ABSTRACT

RHOADES, ELIZABETH RENEE. Product Development of a Nutraceutical Beer via Biological Isolates of Soy Kefir. (Under the direction of Dr. John Sheppard.)

There are a few main reasons for new product development: a) there is a trend for this particular product or product category or potential for a large market share with this product/product category; or b) it fills or has the potential to fill a consumer need. A nutraceutical beer via biological isolates of soy kefir has the potential to be a very viable product in the current market considering these reasons: beer, and furthermore craft beer, has proved to be a mainstay in modern society with an 11% increase in production volume in 2010 in the United States (Brewer's Association, 2011); the Global Nutraceutical Beverage market is expected to grow to 71.3 billion dollars by 2013, a nearly 40% increase from 2008 (Roberts, 2009). Due to the addition of the biological isolates of soy kefir, or Soy Kefir Powder (SKP)- spray dried soy kefir liquid- this product can also help to fill the needs of an intended target audience of "baby boomers," 26.5% of the U.S. population, by providing relief from pain, fatigue, inflammation, and hypertension (which are some physiological factors of getting older). The soy kefir is beneficial mainly due to their possession of isoflavones, daidzin, genistin, daidzein, and genistein, and small peptides. However, while the flavor profiles of the two main components of this product, soy kefir and beer, may share a few similar attributes, such as ethanol and some fruitiness, most of their flavors or tastes that are considered desired in each product, differ. Therefore, work was done to be able to produce a quality, palate pleasing nutraceutical beer, or beer-like beverage that consists of a flavor profile most similar to ales.

During the research, several means of analysis were utilized, such as descriptive anal-

ysis, soy isoflavone analysis via HPLC equipped with a Photodiode Array Detector (DAD) (Waters 2998, Milford MA), and fermentation by-product analysis via HPLC equipped with a Refractive Index Detector (Waters 2414, Milford, MA). Kristalkefir (KK) was found to be a better choice as an addition into the beer versus the SKP for a variety of reasons, for example, cost and sensory acceptance. A diacetyl rest on the Kristalkefir was tested to determine if it decreased the amount of perceived diacetyl in the beer treatments, thus improving its acceptance, and to see how its isoflavone content (glycosides and alglycones) was affected. It was found that KK with a 24-hour diacetyl rest significantly decreased the amount of perceived diacetyl, and as rest duration increased, glycoside concentration decreased and alglycone concentration increased. While IWA, or India Wheat Ale, was the initial ale style selected for the nutraceutical beer, an Aardbeien Lambic ale style containing liquid soy kefir at a concentration of 25%, which was previously heat-pasteurized at 60° C for 20 minutes, was ultimately selected because it proved to satisfy the feasibility objectives. Despite heat-pasteurization showing to be deleterious to the soy isoflavones, they were still within the matrix at a moderate amount, allowing the alglycones, to be present within the product in a sufficient amount, at 8.60% of the Soy Kefir Powder published data. Withstanding this, one 12 oz or 355 ml serving of this product provides the consumer with 18.74% of the Recommended Daily Intake (RDI) of SKP, at 30 g/day, thus allowing the product to have the potential to supply some therapeutic effect, based on the previous SKP clinical trials (Kubow and Sheppard, 2009). The data from the Accelerated Shelf-life Study implicates that this product would have a shelf-life of up to three months, based upon the sensory and isoflavone data, and it would only add $0.09/355 ml serving to the production cost.

Product Development of a Nutraceutical Beer via Biological Isolates of Soy Kefir

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Food Science

Raleigh, North Carolina

2012

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BIOGRAPHY

Elizabeth Renee Rhoades was born on September 12th 1987 in Carbondale, IL, and she calls home Champaign-Urbana, IL- "the Cham-ban," where she spent most of her life. She attended Dominican University in River Forest, IL for her undergraduate education, and obtained a B.S. in Food Science and Nutrition with a minor in Vocal Performance in May of 2009. It was here where she discovered her eventual career path, and tabooed love affair, of beer science. She participated in undergraduate research, under the direction of Dr. Judith Beto, exploring the product development of nutraceutical beers, and brewed her first batch of beer at the age of 19 (which is legal). In the fall of 2009, she continued on in her educational career path to North Carolina State University, where she connected with Dr. John Sheppard and had the privilege of working under his direction for her Master's work- *Product Development of a Nutraceutical Beer via Biological Isolates of Soy Kefir*.

AKNOWLEDGEMENTS

First, I would like to thank Dr. Gabriel Keith Harris and Dr. Trevor Phister for serving on my committee, and I would also like to thank Dr. John Sheppard for allowing me to pursue my Master's research. Much thanks and appreciation goes out to Mara Massel, the Phister/Sheppard Research Technician and Lab Manager- I have learned an incredible list of things from you and appreciate all of our encounters and conversations, from HPLC columns to Bob Fosse…thank you! In addition, I would like to thank the NC State Music Department and Dr. Nathan Leaf for allowing me to scratch my musical performance itch, and for helping me keep my "pipes" in tune over my graduate career. Lastly, I would like to thank Brad Wynn, Geoff Lamb, and everyone at Big Boss Brewing Co. for allowing me to work at Big Boss, and supporting me in my research over the past year and a half; I have learned a great deal about beer, from barley to bottle, and the experience has allowed me to actively engage myself in the incredible world of craft beer and fermentation- Thank you!

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OBJECTIVE(S)

To develop a feasible beer or beer-like product with added biological isolates of soy that would allow for some added health benefit to the consumer; in which feasibility is defined as:

a) It is sensorally pleasing as, and/or contains a sensory profile similar to that of ales

b) Biological isolates of soy, and more specifically soy isoflavones, are present within the product at sufficient amounts allowing for some therapeutic effect

c) The product has some shelf stability and similar shelf life/conditions to that of an ale

d) Has the potential to be a successful product in the current marketplace, withstanding its price point and intended target audience

CHAPTER 1. LITERATURE REVIEW

Beer is quite arguably one of the oldest and most historically rich beverages of global society known by man. Beer brewing can be dated back to 6000 B.C. in the ancient Middle East region, known as Mesopotamia (Freemantle, 2006). According to Hornsey (2003), author of *A History of Beer and Brewing*, Ancient Egypt during the Predynastic Era (5500-3100 B.C.), has also proved to be another historical hub for this beloved beverage. During these times, beer was brewed in order for water to be safely consumed- like many ancient fermentations, especially in regions with intensely warm climates, such as the Near and Middle East regions. Beer consumption was more-or-less a necessity, but it was regarded as an activity of pleasure as well:

> [it] was consumed by all social classes in the community, including women. It was also interlinked with mythology, religion, and medicine, and its consumption was synonymous with happiness and a civilized life (Hornsey, 2003).

However, like many pre-historic ideals and ways of life, time will prove to change these notions and the societal perception of beer consumption. One of the largest historical examples of this paradigm shift, occurred in the early 20th century in the United States of America (1920-1933), where the production, sale, and transportation of all alcoholic beverages, including beer, was prohibited. Although this prohibition was just a short period of time, merely a slight hiccough in global society, it has more-or-less, greatly impeded the craft and science of beer development and appreciation, and furthermore, has created a means of controversy.

