>5</td>
<td>2</td>
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<td>2-phenethyl acetate</td>
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<td>46</td>
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<td>93</td>
<td>86</td>
<td>16</td>
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<tr>
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<td>acetaldehyde</td>
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<td>14</td>
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<td>22</td>
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<td>111</td>
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<td>36</td>
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<td>63</td>
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<td>dimethyl disulphide</td>
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<td>53</td>
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<td>7</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
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<td>0</td>
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Continued
Table 4.2 continued

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<th>Don Homero</th>
</tr>
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<td>ethanol</td>
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<td>84</td>
</tr>
<tr>
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<td>109</td>
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<tr>
<td>furan</td>
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<td>6</td>
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<tr>
<td>indole</td>
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</tr>
<tr>
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<td>70</td>
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<tr>
<td>isobutyI acetate</td>
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<td>0</td>
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<td>n-amyl alcohol</td>
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<td>3</td>
</tr>
<tr>
<td>nonanal</td>
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<td>19</td>
</tr>
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<td>49</td>
</tr>
<tr>
<td>phenylacetic acid</td>
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<td>15</td>
</tr>
<tr>
<td>sotolone</td>
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<td>43</td>
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<td>4</td>
</tr>
<tr>
<td>toluene</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>trimethylpyrazine*</td>
<td>11</td>
<td>84</td>
</tr>
<tr>
<td>vanillin</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

* Compounds with significant difference between Arriba and Don Homero beans.
Table 4.3. Roasting time (min) to peak concentration for Don Homero beans.

<table>
<thead>
<tr>
<th>Temperature (C)</th>
<th>120</th>
<th>150</th>
<th>170</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.8</td>
<td>7.6</td>
<td>8.6</td>
</tr>
<tr>
<td>Dimethylpyrazine</td>
<td>–</td>
<td>11.5</td>
<td>–</td>
</tr>
<tr>
<td>Trimethylpyrazine</td>
<td>11.8</td>
<td>11.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Tetramethylpyrazine</td>
<td>12.5</td>
<td>–</td>
<td>7.2</td>
</tr>
<tr>
<td>Total pyrazines</td>
<td>13.5</td>
<td>11.3</td>
<td>5.9</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>12.8</td>
<td>9.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Methylbutanal</td>
<td>12.1</td>
<td>9.3</td>
<td>3.5</td>
</tr>
<tr>
<td>Total aldehydes</td>
<td>12.7</td>
<td>9.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Ketones</td>
<td>12.6</td>
<td>11.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Acids</td>
<td>13.2</td>
<td>12.5</td>
<td>5.9</td>
</tr>
<tr>
<td>Alcohols</td>
<td>14.8</td>
<td>10.5</td>
<td>4.9</td>
</tr>
</tbody>
</table>

-Time to peak concentration can’t be determined.
Figure 4.1. Total concentration of each volatile category in the headspace of unalkalized Don Homero beans for 60 min roasting at 150 °C.
Figure 4.2. Dimethylpyrazine (DMP) in the roaster headspace at different roasting temperatures.
Figure 4.3. Trimethylpyrazine (TrMP) in the roaster headspace at different roasting temperatures.
Figure 4.4. Acetaldehyde in the roaster headspace at different roasting temperatures.
Figure 4.5. Methylbutanal in the roaster headspace at different roasting temperatures.
Figure 4.6. Maillard reaction pathways (adapted from Martins and others 2001)
Figure 4.7. Dimethylpyrazine (DMP) in the real-time roasting Don Homero cocoa beans with different pH values at 150 °C.
Bibliography


Appendix A: Coating method and charge to mass ratio measurement

Figure A.1. Food powder nonelectrostatic and electrostatic coating setup.
q/m = charge measured by electrometer (µQ) / mass of powders on the foil (g)

Figure A.2. Charge to mass ratio measurements
Appendix B: pH change in terms of alkali concentrations and alkalization order

To investigate the relationship between the concentration of the alkali added and the final pH values of the cocoa liquor, K₂CO₃ in different concentrations (0, 42, 70, 95, 114, 150, 168, 186 g/L) were prepared as alkali solutions to soak the cocoa beans before roasting. The final pH value increased with increasing alkali concentration, but the pH value was not linearly correlated to the alkali concentration (Figure C.1). The pH values of the cocoa liquors alkalized by alkali concentrations below 114g/L increased more rapidly than that of the ones alkalized by alkali concentrations above 114g/L. The natural (unalkalized) cocoa is normally acidic because there are many acids formed during the fermentation of cocoa beans. When K₂CO₃ solution was added, it ionizes to KHCO₃, K⁺ and OH⁻ due to the existence of extra H⁺ from the acids. As the neutralization reaction between H⁺ and OH⁻ happened very fast, a rapid increase of pH value was observed. If most extra H⁺ had been neutralized, in this case pH value was very close to 7.0, the increase of pH slowed down.

