CONSUMER ACCEPTABILITY OF A KOMBUCHA COFFEE *(COFFEA)* PROTOTYPE WITH TRADITIONAL COFFEE CHARACTERISTICS

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ABSTRACT

CONSUMER ACCEPTABILITY OF A KOMBUCHA COFFEE *(COFFEA)* PROTOTYPE WITH TRADITIONAL COFFEE CHARACTERISTICS

By

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Coffee and Kombucha tea are both beverages that have been consumed for many years, with a current increased momentum in consumption due to many correlations with beneficial health aspects. The objective of this study was to assess the consumer acceptability of a Kombucha Coffee which tastes more like traditional coffee. A Kombucha Coffee prototype "BubbLê," was created and compared to a market Kombucha Coffee via a hedonics evaluation, food action rating scale (FACT), and a paired-comparison ranking test. Participants rated the market Kombucha Coffee significantly higher than BubbLê Kombucha Coffee in all sensory aspects for flavor (6.84 ± 1.82; 4.46 ± 2.48; $p < 0.001$), sweetness (7.11 ± 1.63; 4.65 ± 2.33; $p < 0.001$), tartness (6.27 ± 1.77; 4.72 ± 2.55; $p < 0.001$), aroma (6.30 ± 1.82; 5.55 ± 2.59; $p = 0.018$), mouthfeel (6.87 ± 1.62; 5.36 ± 2.64; $p < 0.001$), and overall likeability (6.90 ±1.76; 4.59 ± 2.43; $p < 0.001$) in the hedonics evaluation. The FACT test indicated that participants would more likely drink the market alternative compared to the prototype (5.42 ± 1.96; 3.62 ± 2.29; $p < 0.001$). The majority of participants (80%) chose the market Kombucha Coffee over the more traditional coffee flavored Kombucha Coffee prototype. It is noted that flavor scored the lowest in sensory evaluation for the prototype, therefore, reevaluation of flavor by means of adding coffee enhancing notes is needed for further development of a Kombucha Coffee with a more traditional coffee flavor profile.

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CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD), cancer, and diabetes are among the leading causes of human death in the United States with a total death rate of 1,282,535 in 2014 (Centers for Disease Control and Prevention [CDC], 2015). Cardiovascular disease is an umbrella term for a range of diseases the affect the heart and blood vessels (World Heart Federation, n.d.). Diabetes is a type of disease in which the body is unable to use blood glucose for energy, resulting in hyperglycemia (American Diabetes Association , 2014). Cancer is a term for diseases in which abnormal cells divide without control which can invade other tissues leading to complications (National Cancer Institute, n.d.). The World Health Organization (WHO), Food and Agriculture Organization (FAO) and the CDC are in agreement that these various diseases can be prevented with proper diet and lifestyle (CDC & U.S. Department of Health and Human Services, 2008; WHO/FAO, 2002). Many of these diseases are thought to stem from free radicals and reactive oxygen species (ROS), which compromise the human body's cells and tissues (Lobo, Patil, Phatak & Chandra, 2010). Both of these unstable and reactive compounds derive from normal metabolic processes within the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals (Bagchi & Puri, 1998). In low concentrations, free radicals and ROS do have beneficial roles, such as human defense mechanism of destroying invading pathogenic microbes (Dröge, 2002; Young & Woodside, 2001). In excessive amounts, free radicals and ROS in the human body lead to a phenomenon known as oxidative stress which can dramatically alter cell membranes, resulting in the development of those chronic and degenerative diseases such as cancer and CVD. The human body defends itself against oxidative stress by utilizing antioxidants, which are known to combat

against free radicals and ROS (Pham-Huy, He, & Pham-Huy, 2008).

Coffee consumption has been associated with the possibility of reducing the risks for diseases, due to high amounts of dietary antioxidants present (Andersen, Jacobs, Carlsen, & Blomhoff, 2006). Epidemiological studies suggest that coffee consumption is associated with the prevention or delay of degenerative diseases, which include diabetes, CVD and cancer (Zhang, Lopez-Garcia, Li, Hu, & Dam, 2009). Deoxyribonucleic acid (DNA) protective properties of coffee reduce free radical and ROS cellular damage, which may be implicated in cancer development (Bakuradze et al., 2011). These beneficial effects have been partly attributed to the antioxidant activity present in coffee consumption (Hoelzl et al., 2010).

Coffee (*coffea*) is one of the most popular beverages consumed around the world with increasing consumption of Gourmet Coffee Beverages (GCB). More than half of the American adult population consumed this beverage daily in 2015 (National Coffee Association USA [NCA], 2016b). A traditional cup of coffee is depicted by a flavor complexity of sensations described as a combination of aroma, taste, texture and mouthfeel (Taylor & Roozen, 1996). Among the sensations, aroma is notably the most important (Cliff & Green, 1994).

There is currently a trending increase in consumption of GCB, defined by the NCA (2016a) as specialty coffee including gourmet traditional coffee, espresso-based beverages, along with iced or frozen drinks. More specifically, gourmet coffee derives from premium grade coffee beans of specific species, such as the Arabica, which are more scarce due to difficulty to grow in specific geographical regions. In 2012, 44% of coffee consumers chose GCB over non-GCB, which increased to 54% in 2014 (Murray, 2014). Within the GCB category, espresso-based beverages also showed a steady increase in consumption. Espresso-based beverages are variations of coffee drinks with at least one shot of espresso present. Of the GCB consumers,

espresso-based beverages made up 35% of the consumption in 2012 and increased to 52% of the GCB consumption in 2014 (Murray, 2014).

Similarly to coffee, another beverage that has many beneficial health properties is Kombucha tea (KT). Kombucha tea is traditionally a sweetened black tea that is fermented with a symbiotic culture of yeast and bacteria to produce a mild vinegar taste with an effervescent finish (Marsh, O'sullivan, Hill, Ross,& Cotter, 2014; Watawana, Jayawardena, Gunawardhana, & Waisundara, 2015). This tea has been brewed in China for over 2,000 years and has acted as a functional beverage due to its ability to combat chronic illnesses such as CVD, cancer and type 2 diabetes (Marsh et al., 2014; Watawana et al., 2015).

There are many different techniques to produce brewed coffee with varying amounts of antioxidants (Babova, Occhipinti, & Maffei, 2016; Lopez-Galilea, Peña, & Cid, 2007; Ludwig, Bravo, Peña, & Cid, 2013; Yilmaz, Hacibekiroglu, & Kolak, 2014). These brewing techniques can be generally characterized by brewing pressure, brewing process, extract volume, and solid content (Parenti et al., 2014). The current brewing method that results in highest antioxidant capacity when compared to other conventional brewing techniques is the espresso method, obtained via percolation of hot water under pressure through compacted roasted ground coffee (Illy & Viani, 2005; Lopez-Galilea et al., 2007; Ludwig et al., 2012). Another brewing technique commonly used is cold brewing, which uses coarsely ground coffee mixed with cold water that is left in a refrigerator for at least 12 hours (Perratore, 2016). This novel form of brewing coffee can lead to potentially higher extract of antioxidant levels.

Justification of Study

Although there is a vast amount of research on both beverages, there is limited research on the potential benefit of amalgamating the brewing and fermenting techniques of coffee and

KT in order to create a highly nutrient dense functional beverage with traditional coffee characteristics. These characteristics include the visual appearance of coffee along with taste. Coffee's appearance is characteristically marked by the rich dark brown color melanoidins (Shibamoto, 1983). Traditional flavor and mouthfeel of coffee is depicted as sweet-caramel, astringent, bitter flavor, earthy, roast/sulfur and smoky (Bicho, Leitão, Ramalho, de Alvarenga, & Lidon, 2013; Czerny, Mayer & Grosch, 1999; Mayer, Czerny & Grosch, 2000). Lexicon on the most important characteristic of coffee, the aroma, includes sweet aromatic, sour aromatic, roasted, burnt/acrid, nutty, cocoa, musty/earthy, floral, fruity and pungent (Bhumiratana, Adhikari, & Chambers, 2011).

Currently there are minimal commercial products of Kombucha Coffee, the only option available does not contain the aforementioned traditional coffee characteristics. A food product, which combines the brewing techniques of coffee and its traditional characteristics, with the fermentation techniques of KT, can offer an attractive alternative to carbonated beverages with the added health benefits of both coffee and KT. While further studies are needed to understand if there is a synergistic effect of combining coffee and the techniques of fermenting KT, food scientists along with nutritionists can still design products aimed to provide consumers with a functional beverage to promote general health. Due to the long consumption history of both of these beverages, there is a potential for long-term acceptance of this beverage.

Objectives of Study

The objective of this thesis is to evaluate the consumer acceptability and preference of a Kombucha Coffee prototype (BubbLê) versus commercial type using:

1. Determine whether subjects prefer BubbLê to the market alternative using the Paired-Comparison Test.

2. Compare the consumer's degree of likeability of BubbLê with the market alternative using the Hedonics Preference Test.
3. Evaluate BubbLê's acceptance by measuring the frequency of the consumers' desire to consume BubbLê using the Food Action Rating Scale (FACT).

Definitions of Terms

Antioxidant: Are man-made or natural substances that may prevent or delay some types of cell damage (U.S. National Library of Medicine, MedlinePlus, 2016).

Cold brew: A coffee brew that uses more coarsely ground coffee and is mixed with cold water and left to sit in a refrigerator or at room temperature for at least 12 hours (Perratore, 2016).

Espresso: A coffee brew that is obtained by percolation of hot water under pressure through compacted roasted ground coffee (Illy & Viani, 2005).

Filter coffee: Method of brewing coffee with coffee grounds put in a paper filter and is extracted with boiled water by conventional percolation coffee machine. The brew is dripped into a heated pot within 2-3 minutes (Moeenfard, Rocha, & Alves, 2014).

Free radical: An unstable and highly reactive molecular species capable of independent existence that contains an unpaired electron in an atomic orbital (Lobo et al., 2010).

Hedonic test: A 9 - point scale that is widely used for measuring food acceptability (Peryam & Pilgrim, 1957).

Kombucha: A carbonated beverage obtained by the fermentation of sugared tea with a symbiotic culture of acetic bacteria and fungi (Dufresne & Farnworth, 2000)

Mocha: Method of brewing coffee using an aluminum pot with ground coffee placed in filter cup, filled with water then heated until the water reservoir is empty (Moeenfard et al., 2014).

Principle display panel (PDP): The visual appearance of a package, which is the part of a label, that is most likely to be displayed, presented, shown, or examined under customary conditions of display for retail sale (U.S. Food and Drug Administration, 2015).

Reactive oxygen species (ROS): These are chemical compounds, which donate oxygen to other substances leading to instability (Lobo et al., 2010).

Limitations

The major limitation of this study is that the sensory evaluation used subjects recruited from the student body and staff within the Family and Consumer Sciences (FCS) department at California State University, Long Beach (CSULB); therefore the sample may not be an overall representation of the target population. Novelty and taste of the fermented coffee product may not be accepted which can result in lower scores during sensory evaluation. These limitations may lead to bias in the outcome of the results.