Even after the 18th Amendment was repealed, federally legalizing alcohol once

again, many state governments enacted strict legislation on the production and sale of such alcoholic beverages. For example, Kansas still prohibited alcohol sales until 1948, one and one-half decades after the federal lift (Heller, 1992). Home-brewing was not federally legalized until President Jimmy Carter signed bill H.R. 1337 in October of 1978. In addition, in North Carolina state legislation forbade the production, distribution, and sell of beer with an Alcohol by Volume (ABV) concentration of greater than 6.0% until August 2005. The "Pop the Cap" grassroots campaign led to the House Bill 392 to be passed, now allowing beer up to 15.0% ABV to be produced, distributed, and enjoyed across North Carolina (LeClaire, 2010). Legality, mainly rooted in religion, however, is not the only culprit behind the negative stigma of beer drinking, but can also be attributed to the influence of social status and health.

Morris states that "drinks are classified in terms of their social meaning, and the classification of drinks is used to define the social world. Few, if any, alcoholic beverages are socially neutral: every drink is loaded with symbolic meaning, every drink conveys a message [… it is] also a significant indicator of social status" (1998). In a 1983 study on the "multifaceted nature of drinking in U.S. society," Kilty discovered that not only did distinct categories of drinkers exist, but there was also a social status divide between beverage choice, within relation to activity or behavioral pattern. He noted that one class was categorized as "middle-class men beer drinkers," and beer was strongly associated with passive activities such as watching television or sporting events, whereas another notable group was categorized as "sophisticated middle-class women drinkers" who drank wine and distilled spirits. The latter category largely identified and associated their alcohol consumption with

lifestyle, and that there drinking (beverage choice included) was "stylish." Moreover, this study implicated wine drinking, not beer drinking, as an act of sophistication, while beer drinking was associated with the "average man" participating in "average activities." (Kilty, 1983) Another U.S. study, researching perceived appropriateness of alcoholic beverage choice across varying social scenarios (Klein, et al, 1991), found that for celebratory or special occasions, wine and spirits were very appropriate, whereas beer was not, nor appropriate as an accompaniment to a nice dinner. Beer was, however, found to be very appropriate in an informal or relaxation-geared scenario (again, such as a ballgame), whereas wine and spirits were not.

Morris (1998) also notes that "foreign" or non-native beverages have a higher status than their "local" or native counterparts. She also states that in France, "where wine-drinking is commonplace and confers no special status, the young elite" are selecting beer as their beverage of choice, whereas in Poland, wine is considered "high-class," and vodka is for the "working-class" (Morris, 1998). In the early sixteenth century, the "New World" settlers brought beer with them on their voyage, consequently making it a commodity in this new society. The Pilgrims set-up camp in Plymouth Rock, instead of their intended geographical arrival, the Hudson River, due to there being fear of shortage of this fermented barley beverage (Mittelman, 2008). Even in the very conception of the nation, the Founding Fathers were fueled by beer, turning to it for encouragement, solace, and, naturally, pleasure. Withstanding this, there is something to be said about beer in America…it is a beverage symbolizing our heritage, and furthermore, deeming it a "native" or "local" beverage, or perhaps, "working-class" beverage. However, this does not paint the whole picture in modern society.

Society has progressed. As any beer connoisseur would proclaim, beer is as diverse as pickles and ice cream…in other words, the category of "beer" is multi-dimensional, accepting different flavors, colors, and depths into its vast repertoire. And more importantly, society is beginning to pick-up on these nuances, allowing for inter-categorical social association. Antin et al (2010) found in a recent study that consumers do coherently identify classifications amongst the beer category when speaking in terms of quality. It was found that consumers associated "specialty beers or microbrews" with process-oriented drinking, or "beverages that you would drink to appreciate, not drink to get drunk." This demonstrates this new notion of inter-categorical differentiation- a higher quality, higher-class beer, versus a lesser quality, "working-class" beverage. In addition, "cheap wine" was included as an outcome-oriented beverage (drank to feel the outcome of alcohol…"drinking to get drunk"), along with the lesser quality/average beer (Antin, et al, 2010). These societal differentiations are important to beer consumption, and to the craft and science of beer development and appreciation; however, social perception is not the only possible factor, but beer consumption on health, is also a variable of consumer concern as well.

When consumers think about beer and health, the majority of society most likely envisions the notorious "beer belly." In continuation, this so-called "beer-belly," in other words, a centrally located abundance of adipose tissue, contributes negatively on health, including increased risk for cardiovascular disease, stroke, and type II diabetes (Dorn et al, 2003). Dorn et al also notes that:

> central adiposity has also been positively associated with blood pressure, total cholesterol, LDL cholesterol [(low-density lipoprotein, or "bad cholesterol")], triglyceride

> levels, diabetes mellitus and inversely with HDL cholesterol [(high-density lipoprotein, or "good cholesterol")] (2003).

Bes-Rastrello et al (2011) states that alcohol "cannot be stored by the body, meaning it has priority for oxidation compared with fat and carbohydrates." While some studies have scientifically proven the link between beer or alcohol consumption on adiposity (Duncan et al., 1995; Shaper et al., 2005), other studies have contradicted these findings, and possible societal misperception (Bobak et al., 2003; Halkjaer et al., 2006). The underlying problem, however, is not alcohol consumption, it is heavy or excessive alcohol consumption- much like many foods or beverages, moderation is the key for healthy living. There is evidence that heavy daily consumption of alcohol, greater than 30 g/day, or greater than 2 servings of alcohol[1]/day, is linked with increased body weight and Body Mass Index (BMI) (Shaper and Wannamethee, 2003), but moderate consumption of alcohol (up to a daily intake of two servings) can actually be beneficial to one's health.

A study conducted by Kemper et al (2005) found that "moderate alcohol consumption [… is] positively related with the levels and changes in high-density lipoprotein cholesterol in healthy adult men and women." In a different study researching the effects of alcohol consumption and adiposity, the following information was discovered:

> […] among both women and men, daily drinkers of alcohol had smaller [abdominal heights] than less frequent drinkers. When frequency and intensity of drinking were combined, small amounts of alcohol on a regular basis were associated with the smallest abdominal heights, whereas participants with the most intense drinking (3– 4+

[1] One serving of alcohol is equal to 14 g or 1/2 oz of pure ethanol; it is also the equivalent of 12 oz of beer, 5 oz of wine, and 1.5 oz of 80 proof distilled spirits (NIAAA, 2011).

drinks/drinking day) but on a sporadic basis (<weekly) had some of the largest abdominal heights (Dorn et al, 2003).

Furthermore, moderate beer consumption has much more to offer, for example, antioxidant capabilities in dark beer (Bose et al, 2003). While beer may possess lesser amounts of polyphenol antioxidants than red wine, beer is shown to still be able "to improve plasma antioxidant capacity without the negative effects produced by high doses of ethanol […] probably through the increase of the absorption of phenolic compounds" (Fantozzi, 2000). In addition to these phytochemicals, other micronutrients exist in beer as well, such as B-vitamins and minerals. These nutrients include B12, Riboflavin, B6, Niacin, Folate, Magnesium, and Potassium (Table 1). Lastly, although this is widely dependent upon the style of beer, specific malt utilized in the formula, and filtration techniques applied throughout various stages in the brewing and cellaring processes, the majority of beers have a protein content ranging from 1.0- 2.0 g, which is equivalent to approximately 2-4 % of the Daily Value based on a 2,000 calorie diet (MillerCoors, 2011). Again, in moderation (two 12 oz servings), beer is not deleterious to one's health, and can even offer some health benefits.

