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TOWARDS A HEALTHIER CHOCOLATE FORMULA WHICH IS RICH IN POLYPHENOLS AND LOW IN FAT

Lakmani Tharaka Galaniha

University of Kentucky, galaniha.tharaka@gmail.com

Author ORCID Identifier:

https://orcid.org/0000-0002-1869-0834

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Lakmani Tharaka Galaniha, Student
Dr. Sandra Bastin, Major Professor
Dr. Alison Gustafson, Director of Graduate Studies
TOWARDS A HEALTHIER CHOCOLATE FORMULA WHICH IS RICH IN POLYPHENOLS AND LOW IN FAT

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Nutrition and Food Systems in the College of Agriculture, Food and Environment at the University of Kentucky

By

Lakmani Tharaka Galaniha

Lexington, Kentucky

Director: Dr. Sandra Bastin, Department Chair/Professor Of Dietetics and Human Nutrition

Lexington, Kentucky

2018

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https://orcid.org/0000-0002-1869-0834
ABSTRACT OF THESIS

TOWARDS A HEALTHIER CHOCOLATE FORMULA WHICH IS RICH IN POLYPHENOLS AND LOW IN FAT

Chocolate is a food that is craved by many in the world and healthy chocolates have become a current topic in the healthy eating trend. The healthiness of a chocolate depends mainly on the polyphenol, fat and sugar contents. Although the literature serves several interventions to reduce the fat content and total calorie content of chocolates, it does not provide many interventions that consider both improving the polyphenol and reducing the fat content simultaneously. Considering this gap in the literature, this research project sought to develop a chocolate that is both low in fat and high in polyphenol content, without sacrificing the taste that consumers would expect in a good dark chocolate. The research resulted in three chocolate formulas that consists of 60% cocoa and 30% fat (formula A, formula B and formula C). They differed from each other in the polyphenol content due to the changes in the cocoa powder type and the presence or absence of freeze-dried blueberry powder. Formula A and formula B mainly differed in the percentage of each type of cocoa powder, both alkalized and non-alkalized. Formula C differed from the other two formulas because there was no freeze-dried blueberry powder. The resulted chocolates were evaluated for the sensory characteristics using paired preference tests and consumer-oriented attribute diagnostic tests (color, flavor, melting properties, overall preference, likelihood to buy). They were analyzed for polyphenol content using Folin-Ciocalteu assay. The chocolates were compared with a well-established commercial chocolate (formula D) of a 60% cocoa. Sensory evaluation tests revealed that formula C was superior and comparable to the commercial chocolate D in all the tested attributes except for color. Polyphenol analysis confirmed that all three developed formulas had a higher polyphenol estimate than formula D. Consumers preferred to buy each chocolate type regardless of the differences in their preference for the other attributes, if the chocolates were known to be healthier. Formula C was found to be the best prototype formula among the developed formulas and it can further be developed to enhance the color and other attributes in future work.

KEYWORDS: Cocoa-polyphenols, Healthy Chocolates, Sensory Evaluation, Chocolate processing, Paired Preference Test, Attribute Diagnostic Test

Lakmani Tharaka Galaniha

09/27/2018

Date
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By

Lakmani Tharaka Galaniha

Dr. Sandra Bastin, PhD, RDN, LD
Director of Thesis

Dr. Alison Gustafson, PhD, MPH, RD, LD
Director of Graduate Studies

09/27/2018
Date
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Background

Chocolate, which is made from the seeds of the cocoa tree, was considered the ‘Food of Gods’ in ancient history. This association was the reason behind the rise of the scientific name *Theobroma cacao* for the cocoa tree from the Greek words “theo” which means God and “broma” which means food. (Dillinger et al., 2000) The term ‘cacao’ refers to less processed and the purest form. The term ‘cocoa’ refers to the heated form of cacao. The solid chocolate industry began in the 19th century after the invention of the method for the cocoa butter extraction by Coenraad Johannes van Houten. (Coe, Coe, & Huxtable, 1996) Since then, the chocolate industry has developed different methods to process cocoa seeds that has results in a wide variety of chocolate products. Starting as a luxury item, the chocolates have made their way to one of the most craved food in the world.

Chocolate is craved by many in the world because of chocolate’s many appealing sensory characteristics. According to the general review of cravings (Weingarten & Elston, 1990), the sensory characteristics of food products account for most cravings. Thus, chocolate has the chemosensory characteristics of sweetness, texture and attractive aroma that motivates the consumers to have an inherent craving for chocolates (Rozin, Levine, & Stoess, 1991). The ability of cocoa butter to melt at body temperature gives a distinctly pleasant oral sensation, which is a major attraction to consumers. Combined with the distinct chocolate aroma, consumers ingest chocolate despite of the high content of fat and sugar. A study has even reported that 75% of male and 77% of female chocolate cravers say there is no other non-chocolate substitute for their craving for chocolate (Rozin et al., 1991).

There are various health benefits of chocolates which may motivate consumers to consume chocolate other than for its appealing sensory characteristics. Chocolates have had medical uses since the Mesoamerican civilization. (Lippi, 2013) Therefore, it is helpful to build a bridge to the past to further explore the potential health benefits of chocolate. The nutritional connection to these benefits of chocolates are the flavonoids which are a group of polyphenols. Compared to vitamins and other bioactive substances polyphenols are consumed in substantial quantities such that the estimated adult consumption is about 1,000 mg per day, while Vitamin E accounts for only 12 mg, Vitamin C for 90 mg and carotenoids for only 5 mg per day. Flavanols make a significant portion of the total polyphenol consumption of a human. Among the numerous polyphenol sources are tea, wine and certain fruits like apples, red grapes and cranberries. Cocoa contains a high concentration of polyphenols and compared to red wine, green tea and black tea, cocoa is greater in flavonoid and antioxidant content. There are three types
of flavonoids in cocoa, including flavanols, anthocyanins and flavones, with flavanols being the most abundant. (Lee, Kim, Lee, & Lee, 2003)

Recent clinical studies support the reduction of chronic disease risk factors from the consumption of cocoa flavanols. It is mainly due to the antioxidant and antiradical properties of cocoa flavanols. Prevention of the Low-density lipoprotein (LDL) oxidation and antiplatelet effects of cocoa polyphenols are related to the protective mechanisms against heart disease. Cocoa polyphenols are also able to modulate the immune response and their anti-inflammatory and anti-carcinogenic properties. (Andujar, Recio, Giner, & Rios, 2012) Recent studies have found that cocoa polyphenols could improve the cognitive functions of the elderly. (Blumberg, Ding, Dixon, Pasinetti, & Villarreal, 2014) Cocoa intake may also result in increased cerebral blood flow of the young, which suggests the role in treatments of dementia and stroke. (Francis, Head, Morris, & Macdonald, 2006) Due to these health benefits, there are individuals that consume chocolates as a self-medication.

Problem Statement

Considering the health benefits of cocoa polyphenols and the cravings of people towards chocolate, the importance of developing a chocolate that is high in cocoa polyphenols has emerged. Even though cocoa polyphenols are highly beneficial, the health benefits of chocolate should be regarded in the context of the other ingredients. Certainly, when it comes to chocolate, a high concentration of refined sugars and a high fat content would be very common. Therefore, the importance of reformulation of a chocolate to be low in sugar and fat content is emphasized when the net benefit of cocoa is weighted against other components in a chocolate.

Purpose

The accumulating evidences of cocoa polyphenols supports a variety of health benefits including, the cardiovascular disease risk reduction, anti-carcinogenic properties, and cognitive function enhancement of the elderly. However, the health benefits of cocoa polyphenols should be weighed against the other ingredients when it comes to a healthier chocolate. When considering the high fat and energy content of the regular chocolate, developing a healthier chocolate would be appealing for consumers. Thus, this research project emphasizes developing a chocolate that is high in polyphenols but low in fat without compromising the taste that consumers have come to expect in a good dark chocolate.

Research Questions

1. Would there be a significant consumer preference for one chocolate bar over the other based on the taste. (Among the two chocolate bars which are the “60% cacao” chocolate bar made with a greater percentage of non-alkalized cocoa
powder using Formula A and the “60% cacao” chocolate bar made with a greater percentage of alkalized cocoa powder using Formula B).

2. Would there be a significant consumer preference for one chocolate bar over the other based on the taste. (Among the chocolate bar that includes Blueberry fruit powder made with Formula A and the chocolate bar of the same cocoa percentage that excludes Blueberry fruit powder made with Formula C).

3. Would the addition of Blueberry fruit powder increase the total polyphenol content of the chocolate bar without compromising the overall consumer preference?

Research Hypotheses

1. It is possible to find a significant consumer preference for one chocolate bar over the other based on the taste. (Among the two chocolate bars which are the “60% cacao” chocolate bar made with a greater percentage of non-alkalized cocoa powder using Formula A and the “60% cacao” chocolate bar made with a greater percentage of alkalized cocoa using Formula B).

2. It is possible to find a significant consumer preference for one chocolate bar over the other based on the taste. (Among the chocolate bar that includes Blueberry fruit powder made with Formula A and the chocolate bar of the same cocoa percentage that excludes Blueberry fruit powder made with Formula C).

3. The addition of Blueberry fruit powder increases the total polyphenol content of the chocolate bar without compromising the overall consumer preference.

Justification

The literature serves several interventions that have taken place to reduce the fat and total calorie content of chocolates. However, the literature doesn’t provide methods to produce chocolates with a reduced fat and an increased polyphenol content at the same time. There are chocolates which contains a high polyphenol content, but which are extremely bitter, reducing the likelihood of consumer purchase solely for the polyphenolic benefits. Those chocolates which are higher in cocoa percentage are also high in fat content which causes a negativity on the overall health benefits. Considering these limitations in current chocolate products, this research project is expected to develop a chocolate that is both low in fat and high in polyphenol content without sacrificing the good taste that consumers have come to expect in a good dark chocolate.
CHAPTER 2: LITERATURE REVIEW

Introduction

Accumulating evidence suggests that cocoa polyphenols provide sustained health benefits, especially in resisting hypertension and cardiovascular disease. However, the health benefits of cocoa polyphenols should be regarded in the context of other ingredients when it comes to chocolate. A regular chocolate is a high-energy food with a high fat content of about 45% by weight which provide about 53% of an energy value. According to the internationally accepted nutritional guidelines, fat should provide no more than 30% - 35% of energy(Zumbe, 1999). Therefore, there is a need to reduce the fat and energy content of chocolate while increasing the polyphenol content. Prior to the chocolate reformulation it is important to understand the physical and chemical properties of each ingredient associated with chocolates. Identifying the steps which could change in chocolate processing would allow for the development of a healthier product without sacrificing positive sensory attributes.