Assumptions

This research assumes that the respondents will answer truthfully to sensory evaluation tests. Other products offered during taste test will help in determination of acceptability of prototype. Another assumption is the validity of tools used for sensory evaluations and antioxidant assessment is accurate and will work. Also it is assumed that the combination of use of cold brewing coffee technique and the fermentation process of KT will yield a highly nutrient dense product. There is also an assumption that the product produced will always result in a consistent product with same quality, freshness and flavor profile.

CHAPTER 2

REVIEW OF LITERATURE

Coffee and Kombucha Tea

The consumption of coffee dates back to many centuries ago with a history that started in Abyssinia and Arabia. It was used for years in the classical period of Arabian medicine before the cultivation of the plant spread throughout the tropics (Ukers, 1922). The progression of coffee cultivation dates back to 575 A.D., but this progression was slow until the 15th and 16th centuries where the use of coffee grew in the Yemen district of Arabia, which led to the spread of coffee cultivation around the globe (Ukers, 1922). This popular beverage is now consumed daily by more than half of the adult population in the United States (NCA, 2016b).

Over the years, there has been a shift in coffee consumption among younger adults choosing more GCBs (Table 1). According to the NCA's (2016a) the National Coffee Drinking Trends (NCDT) 2016 showed that over the last 8 years, daily consumption of espresso-based beverages have nearly tripled since 2008. In 2008 GCB consumption among 18-24 year old individuals was 13% whereas in 2016 at 36%, and among 25-39 year olds it was 19% in 2008 and at 41% in 2016. For espresso-based beverage consumption, there was an increase of 13% for 18-24 year olds and 21% increase for the 25-39 year olds from 2008 to 2016 (NCA, 2016a). There were three new coffee beverages added to the GCB category: flat whites, cold brew and iced coffee infused with nitrogen. Of the three newly added GCBs, cold brew had the highest consumption with increased sales of 115% from the year 2014 to the year 2015 (NCA, 2016a; Perratore, 2016).

Kombucha tea (KT) has been consumed around the world for many years with its origins dating over 2,000 years ago in China (Marsh et al., 2014). It has been known by various names

TABLE 1. List of Gourmet Coffee Beverages

Type	Definition
Traditional Coffee	Traditional Coffee drunk hot or iced that is brewed from premium whole bean or ground varieties
Espresso-based beverages	These beverages have at least one espresso shot mixed. • cappuccino • espresso • latte • café mocha • macchiato • flat white • Americano
Iced/Frozen blended coffee	These coffee beverages are blended with ice before consumption.
Cold brew coffee	This coffee requires for the coffee grounds to be brewed in water without heat for a long period of time
Iced coffee infused with Nitrogen	This is iced coffee that has been infused with nitrogen to create a carbonated coffee drink.

Note: Adapted from *National Coffee Drinking Trends*, by National Coffee Association. Copyright 2016.

throughout the world such as red tea fungus, Champignon de longue vie, Ling zhi, kocha kinoko, Chainii grib, and Chainii kvass (Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011). It was discovered and used for a long time in China for detoxification and energizing properties (Roche, 1998). It was later used by doctor Kombu who brought the tea fungus to Japan to cure the digestive problems of Japanese emperor, Inkyo (Jayabalan, Malbasa, Loncar, Vitas, & Sathishkumar, 2014). The name "Kombucha" was derived from that physician's name "Kombu" with the Japanese word for tea, "cha," to create the name of "Kombucha" (Roche, 1998). Kombucha tea continued to make its way throughout the world surfacing in Russia where it was also known by many other names such as Japonskigrib or Kambucha. It then made its way through other eastern European countries, showing up in Germany in the 20th century, then in the 1950s KT arrived in France and its dominated regions of North Africa; this beverage continued to increase in popularity during the 1960s after scientific research in Switzerland found that the

benefits of consuming this beverage were similar to the consumption of yogurt (Jayabalan et al., 2014). Kombucha tea is currently sold worldwide in various flavors and continues to build popularity (Dufresne & Farnworth, 2000; Jayabalan et al., 2014).

However, with the changes and demands in food and beverage trends, (1) coffee and KT consumption, due to their affiliated health benefits, has increased along with optimization of (2) current brewing techniques, to (3) application of novel brewing techniques such as cold brewing in new product development.

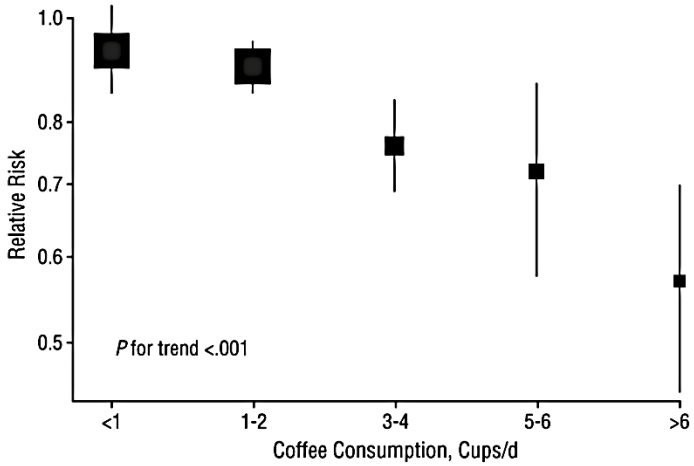
Coffee and Kombucha Tea Consumption and Their Health Benefits

Both coffee and KT have been consumed for long periods of time for their health benefits. Coffee has been consumed for the caffeine content to enhance and stimulate focus and energy, whereas KT has been consumed for centuries due to the testimonials and claimed health benefits (Babova et al., 2016; Jayabalan et al., 2014). Much research has been conducted on both of these beverages for the health effects they have on the human body. Kombucha tea and coffee each have shown to have positive health benefits. Prospective studies have shown beneficial health properties of coffee, as the consumption over time may decrease the risk for CVD and all causes of mortality (Freedman, Park, Abnet, Hollenbeck, & Sinha, 2012; Sugiyama et al., 2010; Zhang et al., 2009).

Coffee Consumption and Health Benefits

Recent studies have shown the potential health benefits of consuming coffee and its relationship to leading causes of human death in America. These health benefits include but are not limited to lowered risks for onset of various diseases such as heart disease and Type 2 diabetes mellitus (T2DM; Cano-Marquina, Tarin, & Cano, 2013; Harvard T.H. Chan School of Public Health, 2010). Huxley and others (2009) further confirmed the relationship of coffee

consumption and risk of development of T2DM, concluding that subjects who drank > 6 cups daily had the lowest risk for T2DM (relative risk [RR]: 0.65; 95% *CI*: 0.54 to 0.78) while there was also a significantly reduced risk in subjects who consumed 4 to 6 cups daily (RR: 0.72; 95% *CI*: 0.62 to 0.83; Figure 1).

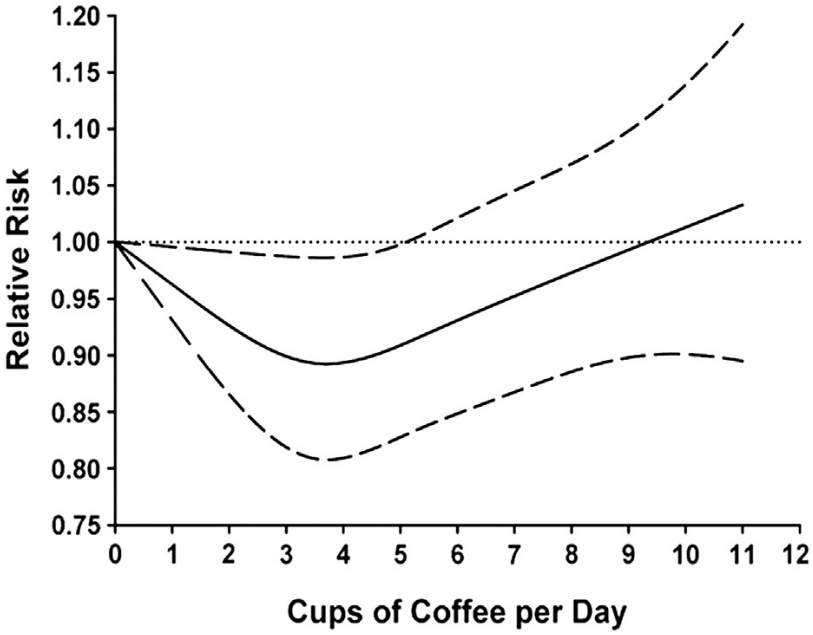


The relationship between coffee consumption and subsequent type 2 diabetes mellitus in different categories of coffee consumption. The center of each black square is placed at the summary point estimate; the area of the square is proportional to the statistical size; and each vertical line shows the 95% confidence interval about the summary estimate.

FIGURE 1. Coffee consumption and relationship to T2DM risk. Adapted from "Coffee, Decaffeinated Coffee, and Tea Consumption in Relation to Incident Type 2 Diabetes Mellitus: A Systematic Review With Meta-Analysis," by R. Huxley, C. Lee, F. Barzi, L. Timmermeister, S. Czernichow, E. Perkovic, … M. Woodward, 2009, *Archives of Internal Medicine, 169*, p. 2059. Copyright 2009.

In a 13 year follow-up of a prospective cohort research of 402,260 subjects, Freedman and others (2012) showed an inverse association between consumption of coffee and many leading causes of death, such as stroke, heart disease, and diabetes. As coffee intake increased the risk for heart failure decreased until the threshold of more than 9 cups per day was reached (Figure 2; Mostofsky, Rice, Levitan, & Mittleman, 2012). High blood pressure (BP), also known as hypertension, is directly related to increasing the heart's workload, leading to heart failure (American Heart Association, 2016). One of coffee's main constituents, caffeine, plays a

hemodynamic role in lowering BP; caffeine has the ability to interfere with action of adenosine on its receptors A1 (vasoconstrictor, increase BP) and A2a (vasodilator, decrease BP; Chrysant,



Relative risk (**solid line**) and 95% confidence interval (**dashed lines**) for the association between heart failure and cups of coffee per day compared with no consumption in a meta-analysis of studies published in 2001 to 2011.

FIGURE 2. Coffee consumption and relationship to risk for heart failure. Adapted from "Habitual Coffee Consumption and Risk of Heart Failure Clinical Perspective: A Dose-Response Meta-Analysis," by E. Mostofsky, M. Rice, E. Levitan & M, Mittleman, 2012, *Circulation: Heart Failure, 5,* p. 404. Copyright 2012.