Table 1. Average B-Vitamin and Mineral Content in Beer (Witheridge, 2004)

	Per half liter serving		**Per 12 oz serving**	
Vitamin/Mineral	**Amount**	**Europe % DV**	**Amount**	**U.S. % DV**
Cobalamin (B12)	.9 μg	50	.64 μg	27
Riboflavin (B2)	150 μg	8	107 μg	9
Pyridoxine (B6)	150 μg	8	107 μg	8
Niacin	1.5 mg	7	1.1 mg	7
Folate (B9)	20-60 μg	5-22	14-43 μg	4-11
Magnesium	50 mg	12	36 mg	10
Potassium	200 mg	12	142 mg	3

In continuation, all of these aforementioned historic hurdles and perhaps misguided beliefs briefly outline the struggle that beer development has undergone over the years throughout American history; however, although these laws have impeded said development, beer, and furthermore craft beer, has proved to be a mainstay in modern society once again.

In 2009, the Beer, Cider, and Flavored Alcoholic Beverage (FAB) segment accounted for 34.5% of the global beverage share (Datamonitor, 2010). Also in 2009, the United States was ranked thirteenth in the world for per-capita beer consumption, at roughly 225-twelve ounce bottles of beer consumed per person of the legal drinking age, with a global market share of 13.8% (Table 2) (Kirin Holdings Company, LTD, 2010). In the United States, the craft beer industry grew 11% in production volume in 2010 (Brewer's Association, 2011), and has a combined projected annual growth rate (CAGR) of 7.9% (Cioletti, 2011). According to the Brewer's Association, craft beer can be defined as any beer produced by either:

> [a) a] microbrewery: A brewery that produces less than 15,000 barrels (bbl) (17,600 hectoliters) of beer per year with 75% or more of its beer sold off site; [b) a] brewpub: A restaurant-brewery that sells 25% or more of its beer on site; [c) a] contract brewing company: A business that hires another brewery to produce its beer. It can also be a brewery that hires another brewery to produce additional beer; [or d) a] regional craft brewery: an independent regional brewery [producing 15,000- 6,000,000 bbl] who has either an all malt flagship or has at least 50% of its volume in either all malt beers or in beers which use adjuncts to enhance rather than lighten flavor. (2011)

Table 2. Leading Countries in Global Beer Consumption in 2009

2009 Ranking	Country	Volume Consumed (thousand kiloliters)	% Share in Global Market
1	China	42,194	23.8
2	United States	24,513	13.8
3	Brazil	10,489	5.9
4	Russia	10,005	5.6
5	Germany	8,985	5.1
6	Mexico	6,406	3.6
7	Japan	5,982	3.4
8	United Kingdom	4,682	2.6
9	Spain	3,320	1.9
10	Poland	3,225	1.8

It is clear to see that beer is gaining back the respect, and demand, it once held some thousands of years ago…intertwining people across different social classes, and, when drank in moderation, a refreshment of health. However, this begs the question, is it possible to expand this market, reaching out to more consumers, and perhaps shift the paradigm once again, but for the better? Perhaps it could; there is the potential for much money to be made with the prospective, yet untapped product category of Nutraceutical Beer, a beer that may not merely offer some health, but promote it in some capacity.

Nutraceutical Beer

While the term "nutraceutical" itself does supply some ambiguity, DeFelice, founder and chairman of the Foundation for Innovation in Medicine (FIM), describes it as "[…] any

substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease" (DeFelice, 2011). Withstanding this, a nutraceutical beer can be described as any substance that is part of a beer or beer-like product, which is innate or supplied from a food or part of a food, and provides medical or health benefits, including the prevention and treatment of a disease. In continuation, it is easy to see how this product category could become diverse in itself, manifesting itself in several variations of the theme; however, the particular product of focus here is the development of a nutraceutical beer via biological isolates of soy kefir. While this product, and product category, seems interesting and decently innovative, is it a viable or necessary creation? To be able to answer this concern, the situation must be examined further.

Reasons for Product Development

There are a few main reasons for new product development: a) there is a trend for this particular product or product category or potential for a large market share with this product/product category; or b) it fills or has the potential to fill a consumer need. In the current market, the functional beverage category[2] holds quite a presence, at approximately 9 billion dollars in sales in 2009, and is expected to steadily grow over the next few years with an average CAGR of 3.5% (Figure 1) (Mintel, 2009). In addition, the Global Nutraceutical Beverage market is expected to grow to 71.3 billion dollars by 2013, a nearly 40% increase from 2008, where it was at 42.8 billion dollars (Roberts, 2009). Withstanding this, coupled with the previously mentioned information of craft beer consumption and growth, it is easy to see how the category of nutraceutical beer would have the potential for a large market share.

[2] A functional beverage can be defined as a drink that has been enhanced with added ingredients to provide specific health benefits beyond general nutrition (Palmer, 2008).

However, would this particular product fill, or have the potential to fill, a consumer need?

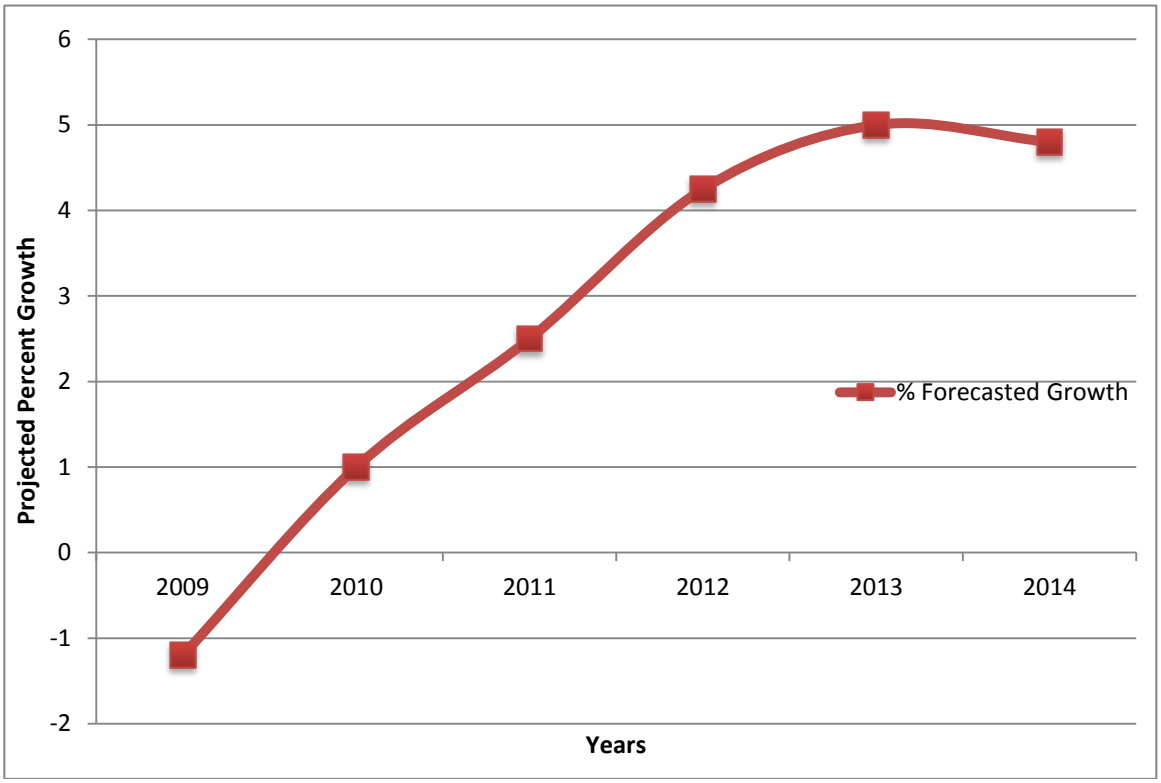


Figure 1. Projected US Functional Beverage Growth Over Five Years (Mintel, 2009)

According to the 2010 U.S. Census, the "Baby Boomer" generation accounts for 26.5% of the US population with ages ranging from 46-64 (Howden and Meyer, 2011). Furthermore, this population accounts for 26.5% of the consumers in the US- consumers who are beginning to feel the physiological effects and characteristics of getting older…pain, inflammation, and fatigue, for example. Due to the addition of the biological isolates of soy kefir, or Soy Kefir Powder (SKP), this product can help to fill this need.