Health Benefits of Chocolate

It is widely recognized that flavonoids derived from vegetables and fruits improve health and reduce the risk of chronic disease. Among the dietary polyphenols, flavonoids comprise approximately two-thirds of the total of this class of bioactive compounds. There are many plants derived foods and beverages that are rich in flavanols, including wine, tea, various fruits and berries and cocoa and cocoa products. Cocoa contains the highest flavanol content, specifically Flavon-3-ol, of all foods on a per-weight basis. This allows for a considerable contribution to the total intake of dietary flavonoids(Blumberg et al., 2014). In fact, Lee et al. found that on a per serving basis, cocoa has a higher flavonoid content and antioxidant capacity than red wine (2 times), green tea (2-3 times) and black tea (4-5 times)(Lee et al., 2003).

Recent clinical interventions in humans suggests that intake of flavonoids, particularly the flavanols from cocoa, is inversely related to the risk factors for chronic diseases, including elevated blood pressure, dyslipidemia, platelet adhesion, insulin resistance, inflammation and glucose intolerance. Also, they influence the improvement of vascular reactivity, vascular relaxation, vascular functions, endothelium dependent vasodilation, immune responses and antioxidant defense system. Further, cocoa polyphenols could decrease the levels of low density lipoprotein (LDL)-cholesterol and its oxidation while increasing the high-density lipoprotein (HDL)-cholesterol. The antioxidant properties and the anti-inflammatory activities of polyphenols provide the positive effects against
various disorders including cardiovascular disease, inflammatory processes and cancer. (Andujar et al., 2012; Keen, Holt, Oteiza, Fraga, & Schmitz, 2005; Schroeter et al., 2006)

The randomized crossover trial done by Grassi et al. (Grassi et al., 2008) using 19 hypertensive patients with impaired glucose tolerance, showed that polyphenols in dark chocolates decreased insulin resistance, systolic and diastolic blood pressure, total cholesterol and LDL-cholesterol and increased insulin sensitivity, β-cell function and flow-mediated dilation. A systematic review and meta-analysis of randomized, controlled trials done by Shrime et al. with an average cocoa flavonoid dose of 400–600 mg/d and with isocaloric comparison as a control has shown that cocoa flavonoid could lower blood pressure and LDL and increase HDL cholesterol, improve insulin resistance and enhance flow-mediated dilation (Blumberg et al., 2014). A systematic review (from 136 publications) about the relationship between chocolates and the risks of cardiovascular disease, suggests that chocolates may have beneficial effects on cardiovascular risk via various mechanisms (Ding, Hutfless, Ding, & Girotra, 2006). These include lowering blood pressure, anti-inflammation and anti-platelet function, LDL oxidation and increasing HDL level. In addition, Buijsse et al. (Buijsse, Feskens, Kok, & Kromhout, 2006) showed a significant inverse association between cocoa intake and reductions of 50% and 47% in cardiovascular disease (CVD) mortality and total mortality, respectively. They studied 470 men in The Netherlands with a highly-detailed assessment of cocoa intake. Further, a systematic review and meta-analysis (7 cohort studies) (Buitrago-Lopez et al., 2011) have shown that higher versus lower amounts of calorie-adjusted chocolate consumption were associated with a 37% reduction in the relative risk of total CVD. Larsson et al. (Larsson, Virtamo, & Wolk, 2012) has also shown a 19% reduction in the risk of stroke in their analysis of calorie-adjusted chocolate and cocoa consumption.

With the many studies found in literature, cocoa polyphenols appear to provide health benefits. Yet, most chocolate bars are also high in fat and sugars which the US Dietary Guidelines recommend ("2015 – 2020 Dietary Guidelines for Americans," December 2015) limiting in the daily diet. Thus, the development of a high polyphenol chocolate formulation with less fat and sugar may offer added health benefits while meeting consumer preferences in eating dark chocolate.

**Cocoa Tree (Theobroma cacao)**

The cocoa tree originated in South and Central America as a commercial crop. It grows in suitable environments between 20° north and 20° south that have a higher average temperature of 27 °C throughout the year. These areas have high humidity because of plentiful rainfall (at least 1500-2500 mm per year). The appropriate soil would be a deep, rich and a well-drained soil for the cultivation and it should also be less than 700 m above sea level to avoid crop damage due to high winds.

The cocoa tree is a relatively small tree of 12-15 m in height. The leaves are ever green and are up to 300 mm in length. The trees start to bear pods after 2-3 years, but it takes up to 6 or 7 years for maximum yield. The pods arise from tiny flowers which grow on the...
branches and trunk of the tree throughout the year taking 5-6 months to develop into mature pods. Mature pods grow between 100 mm and 350 mm long, weigh 200 g to more than 1 kg and exist in a wide variety of shapes and colors depending on the variety. Each pod consists of 30-45 beans (Sheilah Beckett, 2000).

The flavor of cocoa is dependent on the varieties of cocoa. There are four varieties of cocoa: Criollo, Forastero, Trinitario and Nacional. Criollo, originated in South America has a mild flavor but the trees are low yielding. Forastero, originated in West Africa has a pungent aroma and the trees have a vigorous yield. Trinitario, originated in Trinidad and is a hybrid of the other two. Nacional, originated in Ecuador, has a full, smooth cocoa flavor, with additional floral, spicy flavors (Sheilah Beckett, 2000; Notter, 2011). This research was conducted with the cocoa supplies (cocoa powders, cocoa liquor and cocoa butter) of the variety Nacional.

Process and Chemistry of Chocolate Production

The process of making chocolate from cocoa is a complicated process that requires knowledge, specialized equipment and supplies. Detailed information is available in Appendix (Ai). See figure 1 for the process steps required to make chocolate.
Important Ingredients in Making Chocolate

Cocoa Polyphenols

Among the 380 chemicals which cocoa contains, ten compounds are psychoactive. Due to this high concentration of polyphenols, cocoa beans have an extreme bitter flavor which make them virtually inedible in their natural state. (Anduajar et al., 2012) Studies have been conducted since the early 1950’s to investigate cocoa polyphenols. Only three groups of polyphenols have been identified in cocoa beans. They are catechins, anthocyanins and procyanidins. Catechins constitute around 37% of the polyphenol content, anthocyanins about 4% and procyanidins about 58% (Andujar et al., 2012).

The polyphenols are stored in the pigment cells of the cotyledon in the cocoa beans. The amount of anthocyanins of these pigment cells/polyphenol-storage cells determines the color of the cocoa bean, which varies from white to deep purple as the anthocyanin content increases. Cocoa polyphenols mainly include Flavan-3-ols epicatechin, catechin and oligomeric and polymeric procyanidins. (Wollgast, 2005) The most abundant flavonoids in cocoa are the flavanols, which comprise the monomeric flavanols, (+)-catechin and (-)-epicatechin and their oligomeric and polymeric forms (pro-cyanidins). (-)-Epicatechin represents 35% of the total phenolic content as the major monomeric flavanol in cocoa, which is a complex series of procyanidins. (Andres-Lacueva et al., 2008).

The quantitative determination of polyphenols in chocolate includes the assessment of either a sum parameter for total polyphenols or measurements of epicatechin and catechin or even B-type procyanidin contents. The polyphenol content of chocolate confectionaries or cocoa powder is subjected to changes during various processing steps, from harvesting to packaging. Fermentation of cocoa beans leads to the diffusion of polyphenols from their storage cells. These undergo oxidation to form condensed high molecular, mostly insoluble tannins. During the fermentation process, the soluble polyphenol content including epicatechin, reduces about 10-20%, anthocyanidins disappear and the procyanidins decrease 3- to 5-fold. (Andres-Lacueva et al., 2008). Epicatechin and soluble polyphenol content reduction is a result of both the oxidation and fermentation sweating. The anthocyanins are hydrolyzed to anthocyanidins during fermentation, where the anthocyanidins then polymerize along with simple catechin to form complex tannins. Higher processing temperatures and longer processing times cause the reduction of available catechin and procyanidin amounts in cocoa components. Additionally, alkalizing steps could remarkably decrease the catechin and procyanidins. (Andres-Lacueva et al., 2008; Blumberg et al., 2014; Wallace et al., 2009; Wollgast, 2005).
Properties of Cocoa Butter

Cocoa butter is a relatively simple fat which accounts for over 95% fatty acids and is composed of three fatty acids, palmitic (26%), stearic (34%) and oleic (35%). Cocoa butter melts rapidly over a small temperature range. The major triglyceride groups are StOSt, POP and POSSt. These groups are mainly responsible for providing the specific crystallization and melting characteristics in chocolates. These melting characteristics contribute to the characteristic “mouth feel” of chocolate. Upon crystallization, cocoa butter can take six different forms which have different melting temperatures. A high-quality chocolate will contain only type V crystals. This form of crystal is hard with good snap and it gives a glossy appearance with a relatively good resistance to bloom. Only 1% to 2% of cocoa butter is all saturated (StStSt). It melts at a higher temperature than the common StOSt. From 5% - 20% contains two oleic acid molecules (StOO) that remain as liquid at room temperature. The relative hardness level of cocoa butter is affected by the StOSt/ StOO ratio and this ratio differs according to the geographical location of origin (Sheilah Beckett, 2000). As an example, the cocoa butter of the Ivory coast of Ghana contains a significantly lower amount of oleic acid than cocoa butter from South America, whereas cocoa butters from South East Asia are in-between (Lipp & Anklam, 1998).

Properties of Polydextrose

Polydextrose is a polysachcharide made by randomly bonded condensation polymers of glucose containing minor amounts of bound sorbitol and citric acid. It is practically inert to mammalian digestive enzymes. Thus, polydextrose could be used in low calorie products. Polydextrose is a multi-purpose food ingredient used to replace sugar, fat and calories and to increase fiber content of foods. It also functions as a stabilizer and bulking agent and to maintain the ideal moisture in a food ("Calorie Control Council," 2018). Standard toxicology studies have shown that polydextrose does not show any harmful effects even though it is included in the diet at high levels for long periods of time. However, it may show gastrointestinal effects at high ingestion levels. Further clinical studies have also shown that polydextrose does not increase the blood glucose level in diabetic patients and there is no interference with absorption and utilization of essential dietary components like vitamins, minerals and amino acids. (Charalambous, 2012; Jamieson, 2008)

Properties of Erythritol

Erythritol is a sugar that has four-carbons but is approximately 70% as sweet as sucrose ("Calorie Control Council," 2018). It is found in algae, fungi and lichens. It
occurs naturally in several foods including wine, sake, beer, water-melon, pear, grape and soy sauce.

The absorption of Erythritol from the proximal intestine follows passive diffusion similar to the other low molecular weight organic molecules. Since the absorption rate depends on the molecular size, erythritol passes through the intestinal membranes at a faster rate than larger molecules such as mannitol or glucose. This is important for diabetic patients as it has been identified that erythritol is rapidly absorbed and excreted unchanged in the urine. Ingested erythritol is rapidly distributed throughout the body after absorption and it has been reported to occur in hepatocytes, pancreatic cells and vascular smooth muscle cells. (Database, 2018a; Munro et al., 1998)

Properties of Sucrose

Sucrose is a nonreducing disaccharide composed of glucose and fructose. Sucrose is mostly used as a sweetener in foods and drinks. But it is also a chemical intermediate for detergents, emulsifying agents, and other sucrose derivatives. In nature sucrose functions as an energy store for metabolism and as a carbon source for biosynthesis. In addition to its use as a sweetener, it is used in food products as a preservative, antioxidant, moisture control agent, stabilizer and thickening agent(Database, 2018b).