For the samples alkalized with same alkali concentration, the cocoa liquor alkalized after roasting showed a higher pH value than the one alkalized before roasting (Figure C.1). The high temperature during roasting evaporated the acids with low boiling points in cocoa beans (Frauendorfer and Schieberle 2008). The unroasted cocoa liquor has a pH
value of 5.20, while the roasted cocoa liquor has a pH value of 5.32. Thus fewer volume of alkali solution was required to neutralize the residue acids in roasted cocoa beans. Therefore if same volume of alkali was added, the roasted cocoa beans showed a higher pH value than the unroasted ones.
Figure B. 1. pH of cocoa liquors alkalized by different alkali concentrations.
Appendix C: The Creation and Refinement of the Method for SIFT-MS

C.1 Creation of SIFT-MS methods

The software method used by SIFT-MS for cocoa is established based on the literature. The important aroma volatiles mentioned in the literature include pyrazines, aldehydes, acids, ketones, alcohols and esters (Ramli and others 2006, Frauendorfer and Schieberle 2008). These compounds either naturally exist in cocoa beans or are developed during the fermentation and roasting processes. When creating the chocolate method in the SIFT-MS, all these compounds are added to the method from the library of the software. If a compound is missing from the library, the compound should be put into the user library. A pure sample of this compound must be tested by SIFT-MS thus the reaction kinetics and branching ratios can be determined and the compound can be added to the method. Once all the compounds are added in the method, the method is saved under a name starting with the initials of the user, description of the method and the date. Under the settings tab of the software, the parameters are set as: Scan type: SIM scan; Scan by duration: sample scan time 3600 s (cocoa bean roasting), settle time 5 s; Display units: ppb; Measurement time limits: product 100 ms, reagent 20; Quadrupole setting
times: upstream 25 ms downstream 10. Under compound calculation, secondary chemistry and wet are checked when all masses are showing.

**C.2 Determination of precursor ions to scan and calculate**

The reaction of H$_3$O$^+$ with the large majority of organic compounds proceeds via proton transfer and the great advantage to SIFT-MS analyses of this proton transfer is that they frequently proceed with unit efficiency and usually result in only one or two product ions (Smith and Spanel 2005). The product ion of the proton transfer reaction of H$_3$O$^+$ with molecule M is MH$^+$, whose mass is the mass of the reactant molecule plus 1. So the precursor ion H$_3$O$^+$ is not good to separate isomers because they produce the same mass spectra. When ions are introduced into humid air, clustering H$_3$O$^+$(H$_2$O)$_n$ may occur to the abundant water molecules (Smith and Spanel 1996). The presence of these cluster ions in the carrier gas of the SIFT-MS apparatus must be taken into account in the analysis. They can be used as an additional analytical tool due to their rapid reaction with many gas molecules, especially with polar molecules (Spanel and Smith 1995). Another important possible reaction is that the product ions such as MH$^+$, cluster with the abundant H$_2$O in wet air, producing a secondary reaction, which obviously influences the product ion signal distributions and intensities in SIFT-MS analyses. Both water clustering reactions produce masses MH$^+$18, MH$^+$36, etc. If M is an alcohol, aldehyde or a carboxylic acid, the dihydrate ions (MH$^+$ (H$_2$O)$_2$) readily form, but if M is a ketone, ester or ether, only the monohydrate ions (MH$^+$ H$_2$O) readily form. Protonated phenols form mostly monohydrates and very small fractions of dihydrates (Wang and others
The increase of the number of final product ions generates more conflicts with other masses. Thus \( \text{H}_3\text{O}^+ \) reaction masses should be avoided for these compounds.

The reactions of \( \text{NO}^+ \) with organic molecules are more varied than those of \( \text{H}_3\text{O}^+ \) but they usually result in one or two product ions. Several reaction processes occur in \( \text{NO}^+ \) reactions with organic molecules \( \text{M} \), including charge transfer producing \( \text{M}^+ \) ions, hydride ion transfer producing \( \text{MH}^+ \), and ion-molecule association producing \( \text{NO}^+\text{M} \) ions. Charge transfer can only happen if the ionization energy (IE) of \( \text{M} \) is less than the IE of \( \text{NO} \). Hydride ion transfer occurs in the reactions of \( \text{NO}^+ \) with aldehydes and ethers (Spanel and others 1997, Spanel and Smith 1998). Ion-molecule association is common in the reactions of \( \text{NO}^+ \) with some types of polar organic molecules at thermal energies (internal energy from the random movements of atoms and molecules), especially carboxylic acids and esters (Spanel and Smith 1998) and ketones (Spanel and others 1997). Although several reaction processes are represented in \( \text{NO}^+ \) reactions, usually the reactions of \( \text{NO}^+ \) with organic species proceed via only one of these processes. Therefore, \( \text{NO}^+ \) is a very valuable precursor ion for SIFT-MS analyses. However, small compounds such as methanol react very slowly with \( \text{NO}^+ \), which means those compounds have very high counts with \( \text{H}_3\text{O}^+ \) but 20 to 50 times lower counts with \( \text{NO}^+ \), so the \( \text{NO}^+ \) is not suitable for their quantification.