Health Benefits of Soy Kefir and Soy Kefir Powder

Kefir dates back a few centuries to the Caucasus Mountains in Russia. These people produced kefir by daily pouring milk into leather satchels. They placed these bags either on horseback, or dangling in doorways. As people came and went, they would knock the bag- allowing for agitation, and the same logic applied to the jostling bags via horseback (Farnworth, 2003). Then, it was a natural fermentation, but today, the process is instigated from a starter culture known as kefir grains. The kefir grain is an extremely microbiological-dense, globular package (resembling a cauliflower floret) encased in a polysaccharide "mucus," known as kefiran (Figure 2).

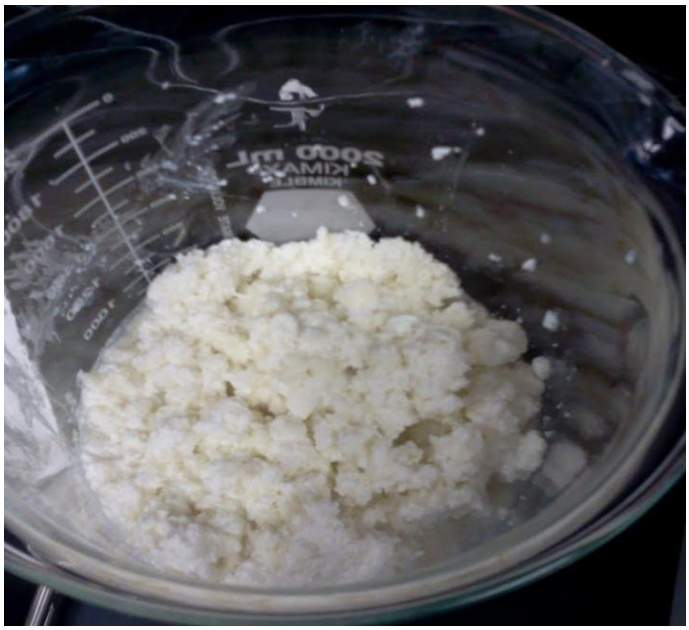


Figure 2. Kefir Grains

The kefiran is produced by the Lactobacilli from the grain (Lin and Liu, 2000), and has been proposed to have a branched hexa- or heptasaccharide repeating unit structure of D-glucose

and D-galactose in a 1:1 ratio (Farnworth, 2005). One of the interesting facts about kefir is that it is a result of both an ethanol and lactic acid fermentation- meaning it contains both lactic acid bacteria and yeast strains, which can be broken into five main sections of species: Lactobacilli/coccus, Leuconostoc, Streptococci, acetic acid bacteria, and yeasts.

Table 3. A List of Bacteria and Yeasts Found in Kefir Grains (Farnworth, 2005)

Lactobacilli spp.		**Streptococci**	**Leuconostocs**	**Acetic Acid Bacteria**	**Yeasts**	
Lactobacilli	**Lactococci**	*S. thermophilus*	*Ln. mesenteroides*	*Acetobacter aceti*	**Lactose-fermenting**	**Non lactose-fermenting**
Heteroferm. *L. kefir*	*L. lactis ssp lactis*	*S. lactis*	*Ln. sp.*	*Acetobacter rancens*	*Candida kefir*	*Saccharomyces cerevisiae*
L. brevis	*L. cremoris*				*Candida pseudotropicalis*	*Saccharomyces unisporus*
L. parakefir	*L. lactis ssp diacetylactis*				*Kluyveromyces marxianus*	*Saccharomyces exiguus*
L. hilgardii					*Kluyveromyces lactis*	*Saccharomyces turicensis*
L. fermentum					*Torula kefir*	*Saccharomyces delbrueckii*
L. viridescens						*Saccharomyces dairensis*
L. fructivorans						*Pichia fermentans*
Homoferm. *L. acidophilus*						*Candida friedichii*
L. delbrueckii						*Candida tenuis*
L. rhamnosus						*Candida lambica*
L. casei						*Candida maris*
L. paracasei						*Candida valida*
L. helveticus						
L. kefirgranium						
L kefiranofaceins						
L. plantarum						

Withstanding this information, it is known that the kefir fermentation process has three major metabolic pathways: homofermentative pathway, heterofermentative pathway, and non-lactose fermenting yeast pathway. The typical process of kefir making consists of pasteurizing milk, and then cooling this milk to 22 °C. Once at this temperature, the milk is then inoculated with the kefir grains and fermented for approximately 24 hours at a temperature of 22-25 °C. Soymilk kefir, however, is slightly different, but is exactly as it sounds- kefir grains inoculated into soymilk versus cow's milk. The soy kefir fermentation process is altered slightly, but similar production is found because of the microorganisms' ability to use the sugars found in soymilk- sucrose, raffinose, and stachyose (Lin and Liu, 2000). The Soymilk kefir is superior to its cow's milk counterpart due to its anti-inflammatory properties, anti-carcinogenic, and heart disease suppressant capabilities (Lin et al, 2002). The soy kefir fermentation process, although, may take slightly longer, but the addition of other fermentables- such as glucose and lactose- may be added to aid in growth and production of biochemical end-products, and the end-products are the focus here. SKP is a powder created by spray-drying the soy kefir liquid after fermentation and maturation has been completed- thus concentrating these end-products. Some main end-products in soy kefir are isoflavones.

Soy Isoflavones. Isoflavones are phytochemicals derived from soybeans, which are categorized into two main categories- glycosides, and their conjugates, alglycones. Kubow and Sheppard (2009) note that isoflavones possess anti-hypertensive, anti-inflammatory, and anti-oxidative properties. The isoflavones in native form are the glycosides, for example genistin, daidzin, and glycetin; however, in this native form, they are biologically inactive due to them being bound to a glucose molecule. During fermentation, the probiotic bacteria,

found within the kefir grains, hydrolyze the glycosides to transform them into their active alglycone counterparts, for example, genistein, daidzein, and glycetein, respectively. This is made possible widely due to their possession of the β-glucosidase, β-galactosidase, and α-galactosidase enzymes (Tochikura et al., 1986). Kubow and Sheppard (2009) found a three- to four-fold difference in genistein and daidzein content in the SKP in comparison to its content in unfermented soymilk. They also found that "soy kefir powder of the present invention has beneficial effects on chronic pain. Patients suffering from pain that are not adequately treated by conventional medicine, such as fibromyalgia, may be a good population" (Kubow and Sheppard, 2009). In addition, it was reported by Campbell et al (2002) that the alglycones, genistein and daidzein, demonstrated a decrease in neuropathic pain in rats. They "identified soy as a novel dietary ingredient that markedly suppresses allodynia and hyperalgesia in a model of neuropathic pain produced in rodents after partial sciatic nerve injury" (Campbell et al, 2002). In another isoflavone study, Breitkopf et al (2001) found that in hypertensive rats, genistein showed anti-hypertensive activity.