Role of Emulsifiers in chocolates

An Emulsifier acts to form a barrier between two immiscible substances using its’ “head” and “tail” which have hydrophilic and lipophilic properties. In chocolates there are sugar and other solid particles that are hydrophilic but are lyophobic. Thus, the emulsifiers coat the solid surface and foam a boundary layer between it and the fat. There are many types of emulsifiers used in chocolates. Some contain a larger “head” which bind very strongly to the sugar. Similarly, there are different “tail” lengths which affect the flow properties in different ways. Which means that the type of emulsifiers used in the chocolate effects the yield and the plastic viscosity. It is important to take note that emulsifiers that are beneficial in the yield value may be poor regarding the plastic viscosity and vice versa. Also, the chocolates made with emulsifiers are able to tolerate higher levels of moisture than emulsifier free ones. This is very important as water is very detrimental to the viscosity of the chocolate.

Properties of Blueberry

Raw blueberries (Vaccinium spp.) have polyphenols of the classes anthocyanidins, flavan-3-ols and flavanols. Among the anthocyanidins, cyanidin, delphinidin, malvidin, peonidin and petunidin are present. Among flavonols, kaempferol, myricetin and
quercetin are present. Most importantly, similar to cocoa polyphenols blueberries have a higher flavan-3-ols content where (+)-catechin is predominant with a 98.47 mg per 100 g edible portion and (-)-epicatechin has 25.66 g per 100 g of edible portion. (Bhagwat, Haytowitz, & Holden, 2014)

**Chocolate Standards as per FDA**

There are specific standards or lack thereof for a product to be called chocolate, determined by the Food and Drug Administration. Some are included here.

- Chocolate shall contain, on a dry matter basis, not less than 35% total cocoa solids, of which not less than 18% shall be cocoa butter and not less than 14% fat free cocoa solids.
- Milk chocolate shall contain not less than 3.39% by weight of milkfat and not less than 12% by weight of total milk solids based on the specified dairy ingredients. This is exclusive of any added sweetener or other dairy-derived ingredient that is added beyond that amount that is normally present in the specified dairy ingredient.
  - Specified dairy ingredients: cream, milkfat, butter, milk, concentrated milk, evaporated milk, sweetened condensed milk, dried milk, skim milk, concentrated skim milk, evaporated skim milk, sweetened condensed skim milk, nonfat dry milk
- Low-fat cocoa should confirm to the definition and standard of identity and is subjected to the requirements for label declaration of ingredients for breakfast cocoa, except that cocoa fat content should be less than 10% by weight.
- There are no standards given specifically for dark chocolates by FDA or in the Codex Alimentarius (Alimentarius, 2016)

**Dried Fruit Powder as a Flavor Enhancer**

The flavor molecules of fruits are water soluble and chocolate is an emulsion with a fat-based continuous phase. Therefore, the ability of flavor molecules to be infused into the corresponding fat base continuous phase of chocolate is greatly hindered. Fruit powders with a reduced particle size that are too small for the tongue to detect them, can be blended with cocoa butter into a smooth paste. Freeze drying will result in a reduced particle size and the anhydrous state will allow the fruit powder to be incorporated into a naturally hydrophobic lipid base. (Crowley & Najmeddine, 2011; Given Jr & Arciszewski, 1989)
Polyphenol Analysis

Colorimetric methods are widely used for the rough quantification of polyphenols mainly due to their high sensitivity and simplicity. These include Folin-Ciocalteu and Prussian-Blue methods for total polyphenols, the vanillin-HCl assay for catechins and butanol-HCl assay for proanthocyanidins. All the methods produce quantitative estimates given as equivalents of one standard phenolic compound such as gallic acid or catechin. This research project utilized Ultraviolet-Visible (UV-Vis) spectroscopic technique as the standard colorimetric method. UV-Vis spectrophotometer quantifies the optical properties of samples in the ultraviolet and visible wavelength ranges of light (typically 190 to 900/1100 nm). Specifically, UV-Vis spectrophotometers determine how much light of a given wavelength passes through a sample, and how much is absorbed. This information can be diagnostic of concentration utilizing the Beer-Lambert’s law.

Polyphenol analysis in chocolate and chocolate raw products include sample extraction techniques, techniques for isolation and purification of polyphenols from the food (Wollgast & Anklam, 2000).

Both Folin-Ciocalteu and Prussian-Blue assays are based on redox reactions. A reduction of ferric to ferrous ions is followed by the formation of the deep blue hexacyanoferrate-(II)-chelate in the Prussian-Blue assay. While in the Folin-Ciocalteu assay, complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids are reduced by phenolic compounds and other reducing compounds forming a complex molybdenum-tungsten blue (Singleton & Rossi, 1965). In both assays, the yielding color depends on the redox potential of the reference standard used and the phenolic compounds as well as other interfering cocoa matrix compounds such as aromatic amines, carbohydrates or Maillard reaction products formed during the chocolate manufacturing.

One limitation of the above-mentioned techniques is that it is unable to specify the type of the flavonoids present. More specific results can be obtained using chromatographic techniques, such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC)- and more recently capillary electrophoresis (CE). These techniques allow for qualitative and quantitative analysis as well as isolation and purification procedures.
CHAPTER 3: MATERIALS AND METHODS

Materials

Ingredients used for the chocolate formulation

The following materials were used to meet the objectives of this study. Please see Appendix (Aii) for where these were purchased.

- Cocoa liquor
- Natural pure prime pressed cocoa butter
- Non-alkalized cocoa powder
- Alkalized cocoa powder
- Non-GMO organic Erythritol
- Polydextrose
- Sucrose
- Emulsifiers: Polyglycerol polyricinoleate (PGPR), Ammonium phosphatide (AMP)
- Freeze dried organic Blueberry powder

Chemicals used for the polyphenol analysis

- Sodium Carbonate
- Ethanol
- Folin-Ciocalteu's Phenol reagent
- Gallic acid (fine Powder)

Equipment

- KitchenAid Tilt-Lift Precise Heat Mixing Bowl (used for the mixing of the dry ingredients and for the conching process)
- Melangers' premier chocolate refiner (used for the chocolate refining process)
- The UV-vis specrophotomer (Ultra Violet- Visible spectrophotometer) of the model Agilent 8453 (utilized for the polyphenol analysis)
Methods

Chocolate Formulation

This research project followed an experimental study design and developed three formulas to achieve the research intention. The ingredients and the quantities required for each formula is listed in the table 1 and each formulation was done using the following procedure.

- The dry ingredients (Erythritol, Polydextrose, Sucrose and Cocoa Powders) were mixed together at the speed of one in a jacketed mixing bowl for 10 minutes while controlling the temperature between 48-50 °C.
- Next, cocoa butter and cocoa liquor were melted at 49 °C and were added to the mixture of dry ingredients in the mixing bowl.
- Then the mixing was continued at the same temperature (49 °C) to produce a consistent paste.
- The resulting paste was then roll refined for one hour using the chocolate refiner.
- Next, the freeze-dried blueberry powder was added, and the refining process was continued for another 1½ hours to reduce the particle size to 18-20 μm (measured using a digital micrometer screw gauge).
- Next, the emulsifiers (AMP and PGPR) were added to the refined mixture and was conched in the jacketed mixing bowl for one hour at 48-50 °C at the speed of one.
- At the end, the mixture was hand tempered and molded in to bars.

Table 1: Ingredients for each formulation

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight percentage (%) of final product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formula A</td>
</tr>
<tr>
<td>Cocoa Butter</td>
<td>13.31</td>
</tr>
<tr>
<td>Cocoa Liquor</td>
<td>18.75</td>
</tr>
<tr>
<td>Erythritol</td>
<td>19.02</td>
</tr>
<tr>
<td>Polydextrose</td>
<td>6.79</td>
</tr>
</tbody>
</table>
Polyphenol Analysis

Folin-Ciocalteu spectrophotometric method was used to determine the total phenolic content and gallic acid was used for the calibration curve. The results were presented as gallic acid equivalents. The polyphenol extraction was done as described by Wollgast, D.J. with slight modifications as described below. (Wollgast, 2005)

-Procedure for the extraction of polyphenols from chocolate-

One gram of the chocolate sample was defatted twice with 10 ml of n-hexane for 5 minutes in an ultrasonic bath at 30 °C, then it was centrifuged for 10 minutes at 3000 x g. Next the sample was air dried and the polyphenols were extracted with 10 ml of a mixture of acetone, water, and acetic acid (70+ 29.8+0.2, v/v/v) for 10 minutes at 30 °C in an ultrasonic bath. The sample was filtered out using a folded filter paper and the organic solvent was removed using rotary evaporation under reduced pressure at 30 °C. Finally, the remaining aqueous extract was transferred quantitatively to a 50 ml volumetric flask and filled the volume up with distilled water for the Folin-Ciocalteu assay.

-Folin-Ciocalteu Assay-

The total phenolics were colorimetrically assayed as follows:

20 μl from each polyphenol extract were added into 1cm, 2 ml glass cuvettes. Then 20 μl of gallic acid calibration standard, and 20 μl of blank (distilled water) were added into the same glass cuvettes. Next, 1.58 ml water were added followed by 100 μl Folin-Ciocalteu(FC) reagent to each cuvette and were mixed thoroughly by inverting. Then they were incubated one to eight minutes. Next, 300 μl of sodium carbonate solution was added, and mixed and incubated for 30 minutes at room temperature. Finally, the sample absorbances were measured at 765 nm using the UV-Vis spectrophotometer (UV-Vis spectrum is shown in Figure 2) and the polyphenol concentration of each chocolate sample was calculated using the Beer-Lambert’s law (the obtained polyphenol estimates are shown in Table 3).

-Gallic acid calibration standards-

<table>
<thead>
<tr>
<th></th>
<th>1.09</th>
<th>1.09</th>
<th>1.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>1.09</td>
<td>1.09</td>
<td>1.10</td>
</tr>
<tr>
<td>PGPR</td>
<td>0.27</td>
<td>0.27</td>
<td>0.28</td>
</tr>
<tr>
<td>Non-Alkalized cocoa powder</td>
<td>16.57</td>
<td>10.87</td>
<td>16.62</td>
</tr>
<tr>
<td>Alkalized cocoa powder</td>
<td>10.87</td>
<td>16.57</td>
<td>10.80</td>
</tr>
<tr>
<td>Freeze Dried Blueberry Powder</td>
<td>6.52</td>
<td>6.52</td>
<td>N/A</td>
</tr>
<tr>
<td>Sucrose</td>
<td>6.79</td>
<td>6.79</td>
<td>9.69</td>
</tr>
</tbody>
</table>
0.5 g of gallic acid was dissolved in 10 ml of ethanol and then diluted up to 50 ml with water (5 g/liter). Next, 0.25, 0.5, 1.25, and 2.5 ml were diluted to 50 ml with water to create standards with 50, 100, 250 and 500 mg/liter concentrations, respectively.