The reactions of \( \text{O}_2^+ \) with most organic molecules are rapid, and they result in a single production ion or two or more product ions. The ionization energy of \( \text{O}_2 \) molecules is greater than most organic molecules (Lias and others 1988), which ensures that their
reactions with O$_2^+$ ions proceed either via simple non-dissociative charge transfer or dissociative charge transfer reactions resulting in two or more fragment ions (Anicich 2003). O$_2^+$ has higher energy than H$_3$O$^+$ or NO$^+$ so frequently splinters the compounds into the same fragments. The reactions of O$_2^+$ with some aromatic organic compounds result in a single product ion, which is valuable for detection and quantification of these compounds. For instance, alkylpyrazines are best measured under O$_2^+$ because they don’t fragment but other compounds do. O$_2^+$ precursor ions are also valuable for the detection and quantification of small molecules such as NO, NO$_2$ and CS$_2$ that do not react with either H$_3$O$^+$ or NO$^+$ ions (McIntosh and others 1988, Hunter and Lias 1998). However, for O$_2^+$ reactions with polyatomic aliphatic hydrocarbons, several product ions can result thus the mass spectra are very complex. This limits the usefulness of O$_2^+$ precursor ions in SIFT-MS analyses.

**C.3 Refinement of the SIFT-MS methods**

The refining of a method is to determine which masses are used for scanning and calculating so that the measurements have fewer conflicts and are most accurate. The masses that Table C.1 lists should be removed because they are conflicting with the precursors. Table C.2 lists the types of conflicts.

After removing the masses having conflicts with the precursor ions, the method needed to be run on relatively clean samples to identify any masses that can obviously be eliminated because they don’t perform well. The reactions of the three precursor ions (H$_3$O$^+$, NO$^+$ and O$_2^+$) with the analytes produce multiple masses, but only one product ion
from one precursor ion can be used for each compound and the excess masses must be removed. The samples must be run at extreme experimental conditions, i.e., highest and lowest roasting temperature with longest and shortest roasting time for the cocoa bean roasting method so that all possible conflicts can occur. Ideally, the masses that have high branching ratio and no conflicts should remain. Branching ratio is the percentage of the product ions produced from the same precursor ion. The sum of all the branching ratios for a single precursor ion should be 1. Products with low branching ratio, i.e. 5% or less should be removed because they are less accurate and have more interference from conflicts.

In all the product masses of each compound, if the calculated concentration for one or more product ions is significantly higher than that of other product ions, the calculation option can be turned off for that ion, or the mass can be completely turned off for the primary product. The masses that are obviously in great conflict (i.e. the concentrations are more than 100 times higher than the others) should also be removed. The extremely high concentration may be caused by conflict with some high concentration compounds. The concentration is determined for each compound at each remaining mass. If the compound was measured at several masses, the measurements should be about the same for each precursor ion. If one (i.e. H$_3$O$^+$) is significantly higher than the others, it is probably measuring multiple compounds, and the mass should be eliminated from the scan. If the concentration at a mass is much lower than others, this may be caused by: (1) The mass is too large to be measured in the machine. The maximum mass can be measured by SIFT-MS is about 200, and masses above that will be lost. (2) The reaction
chemistry is incorrect or the reaction chemistry in the library is blank. (3) Water chemistry is missing from the library or the method. For example, the acids, acetates, ethanol and acetaldehyde should have water chemistry under NO+ and O2+. If it is not in the method, the value will be too low. Those masses should be deleted or manually added in the water chemistry.

The compounds that are in 100% conflict need to be combined. For instance, in the method for cocoa bean roasting, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine produce exactly the same masses, so only 2,5-methylpyrazine is kept and the other two compounds are removed from the method. The concentration must be reported as a mixture of these compounds.

If the data for a particular compound’s product ion need to be retained, regardless of how it performed, a tolerance ratio in percentage can be set for that parent compound that allows the software to judge case-by-case whether the data should be included in or excluded from averaging. In the method of cocoa bean roasting, the tolerance is set as “50%” for all the compounds, which means if a compound is being measured at multiple masses, the SIFT-MS looks at the lowest calculated concentration, and if any of the other calculated concentrations are more than 50% higher, it discards them.