Branched Chain Amino Acids. Angiotenisin Converting Enzyme (ACE) inhibitory peptides are also one of the main end-products of kefir fermentation, and Kubow and Sheppard (2009) report that fermentation allows for greater digestibility and adsorption of these beneficial peptides. "ACE plays an important role in the renin-angiotensin system (RAS), which regulates both arterial blood pressure and the salt/water balance. […] inhibitors of ACE have been shown to lower blood pressure in hypertensive animals and human beings" (Ding and Wu, 2001). In continuation, Ding and Wu (2001) found that "ACE inhibitory peptides derived from soy protein had a significant hypotensive effect on SHR [(sponta-

neously hypertensive rats), … and since] soy ACE inhibitory peptides have a low molecular weight, they could be dissolved easily in different solutions or added to other food types for functional food components" - such as beer. The peptides and isoflavones are two major benefits of soy kefir, thus helping to combat pain, fatigue, and inflammation; however, there are other potential benefits of soy kefir.

Other potential health benefits of soy kefir consumption. One substantial benefit of soy kefir, and kefir in general, is its probiotic effect. A probiotic can be considered as such if it contains 10^8 cfu/ml of probiotic bacteria. Kefir as a probiotic can be defined as "a live microbial food that, when ingested, exerts a positive influence on the health or physiology of the host" (Aranguren, et al, 2007). Probiotics supply our bodies with a whole host of benefits. Population of healthy gut flora, and consequently, gastrointestinal health, is one benefit. As these helpful microorganisms populate the intestinal tract, they take-up "real estate," therefore not allowing harmful bacteria to attach to the intestinal lining. In addition, kefir is easily digested- because much of the milk proteins and sugars are broken down into smaller compounds by the grain biomass prior to ingestion- and, aids in digestion; digestion is aided by the microorganisms themselves, as well as in the case of lactose-intolerant individuals (with cow's milk kefir), their digestion is aided by the β-galactosidase produced by the Lactobacilli, and furthermore, allowing for a full breakdown of lactose. Kefir is also shown to stimulate the immune system by means of bio-active peptides that are produced during fermentation, as well as the exopolysaccharides- such as kefiran- that are being produced (Farnsworth, 2005).

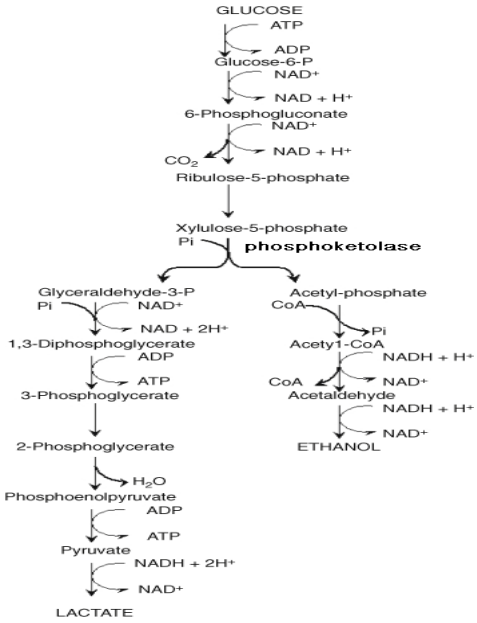
Furthermore, it is easily understood why a nutraceutical beer via biological isolates of

soy kefir would be a very viable product in the current market; there is a trend in the market for nutraceutical beverages, and this particular nutraceutical beverage would fill, or have the potential to fill a consumer need, more specifically, the needs of 26.5% of the US consumers- "baby boomers," by offering added health benefits such as relief from pain, fatigue, hypertension, and inflammation. While this product may appear to have the potential to be a supermarket sell-out, one major factor, or product attribute, has been overlooked thus far..."taste." Any product could be purchased once, but it is when consumers subject the product to repeat purchases, is what signifies as a successful product. In continuation, one main driver of repeat purchases is sensorial perception- did the consumer like it and did he/she accept its sensory profile. The two main components of this product- soy kefir and beer- should be examined further to gain insight into its possible sensory profile. How are the two similar, and how do they differ?

Sensorial Characteristics of Soy Kefir

As mentioned previously, the typical process of kefir making consists of inoculating milk with the kefir grains and fermenting this mixture for approximately 24 hours at a temperature of 22-25 °C, and that it is both an ethanol and lactic acid fermentation. Fermentation time, however, varies, but the main concept to take away from the process is that the longer the fermentation time, and the more kefir grains inoculated, the more acidic/sour and effervescent the product is going to be. During the first 24 hours, the pH decreases markedly by 2.5 units from approximately 6.68 to 4.24; the cause of this drop in pH is a result of lactose consumption, or glucose consumption in the case of soy kefir, and consequently lactic acid, and other organic acid, production (Lactobacilli favor this pH). As acid accumulates,

this allows for coagulation of the milk, which helps give kefir its viscous nature. After 24 hours of fermentation, lactic acid production still occurs, but is slowed. The Leuconostocs are present in much lower levels than the other lactic acid bacteria, in general, and as pH drops. These "heterofermentative lactic acid bacteria [Leuconostoc mesenteroides and others] produce aromatics, can degrade lactose to lactic acid, acetic acid, ethanol and carbon dioxide, and degrade citric acid into diacetyl, endowing good flavor" (Dong et al, 2006) (Figures 3 and 4). The combination of these organisms results in the niche (and slightly jarring) flavor of kefir- including volatiles such as acetaldehyde and diacetyl (Bodine et. al., 2000). These compounds are perceived by the palate as green apple/fruity and butter respectively (Greene et al, 2000).



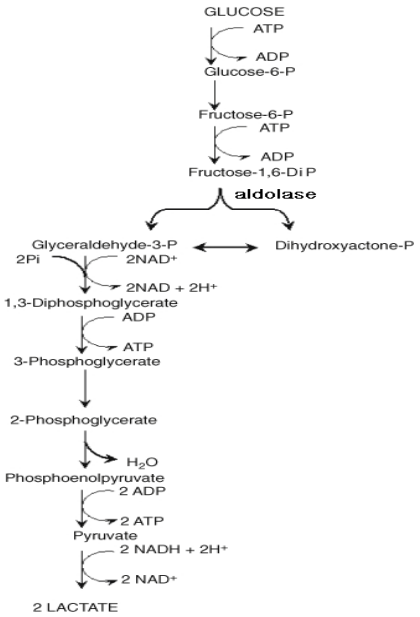


Figure 3. and Figure 4. Heterofermentative and Homofermentative Metabolic Pathways (Dong et al, 2006)

The flavor profile, however, can be different with a shorter fermentation time, but these are the typical compounds detected for an average fermentation time of 24 hours or more. Yeast growth also contributes to acid production, as well as other sensorally perceived attributes.

Yeast growth and ethanol production, however, is not significant until later in the fermentation process (Carballo et. al., 2005); despite its delay, ethanol can be produced up to 1.0% by volume (Cole and Marshall, 1985), and carbon dioxide can be present in levels around 3.0% (Assadi, et al, 2000), thus making a effervescent and slightly alcoholic product. The reasoning behind the slow reaction rate may be partly due to the yeasts locale in the middle of the kefir grain (Farnworth, 2003). This centralization of the yeasts delays contact with the milk (note: microorganisms dispense into the milk as it is fermented). It may also be due to the non-lactose fermenting yeasts waiting for the bacteria to break down lactose into glucose and galactose via their β-galactosidase activity. The yeasts (Figure 5) are then able to ferment these sugars and produce ethanol, carbon dioxide, and other important metabolites and growth nutrients for the lactic acid bacteria, such as amino acids and vitamins, as well as other flavor contributions (Păucean and Socaciu, 2008). The two microbiological groups here act in symbiosis- meaning they, (the bacteria and yeasts) rely on each other for survival and/or growth.