_Sodium carbonate solution_

2.0 g of anhydrous sodium carbonate were dissolved in 8.0 ml of water to result in a homogenous solution.

_Sensory Evaluation_

All sensory evaluation tests were consumer-oriented and included 55 participants. The participants were current students, staff and faculty members of University of Kentucky. They were age 18-64, who had not been trained for sensory evaluation and volunteered. The tests were conducted in a foods laboratory at the Department of Dietetics and Human Nutrition. The affective tests followed a quantitative method to determine the overall preference for the obtained products and to determine the preference for the important sensory attributes.

The panelists were required to sign an informed consent document before the evaluation, as required by the Institutional Review Board of the University of Kentucky. Participants were asked to complete a short questionnaire including sex, age, chocolate consumption patterns and preferences. Prescreening questions were also included in the questionnaire to screen the panelists who may have health issues such as oral or gum disease, food allergies, fever or common cold and other diseases which could affect the senses of the panelists. Apart from the diseases, those who take medications which affect the senses, were also screened using questions which allowed the participants to mark yes or no without referring to further details to avoid the sensitivity of such questions.

Terms/attributes, references and rinsing protocols were explained thoroughly to the participants before they started the testing. Qualtrics Survey software was used for data collection. Appearance was evaluated under incandescent light and ambient temperature and relative humidity, while the melting properties of the chocolate was analyzed by placing 1/8 of the sample between the tongue and roof of the mouth and waiting over 4 min or until the sample has completely melted. Samples were masked for visual aspects other than color and all the samples were encoded using the table of three-digit random numbers. Before each sensory evaluation session, the participants were given a brief introduction to the methodology and the procedures of paired preference tests and rating tests (Meilgaard, Carr, & Civille, 2006).

The sensory evaluation test was done in three rounds, where the first two rounds were given a paired preference test and the final round was given acceptance tests aiming the
attribute diagnostics. In one of the paired preference tests, the participants were given encoded samples from the blueberry developed formulas, formula A (60% cacao, blueberry, high in non-alkalized cocoa powder) and formula B (60% cacao, blueberry, high in alkalized cocoa powder). In the other paired preference test, the participants were given encoded samples of formula C (60% cacao, non-blueberry, high in non-alkalized cocoa powder) and the chocolate bar made using formula A. In each round, the participants were instructed to choose the most preferred sample considering the taste of the product.

In the third round, the developed formulas were compared with a well-established commercial chocolate brand of the same cocoa percentage to identify where the consumer preference falls compared to the commercial products. The participants were given the encoded chocolate samples formula A and C and the samples of “Ghirardelli-60%” (formula D). Then an attribute diagnostics questionnaire was given using a five-point hedonic rating scale, emphasizing the color, flavor, melting properties and the panelists' personal overall preference for the chocolates. As the final step of the third round, the panelists were told about the health benefits of polyphenols and were asked to evaluate the likeliness of purchasing these chocolates if they knew that each chocolate was low in fat and high in polyphenol content on a five-point hedonic scale. (Survey questions are attached in the appendix: A2)

**Statistical Analysis**

The first two paired preference tests were analyzed using a two tailed binomial distribution table (Lawless & Heymann, 2010) to determine whether the results of the study were due to chance or whether the panelists preferred one sample over the other. The final attribute diagnostic tests were analyzed using univariate analysis of variance (ANOVA) where the data were analyzed to determine the differences in consumer preference for each attribute (color, flavor, melting properties, and overall preference) and for their likeliness to buy the chocolate if they knew the chocolates were healthier. Each factor; color, flavor, melting properties, overall preference and the likeliness to buy the chocolates were analyzed separately using a five-point hedonic scale. These hedonic results were numbered from 1 to 5 to aid the statistical analysis (1 represented most preferred and 5 represented least preferred).

The data gathered during the attribute diagnostic tests were analyzed using the IBM SPSS software version 24. Since no significant differences in sensory perception were observed in the panel, all the data were pooled for the analysis. Significant differences detected by Levene test (p<0.05) were subjected to post hoc Tukey “Honestly Significant Difference” (HSD) multiple comparison to test the means of preference for each factor (color, flavor, melting properties, overall preference, likeliness to buy) at 0.05% significant level. In
addition to the multiple comparison test, descriptive tests were run to determine the means and corresponding standard deviations. The Welch was run to test the equality of means and finally the means plots were obtained for a better visualization of the results.

CHAPTER 4: RESULTS

Chocolate Formulation

The highlighted characteristics of each chocolate bar are listed in the Table 2.

Table 2: Characteristics of major formulas

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Highlighted Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula A</td>
<td>High percentage of non-alkalized cocoa powder with added freeze-dried blueberry powder</td>
</tr>
<tr>
<td>Formula B</td>
<td>High percentage of alkalized cocoa powder with added freeze-dried blueberry powder</td>
</tr>
<tr>
<td>Formula C</td>
<td>High percentage of non-alkalized cocoa powder</td>
</tr>
</tbody>
</table>

Polyphenol Analysis

The total polyphenol estimate of each type of chocolate was colorimetrically determined using the Folin-Ciocalteau (FC) method. The polyphenol estimates were given as gallic acid equivalents since gallic acid was used as the reference standard compound for calibration of all assays (calibration curve is attached in the appendix A1). The precision was determined for the analytical samples by calculating the percent relative standard deviations over the repeated analysis of each sample. Table 3 shows the obtained values of polyphenols estimates of each chocolate formula, with the average and the relative standard deviations, further the supporting UV-vis spectrum is shown in figure 2. Figure 3 gives a better visualization of the polyphenol estimates of each chocolate bar.

Table 3: Polyphenols estimate of chocolate bars made with Formula A, Formula B, Formula C, and the commercial chocolate bar (Formula D) respectively, determined by the Folin-Ciocalteau assay along with the percent relative standard deviations.
<table>
<thead>
<tr>
<th>Chocolate type</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Average ± Standard Deviation</th>
<th>%Relative Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula A</td>
<td>234.55</td>
<td>228.12</td>
<td>227.27</td>
<td>229.98±3.25</td>
<td>1.41%</td>
</tr>
<tr>
<td>Formula B</td>
<td>210</td>
<td>224.55</td>
<td>211.82</td>
<td>215.46±6.47</td>
<td>3.00%</td>
</tr>
<tr>
<td>Formula C</td>
<td>206.36</td>
<td>213.64</td>
<td>208.18</td>
<td>209.39±3.09</td>
<td>1.48%</td>
</tr>
<tr>
<td>Formula D</td>
<td>137.27</td>
<td>150.91</td>
<td>152.27</td>
<td>146.82±6.77</td>
<td>4.61%</td>
</tr>
</tbody>
</table>

Figure 2: UV-vis absorption spectrum (λ= 765 nm) of cocoa polyphenols expressed as Gallic acid equivalents in each chocolate bar made with each formulae A, B, C and the commercial chocolate bar D.
Figure 3: Polyphenols estimate of chocolate bars made with Formula A, Formula B, Formula C, and the commercial chocolate bar (Formula D) respectively, determined by the Folin-Ciocalteu assay (Error bars are represented as standard deviation of the means).

Following table shows the specifics of each chocolate bar made with each prototype formula A, B, C and the commercial chocolate bar-D.

Table 4: Specifics of each chocolate bar developed with formulae A, B, C, and the commercial chocolate bar (D).

<table>
<thead>
<tr>
<th></th>
<th>Cocoa%</th>
<th>Fat %</th>
<th>Total Calories (per 1 g)</th>
<th>Calories from fat (per 1 g)</th>
<th>Polyphenol content (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula A</td>
<td>60%</td>
<td>30%</td>
<td>4.51</td>
<td>2.71</td>
<td>229.98</td>
</tr>
<tr>
<td>Formula B</td>
<td>60%</td>
<td>30%</td>
<td>4.50</td>
<td>2.69</td>
<td>215.46</td>
</tr>
<tr>
<td>Formula C</td>
<td>60%</td>
<td>30%</td>
<td>3.95</td>
<td>2.71</td>
<td>209.39</td>
</tr>
<tr>
<td>Commercial Chocolate (D)</td>
<td>60%</td>
<td>40%</td>
<td>5.33</td>
<td>3.33</td>
<td>146.81</td>
</tr>
</tbody>
</table>

Fat percentage was calculated considering the fat content in 100 g of chocolate.
Sensory Evaluation

Several questions were included in the pre-survey to collect details about the panel members, including demographics (age and sex), sensory facts (whether they had any conditions or taking any medications which affect their smell and taste) and chocolate dietary habits (chocolate eating frequency and their preference order for dark, milk and white chocolates). Figure 4. represents the panel preference for different types of chocolates and the rest of the supporting figures are shown in the appendices A3 and A4.

Figure 4: Panel preference for the dark chocolates, milk chocolates, and the white chocolates.

Paired Preference Tests

Sensory evaluation was done in three rounds for the prototype chocolate samples made with formula A, B and C and for the commercial chocolate D. The first two rounds were paired preference tests. The chocolate bars made with formula A and formula B were compared based on taste in the first paired preference test and the results obtained are shown on the Figure 5. The analysis done with the two tailed binomial distribution table revealed that there is not enough evidence to conclude that the chocolate made with the formula B was preferred over the chocolate made with formula A. The second paired preference test compared the chocolate bars made with formula A and formula C based on taste and revealed that the chocolate made with the formula C was preferred over the chocolate made with formula A. The obtained results are shown on the Figure 6 and the statistical calculations done with the two tailed binomial distribution table in both instances are attached in the appendix (A5).
Figure 5: Results obtained from the paired preference test in between the chocolates made with formula A and formula B
(There is no statistically significant difference in between the preference for A and B)

Figure 6: Results obtained from the paired preference test in between the chocolates made with formula A and formula C
(A statistically significant difference exists in between the preference for A and C)
Attribute Diagnostic Tests

The participants were informed of the chocolate health benefits. Using the attribute diagnostic tests, consumer preference for color, flavor, melting properties, overall preference and the likelihood to purchase, the chocolates were assayed using the attribute diagnostic tests and analyzed using ANOVA.