2015). Caffeine acts as an inhibitor of A1 receptors for the afferent arteriole, glomerulus, proximal tubule, and collecting ducts which improves glomerular filtration rate and renal blood flow then causes diuresis and natriuresis, ultimately lowering the BP which has shown to improve the condition of individuals with heart conditions (Cano-Marquina et al., 2013). In addition to this inhibitory factor caused by caffeine and chlorogenic acids, they act as strong antioxidants to improve endothelial and vascular function through increasing nitric oxide (Guessous, Eap, & Bochud, 2014).

Another major health benefit of coffee is its ability to protect DNA from being damaged

by decreasing spontaneous DNA breakage (Bakuradze et al., 2011, 2014). When observing the relationship between coffee consumption and the levels of oxidative stress, there is also an inverse relationship between coffee consumption and derivatives of reactive oxygen metabolites (Ishizaka, Yamakado, Toda, Tani, & Ishizaka, 2013). These health benefits and associations of coffee can be attributed to the antioxidants present in coffee, with its antioxidants showing high potentials to act on free radicals and protect cells (Babova et al., 2016).

Kombucha Tea Consumption and Health Benefits

Kombucha tea has been an accepted beverage for centuries due to the many health benefits with recent studies showing positive effects on diabetes. It has been reported that in diabetic induced rats, KT was more beneficial than black tea at inhibiting α-amylase and lipase activities in the plasma and pancreas while exuding higher suppression of increased blood glucose levels (Aloulou et al., 2012). Additionally, Aloulou and others (2012) also found ameliorative action of KT on the pancreas; moreover, the KT supplemented diabetic rats were observed to have a significant decrease of 50 ± 11% in blood glucose concentration ($p < 0.05$). Additionally, antihyperglycemic properties were shown with the continual consumption of 6 mg/kg body weight KT for 45 days in diabetic induced rats, resulting in a significant decrease in blood glucose levels and glycosylated hemoglobin with increases in plasma insulin and tissue glycogen (Table 2). Table 2 shows that the addition of KT can improve the glycemic state of diabetic induced rats by bringing total hemoglobin and glycated hemoglobin to near normal levels (12.01 +/- 0.84 g/dl; 0.27 +/- 0.03 mg/g Hb, respectively) of the control rats (Srihari, Karthikesan, Ashokkumar, & Satyanarayana, 2013). Other researchers found that KT improved serum glucose levels by 56.4% ($p < 0.05$) in diabetic induced rats compared to the control group (Bhattacharya, Gachhui, & Sil, 2011.

In addition to the health benefits shown in diabetes, KT has antioprolifeative activity on cancer cells of the HeLa cells (cervix epithelial carcinoma), HT-29 (colon adenocarcinoma), and MCF-7 (breast adenocarcinoma; Cetojevic-Simin, Bogdanovic, Cvetkovic, & Velicanski, 2008). Prostate cancer cells significantly decreased in survival with the presence of KT extract due to the downregulation of angiogenesis stimulators such as matrix metalloproteinase, cyclooxygenase-2, interleukin-8, endothelial growth factor, and human inducible factor-1α (Srihari, Arunkumar et al.,2013)

TABLE 2. Effects of Kombucha on the Lab Levels of Normal and Experimental Rats

Groups	Plasma Glucose (Mg/Dl)	Plasma Insulin (µu/Ml)	Total Hemoglobin (G/Dl)	Glycated Hemoglobin (Mg/G Hb)
Normal	92.67 ± 7.23^{a}	12.82 ± 1.02^{a}	12.01 ± 0.84^{a}	0.27 ± 0.03^{a}
Normal + Kombucha (12 mg/kg)	93.78 ± 8.64^{a}	11.96 ± 0.98^{a}	12.58 ± 0.92^{a}	0.26 ± 0.02^{a}
Diabetic	278.36 ± 25.72^{b}	3.92 ± 0.31^{b}	8.02 ± 0.59^{b}	0.74 ± 0.06^{b}
Diabetic + Kombucha (3 mg/kg)	222.33 ± 20.18^{c}	5.97 ± 0.46^{c}	8.88 ± 0.48^{c}	0.62 ± 0.05^{c}
Diabetic + Kombucha (6 mg/kg)	120.01 ± 10.86^{d}	9.22 ± 0.64^{d}	10.58 ± 0.98^{d}	0.36 ± 0.03^{d}
Diabetic + Kombucha (12 mg/kg)	163.31 ± 14.84^{e}	7.18 ± 0.53^{e}	9.42 ± 0.38^{e}	0.56 ± 0.05^{e}

Values in each group are represented as means ± S.D. for 6 rats in each group. Values not sharing a common superscript a–e differ significantly at $p < 0.05$.
Note: Adapted from "Antihyperglycaemic Efficacy of Kombucha in Streptozotocin-Induced Rats," by T. Srihari, K. Karthikesan, N. Ashokkumar, and U. Satyanarayana, 2013, *Journal of Functional Foods,5*, p. 1799. Copyright 2013.

Current Brewing Techniques for Coffee and Kombucha Tea

Production of Coffee and Kombucha Tea

There are many different techniques used to produce brewed coffee and tea. The brewing of coffee can be generally characterized by the brewing pressure, brewing process, brew volume,

and solid content (Parenti et al., 2014). The most conventional products of coffee brewing are: filtered coffee, espresso, and cold brew (Illy & Viani, 2005; Moeenfard et al., 2014; Perratore, 2016). These various brewing techniques widely affect the flavor profile and chemical compounds of coffee (Babova et al., 2016). Similarly to coffee, there are various ways to brew tea (Jayabalan et al., 2014). After the tea is brewed, additional steps of fermentation are needed to produce KT, which will be discussed in detail in the review of literature that follows.

Conventional Methods of Brewing Coffee

There are multiple ways to conventionally brew coffee for consumption, which includes filtration, espresso and full immersion. The most common and conventional form of coffee brewing is the use of the filter coffee technique. This is a method of brewing in which coffee grounds are placed in a paper filter then boiled water percolates into a coffee machine and then is dripped into a heated pot (Moeenfard et al., 2014). Second, another conventional form of brewing is espresso ; this method is done via percolation of hot water under pressure through compacted roasted ground coffee (Illy & Viani, 2005). Other methods used for brewing coffee are the following: boiling; French pressing, and cold brewing, all of which require complete submersion of the grounds in hot or cold water for an extended period of time until the liqueur (i.e., brew) is separated from the solids (Moeenfard et al., 2014; Perratore, 2016). Cold brewing is a novel technique of brewing coffee, which requires the use of more coarsely ground beans, mixed with cold water and left in a refrigerator for at least 12 hours to slowly extract the plant solubles (Perratore, 2016).

Conventional Brewing of Kombucha Tea

The traditional preparation of KT consists of three basic ingredients which are: brewed tea, sugar and the symbiotic culture of acetic acid bacteria and various species of yeast

(Jayabalan et al., 2014; Liu, Hsu, Lee, & Liao, 1996). The production of carbonated KT requires two-step process of tea fermentation at between 20°C - 30°C (Figure 3). In the first step, KT is prepared under aerobic conditions in which the yeast cells hydrolyze sucrose ($C_{12}H_{22}O_{11}$) into

FIGURE 3. Kombucha fermentation. Photo courtesy of Linh Le. Copyright 2017.

glucose ($C_6H_{12}O_6$) and fructose ($C_6H_{12}O_6$; Reiss, 1994; Sievers, Lanini, Weber, Schuler-Schmid, & Teuber, 1995). The acetic acid bacterium converts those substrates into gluconic acid ($C_6H_{12}O_7$) and acetic acid ($C_2H_4O_2$; Balentine, Wiseman, & Bouwens, 1997). The presence of acetic acid creates a cyclic affect, which stimulates the yeast to produce ethanol (C_2H_6O), and the alcohol then aids in the growth of acetic acid bacterium, which then produces more of itself (Liu et al., 1996). The end product results in total consumption of sucrose after 2 weeks of fermentation with the production of ethanol, acetate and a Kombucha cellulose pellicle (Kallel, Desseaux, Hamdi, Stocker, & Ajandouz, 2012). The second step of fermentation involves the development of naturally occurring carbon dioxide (CO_2), which occurs in an anaerobic

environment in which the yeast continues to ferment the sugars resulting in a carbonated beverage (Chen & Lui, 2000).

Cold Brewing Coffee and Tea

Cold brewing is an emerging technique used to brew coffee or tea in cold water for a prolonged period of time which can lead to enhanced product yield. When this procedure is applied in tea brewing (room temperature [25°C] for 2 hours), higher antioxidant activity is observed in cold brew teas compared to hot brewed teas (Figure 4), with white tea having the ability to more than double the lag time of copper-induced low density lipoprotein (LDL) oxidation compared to the control (Venditti et al., 2010). The conditions for this system under which coffee is brewed is as follows: 3.3°C for at least 12-18 hours (Perratore, 2016). The soluble solids present in coffee grounds decrease in extractability as the temperature of the solvent decreases, therefore, requiring longer brewing times for this method (Strumpf, 2015).

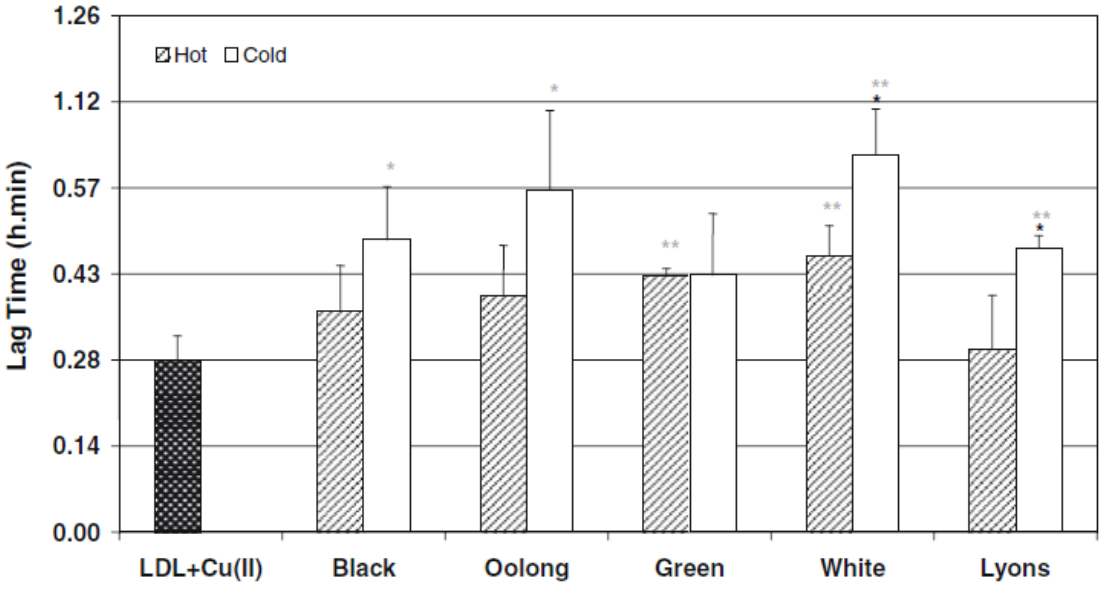


FIGURE 4. Antioxidant effect of cold versus hot tea, shown as the lag time, on LDL oxidation. Adapted from "Hot Vs. Cold Water Steeping of Different Teas: Do They Affect Antioxidant Activity?" E. Venditti, T. Bacchetti, L. Tiano, P. Carloni, L. Greci, and E. Damiani, 2010, *Food Chemistry, 119,* p. 1601. Copyright 2010.