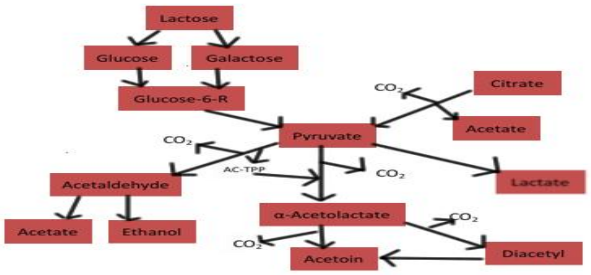


Figure 5. Yeast metabolism in kefir (Păucean and Socaciu, 2008)

Withstanding this, it can be inferred that these said reactions are quicker, and that the respective fermentations are perhaps "sped-up" in the soymilk kefir because there is not any lactose within the inoculating matrix, and furthermore, the fermentation is fueled by glucose. Therefore, within a twenty-four hour period, more organic acids and volatiles could be produced. In addition, other aromatics are present within the soy kefir matrix that are not present in the cow's milk version, such as grassy and beany notes; this is due to these notes being present in the soybeans themselves, which are carried through into the final product. While there are a whole host of microorganisms that are a part of the kefir fermentation, there is only one main microorganism (a yeast) that ferments wort (the inoculating matrix that becomes beer) in ale[3] fermentation- *Saccharomyces cerevisiae*. While this yeast is a part of kefir fermentation, it plays a very small role in the supporting cast, and the flavor profile of an ale, is quite different than soy kefir because of this fact, as well as many other contributing factors.

Sensorial Characteristics of Beer

The truth is, however, that the category of ale is really quite diverse in itself. According to the German Purity Law, beer should only consist of these three things (excluding the obvious- water): grain, hops, and yeast. This regulation seems rather limiting to an outsider, but within these seemingly limited items, numerous subcategories of ales can be created that all impart vastly different flavor profiles. In other words, all three of these ingredients contain many variables, change any one of these variables, and a product with a different flavor profile and sensory experience is created. The Brewer's Association recognizes approx-

[3] There are two main categories of beer: lager and ale. The product in research will be of the ale category; therefore, only ale production will be examined and discussed.

imately fifty different ale styles, which can be broken down into seven main categories: 1) Blonde/Pale/Golden, 2) Amber/Red, 3) Brown, 4) Wheat/Weizen, 5) IPA, 6) Stout, and 7) Porter. Each one of the previously mentioned ingredients either contributes to the differentiation of these categories, or allows for an inter-categorical differentiation. For example, all of the categories except IPA are defined by the grain variable, whereas IPA is defined by hops. To further understand the flavor differentiations, each variable should be examined. Every formula is usually comprised of a grain bill of approximately 80% base malt, which is a very light colored malted barley that basically just supplies the matrix with fermentable substrate, glucose and maltose, and not much flavor. The other 20%+/- of the grain bill is what helps to define much of its flavor. This malt contributes attributes such as caramel, honey, roasted, biscuit-like, molasses, toasted, hay-like, acrid, chocolate, coffee-like, wheaty, corny, burnt, and just simply, malty. The next categorical ingredient, hops (*Humulus lupulus L.*), plays an integral role in the sensory profile as well.

Hops can be defined into three main subcategories: bittering hops, aromatic hops, and multi-purpose hops. Bittering hops are dubbed as such due to their high concentration of alpha acids (8% plus) - humulone, cohumulone, lupulone, and colupulone (Goldstein and Ting, 1996), which largely contributes to the bitterness in the final beer. Bitterness can be quantified in terms of International Bitterness Units (IBU), which is defined as mg of isomerized alpha acids per liter of beer. Aromatic hops are characterized as such because they contain a fair amount of hop volatiles/essential hop oils, and a modest amount of alpha acids. These aromatic chemicals can be broken into a few main groups: monoterpene hydrocarbons, ses-

quiterpene hydrocarbons, oxygenated sequiterpenoids, oxygenated monoterpenoids, aliphatic ketones, aliphatic alcohols, carboxylic esters, carboxylic acids, and aldehydes (Nance and Setzer, 2011). As the name suggests, multi-purpose hops can be used for their bittering capabilities, or for their aromatics, and they usually contain a tempered amount of both alpha acids and hop volatiles. Hops are typically added into the brewing process in the kettle where the wort is boiled for sixty minutes, or more, to sterilize it; several factors come in to play here, which ultimately shapes the intended beer's flavor profile. These variables include alpha acid %, amount of hops used, if the hops are of the whole or pellet variety (a pellet hop is a ground and concentrated form of the whole hop version, once added to the boiling wort, the pellet breaks open, dispersing hop particles throughout the matrix- thus allowing for more surface area and better extraction of alpha acids and hop volatiles), type or particular cultivar of hop utilized, boil time/contact time for each hop, and even the design of the brew kettle. As mentioned previously, bittering hops tend to be categorized as such due to their higher concentrations of alpha acids; however, the alpha acids themselves are actually not bitter. During the boil, these acids are isomerized as heat is applied; this reaction produces iso-alpha acids, and these isomerized alpha acids are the causative agents behind bitterness and the IBU[4]. These bittering hops are typically added at the beginning of the boil (sixty minutes plus) to not only allow for a full isomerization of the alpha acids, but to allow for ample contact time to ensure a full dispersion of the iso-alpha acids throughout the wort. Hops can be added in various stages throughout the boil to create various levels of bitterness, but the true

[4] IBU can be mathematically defined as: IBU= (W)(AA%)(U%)(7489)/ (V)(GC); where W= weight of hops in ounces, AA%= alpha acid percent as a decimal, U%= utilization percent as a decimal, which is based off boil time and whether the hop is of the whole or pellet variety, V= boil volume in gallons, and GC= gravity correction factor

aroma hops are not added until the last fifteen minutes of the boil, or less. Volatile and aromatic compounds are heat sensitive, therefore, if these aromatic hops are added too early (more than a fifteen minute boil time), then these important chemicals can be destroyed, and furthermore, not contributing a hoppy flavor, but just a bitter taste. Withstanding this, hops can play a very large role in beer flavor, which includes bitter taste, ranging from an IBU, for example, of 3 in a sour weizen or weisse, to a value of 120 in an imperial IPA, and, hoppy aromatics. These aromatics include earthy, herbal, woody, or spicy aromas- contributed predominately by the sesquiterpene hydrocarbons, fruity or citrusy- delivered via monoterpenoids, floral- contributed by the geranyl esters (Nance and Setzer, 2011), and many more including grapefruit, piney, lemon, rose-like, green, vegetal, and in the case of old or temperature abused hops, butyric/isovaleric (rancid or cheesy) or oxidized/papery (Meilgaard, 1982). While the malt and hop ingredient categories greatly influence the flavor profile in ales, yeast, *Saccharomyces cerevisiae*, plays a substantial role as well.