Levene statistics revealed a statistically significant difference in consumer preference for the **color** among the three types of chocolates; chocolates made with formula A, formula C and the commercial chocolate D (p = 0.017). It was further confirmed by the Welch’s ANOVA test (p= 0.028); F (2, 162) = 3.497, p < 0.05, n²p = 0.041. Post hoc testing further revealed a significant difference in the preference for the color between the chocolates made with formula C and the commercial chocolate D (p=0.036). The color of the commercial chocolate D (mean: 1.65, standard deviation: 0.799) was preferred over formula C (mean: 2.02, standard deviation: 0.707). However, there was no difference in the preference for the color of the chocolate made with formula A (mean: 1.73, standard deviation: 0.781) over formula C or the commercial chocolate D.

When considering **flavor**, Levene statistics confirmed the homogeneity of variance among the three types of chocolates; chocolates made with formula A, formula C and the commercial chocolate D (p=0.277). But Welch’s ANOVA showed statistically significant differences in the means of preferences for flavor among the three types of chocolates (p=0.002); F (2, 162) = 6.432, p< 0.05, n²p = 0.073. Further, post hoc testing revealed that the preference for the flavor were significantly different between the chocolates made with formula A and D (p=0.002) and A and C (p=0.034). There was no significant difference in the flavor preference formula C and D. Overall, participants preferred the flavor of the chocolate of formula C (mean: 2.02 standard deviation: 0.933) and D (mean: 1.85 standard deviation: 0.931) over the chocolate made from the formula A (mean: 2.45, standard deviation: 0.857).

Levene statistics confirmed the homogeneity of variance for **melting properties** (p=0.313), but Welch’s ANOVA showed statistically significant differences in the means of preferences for melting properties between the three types of chocolates (A, C and D) (p=0.001); F (2, 159) = 7.204, p< 0.05, n²p = 0.083. Post hoc testing revealed that the preferences for melting properties were significantly different between the chocolates made with formula A and C (p=0.003) and A and D (p=0.004). There was no significant difference for the flavor preference between the chocolates made with formula C and D (p=0.995). In overall, the panelists preferred the melting properties of C (mean: 1.96, standard deviation: 0.910) and D (mean: 1.98, standard deviation: 1.065) more than the chocolate made from the formula A (mean: 2.56, standard deviation: 0.834).

Although the homogeneity of variance for **overall preference** was confirmed by the Levene statistics (p=0.981), the Welch’s ANOVA for overall preference showed statistically significant differences in the means of overall preference for each type of
chocolate \((p=0.004)\); \(F(2, 162) = 5.508, p< 0.05, n^2_p= 0.083\). Further clarifications were provided with the post hoc analysis and it revealed that the overall preference was significantly different between the chocolates made with formula A and D \((p=0.003)\). But there was no significant difference for the overall preference in-between the chocolates made with formula A and C \((p=0.246)\) and C & D \((p=0.203)\). In overall, the panelists preferred the commercial chocolate D (mean: 1.82, standard deviation: 0.863) more than the chocolate made from the formula A (mean: 2.38, standard deviation: 0.850).

The final attribute diagnostic test was to evaluate the** likeness of the panelists to buy each type of chocolate if they thought the chocolates were healthy.** Levene statistics confirmed the homogeneity of variance for likeliness to buy \((p=0.861)\) and it was further confirmed by the Welch’s ANOVA as it didn’t show a statistically significant difference in the means of likeliness to buy between all the three types of chocolates \((p=0.160)\); \(F(2, 162) = 1.876, p< 0.05, n^2_p= 0.023\).

**Table 5: The summary of the results obtained from the statistical analysis of the attribute diagnostic sensory evaluation of each type of chocolate including the chocolates made with formula A C and D**

<table>
<thead>
<tr>
<th>Consumer Preference for the Attribute</th>
<th>Significant differences observed in between</th>
<th>No significant differences observed in between</th>
<th>Panelists’ Preference order for each chocolate type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>C and D</td>
<td>A and C, A and D</td>
<td>D&gt;C</td>
</tr>
<tr>
<td>Flavor</td>
<td>A and C</td>
<td>C and D</td>
<td>C &amp; D &gt; A</td>
</tr>
<tr>
<td>Melting Properties</td>
<td>A and C</td>
<td>C and D</td>
<td>C &amp; D &gt; A</td>
</tr>
<tr>
<td>Overall Preference</td>
<td>A and D</td>
<td>A and C, C and D</td>
<td>D&gt; A</td>
</tr>
<tr>
<td>Likeliness to Buy (If known to be healthy)</td>
<td>None</td>
<td>A, C, and D</td>
<td>Similar for all</td>
</tr>
</tbody>
</table>

A: 60% cacao chocolate bar made with a greater percentage of non-alkalized cocoa powder, including freeze dried blueberry powder using Formula A  
C: 60% cacao chocolate bar made with a greater percentage of non-alkalized cocoa powder, excluding freeze dried blueberry powder using formula C  
D: 60% cacao chocolate bar of a well-established commercial brand.
Table 6: Descriptive statistics of the preference for each attribute including color, flavor, melting properties, overall preference and the likeliness to purchase (if the health benefits were known) of chocolates made with formula A, C and D

<table>
<thead>
<tr>
<th>Mean values of consumer preference</th>
<th>Color</th>
<th>Melting Properties</th>
<th>Flavor</th>
<th>Overall Preference</th>
<th>Likeliness to Buy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula A</td>
<td>1.73(0.781)</td>
<td>2.56(0.834)</td>
<td>2.45(0.857)</td>
<td>2.38(0.850)</td>
<td>2.24(1.138)</td>
</tr>
<tr>
<td>Formula C</td>
<td>2.02(0.707)</td>
<td>1.96(0.910)</td>
<td>2.02(0.933)</td>
<td>2.11(0.956)</td>
<td>2.04(1.186)</td>
</tr>
<tr>
<td>Chocolate D</td>
<td>1.65(0.799)</td>
<td>1.98(1.065)</td>
<td>1.85(0.931)</td>
<td>1.82(0.863)</td>
<td>1.80(1.223)</td>
</tr>
<tr>
<td>Levene Statistic</td>
<td>*0.017</td>
<td>0.313</td>
<td>0.277</td>
<td>0.981</td>
<td>0.861</td>
</tr>
<tr>
<td>Welch Statistic</td>
<td>*0.028</td>
<td>*0.001</td>
<td>*0.002</td>
<td>*0.004</td>
<td>0.160</td>
</tr>
</tbody>
</table>

Scale for color: 1=Excellent, 3=Average, 5=Terrible.
Scale for melting properties: 1=Extremely good, 3=Neither good nor bad, 5=Extremely bad
Scale for flavor: 1=Delightful, 3=Average, 5=Terrible
Scale for overall preference: 1=Excellent, 3=Fair, 5=Awful
Scale for likeliness to buy: 1=Extremely likely, 3=Neither likely not unlikely, 5=Extremely unlikely

*Means of 55 panelists, bMeans of 54 panelists, cMeans of 53 panelists; numbers in parenthesis refer to standard deviation.

*The mean/variance difference is significant at the 0.05 level

Multiple Comparisons

<table>
<thead>
<tr>
<th>Mean Difference (I-J)</th>
<th>(I) Chocolate sample</th>
<th>(J) Chocolate sample</th>
<th>Color</th>
<th>Melting Properties</th>
<th>Flavor</th>
<th>Overall Preference</th>
<th>Likelihood to Buy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tukey HSD</td>
<td>Formula A</td>
<td>Formula C</td>
<td>-.291(.146)</td>
<td>.601(.180)*</td>
<td>.436(.173)*</td>
<td>.273(.170)</td>
<td>.200(.226)</td>
</tr>
<tr>
<td></td>
<td>Chocolate D</td>
<td>Formula A</td>
<td>.073(.146)</td>
<td>.583(.181)*</td>
<td>.600(.173)*</td>
<td>.564(.170)*</td>
<td>.436(.226)</td>
</tr>
<tr>
<td></td>
<td>Formula C</td>
<td>Chocolate D</td>
<td>.291(.146)</td>
<td>-.601(.180)*</td>
<td>-.436(.173)*</td>
<td>-.273(.170)</td>
<td>-.200(.226)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chocolate D</td>
<td>.364(.146)*</td>
<td>-.018(.182)</td>
<td>.164(.173)</td>
<td>.291(.170)</td>
<td>.236(.226)</td>
</tr>
<tr>
<td>Chocolate D</td>
<td>Formula A</td>
<td>Formula C</td>
<td>-.073(.146)</td>
<td>-.583(.181)*</td>
<td>-.600(.173)*</td>
<td>-.564(.170)*</td>
<td>-.436(.226)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Formula C</td>
<td>-.364(.146)*</td>
<td>.018(.182)</td>
<td>-.164(.173)</td>
<td>-.291(.170)</td>
<td>-.236(.226)</td>
</tr>
<tr>
<td>Games-</td>
<td>Formula A</td>
<td>Formula C</td>
<td>-.291(.142)</td>
<td>.601(.167)*</td>
<td>.436(.171)*</td>
<td>.273(.172)</td>
<td>.200(.222)</td>
</tr>
<tr>
<td>Howell</td>
<td>Chocolate D</td>
<td>Formula C</td>
<td>Forma A</td>
<td>Chocolate D</td>
<td>Formula A</td>
<td>Chocolate D</td>
<td>Formula C</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
<td>-----------</td>
<td>---------</td>
<td>-------------</td>
<td>-----------</td>
<td>-------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Formula C</td>
<td>0.073 (.151)</td>
<td>0.583 (.184)*</td>
<td>0.600 (.171)*</td>
<td>0.564 (.163)*</td>
<td>0.436 (.225)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forma A</td>
<td>0.291 (.142)</td>
<td>-0.601 (.167)*</td>
<td>-0.436 (.171)*</td>
<td>-0.273 (.172)</td>
<td>-0.200 (.222)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate D</td>
<td>-0.364 (.144)*</td>
<td>-0.018 (.192)</td>
<td>0.164 (.178)</td>
<td>0.291 (.174)</td>
<td>0.236 (.230)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate D</td>
<td>0.073 (.151)</td>
<td>-0.583 (.184)*</td>
<td>-0.600 (.171)*</td>
<td>-0.564 (.163)*</td>
<td>-0.436 (.225)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula C</td>
<td>-0.364 (.144)*</td>
<td>0.018 (.192)</td>
<td>-0.164 (.178)</td>
<td>-0.291 (.174)</td>
<td>-0.236 (.230)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 7: Post hoc analysis of the preference for each attribute including color, flavor, melting properties, overall preference and the likeliness to purchase (if the health benefits were known) of chocolates made with formula A, C and D*

Scale for color: 1=Excellent, 3=AVERAGE, 5= Terrible.
Scale for melting properties: 1=Extremely good, 3= Neither good nor bad, 5=Extremely bad
Scale for flavor: 1=Delightful, 3= Average, 5=Terrible
Scale for overall preference: 1=Excellent, 3=Fair, 5=Awful
Scale for likeliness to buy: 1=Extremely likely, 3=Neither likely not unlikely, 5=Extremely unlikely
Numbers in parenthesis refer to the standard error; *The mean differences are significant at the 0.05 level
The intention of this research was to develop a healthier chocolate formula that is high in polyphenol content and low in fat content, thus three formulas (formula A, B and C) were developed. The resulting chocolates from these formulas were compared with a commercial chocolate bar of a well-established brand (Ghirardelli) for the polyphenol content and for some selected sensory attributes (color, flavor, melting properties and overall preference).