Cold brewing can offer a better extraction of active components and results in a unique flavor profile that is less acidic and bitter compared to its counterparts (Phung, 2014; Wang, Xu, Feng, Yang, & Qian, 2011).

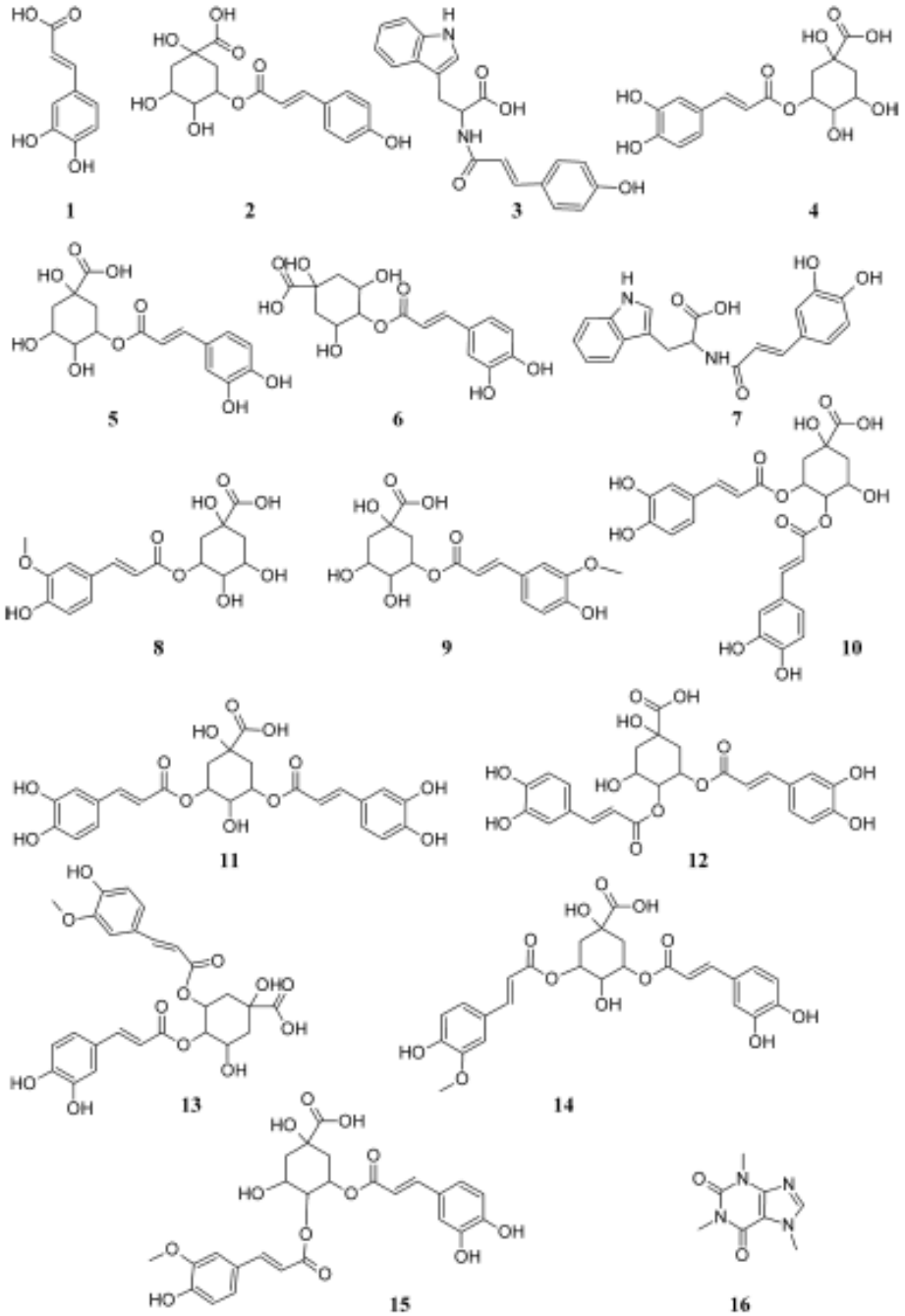
Brewing Effects on Antioxidants Extracted from Coffee

Among the various coffee brewing techniques, studies have shown that levels of antioxidants can vary depending on the coffee brewing method used. In addition to the commonly known compound found in coffee, caffeine, there are also abundant amounts of beneficial health components such as phenolic compounds, tocopherols, neochlorogenic acids and chlorogenic acids (Figure 5), within this beverage that play a role in antioxidant activity and human health (Babova et al., 2016; Herman & Herman, 2013). In a study led by Lopez-Galilea and others (2007), espresso showed to have the highest antioxidant capacity when compared to other brewing techniques of filter, mocha and plunger: (espresso > mocha > plunger > filter). Furthermore, other studies also show espresso coffee being superior in obtaining chlorogenic acids compared to that of the filter coffee brewing method. For example when looking specifically at 5-caffeoylquinic acid the concentrations in espresso was 201.1 ± 1.6 mg/100 mL where as in filter coffee it was 70.1 ± 0.3 mg/100 mL. Table 3 shows the differences in chlorogenic acids extracted (Ludwig et al., 2012).). Optimum tocopherol extraction from coffee beans depends on the pressure that exists at the beginning of the extraction phase present in the espresso extraction phase (Alves, Casal, & Oliveira, 2010).

New Developments in Product Development

Beverage Trends - Kombucha

The consumption of KT has increased over the last several years. In the 2016 Foodservice Trends and Carbonated Soft Drinks US 2015 reported that 51% of U.S. adults



(1) Caffeic acid, (2) p-coumaroylquinic acid, (3) p-coumaroyl-Ntryptophan, (4) chlorogenic acid (3-O-caffeoylquinic acid), (5) neochlorogenic acid (5-O-caffeoylquinic acid), (6) cryptochlorogenic acid (4-O-caffeoylquinic acid), (7) caffeoyl-N-tryptophan, (8) 3-O feruloylquinic acid,(9) 5-O-feruloylquinic acid, (10) 3,4-O-dicaffeoylquinic acid, (11) 3,5-O-dicaffeoylquinic acid, (12) 4,5-O-dicaffeoylquinic acid, (13) 3-O-feruloyl-4-caffeoylquinic acid, (14) 3-O-feruloyl-5-caffeoylquinic acid,(15) 4-O-feruloyl-5- caffeoylquinic acid, and (16) caffeine.

FIGURE 5. Structures of identified extracted coffee compounds. Adapted from "Chemical Partitioning and Antioxidant Capacity of Green Coffee (Coffea Arabica and Coffea Canephora) of Different Geographical Origin," by O. Babova, A. Occhipinti, and M. Maffei, 2016, *Phytochemistry, 123,* p.35. Copyright 2016.

between the ages of 25-34 already consumed the KT beverage (Menayang, 2016). There is increased consumer interest in KT, which has forced retailers to stock their shelves with a variety of KT products. Sales from one company (KeVita) alone surged by 6,682% from January of 2015 compared to January of 2016 (Crawford, 2016). There has been an increase in sales for kombucha and fermented beverages by 32% since October 2015 to October 2016 (Watson, 2016). The kombucha market is predicted to have a large growth rate of 25% each year until the year 2020 (Conick, 2016; Markets and Markets, 2015).

TABLE 3. Comparison of Chlorogenic Acids (Caffeoylquinic Acid [CQA] Expressed in Mg/100 Ml) Extracted Via Espresso Versus Filter Method

Method	3-CQA	4-CQA	5-CQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
Espresso	91.3±1.3	114.6±0.6	201.1±1.6	9.8±0.2	4.2±0.1	9.6±0.4
Filter	31.0±0.3	40.9±0.1	70.1±0.3	6.1±0.3	2.9±0.1	6.0±0.0

Note: Adapted from "Extraction of Coffee Antioxidants: Impact of Brewing Time and Method," by I. Ludwig, L. Sanchez, B. Caemmerer, L. Kroh, M. Peña, and C. Cid, 2012, *Food Research International, 48,* pp.62-63. Copyright 2012.

Cold Brew Coffee

Cold brew coffee is an emerging new technique in the brewing coffee industry that has gained momentum in popularity. The NCA has added cold brew to the GCB category in 2016 (2016a). In 2015, the coffee trends report showed that 15% of coffee drinkers tried cold brew coffee. There was an estimated 115% growth in sales for cold brew in 2015 (NCA, 2016b). Researchers also found that 37% of U.S. consumers between the ages of 29-38 years old have an interest in cold brew coffee because they enjoy trying new styles of coffee preparation (Sisel, 2015). The continuation of brewing novelty will increase sales of this brewed coffee over the next several years.

Beverage Trends in New Food Product Development: Kombucha Coffee

Coffee and KT have both shown an enormous growth in popularity over the years, but

there is still a need to produce a high quality product that combines the potential health benefits of both products. In the United States, it was reported that 42% of the consumers would like to see added nutritional benefits of coffee consumption with possibly the addition of probiotics (Sisel, 2015). A fermented coffee using the techniques of kombucha brewing has produced naturally occurring probiotic rich beverage which can produce a functional beverage that will satisfy consumer's need for increased functional coffee beverages. Although, consumers can find Kombucha Coffee in the local health food stores, the options are limited to one commercial brand which uses the inferior antioxidant and flavor extraction method of filter coffee brewing. With the rising popularity of cold brew coffee, a Kombucha Coffee using the cold water brewing method can offer more high quality options for consumers. The main limitation of traditional cold brewing is the long process needed to yield the coffee product.

Summary

As discussed, both KT and coffee consumption have several health beneficial aspects and have been accepted for long periods of time. These health benefits of the two beverages include a wide range of DNA protective properties along with heart disease prevention, antihyperglycemic effects in diabetics, cancer cell degradation and organ restorative aspects. The techniques of brewing these beverages widely affect the antioxidant profile extracted, which can disrupt those beneficial health properties. The non-thermal and effective way of brewing coffee to preserve those health properties can be attained with the use of cold brewing. With the aging of millennials, there has been a shift in consumption of the two beverages which has proven to have increased in demand (Menayang, 2016; NCA, 2016b). The need to use novel techniques like the application of cold brewing with Kombucha fermentation to obtain these beverages will keep the interest of millennials (Sisel, 2015).

CHAPTER 3

METHODOLOGY

Coffee Brewing Techniques

The brewing of coffee using cold methods was performed according to procedure of Temple Coffee Roasters. In preparation of the coffee brew, high quality coffee beans (donated from Temple Coffee Roasters, Sacramento, CA, USA) were used. Each coffee brewing technique was conducted in triplicates and stored in the refrigerator until analysis.