This yeast is really the only ingredient that soy kefir and ale fermentations share (besides glucose), and while it may produce similarities within the different matrices, differences exist as well. First, since beer is a yeast fermentation, it also produces ethanol and carbon dioxide, however in much larger quantities; on average, most ales have an ABV of around 5.0%. Both of these by-products allow for a competitive edge by inhibiting growth and/or killing competing bacteria that are ethanol intolerant, and by also allowing for an anaerobic environment. Much larger quantities are produced because all of the substrate is consumed by the *Saccharomyces cerevisiae*, as opposed to a slew of other microorganisms, and there is

more available substrate- glucose- to begin with. In addition, in kefir fermentation, bacteria and yeast have a symbiotic relationship and in beer fermentation they do not- only *Saccharomyces cerevisiae* should be a part of the process- in other words, the yeast does not want to kill bacteria in kefir fermentation, and *Saccharomyces cerevisiae* does want to kill/limit growth of bacteria and wild yeasts in beer fermentation. As mentioned previously, the average ABV is 5.0%, however, varying concentrations of ethanol are produced throughout ale fermentations, for example, from 3.0% to 15.0%, which is inoculated with very ethanol tolerant strains of this yeast; however, at high concentrations, this affects the flavor profile as well, such as imparting an "alcoholic" flavor component. With ethanol production, other higher alcohol volatiles are produced, which impart flavors such as solvent-like or fruity (ethyl acetate) and banana (isoamyl acetate) (Kobayashi et al, 2008). Ethyl esters are considered to be the most abundant here because they utilize ethanol as a substrate (Boulton and Quain, 2006). In addition to ethanol and carbon dioxide, both fermentations also include some acid production. An average beer typically starts its fermentation with a pH of about 5.2.

During fermentation, a small amount of different organic acids are produced, such as acetic (10-50 ppm), pyruvic (100-200 ppm), citric (100-150 ppm), malic (30-50 ppm), succinic (50-150 ppm), and lactic (50-300 ppm) (Boulton and Quain, 2006) which contributes to yet another "hurdle" for competing microorganisms, and over the course of the fermentation, the liquid drops one whole pH unit to about 4.2. This is, however, very similar to the pH of the kefir. Although, all of these organic acids contribute differently to an overall sen-

sory perception of sourness, and can also contribute to other flavor sensations such as salty or bitter, in the case of succinic (Boulton and Quain, 2006), or vinegary/pungent with acetic acid (Meilgaard, 1982). Pungent acidity, especially a noticeable lactic note, in beer is not desired typically and would be noted as an off-flavor; this note would often be indicative of bacterial contamination, with lactic acid bacteria as the main culprit, which as noted, is one of the main microorganisms in kefir. Another parameter that affects the ale flavor profile is fermentation temperature, which can range from 56 °F, with a Kölsch ale yeast, to 95 °F, with a Belgian Saison yeast (Wyeast Laboratories, 2011). Most of the aromatics that are affected by temperature are esters and higher alcohols, which are produced more at higher fermentation temperatures (Boulton and Quain, 2006). Again, these compounds can affect the flavor profile of beer by creating floral (rose), fruity (banana, pear, pineapple, etc), solvent-like, and honey-like notes. Other yeast metabolites that affect ale flavor are flavor-active[5] phenolic compounds, which include vanillin, eugenol, 4-vinylguaiacol and 4-vinylphenol; these compounds create vanilla, smokey, or clove-like aromatics. Delvaux et al (2008) reports that these compounds are produced through yeast metabolism "by enzymatic decarboxylation during fermentation, by phenylacrylic acid decarboxylase activity of top-fermenting yeasts strains (Pad1 enzyme)." Other flavor-active phenolics may be present in beer originating from other sources that may intensify these flavors, resulting in a medicinal, or "band-aid" note. These notes may be present due to the accidental addition of residual sanitizer, moreover, chlorine based chemicals leading to the formation of chlorophenol, or through "contaminating micro-organisms, like Enterobacteriaceae, lactic acid bacteria, acetic acid bacteria and

[5] Other phenolic compounds exist throughout the brewing process, and ultimately, in beer, however, many phenolic compounds are not of the flavor-active variety.

some wild yeasts, like Brettanomyces/Dekkera spp" (Delvaux et al, 2008). Different strains of yeast also effect end-product flavor profile, most likely due to the presence of different genes/gene expression or different levels of enzymatic activity. For example, Wyeast Yeast Strain 1010, American Wheat, is "ideal for beers when a low ester profile is desirable," whereas its 3638 strain, Bavarian Wheat, "produces apple, pear, and plum esters in addition to the dominant banana character. The esters are complemented nicely by clove and subtle vanilla phenolics" (Wyeast Laboratories, 2011). Another gene-linked flavor-active yeast metabolite is dimethyl sulfide (DMS), which imparts a cooked corn flavor in beer. This aromatic, however, is not ideal or favored typically in beer. Its precursor, dimethyl sulfoxide (DMSO), is attributed by the malted barley, and much of it is driven-off during the "whirlpool" in the kettle after boiling has subsided; however, if DMSO is still present within the matrix after this step, it is converted to DMS downstream, which is an act of the MXR1 gene.

> Saccharomyces yeasts contain an enzymatic activity that reduces DMSO to DMS in an NADPH-dependent manner, and a so-called MetSO (methionine sulfoxide) reductase isolated from yeast was suggested to be identical to the DMSO reductase (Bech et al, 2002)

Other flavor-active compounds manufactured by the yeast that are not always desired in beer are some carbonyls (mainly acetaldehyde), sulfur containing compounds (hydrogen sulfide, sulfur dioxide, and mercaptans), and vicinal diketones (VDK), 2,3-butanedione (also known as diacetyl) and 2,3-pentanedione.

> Although a small amount of acetaldehyde may be desired in some styles of ale, im-

parting a fresh apple/green apple flavor, it is not largely desired across the spectrum of ales, and definitely not in large amounts. Acetaldehyde is an intermediate metabolite, therefore higher concentrations of it in finished beer is indicative of unhealthy, dead, or dormant yeast, or yeast which has just flocculated out of the beer matrix, which in turn resulted in a poor and insufficient conversion of this compound due to slowed/reduced activity of acetaldehyde and alcohol dehydrogenase enzymatic activity (Boulton and Quain, 2006). According to the Brewing Judge Certification Program (BJCP) Style Guidelines, some ale styles are expected to have, or accepted with, some slight sulfury notes, such as a Düsseldorf Altbier, Kölsch, or blonde ale (Bach et al, 2008); however, sulfur notes are deemed inappropriate in most ale styles. In beer, these flavor-active compounds- hydrogen sulfide and sulfur dioxide- create notes such as burnt rubber, rotten-egg, mineral-like, yeasty, and meaty. Boulton and Quain (2006) state that these sulfur-containing components are created from two paths:

> First, from the dissimilation of complex organic molecules such as sulphur-containing amino acids and vitamins and, second, from assimilatory reactions involving inorganic sulphur-containing nutrients (2006).