For the conflicts which can’t be eliminated, the concentration of the compound can be determined by subtracting out the other compounds in conflict. The method firstly determines the branching ratios of other compounds respectively, then multiples the ratios by the concentrations determined at the other masses and subtracts them from the
total. The equation is:

$$Ca = Cm - \sum_{i=1}^{n} \left( Ci \ast \frac{BR_i}{BR} \right)$$

Where Ca is the adjusted concentration, Cm is the measured concentration, Ci is the measured concentration for other compound, BRi is the branch ratio of other compound, BR is the branch ratio of the adjusting compound. This adjustment can be done manually only if the branch ratio and reaction kinetics are exact, or there may be interactions that change these values. If the compounds concentration changes very little after subtracting, the conflict can be ignored. The kinetics values and branch ratio are only to two significant digits so any changes beyond that are not important. If the concentration changes greatly after subtracting, which means the conflict can’t be ignored, either the concentration must be reported as a mixture or it should not be reported at all. If the concentration changes slightly after subtracting, the subtraction should be made and reported in the method, but this should only be done when this adjustment is very important.

**C.4 References**


Table C. 1. Masses subject to interference in SIFT-MS methods.

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<tr>
<td>39</td>
<td>H$_3^{18}$O$^+$.H$_2$O</td>
<td>Isotope</td>
</tr>
<tr>
<td>48</td>
<td>NO$^+$.H$_2$O</td>
<td>Precursor</td>
</tr>
<tr>
<td>50</td>
<td>Unidentified</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>H$_3$O$^+$.2H$_2$O</td>
<td>Precursor</td>
</tr>
<tr>
<td>57</td>
<td>H$_3^{18}$O$^+$.2H$_2$O</td>
<td>Isotope</td>
</tr>
<tr>
<td>66</td>
<td>NO$^+$.2H$_2$O</td>
<td></td>
</tr>
</tbody>
</table>

Continued
Table C.1
Continued

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td></td>
<td>H$_3$O$^+$.3H$_2$O</td>
<td>Precursor</td>
<td>Check</td>
<td>Precursor</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>H$_3^{18}$O$^+$.3H$_2$O</td>
<td>Isotope</td>
<td>Check</td>
<td>Isotope</td>
<td></td>
</tr>
<tr>
<td>91</td>
<td></td>
<td>H$_2$O$^+$.4H$_2$O</td>
<td>High humidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>104</td>
<td></td>
<td>Unidentified</td>
<td></td>
<td></td>
<td></td>
<td>Chemical</td>
</tr>
<tr>
<td>131 - 134</td>
<td></td>
<td>Xenon ions</td>
<td>Carrier Gas</td>
<td>Carrier Gas</td>
<td>Carrier Gas</td>
<td></td>
</tr>
</tbody>
</table>

CB-Chemical Background; CG-Carrier Gas. (from Syft Technologies Inc.)
Table C. 2. Type of conflicts.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Usability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precursor</td>
<td>An ion used as a precursor ion in SIFT-MS or a water cluster of one of these</td>
<td>Cannot be used for detecting product because signal is overwhelmed by the precursor ion signal</td>
</tr>
<tr>
<td>Isotope</td>
<td>The $^{18}$O isotope peak of the precursor ion or a water cluster.</td>
<td>Can often be used if the analyte concentration is moderate and a correction for the precursor intensity is applied</td>
</tr>
<tr>
<td>Check</td>
<td>A mass which is potentially subject to interference from a precursor ion or its isotope</td>
<td>In most instances, these masses are actually useable. On first running the method, check carefully to confirm that this is the case</td>
</tr>
<tr>
<td>High humidity</td>
<td>A precursor peak that may occur when very humid samples are analyzed</td>
<td>Renders mass unusable in the situation</td>
</tr>
<tr>
<td>CB (Chemical Background)</td>
<td>A compound intrinsic to an instrument component occurs at this mass at moderate levels</td>
<td>This mass is not well suited to trace analysis, but can be used for target compounds at moderate levels</td>
</tr>
</tbody>
</table>

Continued
Table C.2
Continued

<table>
<thead>
<tr>
<th>CG (Carrier Gas)</th>
<th>These mass sometimes arise due to a low-level xenon impurity in the carrier gases</th>
<th>Can often be accounted for in trace work if a suitable background scan is run that can be reliably subtracted from the sample signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Blank]</td>
<td>No occurrence expected under normal operational conditions</td>
<td>Completely usable</td>
</tr>
</tbody>
</table>
Appendix D: Experimental setup for real-time monitoring of cocoa bean roasting by SIFT-MS.

Figure D. 1. Experimental setup for real-time monitoring of cocoa bean roasting.