Cold Brewing

Five-hundred grams of coffee was coarsely ground using a Baratza Encore 485 Coffee Grinder set at the 27-grind setting (Figure 6). The ground coffee was immersed in 4,000 mL of cold water, then placed in a refrigerator set at 3.3°C (38°F) for 18 hours (Figure 7). The brewed coffee was filtered from the coffee grounds with a metal mesh strainer placed in a Chemex (Figure 8). The process was repeated two more times.

FIGURE 6. Encore 485 grinder set at 30 and coffee grounds. Photo courtesy of Linh Le. Copyright 2017.

FIGURE 7. Cold brew coffee before refrigeration. Photo courtesy of Linh Le. Copyright 2017.

FIGURE 8. Chemex with metal mesh strainer. Photo courtesy of Linh Le. Copyright 2017.

Fermented Coffee Formulation

The fermented coffee formulation was produced in similar conditions as described by Marsh and others (2014), with the exception of coffee being used in place of tea. A Kombucha cellulose pellicle starter Kombucha culture was used. The coffee was brewed and weighed for determination of sugar (10%). Fermentable sugar was determined by multiplying total brewed coffee by 10% and divided in half for first and second ferment. The sugar was dissolved in 500mL of hot water and cooled then added to the brewed coffee in a sterile glass container (Ball® Mason Jar). The Kombucha cellulose pellicle was added to the coffee solution (Figure 3). The container was covered with a 100% cotton towel and an elastic band was fixed on. The coffee mixture and Kombucha cellulose was placed in a dark space at room temperature (~23 °C) and fermented for 5 days. The second half of sugar was dissolved in 500mL of hot water and cooled then added to the fermented coffee to be bottled in airtight 16 oz bottles for second fermentation of 14 more days. After the second fermentation the developed BubbLê product was refrigerated at 4°C until sensory analysis is performed.

Subject Recruitment, Evaluation, and Consumer Acceptability

The consumer acceptability study was submitted and accepted by the Institutional Review Board (IRB) at California State University, Long Beach (CSULB). Consumer testing was conducted in the sensory evaluation laboratory at the CSULB Department of Family and Consumer Sciences (FCS) building. The sensory evaluation testing was performed under controlled conditions—isolated booths, controlled lighting and temperature. Ninety untrained subjects were recruited by flyers and advertisements (Appendix B). Subjects may have mainly consist of staff and students from CSULB. Subjects were required to complete a questionnaire that requested demographic information (age, gender, ethnicity) along with shopping habits prior

to the testing (Appendix C). Subjects in this study were excluded if they: (1) had a food allergy to any of the products tested, (2) did not consume Kombucha or coffee products used in the study for dietary or cultural reasons, and (3) had any professional experience in sensory evaluation. The subjects were free to withdraw from the study without any consequences. Subjects were required to sign an informed consent form and were compensated for their participation in this study (Appendix D). Consumer acceptance was determined using three tests: (1) Ranking Tests, (2) 9-point Hedonic Scale, (3) Food Action Rating Scale (FACT) test.

Objective 1: Paired-Comparison and Ranking Testing

Consumer preference of the prototype was evaluated using a Paired-Comparison Ranking test using consumer panelists. The panel was provided with randomly coded 1 oz. samples of the prototype (Code 604) and a market alternative (Code 247) in 2 oz., clear, plastic cups. The subjects were instructed to enter the sample code they prefer most (Appendix E1). Water and unsalted crackers were supplied to cleanse the palate between samples. The utilization of these measures will provide the statistical data necessary to evaluate the prototype based on individual consumer preferences, as well as analytic measurements to understand how consumers feel the new prototype compares to the market alternative.

Objective 2: Hedonic Testing

Subjects were administered a 9-point hedonic scale for sensory evaluation where: (9 = “like extremely”; 8 = “like very much”; 7 = “like moderately”; 6 = “like slightly”; 5 = “neither like nor dislike”; 4 = “dislike slightly”; 3 = “dislike moderately”; 2 = “dislike very much”; 1 = “dislike extremely”) to rate the prototype for attributes such as flavor, sweetness, tartness, aroma, mouthfeel, and overall preference (Appendix E2). Panelists were provided with two randomly coded samples of the fermented coffee prototype and a control sample (i.e., commercial brand).

The samples were distributed in 2 oz. clear plastic cups. Samples will also be administered randomly to avoid bias. Water and unsalted crackers were supplied to the panelists to cleanse their palate between samples.

Objective 3: The Food Action Rating Scale (FACT) Test

The FACT test will measure the fermented coffee prototype for the incidences of drinking measurement as described by Ramcharitar and others (2005). A 9-point scale were used and is as follows: 9 = “I would drink this every opportunity I had”; 8 = “I would drink this very often”; 7 = “I would frequently drink this”; 6 = “I like this and would drink it now and then”; 5 = “I would drink this if available but would not go out of my way”; 4 = “I do not like this but would drink this on occasion”; 3 = “I would hardly ever drink this”; 2 = “I would drink this if there were no other beverage choices”; 1 = “I would drink this only if forced”. Additionally, subjects were asked how much they would be willing to pay for the product (Appendix E3).

Statistical Analysis

Descriptive statistics, including frequencies, percentages, and variance, were calculated for scorecard ratings. Means, standard deviations (SD), and standard errors mean (SEM) were calculated for each of the sensory attributes and Hedonic rating scores. Overall acceptability and Hedonic ratings for the Kombucha Coffee prototype were tested using paired t-tests, via the International Business Machine Statistical Package for Social Sciences (IBM SPSS) Statistics 22 for Windows. Correlations between overall acceptability and other sensory attributes were computed, and coefficients of determination were reported. All analysis tests were performed using a significance of at least $p < 0.05$.

APPENDICES

APPENDIX A

FERMENTED COFFEE FORMULATION, FLOW CHART AND 5 D'S OF PRODUCT DEVELOPMENT

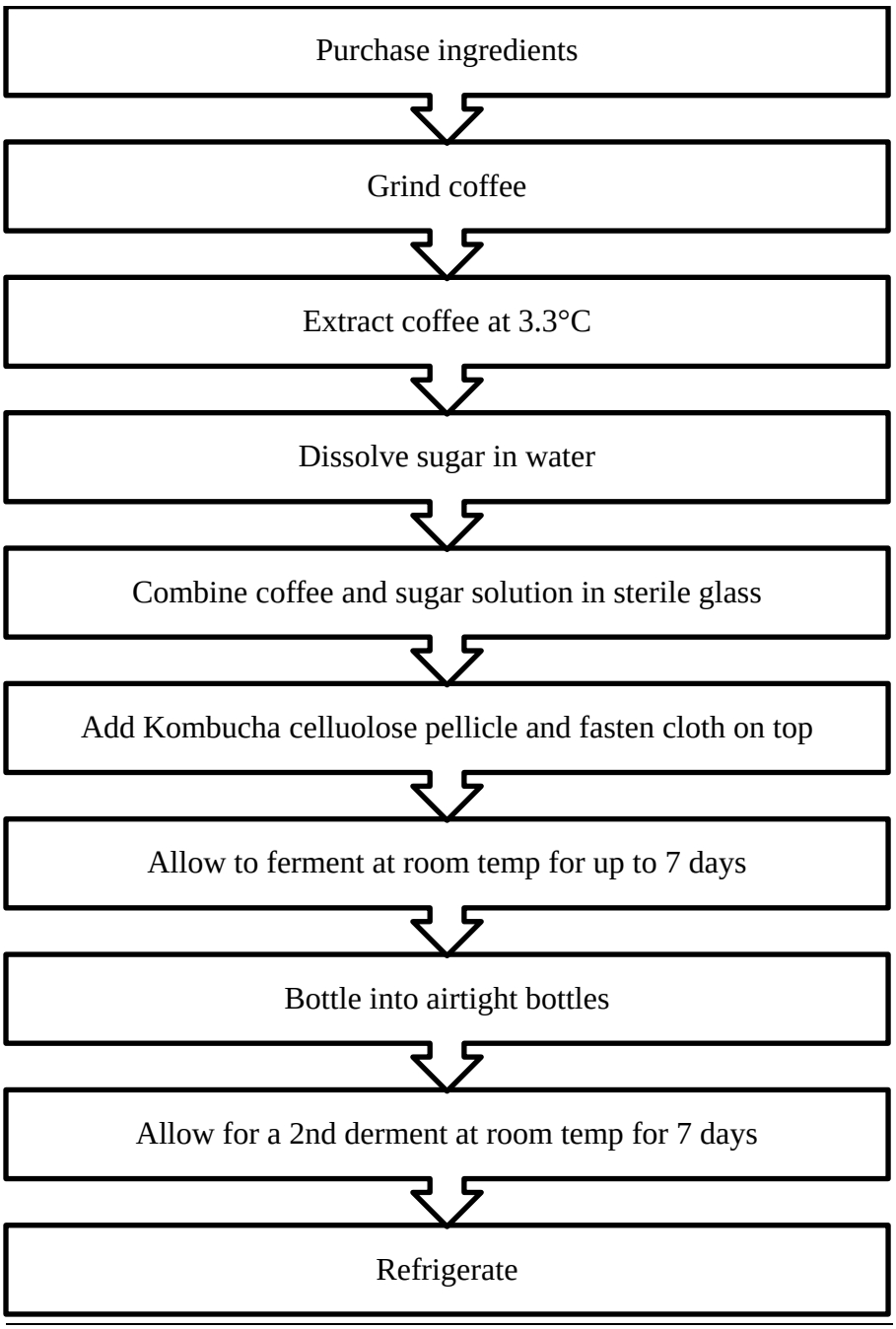
Appendix A1: Fermented Coffee Formulation

Fermented Coffee Formulation

Formulation to yield 3350mL Fermented Coffee

Ingredient	Grams	Percent of total formulation	Functionality
Kombucha Starter Culture	335	10%	Inoculation of kombucha bacterium into product and acidify
Extracted Coffee	1340	40%	Provides caffeine for production of kombucha
Boiling Water	1340	40%	Sweetener solvent
Organic Raw Cane Sugar	335	10%	Sugar needed to promote bacterium growth and fermentation
Kombucha cellulose pellicle	70	N/A	Culture byproduct of kombucha (scoby)

Appendix A2: Process Flow Diagram of Fermented Coffee



Process Flow Diagram of Fermented Coffee

Appendix A3: 5 D's of product Development

5 D's of product Development

Stage	Explanation	Things to Think About
Decide Fermented coffee	Brainstorming of ideas and what product to create, what's needed and its concept	What is currently in the market? Do consumers need or want this product? What are the current health trends?
Discover Antioxidant Capacity	High cases of CVD among other leading causes of death which determines a need for higher antioxidant rich foods.	Target audience Age Gender Geographical location
Define Coffee and Kombucha consumers. Health conscious	Formulation ad packaging of fermented coffee.	Materials Ingredients Techniques to make kombucha
Develop Product will be a healthy Fermented coffee Product called BubbLê	Assemble a fermented coffee prototype with the packaging design, sensory analysis and product.	Type of packaging Label Graphic design
Deploy The product goes into the market.	Create a marketing plan. Decide on pricing, storage, promotion and advertising.	Social media Coffee shop partnerships Retailers

APPENDIX B

SENSORY EVALUATION FLYER


Do you like
COFFEE & KOMBUCHA?
Then come try some!
What: Taste an antioxidant rich carbonated beverage and participate in a sensory evaluation project
Compensation: $5 gift card
Date: 4/24/17
Time: 11am-1:30pm
Location: Sensory Evaluation Lab (FCS building, room 112B)
If interested in participating in this research, please email: lmyle13@gmail.com

APPENDIX C

DEMOGRAPHIC QUESTIONNAIRE

Demographic Questionnaire

Answer the following questions to the best of your ability.