The first research objective was to find out whether there is a significant consumer preference for one chocolate bar over the other based on taste among the formula A and B. Formula A and B were similar with the only difference between the type of cocoa powder used. Formula A had a higher percentage of non-alkalized cocoa powder whereas formula B had a higher percentage of alkalized cocoa powder. The first paired preference test resulted in no significant difference between formula A and B based on taste. As shown in Figure 3, formula A has a higher polyphenol estimate than formula B. The reason for this is the differences in the alkalized and non-alkalized cocoa powder content used in each formula. Among alkalized cocoa powder and non-alkalized cocoa powder, the latter has a higher percentage of cocoa polyphenol due to the absence of alkalization process. Alkalization process is known to remarkably decrease the catechin and procyanidins which causes the reduction of total polyphenols (Wollgast & Anklam, 2000). This theory can further explain the observed polyphenol estimate differences between the chocolate bars made with formula A and B.

The second research objective was to find out the existence of a significant consumer preference for one chocolate bar over the other based on taste among the formula A and C. Formula A and C were also mainly similar except for the absence of freeze-dried blueberry powder in formula C. This comparison allowed the determination of whether the addition of blueberry powder (which increased the polyphenol content), made a difference in consumer preference. The second paired preference test indicated a significant difference in consumer preference between the chocolate bars made with formula A and C. Formula C was preferred the most. Since the only difference between formula A and C was the absence of freeze-dried blueberry powder in formula C, it is possible to interpret that consumers did not prefer the astringent flavor resulted from the blueberry powder in formula A.

The third research objective was to find out whether the addition of blueberry powder increases the total polyphenol content of the chocolate bar without compromising the overall consumer preference. The folin-ciocalteu assay determined that blueberry powder increased the total polyphenol estimate. But the results of the paired preference test in between the formula A and C confirmed that the addition of blueberry powder compromises the consumer preference for taste, as the panelists preferred the formula which did not have blueberry (formula C). The analysis of each factor (color, flavor, melting properties, overall preference and the likeliness to buy the chocolates if known to
be healthy) further provide the supporting evidences for the first two paired preference tests as to why the panelists preferred each chocolate type. (All the supporting statistical plots for each factor are attached in the appendix separately: A6)

Based on participant preferences, formula C was the best formulation as compared with the commercial chocolate D. When considering the results (summarized in the table 5), it is obvious that there was no significant difference between the chocolate made with formula C and the commercial chocolate D regarding each attribute (flavor, melting properties, overall preference and likeliness to buy), except for the preference for the color. Additional research would be needed to improve the color of formula C without changing the other well-established important attributes. Formula C could be marketed for its health value of a higher polyphenol content (42.6% greater) and a lower fat content (10% lower) as compared to commercial chocolate.

The other most important implication that this analysis gave was the attitude of the consumers towards a healthy chocolate formula. This analysis clearly showed that consumers would prefer to purchase a healthy chocolate regardless of their preferences to other attributes like color, flavor, melting properties as well as their overall personal preference. Which gives a very good measure of the acceptability and the success of the prototype chocolate formula C. Even though formula C did not contain any freeze-dried blueberry powder in it, the polyphenol content was not much contrasting to that of the formula A which had freeze-dried blueberry powder (8.9% higher polyphenols in formula A than formula C). The results imply that the consumers did not prefer the astringent flavor in the chocolate which resulted from the blueberry powder. Therefore, it is possible to conclude that the prototype formula C is superior to the prototype formula A and is also comparable to the commercial chocolate D in flavor, melting properties and the overall consumer preference. Most importantly as consumers preferred to purchase the chocolates considering the health benefit regardless of their taste, it possible to imply that formula C would be able to compete with the commercial chocolate D due to the higher health benefits and its’ comparable attributes to the commercial chocolate D. Therefore, the formula C could be considered as the best prototype formula among the developed three formulae (A, B and C) and it can be further developed to enhance the color and other attributes in future work.
CHAPTER 6: CONCLUSION

There was no significant difference in-between the consumer preferences for the “60% cacao” chocolate bar made with a greater percentage of non-alkalized cocoa powder using Formula A and the “60% cacao” chocolate bar made with a greater percentage of alkalized cocoa using Formula B. But a significant difference in consumer preference was found in between the chocolate bar that includes Blueberry fruit powder (made with Formula A) and the chocolate bar of the same cocoa percentage that excludes Blueberry fruit powder (made with Formula C). When the chocolate bars made with formula A and formula C were compared with the commercial chocolate bar D, the chocolate bar made with the formula C became superior to the chocolate made with formula A and was found to be comparable with the commercial chocolate D regarding the consumer preference for each attribute; flavor, melting properties, and their personal overall preference except for the preference for the color. These results confirmed that the addition of blueberry fruit powder increases the total polyphenol content of the chocolate bar, but it compromises the consumer preference. The results also revealed that the consumers would prefer to purchase each type of the chocolate bars, including chocolates made with formula A, C and the commercial chocolate bar-D, considering the health benefit regardless of their taste (After the consumers were acknowledged about the health benefits of polyphenols and low fats). From the developed chocolate formulae, the formula C could be chosen to develop further as it was superior to the rest of the formulae and was comparable to the selected well-established commercial chocolate.
A1. Calibration curve used for the polyphenol analysis via Folin-Ciocalteu assay.

\[ y = 0.0011x + 0.0147 \]
\[ R^2 = 0.9982 \]
A2. Survey questions used for the sensory evaluation study.

Demographics

Sex

- Male
- Female

Age

- <18
- 18-30
- 31-40
- 41-50
- 51-64
- >64

Sensory Properties

Do you have any of the following conditions which may affect taste or smell?
If you do, please check “yes”
- Diabetes, Hypertension, Oral or Gum diseases, Fever or Common Cold

- Yes
- No
Do you have any food allergies for the followings? If you do, please check "Yes"

Milk, Cocoa, Blueberry, Soy, Erythritol, Maltitol, Maltodextrin, Polydextrose

☐ Yes
☐ No

Do you take any medications, which may affect your senses, especially taste, and smell?

☐ Yes
☐ No

**Eating Habits**

How often do you consume chocolate per week?

☐ 0
☐ 1-3
☐ 4-6
☐ 7

Please rank your preference for the following three types of chocolates in the order of 1-3 where, 1 being most preferred and 3 being least preferred. Drag the boxes and drop them in the order of your preference from top to bottom.

Dark Chocolate
Milk Chocolate

White Chocolate

Sensory Evaluation

Consider the samples on plate "A"

You are given two samples of chocolate. Taste them from left to right and indicate your most preferred sample based on the "taste", by checking the appropriate code number.

(Rinse your palate with water, and wait for half a minute between tasting samples)

463

☐

189

☐

Please comment on the reasons for your choice of sample on "Plate A"
Consider the samples on plate "B"

You are given two samples of chocolate. Taste them from left to right and indicate your most preferred sample based on the "taste", by checking the appropriate code number.

(Rinse your palate with water, and wait for half a minute before and in between tasting samples)

<table>
<thead>
<tr>
<th>197</th>
<th>201</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please comment on the reasons for your choice of sample on "Plate B"


Consider the samples on plate "C"

You are given three samples of chocolate. Observe the "color" of each sample and select the most appropriate phrase based on your preference for the "color" of each sample separately.

(Please evaluate each sample independently without comparing the samples to one another)

Please don’t taste the sample "458" of the following samples if you are allergic to milk or soy. If so please continue with the other samples and complete your questionnaire leaving the response to the sample 458 as blank
<table>
<thead>
<tr>
<th></th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Excellent</td>
</tr>
<tr>
<td>327</td>
<td></td>
</tr>
<tr>
<td>613</td>
<td></td>
</tr>
<tr>
<td>458</td>
<td></td>
</tr>
</tbody>
</table>

Consider the samples on plate "C"

Take one sample at a time and place 1/8th of the sample between the tongue and roof of the mouth over 4 minutes or until the sample has completely melted. Select the most appropriate phrase based on your preference for the "melting properties" of each sample separately.

(Please evaluate each sample independently without comparing the samples to one another)

<table>
<thead>
<tr>
<th></th>
<th>Melting properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extremely good</td>
</tr>
<tr>
<td>327</td>
<td></td>
</tr>
<tr>
<td>613</td>
<td></td>
</tr>
<tr>
<td>458</td>
<td></td>
</tr>
</tbody>
</table>

Consider the samples on plate "C"

You are given three samples of chocolate. "Sniff and taste" each sample and select the most appropriate phrase based on your preference for the "flavor" of each sample separately. Make sure to rinse your palate with water, and wait for half a minute before and in between tasting samples.
(Please evaluate each sample independently without comparing the samples to one another)

<table>
<thead>
<tr>
<th></th>
<th>Delightful</th>
<th>Good</th>
<th>Average</th>
<th>Poor</th>
<th>Terrible</th>
</tr>
</thead>
<tbody>
<tr>
<td>327</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>613</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>458</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Consider the samples on plate "C"

Based on the color, melting properties and flavor of each chocolate sample, select the most appropriate phrase which defines your "overall preference" towards each sample separately.

(Please evaluate each sample independently without comparing the samples to one another)

<table>
<thead>
<tr>
<th></th>
<th>Excellent</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
<th>Awful</th>
</tr>
</thead>
<tbody>
<tr>
<td>327</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>613</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>458</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Consider the samples on plate "C"

How likely would you "buy" each of these chocolates, if it is healthy, more specifically if they are low in fat content and high in polyphenol content?

**Health benefits of polyphenol are listed below**

- Protective against elevated blood pressure, dyslipidemia, platelet adhesion, insulin resistance, inflammation, glucose intolerance, heart disease and cancer.
- Improve the immune responses, antioxidant defense system, and cognitive functions of elderly.
- Having a suggested role in treatments of dementia and stroke.
- Reduces LDL cholesterol (which are bad for your body) and increase HDL cholesterol (which are good for your body)

(Please evaluate each sample independently without comparing the samples to one another)

<table>
<thead>
<tr>
<th></th>
<th>Extremely likely</th>
<th>Somewhat likely</th>
<th>Neither likely nor unlikely</th>
<th>Somewhat unlikely</th>
<th>Extremely unlikely</th>
</tr>
</thead>
<tbody>
<tr>
<td>327</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>613</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>458</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please list the additional comments you have for the chocolate samples on the "plate C"
A3. Demographic information of the sensory evaluation panel.

A3.1. The percentage of panelists of each sex

A3.2. Information on the age of the panelists
A4. The chocolate consumption frequency of the sensory evaluation panel

X axis: Age of the panelists  
Y axis: Chocolate consumption frequency.