1. Gender:
   ☐Male ☐Female ☐Other, please specify_____
2. Age _____
3. In which group do you mostly place yourself? Check all that apply.
   ☐African-American/ Black ☐American Indian/ Alaska Native
   ☐Asian/ Pacific Islander ☐Caucasian
   ☐Hispanic/Latino ☐Other, please specify_____
4. What is your current weight (in pounds)? _____
5. What is your current height? _____Feet _____Inches
6. Do you have any food allergies?
   ☐Yes ☐No
   a) If yes, please list:_____
7. How often do you drink coffee?
   ☐Every day
   ☐Several times a week
   ☐About once a week
   ☐Once or twice a month
   ☐A few times a year
   ☐Never
8. How health conscious do you consider yourself?
   ☐Extremely
   ☐Very
   ☐Moderately
   ☐Slightly
   ☐No at all
9. Have you tried kombucha?
   ☐Yes ☐No
10. Are you the primary food or grocery shopper in your household?
    ☐Yes ☐No

APPENDIX D

INFORMED CONSENT FORM

CONSUMER ACCEPTABILITY OF A KOMBUCHA COFFEE *(COFFEA)*

PROTOTYPE WITH TRADITIONAL COFFEE CHARACTERISTICS

You are asked to participate in a research study conducted by Linh Le, from the Department of Family and Consumer Sciences at California State University, Long Beach. The results of this study will be contributed to my thesis for Master of Science in Nutritional Science. You were selected as a possible participant in this study because you are the at least 18 years of age and a student, faculty or staff enrolled or working at California State University, Long Beach.

PURPOSE OF THE STUDY

The overall objective of this study is to evaluate to evaluate the consumer acceptance of the BubbLêcoffee prototype produced using a novel technology and compared to traditional brewing techniques such as cold and hot brewing.

PROCEDURES

If you volunteer to participate in this study, you will do the following steps:

Step 1). Complete a demographic questionnaire

Step 2). Participate in a sensory evaluation study in which you will:

a. Choose between a commercial brand of fermented coffee versus the fermented coffee prototype in a paired-comparison ranking test on various attributes.
b. Rate fermented coffee prototype on a hedonic rating scale about whether you like the product on certain attributes
c. Rate a fermented coffee for the incidence of eating measurement.
d. Rank principle display panels of the fermented coffee prototype based on preference.

The testing will be conducted in the sensory evaluation lab in the FCS department in room 112B. Water will be available throughout the study to cleanse your palate as needed. Testing will take about 7-15 minutes, depending on the amount of time you need to sample the product and evaluate. Once you have completed all of the tests, you will submit the questionnaire to the researcher and are free to leave.

POTENTIAL RISKS AND DISCOMFORTS

Potential risks and discomforts include consumption of a fermented product, which may cause gastric upset. Panelists are free to withdraw from the study at any point. The Student Health Center number (562) 985-4771) will be provided to participants if requested.

POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY

As a participant in this study you will gain formal training experience as a sensory food panelist. Additionally, you will get to try a new coffee beverage.

Potential benefits to this study include introduction of a functional beverage with high antioxidants and various health benefits. Such a product may be beneficial to the promotion of general health in the general population.

PAYMENT FOR PARTICIPATION
Participants will receive compensation in the form of a gift card (value of $5.00) for participation in this study. Compensation will be provided as you exit the testing room. Compensation will not be provided if you quit the study.

CONFIDENTIALITY
Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law.

PARTICIPATION AND WITHDRAWAL
You can choose whether to be in this study or not. In order to avoid a potential sense of coercion, if you volunteer to be in this study, you may withdraw at any time. Participation or non-participation will not affect you or any other personal consideration or right you usually expect. You may also refuse to answer any questions you do not want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise which in the opinion of the researcher warrant doing so.

IDENTIFICATION OF INVESTIGATORS
If you have any questions or concerns about the research, please feel free to contact the principal Investigator, Linh Le at lmyle13@gmail.com or the research advisor, Dr. Cheryl Rock at cheryl.rock@csulb.edu

RIGHTS OF RESEARCH SUBJECTS
You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. If you have questions regarding your rights as a research subject, contact the Office of University Research, CSU Long Beach, 1250 Bellflower Blvd., Long Beach, CA 90840; Telephone: (562) 985-5314. Email: ORSP-Compliance@csulb.edu

SIGNATURE OF RESEARCH SUBJECT OR LEGAL REPRESENTATIVE
I understand the procedures and conditions of my participation described above. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____
Printed Name of Subject

_____ _____
Signature of Subject Date

APPENDIX E

SENSORY EVALUATION SCORE CARDS

Appendix E1: Paired-Comparison Score Card: Product Ranking

Bubble

Name: _____

You are presented with two coded samples.

Please taste the samples on the left first.

Circle the sample that you prefer. You must make a choice.

604 247

Appendix E2: Hedonic Rating Score Card

Bubble

Name:_____

Taste each sample and tick ✓ how much you like or dislike each of the characteristics in the columns below on a scale of "1" to "9". Choose 1 option for each descriptor of each sample.

You may taste the samples more than once.

Please cleanse palate with some cracker and water before each taste.

Descriptors	Score	Flavor		Sweetness		Tartness		Aroma		Mouthfeel		Overall	
Sample number		604	247	604	247	604	247	604	247	604	247	604	247
Like Extremely	9												
Like Very Much	8												
Like Moderately	7												
Like Slightly	6												
Neither Like Nor Dislike	5												
Dislike Slightly	4												
Dislike Moderately	3												
Dislike Very Much	2												
Dislike Extremely	1												

Appendix E3: Food Action Rating Scale (FACT) Score Card

Name:_____

Review the instructions carefully below

Cleanse your palate by taking a small bite of the cracker and sipping a small amount of water
.

Taste the samples and place one tick ✓ in the box that best describes how you feel about each sample.

Feelings about food sample	**Score**	**Sample 604**	**Sample 247**
I would drink this every opportunity I had	9		
I would drink this very often	8		
I would frequently drink this	7		
I like this and would drink it now and then	6		
I would drink this if available but would not go out of my way	5		
I do not like this but would drink this on occasion	4		
I would hardly ever drink this	3		
I would drink this if there were no other beverage choices	2		
I would drink this only if forced	1		
	$2.50	$3.00	$3.50
How much are you willing to pay for **sample 604?**			
How much are you willing to pay for **sample 247?**			

APPENDIX F

FACULTY SUPPORT LETTER

Faculty Supervisor's Statement

TO: Institutional Review Board for the Protection of Human Subjects

FROM: Faculty Supervisor: DR. CHERYL ROCK

Department of: FAMILY AND CONSUMER SCIENCES

Telephone Extension: 562 985- 4497

NAME OF STUDENT: Linh Le

TITLE OF THESIS OR PROJECT: Utilization of Power Ultrasound Technology in the Development of "BubbLê," a fermented Coffee (Coffea) Prototype

(1) Project Risks

As outlined in the IRB proposal, the subjects recruited for the study will be required to sample a food item, involving several Sensory Evaluation techniques (administrative review category 3960). Projected risks may include allergies to food samples being administered, which are kombucha and coffee predominantly.

(2) Mitigation of Risks

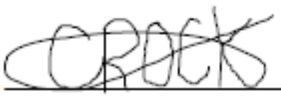
To mitigate any risk encountered in the research undertaken, the student was evaluated on their knowledge about the project was afforded the opportunity to complete a thorough literature review and to complete any foundation course work and training to acquire the knowledge and experience to conduct the proposed research undertaken. This is evidenced by the protocols and procedures that the student researcher has documented in the prepared IRB application. The student researcher also obtained the required IRB certification (Report ID# 18278375, student ID# 5276465) through the on-line training basic course in the CITI basic modules, which included the Belmont Report and CITI introduction, Ethical Principles, Risk Assessment and Federal Regulations, among other components. The student researchers protocols have been thoroughly reviewed by myself the faculty sponsor before the materials were submitted to the IRB. In addition, during the research, I will supervise the student on a regular basis and monitor the progress of the project. Before any research on human subjects is performed, I have instructed the student to only commence their sensory evaluation taste panels once they have received written approval from the IRB. The subjects recruited for the study will also be required to sign an informed consent from which describes all of the possible risk that they may be exposed to and the measures to take should they be exposed to ensure their autonomy as mandated by 45 CFR 46.116 and 117. During the recruitment process, the subjects will also be screened for food allergies using a demographic questionnaire submitted for review by the IRB.

(3) Similar Research

In the Food Science and Nutrition discipline, the Sensory and Food Quality section of the Journal of Food Science (JFS) publishes original and applied research related to the sensory and

quality aspect of foods, beverages, ingredients, and research about the perceptual process which involves affective testing including the 9-point hedonic scale, paired comparison and FACT tests which were all described in the experimental protocols in the IRB applications. In the majority of these tests human subjects are have been used.

(4) Compliance with (a) Federal regulations and (b) University policy with regard to protection of human subjects from potential harms or risk and (c) with respect to the principles of justice, beneficence, and respect for humans in the research was executed by ensuring that the information provided in the IRB Application is accurate and complete and that the project will be approved by the IRB before commencement. As previously mentioned, the student researcher was required to become familiarized with the Belmont Report, acquire IRB approval and to provide an informed consent form to satisfy 45 CFR 46.116 and 117 and the conditions of (a); (b) and (C) with reference to compliance.



11/02/2016

Thesis Chair / Research Supervisor Signature and Date

APPENDIX G

DEPARTMENT PERMISSION LETTER

CALIFORNIA STATE UNIVERSITY, LONG BEACH

FAMILY AND CONSUMER SCIENCES

NUTRITION AND DIETETICS, HOSPITALITY MANAGEMENT, FOOD SCIENCE
FASHION MERCHANDISING AND DESIGN, CHILD DEVELOPMENT AND FAMILY STUDIES
FAMILY LIFE EDUCATION, CONSUMER AFFAIRS, AND GERONTOLOGY

October 21, 2016

IRB Number:	5276465
Title of Study:	Utilization of Power Ultrasound Technology in the Development of "BubbLê," a Fermented Coffee (Coffea) Prototype
Principal Investigator:	Linh Le
Committee Chair:	Dr. Cheryl Rock

To the IRB at California State University, Long Beach:

As a representative of the Department of Family and Consumer Sciences, I confirm that the department grants permission for the proposed research to be conducted once IRB approval has been obtained. I understand the researcher will have contact with students / faculty in the Sensory Evaluation Laboratory in the Family and Consumer Sciences building at California State University, Long Beach. The researcher will recruit students to participate in sensory tests, during which beverage samples will be tasted, food packaging will be evaluated, and respondents' preferences will be recorded as described in the research protocol.

W. Reibolat
Printed Name of Department Official

Chair
Title of Department Official

W. Reibolat
Signature of Department Official

10/24/16
Date

1250 BELLFLOWER BOULEVARD - MS 0501, FCS-001 · LONG BEACH, CALIFORNIA 90840-0501 · 562/985-4484 · FAX 562/985-4414
www.csulb.edu/depts/fcs

APPENDIX H

MANUSCRIPT

RESULTS AND DISCUSSION

CONSUMER ACCEPTABILITY OF A KOMBUCHA COFFEE *(COFFEA)* PROTOTYPE WITH TRADITIONAL COFFEE CHARACTERISTICS

Linh Le[1], Cheryl Rock[1], Rachel Blaine[1], Christine Costa[2] and Wendy Reiboldt[1]

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[2]Department of Nursing, California State University, Long Beach, 90840

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Dr. Cheryl Rock, 1250 Bellflower Boulevard, Long Beach, CA 90840, telephone: (562) 985-4497, fax: (562) 985-4414, cheryl.rock@csulb.edu

Word count: 4101

Short version of title: Kombucha Coffee and Product Development

Choice of journal/section: Sensory and Food Quality

Abstract:

Coffee and Kombucha tea are both beverages that have been consumed for many years, with a current increased momentum in consumption due to many correlations with beneficial health aspects. The objective of this study was to assess the consumer acceptability of a Kombucha Coffee which tastes more like traditional coffee. A Kombucha Coffee prototype BubbLê, was created and compared to a market Kombucha Coffee via a hedonics evaluation, food action rating scale (FACT), and a paired-comparison ranking test. Participants rated the market Kombucha Coffee significantly higher than BubbLê Kombucha Coffee in all sensory aspects for flavor (6.84±1.82; 4.46±2.48; $p<0.001$), sweetness (7.11±1.63; 4.65±2.33; $p<0.001$), tartness (6.27±1.77; 4.72±2.55; $p<0.001$), aroma (6.30±1.82; 5.55±2.59; $p=0.018$), mouthfeel (6.87±1.62; 5.36±2.64; $p<0.001$), and overall likeability (6.90±1.76; 4.59±2.43; $p<0.001$) in the hedonics evaluation. The FACT test indicated that participants would more likely drink the market alternative compared to the prototype (5.42±1.96; 3.62±2.29; $p<0.001$). The majority of participants (80%) chose the market Kombucha Coffee over the more traditional coffee flavored Kombucha Coffee prototype. It is noted that flavor scored the lowest in sensory evaluation for the prototype, therefore, reevaluation of flavor by means of adding coffee enhancing notes are needed for further development of a Kombucha Coffee with a more traditional coffee flavor profile.

Key words: coffee, kombucha, product development, antioxidants, nutrition, health

Practical Application: Coffee and Kombucha tea can be regarded as functional beverages that can promote overall health. Developing products which include traditional coffee flavors with the fermentation techniques of Kombucha tea can lead to increased consumption of this high antioxidant beverage leading to decreased cardiovascular disease, diabetes and cancers.

INTRODUCTION

Cardiovascular disease (CVD), cancer, and diabetes are among the leading causes of human death in the United States with a total death rate of 1,282,535 in 2014 (Centers for Disease Control and Prevention [CDC], 2015). Cardiovascular disease is an umbrella term for a range of diseases the affect the heart and blood vessels (World Heart Federation, n.d.). Diabetes is a type of disease in which the body is unable to use blood glucose for energy, resulting in hyperglycemia (American Diabetes Association , 2014). Cancer is a term for diseases in which abnormal cells divide without control which can invade other tissues leading to complications (National Cancer Institute, n.d.). The World Health Organization (WHO), Food and Agriculture Organization (FAO) and the CDC are in agreement that these various diseases can be prevented with proper diet and lifestyle (CDC and U.S. Department of Health and Human Services, 2008; WHO, 2002). Many of these diseases are thought to stem from free radicals and reactive oxygen species (ROS), which compromise the human body's cells and tissues (Lobo, Patil, Phatak & Chandra, 2010). Both of these unstable and reactive compounds derive from normal metabolic processes within the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals (Bagchi & Puri, 1998). In low concentrations, free radicals and ROS do have beneficial roles, such as human defense mechanism of destroying invading pathogenic microbes (Dröge, 2002; Young & Woodside, 2001). In excessive amounts, free radicals and ROS in the human body lead to a phenomenon

known as oxidative stress which can dramatically alter cell membranes, resulting in the development of those chronic and degenerative diseases such as cancer and CVD. The human body defends itself against oxidative stress by utilizing antioxidants, which are known to combat against free radicals and ROS (Pham-Huy, He, & Pham-Huy, 2008).

Coffee consumption has been associated with the possibility of reducing the risks for diseases, due to high amounts of dietary antioxidants present (Andersen, Jacobs, Carlsen, & Blomhoff, 2006). Epidemiological studies suggest that coffee consumption is associated with the prevention or delay of degenerative diseases, which include diabetes, CVD and cancer (Zhang, Lopez-Garcia, Li, Hu, & Dam, 2009). Deoxyribonucleic acid (DNA) protective properties of coffee reduce free radical and ROS cellular damage, which may be implicated in cancer development (Bakuradze et al., 2011). These beneficial effects have been partly attributed to the antioxidant activity present in coffee consumption (Hoelzl et al., 2010).

Coffee (*coffea*) is one of the most popular beverages consumed around the world with increasing consumption of Gourmet Coffee Beverages (GCB). More than half of the American adult population consumed this beverage daily in 2015 (National Coffee Association USA [NCA], 2016). A traditional cup of coffee is depicted by a flavor complexity of sensations described as a combination of aroma, taste, texture and mouthfeel (Taylor & Roozen, 1996). Among the sensations, aroma is notably the most important (Cliff & Green, 1994).

There is currently a trending increase in consumption of GCB, defined by the NCA (2016a) as specialty coffee including gourmet traditional coffee, espresso-based beverages, along with iced or frozen drinks. More specifically, gourmet coffee derives from premium grade coffee beans of specific species, such as the Arabica, which are more scarce due to difficulty to grow in specific geographical regions. In 2012, 44% of coffee consumers chose GCB over non-GCB,

which increased to 54% in 2014 (Murray, 2014). Within the GCB category, espresso-based beverages also showed a steady increase in consumption. Espresso-based beverages are variations of coffee drinks with at least one shot of espresso present. Of the GCB consumers, espresso-based beverages made up 35% of the consumption in 2012 and increased to 52% of the GCB consumption in 2014 (Murray, 2014).

Similarly to coffee, another beverage that has many beneficial health properties is Kombucha tea (KT). Kombucha tea is traditionally a sweetened black tea that is fermented with a symbiotic culture of yeast and bacteria to produce a mild vinegar taste with an effervescent finish (Marsh, O'sullivan, Hill, Ross,& Cotter, 2014; Watawana, Jayawardena, Gunawardhana, & Waisundara, 2015). This tea has been brewed in China for over 2,000 years and has acted as a functional beverage due to its ability to combat chronic illnesses such as CVD, cancer and type 2 diabetes (Marsh et al., 2014; Watawana et al., 2015).

There are many different techniques to produce brewed coffee with varying amounts of antioxidants (Babova, Occhipinti, & Maffei, 2016; Lopez-Galilea, Peña, & Cid, 2007; Ludwig, Bravo, Peña, & Cid, 2013; Yilmaz, Hacibekiroglu, & Kolak, 2014). These brewing techniques can be generally characterized by brewing pressure, brewing process, extract volume, and solid content (ParentI et al., 2014). The current brewing method that results in highest antioxidant capacity when compared to other conventional brewing techniques is the espresso method, obtained via percolation of hot water under pressure through compacted roasted ground coffee (Illy & Viani, 2005; Lopez-Galilea et al., 2007; Ludwig et al., 2012). Another brewing technique commonly used is cold brewing, which uses coarsely ground coffee mixed with cold water and left in a refrigerator for at least 12 hours (Perratore, 2016). This novel form of brewing coffee can lead to potentially higher extract of antioxidant levels.

Materials and Methods:

Subject Recruitment, Selection, and Training

The consumer acceptability study was submitted and accepted by the Institutional Review Board (IRB) at California State University, Long Beach (CSULB). Consumer testing was conducted in the sensory evaluation laboratory at the CSULB Department of Family and Consumer Sciences (FCS) building. The sensory evaluation testing was performed under controlled conditions—isolated booths, controlled lighting and temperature. Ninety untrained subjects were recruited by flyers and advertisements (Appendix B). Subjects may mainly consist of staff and students from CSULB. Subjects were required to complete a questionnaire that requests demographic information (age, gender, ethnicity) along with shopping habits prior to the testing (Appendix C). Subjects in this study were excluded if they: (1) have a food allergy to any of the products tested, (2) do not consume Kombucha or coffee products used in the study for dietary or cultural reasons, and (3) had any professional experience in sensory evaluation. The subjects were free to withdraw from the study without any consequences. Subjects were required to sign an informed consent form and were compensated for their participation in this study (Appendix D). Consumer acceptance was determined using three tests: (1) Ranking Tests, (2) 9-point Hedonic Scale, (3) Food Action Rating Scale (FACT) test.

Instrumentation and Procedure

Development of BubbLê Kombucha Coffee Prototype

Coffee Brewing Techniques

The brewing of coffee using cold methods were performed according Temple Coffee Roasters. In preparation of the coffee brew, high quality coffee beans (donated from Temple

Coffee Roasters, Sacramento, CA, USA) were used. Each coffee brewing technique was conducted in triplicates and stored in the refrigerator until analysis.

Cold Brewing

Five-hundred grams of coffee was coarsely grounded using a Baratza Encore 485 Coffee Grinder set at the 27-grind setting (Figure 1). The ground coffee was immersed in 4000 mL of cold water, then placed in a refrigerator set at 3.3°C (38°F) for 18 hours (Figure 2). The brewed coffee was filtered from the coffee grounds with a metal mesh strainer placed in a Chemex (Figure 3). The process was repeated 2 more times.