A5. Statistical analysis for the paired preference tests

A5.1. Paired preference test in between the chocolate bar made with formula A and formula B.

Ho: \( p(A) = p(B) = \frac{1}{2} \).  
Ha: \( p(A) \neq p(B) \).

The number of panelists who preferred each formula:

- Formula A: 24/55  
- Formula B: 31/55

Two-tailed binomial table value for 55 participants: 36  
Actual number of the larger of the two segments: 31

The table value for 55 panelists in the two tailed binomial distribution table is 36 with an alpha criterion of 5%. This value is larger than 31 and therefore the consumer panelists did not have a preference for one sample over the other.
A.5.2. Paired preference test in between the chocolate bar made with formula A and formula C.

Ho: $p(A) = p(C) = 1/2$.
Ha: $p(A) \neq p(C)$.

The number of panelists who preferred each formula:

- Formula A: $13/55$
- Formula C: $42/55$

Two-tailed binomial table value for 55 participants: 36
Actual number of the larger of the two segment: 42

The table value for 55 panelists in the two tailed binomial distribution table is 36 with an alpha criterion of 5%. The obtained value of 42 is greater than this minimum (36) and therefore the consumers had a significant preference for sample C, over sample A at alpha of 5%.
A6. Plots obtained from the statistical analysis for the attribute diagnostic tests.

All the plots were derived from the software IBM SPSS version 24.

Mean preference for the color of each chocolate type made with formula A and C and the commercial chocolate D.
Mean preference for the flavor of each chocolate type made with formula A and C and the commercial chocolate D.
Mean preference for the melting properties of each chocolate type made with formula A and C and the commercial chocolate D.
Mean overall personal preference for each chocolate type made with formula A and C and the commercial chocolate D.
Mean likeliness to buy each chocolate type made with formula A and C and the commercial chocolate D, if they were known to be healthy.
Step 1. Fermentation and Bean Cleaning

The cocoa beans are fermented and dried to enable the development of chocolate flavor precursors. Then they are cleaned to remove all extraneous material.

Correct fermentation is important to bring out the flavor in the final chocolate. This process stops ripening thus preventing germination. This reduces the chance of spoilage. There are two main types of fermentation methods: heap and box. In heap fermentation, fresh beans with small amount of white pulp are fermented together in heaps covered with banana leaves. The process usually last 5-6 days and the beans should be turned after 2-3 days. About 25 – 2500 kg of fresh beans could be fermented in heap fermentation method. In box fermentation, wooden boxes that could hold between 1-2 tons of beans are used and these are designed with outlet holes or slits, usually in the base to provide ventilation and to let water from the beans and pulp run away. These boxes could either be 1-meter deep or 250- 500 mm shallow. The beans should be tipped from one box to another each day to increase the aeriation and to give a uniform treatment. The fermentation period is similar to the heap fermentation method.

The fermentation process takes place outside the bean. The sugar rich white pulp allows yeast to react to form acids and ethanol. Ethanol then activates other bacteria such as ethanoic acid and lactic acid bacteria, which then convert it into their respective acids. The ethanol and acid are able to pass through the shell into the bean, which changes the pH level of the bean. The bean can no longer ripen thus preventing germination. Upon the death of the bean, enzymes release and decompose the food reserves and form sugars and flavor precursors. Different fermenting methods give rise to different flavors. In box fermentation aeration of the beans are increased which stimulates bacteria that require oxygen and encourages the production of ethanoic acid. Other reactions that involve yeast are retarded in the presence of oxygen, so the ethanol production is hindered. Thus, in box fermentation, the beans are more likely to taste acidic.

After the fermentation, beans must be dried to prevent molding and then they are transported to the chocolate factories. Over dried beans to a moisture content less than 6% will become very brittle and difficult to process. Beans could either be sun dried or artificially dried. Sun drying has an increase in risk of contamination from insects and rodents. Artificial drying uses forced air to provide more efficient heat exchanger. Before processing, the dried beans must be cleaned to remove the impurities for two major reasons. First is to avoid the damage to the machinery used in bean grinding and next to remove the organic contaminants which will burn during the roasting process and emit gasses that are likely to spoil the cocoa flavor. Impurities like iron could be removed by magnets whereas dust can be drawn off by suction. Stones can be removed due to their difference in densities than the beans by vibrating them together of a grid which is set at an angle to the horizontal.
Step 2. Roasting

The beans are roasted for further chocolate flavor and color development. This step is advantageous as it tends to help the separation of shell from the nib which makes breaking and winnowing easier. In addition, high temperature could also kill any microbial contaminants such as salmonellae. This stage will also serve as a critical control point for hazard analysis to confirm the cocoa beans are safe to consume. Roasting will vary depending on the variety of beans and the appropriate process, temperature, time and degree of moisture.

Cocoa beans come in variety of sizes, depending on the county of origin, climate conditions, seasons where the pod was picked and other factors. If these various sized beans are roasted together on a condition that matches for an average sized bean, the smaller ones will be over roasted whereas the center of the larger beans will not be roasted enough. This prevents the conversion of flavor precursors and result in a lesser chocolate flavor. To overcome this problem, either the cocoa nib roasting or the cocoa liquor roasting is being practiced as an alternative to bean roasting. Both the methods require the shell to be removed before roasting, as it may contain silica particles which could damage the machinery and in addition it could impart an inferior flavor to the chocolate.

The high temperatures and drying during roasting removes many of the volatile acids, especially ethanoic acids which causes the astringency and bitterness of the unroasted beans. The less volatile acids such as ethanedioic (oxalic) and lactic, remains unchanged during the roasting process. One important chemical reaction that happens during roasting is the Maillard Reaction. This is also known as non-enzymatic browning and it gives the products their color and flavors upon baking, toasting or roasting. This is a reaction between reducing sugars, mainly D-glucose, and a free amino acid or a free amino group of an amino acid that is part of a protein chain. At elevated temperatures, the reaction causes decomposition and eventually condensation to insoluble brown products known as melanoidins. The next reaction is the Strecker degradation, which involves the formation of aldehydes by degrading amino acids upon the reaction with carbonyl derivatives from the non-enzymatic browning. The aldehydes contribute to aroma development during the browning reaction. Strecker degradation of each amino acid produces a specific aldehyde with a distinctive aroma (Fennema, 1996).

When the amino acid glycine reacts with glyoxal which is a 1,2-dioxo compound, it eventually forms pyrazines, which is used as a measure in determining the degree of roast of cocoa liquor. The amount of different pyrazine formed depends on the temperature and time of the roasting reaction. The characteristic chocolate smell is a result of the reaction between amino acids such as leucine, threonine and glutamine with glucose, when heated to about 100 °C. The higher the temperature, the more penetrating or pungent the smell would be.

Step 3. Winnowing
Winnowing is the process of separating the shell and some of the germ from the rest of the bean. A winnowing machine is used and leaves only the cocoa nibs which usually contain approximately 53% cocoa butter. The shell is largely fibrous, light and large in surface area; thus, it rises through the nibs when air is drawn upwards through the mixture. The presence of shell in the final chocolate would impair off flavors. The presence of shell in chocolate is legally restricted in most markets.

**Step 4. Grinding of Cocoa Nibs.**

There are two main objectives of grinding cocoa nibs. The first is to reduce the particle size of cocoa and the next is to remove as much fat as possible from the cells within the cotyledons. Once the fat has been removed from the cells, it will coat the solid non-fat particles within the chocolate. The maximum particle size of a nib would be about 0.5 cm but needs to be ground to less than 30 microns, a reduction of 100 times. To achieve this at least two grinding stages are required. First an impacting mill is needed to melt the fat followed by a ball or disc mill.

Impact mills hit the cocoa nibs with fast moving pins or hammers causing the cocoa butter melt. Then the free fat together with smaller particles are passed through a sieve while the larger particles remain on the inside where it will be broken by the next series of pins or hammers. In the disc mills, there are three pairs of carborundum discs which either rotate or remain stationary. When the cocoa liquor and the nibs are fed in to the center of the top set of discs, the cocoa mass is forced through by centrifugal force and then the cocoa liquor runs down a chute. Ball mills only work with liquids. They consist of a large number of balls which are made to bounce against each other. Particles trapped between the balls are broken by crushing or pulling apart due to the shearing of the rotating action.

There is cocoa starch included in the milled cocoa particles, which is about 7% by weight of the cocoa liquor with a particle size of between 2 – 12.5 microns. This is not destroyed by the milling process. Nearly 10% of the liquor is made up of cellulose and protein is present in a slightly larger percentage.

**Step 4. Alkalizing (Dutching)**

Alkalizing was a process developed in the Netherlands in the 19th Century, commonly referred to as the “Dutching process”. This process makes the cocoa powder less likely to agglomerate or sink to the bottom when it is added to a milk or water-based drink. The darker the color, the milder the taste. The cocoa nibs undergo alkalization. The nibs are treated with potassium carbonate. Too much alkali should not be added since saponification of the cocoa butter could occur by reacting with the acids attached to the glycerol backbone of the cocoa butter molecule, which would result in a soapy flavor. To overcome this, small amounts of ethanoic acid or tartaric acid may be added after alkalization to lower the pH. Certain types of beans contain ethanoic acid which make
them taste very acidic. For such beans, mild alkalization is beneficial to neutralize these acids.

The color change occurs due to the reactions based on tannins. The tannins are made up of epicatechin molecules which may join during fermenting, drying and roasting stages and oxidize or react with other chemicals within the cocoa. This increases the number of molecules responsible for color and makes the cocoa much darker. It is possible to produce a wide variety of colors by adjusting the pH, moisture, and roasting temperature and times. Evidence from the literature suggests that the flavanols are substantially reduced when processed with alkali (Miller et al., 2008).

**Step 6. Cocoa Butter Production**

The cocoa liquor is pressed to extract the cocoa butter which leaves a solid mass called a cocoa press cake. The cake contains 8% to 24% fat. Pure pressed cocoa butter has a flavor which will become a part of the whole chocolate. For some products this flavor may be unpleasant and the cocoa butter has to be deodorized by steam distilling the cocoa butter under vacuum. A continuous expeller process usually produces lower quality cocoa butter by pressing the whole cocoa bean including the shell which contains fats other than cocoa butter which get mixed and makes the quality inferior. This effects adversely to the to the hardness and setting properties of the cocoa butter due to its eutectic effect. This lower quality cocoa butter is often cloudy and must be filtered. The maximum free fatty acid content of cocoa butter is usually 1.75% and the maximum saponification value is 0.5%. Free fatty acids effect the setting properties whereas the saponification value ensures that the cocoa butter does not have a soapy flavor.

**Step 7. Cocoa Powder production.**

The cocoa press cake is broken into small pieces less than 3 cm in diameter to form kibbled press cake by two spiked rollers rotating in the opposite directions. Then it is finely grounded by a cooled pin mill and it is further being strongly cooled as it is transported in an air stream to the packing area to solidify the liquid fat to prevent the powder from sticking together. Finally, it is being collected in a cyclone separator with the finer particles that is removed by a filter system.

Manufacturer could control the amount of butter extracted from the liquor to produce press cake with different proportions of fat in order to produce cocoa powders with different fat percentages. Mostly, cocoa powder is having a fat content of 20-22% and lower fat ranges are between 15-17% or 10-12%. Fat free powders are also being produced targeting the low fat or fat free products.

**Step 8. Chocolate production**

Primarily chocolate is produced through the addition of cocoa butter to the cocoa liquor. Depending on the type of chocolate that needs to be made, different proportions of other
ingredients such as sugar, milk, emulsifying agents, and cocoa butter equivalents are added and mixed.

**Step 9. Chocolate Refining/Milling.**

This process is important to remove all the particles larger than 30 microns that adds the chocolate a grittiness. There are two different processes named fine ingredient milling and combined milling. The solid non-fat components are milled separately prior to the addition of cocoa liquor and cocoa butter in the fine ingredient milling where as in the combined milling, the dry ingredients are mixed with the cocoa liquor and some of the other fats before milling takes place. The two processes give different flavor and each process has its own advantages and disadvantages. The number of fine particles could be controlled better in the fine ingredient milling, but at the end of the grinding the particles would be largely fat free. Thus, the fat coating process in the conche would take longer than the combined milling process.

There are two types of mills used in the fine ingredient milling process, the hammer/pin mill and the classifier mill. As the mills generate a lot of heat, some of the sugar will turn from a crystalline in to an amorphous form and the fat present will melt and cause the particle to be sticky. Thus, a cooling process is required if more than 12% of fat is present. At even higher levels, a cryogenic grinding is carried out using liquid nitrogen fed air.

Combined milling is often used in modern chocolate manufacturing industries which uses two roll mills or five roll mills. In two roll refiners, two cylinders which turn in opposite directions are placed horizontally side by side. Five roll mills are for the final grinding and they can reduce the particle size of the paste to about 15-35 microns. They consist of five slightly barrel shapes horizontal cylinders with four of the cylinders placed one above the other. The cylinders can be cooled or heated by the water which flows through the hollows of the cylinder as the temperature is an important factor in the operation of a roll refiner. The temperature could alter the texture/viscosity by changing the flow properties of the fat present and throw the chocolate away from the machine during milling. The shear between the roller during the milling helps the particles to break as well as to coat the newly created surfaces with fat. Additionally, the newly created surfaces would pick up the volatile flavor chemicals from the cocoa particles that were broken nearby at the same time as these surfaces are chemically very reactive.

The fineness of the particles depends on the type of the chocolate product, such that dark chocolate is generally finer than milk chocolate, and chocolate for cookie drops could be coarser than a solid eating chocolate.

**Step 10. Chocolate Conching**

During conching, two distinct processes take place. It is a kneading/smoothing process and also a flavor development process. The flavor components developed during the
fermentation and roasting processes is able to give the chocolate a pleasant taste as well it results some undesirable astringent taste which needs to be removed. The conching process enables this astringency removal as well as further flavor development as required by some products. The kneading/smoothing process of conching involves coating the surfaces of the solid particles with fat which turns the chocolate from a thick dry paste in to a free-flowing liquid that uses to make the final product.

Conching process cannot remove the acids like ethanoic acid and short chain volatile fatty acids such as propionic and isobutyric acids directly by the higher temperature involved in conching as their boiling points are above 118 °C, which is higher than the conching temperatures. But these acids are removing by a type of steam distillation as the moisture is removed during this process. The amounts of volatiles could be removed by about 80% during the first few hours of conching which is also disadvantageous when it comes to the phenolics removal. Care should be taken not to over conch as it would make the flavor very mild at all.

The time and temperature used in conching is critical for chocolate flavor development. In milk chocolate, the cooked flavor begins to develop above 70 °C. Above 100 °C Maillard type flavors would promote above 100 °C but they would not be as strong due to the less water present. The conching temperature should be kept below 50 °C for sugar free chocolates which contains only sugar alcohols, since the high temperature could cause the crystals to be melt and resolidify later causing a grittiness to the chocolate.

Conching causes the physical movement of flavor molecules between different components of the chocolate which result in more uniform cocoa flavor and less sweetness due to the transfer of cocoa flavor and fat on to the sugar surface. This is happening by the initial flavor concentration gradient between different particles or phases. Additionally, the amorphous sugars formed during roll refining helps to absorb the flavor as well. Conching is also important to reduce the viscosity of the chocolate. This is done by the applied shear during conching and higher the shear rate, the thinner would be the chocolate. There is a practical limit to the developing shear in the conche as it requires very large motors and a higher energy. To overcome this, basically two approaches take place to reduce the viscosity. One is to increase the volume of the stirred tank so that only a small amount of chocolate will be sheared in a single moment and since the volume of the tank is larger, lot of chocolate will be inside the tank for several hours allowing enough throughput in the regions of tons per hour. In the other approach, a few kilograms are highly shearing at a time in a continuous processor. Since only a small amount of chocolate would be sheared inside the machine; it can stay there for a fraction of a minute to give the same throughput as a large conche.

At the end of the conching, the chocolate should have the right flow properties for the next processing stage. Therefore, at the final stage of conching emulsifiers should be added and mixing should take place for a little further. After the conching step, the chocolate could be stored in tanks and be transported as a liquid or can be solidified and stored or transported as blocks or small chips before further processes.
Step 11. Chocolate Tempering

Tempering is a heating, cooling, and reheating process, which induces partial pre-crystallization of the cocoa butter, so that crystals form nuclei which further helps in fat set rapidly in the correct form. Uncontrolled crystallization will result in crystals of varying sizes which will cause the surface of chocolate to fat bloom. Tempering prevents the fat bloom as well as discoloration of the product by preventing the development of certain crystalline formations of cocoa butter. During this process, temperature is controlled (β-modification) to leave only the type V crystals among the six different types of crystals of cocoa butter fat which will result in a chocolate with uniform glossy appearance and a good texture that snaps when broken. During this process, chocolate will be heated to 45°C first to melt all the six crystal forms, then type IV and V are allowed to form by cooling to about 27°C. It is then agitated to create small crystal “seeds,” which serve as nuclei to create additional small crystals in the chocolate. The chocolate is then heated to 31°C to eliminate the type IV crystals, leaving only type V crystals. As an alternative method to the tempering process, already tempered solid “seed” chocolate is added to the mixture.

The newly formed crystals are small and easily melted. By stirring and slowly heating, the crystals become more stable with a higher melting point. Therefore, the chocolate is sheared more slowly in machine tempering allowing the crystals to be matured.

Step 12. Molding

After the tempering process, the mixture is fed into molds and cooled in a cooling chamber. Nowadays, mostly plastic molds are used since they are light in weight and less noisy in the process. The molds should be pre-heated a few degrees than the temperature of the tempered chocolate before starting the molding process. This is because some of the fat could set in the wrong form upon the touch of a cold surface. The chocolate should be spread evenly on the mold removing all the air bubbles to avoid the blemishes. This is made possible by vibrating the mold. Vibration provides the energy to separate the particles of the chocolate which are in contact at rest. This lowers the resistance to movement and the yield value.

The chocolate should be solidified after molding. This involves the removal of large amounts of latent heat and a relatively small amount of specific heat. The chocolates drop their temperature in around 10 degrees since the molding to the point of packing. The temperature drop is done by either conduction, convection or by radiation.

Low temperatures could give two problems; Firstly, they may cause the fat to crystalline in the wrong form which will cause fat blooming very quickly and will result difficulties in demolding. Secondly, moisture in the air may condense on cold surface and drip in to the chocolate which will dissolve some of the sugars within it. Then the water would evaporate again once it is re-warmed for packaging, leaving a white powdery surface which is known as sugar bloom.
Step 13. Packaging

The chocolate should be packaged for distribution to retail outlets. The packaging type is varied according to the storage conditions, the type of agents that requires protection against such as dirt, odors and etc, the type of the chocolate product, targeting customers and the cost. The traditional packaging of the chocolate blocks and tablets are the aluminum foil which provides protection against the dirt, insect infestation and taint. The thickness of the foil can be minimized as well as it is easier to recycle. The flow wrap is advantageous as large number of items could be wrapped by a single machine under a short period. They can be a very good barrier to moisture and odors with an appropriate choice of material. There is a wide range of materials that could be used for packaging such as, thin co-extruded of white cavity polypropylene and film foil/ ionomer laminates. During flow wrapping the product is fed in to a tube and cut into the required lengths and finally the open edges are sealed with heat or pressure. The packaged chocolates are placed on card board boxes known as outers in transporting them to the retailers.

(ST Beckett, 1999; Sheilah Beckett, 2000; McHugh, 2016; Organization, 2013)

(Aii). Places where the ingredients and chemicals were purchased

All the cocoa products including the Bergenfield natural cocoa liquor, natural pure prime pressed-cocoa butter, Bergenfield cocoa powder-colonial rosewood(non-alkalized) and Bergenfield Cocoa Powder - Grand Guayacan (alkalized) were purchased online from CocoaSupply.com (Brooklyn, NY 11220). Non-fat skimmed milk powder was from Baker Authority (Borden Ave, Maspeth, NY 11378), non-GMO organic Erythritol was from Now Foods (Bloomingdale, IL 60108), Maltitol was purchased from Prescribed for life (Fredericksburg, TX 78624), Whey protein was purchased online from myprotein.com, GLUCIDEX-Maltodextrin was from Toronto Research Chemicals (Canada), Polydextrose was purchased online from Honeyville.com, anhydrous milk fat was from the company California Dairies (Turlock, CA). All the three emulsifiers used, which were Polyglycerol polyricinoleate(PGPR), Citric acid esters of mono- and diglycerides of fatty acids (CITREM) and Ammonium phosphatide (AMP) were from the company Palsgaard (Morris Plains, NJ 07950) and the freeze dried organic blueberry powder was purchased online from northbaytrading.com (Brule, WI 54820).

Chemicals used for the chemical analysis including anhydrous Sodium Carbonate, Ethanol, Folin-Ciocalteu's Phenol reagent and the Gallic acid (fine Powder) were purchased from VWR.
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Universitätsbibliothek Giessen,
VITA

Lakmani Tharaka Galaniha

Education

University of Kentucky
Graduate Certificate in College Teaching and Learning 2017-2018

University of Peradeniya, Sri Lanka
Postgraduate Diploma in Food Science and Technology 2014-2015

University of Peradeniya, Sri Lanka
B.S. in Science 2010-2013
Areas of Concentration: Chemistry and Botany
Minor: Zoology

Honors and Awards

Graduate Student Research Day-Best Poster Presentation Award 2018
School of Human Environmental Sciences; Master’s Program Category

Conference Proceedings

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