FIGURE 1. Encore 485 grinder set at 30 and coffee grounds. Photo courtesy of Linh Le. Copyright 2017.

FIGURE 2. Cold brew coffee before refrigeration. Photo courtesy of Linh Le. Copyright 2017.

FIGURE 3. Chemex with metal mesh strainer. Photo courtesy of Linh Le. Copyright 2017.

Fermented Coffee Formulation

The fermented coffee formulation was produced in similar conditions as, Marsh and others (2014), with the exception of coffee being used in place of tea. A Kombucha cellulose pellicle starter Kombucha culture was used. The coffee was brewed and weighed for determination of sugar (10%). Fermentable sugar was determined by multiplying total brewed coffee by 10% and divided in half for 1st and 2nd ferment. The sugar was dissolved in 500mL of hot water and cooled then added to the brewed coffee in a sterile glass container (Ball® Mason Jar). The Kombucha cellulose pellicle was added to the coffee solution (Figure 4). The container was covered with a 100% cotton towel and an elastic band was fixed on. The coffee mixture and Kombucha cellulose was placed in a dark space at room temperature (~23 °C) and fermented for 5 days. The 2nd half of sugar was dissolved in 500mL of hot water and cooled then added to the fermented coffee to be bottled in airtight 16 oz bottles for second fermentation of 14 more days. After the second fermentation the developed BubbLê product was refrigerated at 4°C until sensory analysis is performed.

FIGURE 4. Kombucha fermentation. Photo courtesy of Linh Le. Copyright 2017.

Evaluation of Consumer Preference and Acceptability of the Kombucha Coffee

The evaluation of consumer preference and acceptability of BubbLê Kombucha Coffee prototype was conducted using the following sensory analysis tests: (1) A nine-point Hedonics Preference test, (2) the Food Action Rating Scale (FACT) and a (3) Paired-Comparison test. More specifically, acceptability, or likeability, was determined by the Hedonics and FACT test, which identified overall acceptance of the product's sensory characteristics. Preference of BubbLê Kombucha Coffee versus market alternative was analyzed using a pair-comparison test, which does not indicate an overall liking or dislike of the products. All sensory tests were performed at the Family and Consumer Sciences (FCS) sensory evaluation laboratory at CSULB. Water (20°C) and unsalted crackers were provided to participants at the beginning of each test to cleanse their palates between samples during sensory evaluation of the prototype.

9-Point Hedonics Preference Test

Subjects were administered a 9-point hedonic scale for sensory evaluation where: (9 = "like extremely"; 8 = "like very much"; 7 = "like moderately"; 6 = "like slightly"; 5 = "neither

like nor dislike"; 4 = "dislike slightly"; 3 = "dislike moderately"; 2 = "dislike very much"; 1 = "dislike extremely") to rate the prototype for attributes such as flavor, sweetness, tartness, aroma, mouthfeel, and overall preference (Appendix E2). Panelists were provided with two randomly coded samples of the fermented coffee prototype and a control sample i.e., commercial brand. The samples were distributed in 2 oz. clear plastic cups. Samples will also be administered randomly to avoid bias. Water and unsalted crackers were supplied to the panelists to cleanse their palate between samples.

Food Action Rating Scale

The FACT test will measure the fermented coffee prototype for the incidences of drinking measurement as described by Ramcharitar and others (2005). A 9-point scale were used and is as follows: 9 = "I would drink this every opportunity I had"; 8 = "I would drink this very often"; 7 = "I would frequently drink this"; 6 = "I like this and would drink it now and then"; 5 = "I would drink this if available but would not go out of my way"; 4 = "I do not like this but would drink this on occasion"; 3 = "I would hardly ever drink this"; 2 = "I would drink this if there were no other beverage choices"; 1 = "I would drink this only if forced". Additionally, subjects were asked how much they would be willing to pay for the product (Appendix E3).

Paired-Comparison Test

The consumer acceptability study was submitted and accepted by the Institutional Review Board (IRB) at California State University, Long Beach (CSULB). Consumer testing was conducted in the sensory evaluation laboratory at the CSULB Department of Family and Consumer Sciences (FCS) building. The sensory evaluation testing was performed under controlled conditions—isolated booths, controlled lighting and temperature. Ninety untrained subjects were recruited by flyers and advertisements (Appendix B). Subjects may mainly consist

of staff and students from CSULB. Subjects were required to complete a questionnaire that requests demographic information (age, gender, ethnicity) along with shopping habits prior to the testing (Appendix C). Subjects in this study were excluded if they: (1) have a food allergy to any of the products tested, (2) do not consume Kombucha or coffee products used in the study for dietary or cultural reasons, and (3) had any professional experience in sensory evaluation. The subjects were free to withdraw from the study without any consequences. Subjects were required to sign an informed consent form and were compensated for their participation in this study (Appendix D). Consumer acceptance was determined using three tests: (1) Ranking Tests, (2) 9-point Hedonic Scale, (3) Food Action Rating Scale (FACT) test.

Data Analysis

Descriptive statistics, including frequencies, percentages, and variance, were calculated for scorecard ratings. A paired t-test was used to compare means and standard deviations (SD) for each of the sensory attribute of Hedonic rating and FACT scores. Overall statistical tests used for acceptability the Kombucha Coffee prototype was tested via the International Business Machine Statistical Package for Social Sciences (IBM SPSS) Statistics 22 for Windows. All analysis tests were performed using a significance of at least $p < 0.05$.

Results and Discussion

(1) Nine-point Hedonics test

The market Kombucha Coffee, compared to the Kombucha Coffee prototype (BubbLê), scored statistically significantly higher in flavor (6.84±1.82; 4.46±2.48; $p<0.001$), sweetness (7.11±1.63; 4.65±2.33; $p<0.001$), tartness (6.27±1.77; 4.72±2.55; $p<0.001$), aroma (6.30±1.82; 5.55±2.59; $p=0.018$), mouthfeel (6.87±1.62; 5.36±2.64; $p<0.001$), and overall likeability (6.90±1.76; 4.59±2.43; $p<0.001$) in the Hedonics evaluation (Table 1) respectively. On the

Hedonics scale from 1 to 9, the BubbLê Kombucha Coffee overall was rated 4.59±2.43 (dislike very much to like moderately) for overall likeability, and the market Kombucha Coffee was rated 6.90±1.76 (neither like nor dislike to like very much).

It is noted that the highest scoring sensory evaluation for BubbLê Kombucha Coffee was the aroma (5.55±2.59), which was shown in other studies to be the most important aspect of coffee. Aromatic components are particularly important in coffee beverages due to it being the main constituent of the sensory experience for coffee drinkers (Bhumiratana, Adhikari and Chambers, 2011). Participants could have also been influenced by their own experiences and expectations of what coffee should taste like versus what BubbLê Kombucha Coffee tastes like for their scoring of the product (Kemp, Hollywood and Hort, 2009). Although this product was developed with an aim to have more traditional coffee flavors, the process of fermentation changed the flavor profile; however, findings from this research highlights the potential of developing this product further with a focus on enhancing the other traditional attributes of coffee such as flavor, tartness and sweetness.

(2) Food Action Rating Scale (FACT)

The frequency of consumption for the BubbLê Kombucha Coffee was significantly lower ($p<0.001$) compared to the market alternative. On the FACT scale from 1 to 9, the average rating for the BubbLê Kombucha Coffee was 3.62±2.29 (I would drink this only if forced, to, I like this and would drink it now and then), while the market alternative was 5.42±1.96 (I would hardly ever drink this, to, I would frequently drink this) (Table 1). The scores for the frequency of drinking either product was 1 to 9.

Previous researchers have shown the likeability of a traditionally characterized cup of coffee (Cliff and Green, 1994; Taylor and Roozen, 1996); however, the participants in this study

have shown that the hedonic qualities of BubbLê Kombucha Coffee was negatively affected with the more traditional flavors when compared to a market alternative. Researchers have found that the addition of cardamom (*Amonum cardomum*), a popular spice, to traditional brewed coffee, was shown to increase the hedonic score for taste over traditional coffee without cardamom (Febrianto, Rizki and Djumarti, 2015). In order to increase the frequency of consumption of BubbLê Kombucha Coffee, further development of the product using spices such as cardamom to enhance the coffee flavor will increase the likability of the product.

(3) Paired-Comparison test

The Paired-Comparison test showed that 80% of participants preferred the market Kombucha Coffee and 20% the Kombucha Coffee prototype (Table 2). This test aligns with the scores provided by other measures (i.e., Hedonics and FACT test). The market product scored highest on sweetness whereas the prototype's sweetness was second to the lowest score. Considerations in further development of BubbLê Kombucha Coffee can include adjustment of the sweetness level of the product to be sweeter like the market alternative.

In addition to sensory aspects of the study, 33% of participants "never" drinks coffee ranging up to "once or twice a month" and 51% never tried Kombucha prior to the study, showing that this could have led to possible bias in the results. The results were also similar to previous researchers who found lower hedonics scores from participants when the taste of the product was unfamiliar (Febrianto, et al., 2015). Future studies of this product can include, using participants who have already tried Kombucha and enjoy drinking coffee.

Conclusion:

This study examined the consumer overall acceptability of a Kombucha Coffee developed with traditional coffee characteristics compared to a market alternative without those

key characteristics. The findings of this study indicate that consumers preferred the market alternative to the developed BubbLê Kombucha Coffee. It was also observed that the most distinguished characteristic of coffee, aroma, scored highest for consumer acceptability of a more coffee-like Kombucha. Development of another product incorporating key characteristics of coffee may be more successful if the flavor were enhanced to taste more like coffee. For example, one may try different spices such as, cardamom, or increase the amount of naturally flavored extracts to enhance and compliment the flavor profile of coffee. Moreover, the use of novel technologies and varying coffee extraction methods to extract coffee should be explored.

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Tables:

Table 1. Palatability ratings and overall acceptability of Kombucha Coffees

	BubbLê	**Market**	**P value**
Flavor (μ ± SD), n=87	4.46±2.48	6.84±1.82	<0.001
Sweetness (μ ± SD), n=85	4.65±2.33	7.11±1.63	<0.001
Tartness (μ ± SD), n=82	4.72±2.55	6.27±1.77	<0.001
Aroma (μ ± SD), n=86	5.55±2.59	6.30±1.82	0.018
Mouthfeel (μ ± SD), n=86	5.36±2.64	6.87±1.62	<0.001
Overall (μ ± SD), n=87	4.59±2.43	6.90±1.76	<0.001
FACT* (μ ± SD), n=73	3.62±2.29	5.42±1.96	<0.001

Scale for all attributes: Range 9 = “like extremely” to 1 = “dislike extremely” (Peryam & Pilgrim, 1957).
*FACT scale rating: Range 9 = “I would eat this every opportunity that I had” to 1 = “I would eat this only if forced” (Schutz, 1965).

Table 2. Determination of Preference with Pair-Preference test

BubbLê n=18	**Market n=72**	**n=90**
20%	80%	100%

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