These compounds are the most present in green beer, however, as the beer conditions, or matures, these sulfuric notes dissipate widely due to "hydrogen sulphide escaping from the top of the fermentor [or blow off arm/tube] as a gas" (White, 2011). Another reason for their dissipation is due to the formation of "reversible adducts with carbonyl compounds […] which can stabilize beer flavor by binding compounds associated with beer flavor staling such as acetaldehyde and trans-2-nonenal" (which gives off a cardboard or papery note) (Boulton and

Quain, 2006). Other offensive carbonyls in beer are the VDK. Both of these two compounds, diacetyl and 2,3-pentanedione, give off buttery/butterscotch notes, which are widely undesired in beer across the board, or in very minute amounts. The VDK are excreted into the beer, however, they can be converted into alcohols upon uptake back into the cell. It is industry practice to utilize a procedure known as a "diacetyl-rest" to ensure minimal existence of these undesirable compounds in the final product, which includes increasing the temperature of the fermenting vessel to about 75-80 °F (for ales) for a period of 24-48 hours after primary fermentation has finished. This allows for the yeast to become re-activated, in a sense, in order to uptake the VDK and promote activity that will allow for the conversion, as well as to allow for all potential VDK to be produced ensuring it will not be present in the final product if more is produced downstream. The formation and dissimilation of these compounds can be viewed in Figure 6 (Boulton and Quain, 2006).

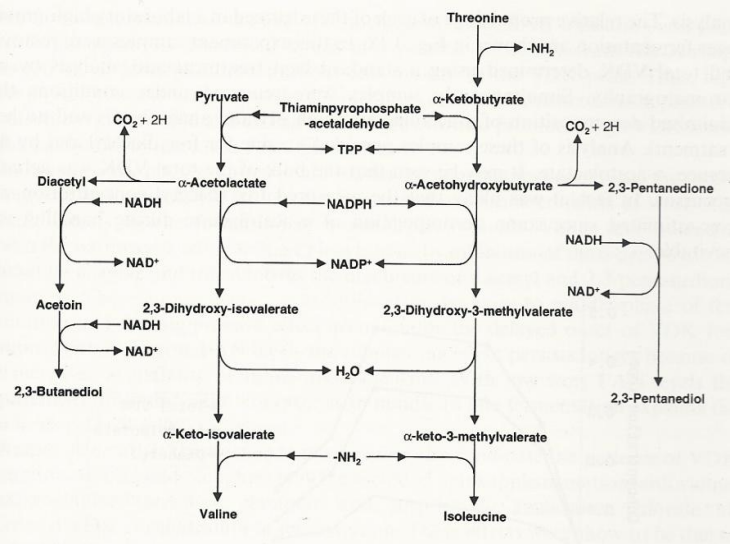


Figure 6. Metabolic Pathways for the Formation and Dissimilation of VDK in *Saccharomyces cerevisiae* (Boulton and Quain, 2006)

Once converted into 2,3-butanediol, the beer does not have an undesirable buttery note. Thus, the flavor profile of ales is a very complex and extensive list, which is heavily influenced by these three ingredient categories: grain, hops, and yeast. Each one of the categorical variables can affect the flavor of beer positively, but also can cause off-flavors as well.

Conclusion

In conclusion, a nutraceutical beer via biological isolates of soy kefir has the potential to be a very viable product in the current market. Beer has been a commodity amongst global society throughout its existence. In 2009, the Beer, Cider, and Flavored Alcoholic Beverage (FAB) segment accounted for 34.5% of the global beverage share (Datamonitor, 2010). Also in 2009, the United States was ranked thirteenth in the world for per-capita beer consumption, at roughly 225-twelve ounce bottles of beer consumed per person of the legal drinking age, with a global market share of 13.8% (Kirin Holdings Company, LTD, 2010). In the United States, the craft beer industry grew 11% in production volume in 2010 (Brewer's Association, 2011), and has a combined projected annual growth rate (CAGR) of 7.9% (Cioletti, 2011). In addition, the Global Nutraceutical Beverage market is expected to grow to 71.3 billion dollars by 2013, a nearly 40% increase from 2008, where it was at 42.8 billion dollars (Roberts, 2009). Furthermore, it is easy to see how the category of nutraceutical beer would have the potential for a large market share.

In addition, this product has the potential to fill a consumer need, moreover, the needs of 26.5% of the US consumers- "baby boomers." This target audience includes consumers with ages ranging from 46-64- consumers who are beginning to feel, or knee-deep in, the physiological factors and characteristics of getting older…pain, inflammation, and fatigue,

for example. Due to the addition of the biological isolates of soy kefir, or Soy Kefir Powder (SKP)- spray dried soy kefir liquid- this product can help to fill this need. Soy kefir can be beneficial mainly due to their possession of isoflavones and small peptides. Isoflavones, and mainly the alglycones, genistein and daidzein, demonstrated a decrease in neuropathic pain in rats (Campbell, 2002) and in hypertensive rats, genistein showed anti-hypertensive activity (Breitkopf et al, 2001). Angiotenisin Converting Enzyme (ACE) inhibitory peptides are also main end-products of kefir fermentation. They help to "regulate both arterial blood pressure and the salt/water balance. [… and] inhibitors of ACE have been shown to lower blood pressure in hypertensive animals and human beings" (Ding and Wu, 2001). In continuation, Ding and Wu found that "ACE inhibitory peptides derived from soy protein had a significant hypotensive effect on SHR [(spontaneously hypertensive rats)] (2001), and fermentation allows for greater digestibility and adsorption of these beneficial peptides (Kubow and Sheppard, 2009). Furthermore, it is easily understood why a nutraceutical beer via biological isolates of soy kefir would be a very viable product in the current market; there is a trend in the market for nutraceutical beverages, and this particular nutraceutical beverage would fill, or have the potential to fill a consumer need, more specifically, the needs of 26.5% of the US consumers- "baby boomers," by offering added health benefits such as relief from pain, fatigue, hypertension, and inflammation. However, one main driver of repeat purchases, and furthermore, product success, is sensorial perception- did the consumer like it and did he/she accept its sensory profile. While the flavor profiles of the two main components of this product, soy kefir and beer, may share a few similar attributes, such as ethanol and some fruitiness, most of their flavors or tastes that are considered desired in each product, differ.

These conflicting volatiles include acetaldehyde, diacetyl, high lactic acid notes, and beany/grassy notes. Furthermore, work needs to be done to be able to produce a quality, palate pleasing, and an acceptable nutraceutical beer, or beer-like beverage that will consist of a flavor profile most similar to ales- hence the research, "Product Development of a Nutraceutical Beer via Biological Isolates of Soy Kefir."

Product Development Stages

Product development includes a variety of stages, which can be broken down into several main steps: 1) Product conception, 2) Design Specifications, 3) Bench Development, which includes three stages that act in consort with one another- 3a) Open Bench-top/ Blind Guidance Testing, 3b) Product Optimization/ Experimental, and 3c) In-house Guidance Testing- which can consist of consumer tests or descriptive analysis (in this case it was via an in-house descriptive analysis panel)- 4) Shelf Life Testing, and 5) Process Development/ Scale-up. Marketing and market research is a large portion of product development as well, but this is not the focus in this particular product development research. This project followed these main stages as guidelines for the development of a nutraceutical beer.

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CHAPTER 2. MATERIALS AND METHODS

A. Sensory Analysis

a) Preliminary Bench Testing

A blind study was conducted within the laboratory setting, including nine panelists from within the lab who were all twenty-one years of age or older, and at least moderate consumers of beer (consumes at least once every other week, see Appendix). Six different treatments were analyzed representing the spectrum of ale styles: Stout, Amber/Red, Brown, Pale/Blonde/Golden, IPA, and Weizen/Wheat. All of the beer was purchased from a local specialty wine and beer shop. The commercial examples- Guinness Dry Stout (Dublin, Ireland), Samuel Smith Russian Imperial Stout (Tadcaster, North Yorkshire, England), New Belgium Fat Tire (Fort Collins, CO), Carolina Blonde Ale (Holly Spring, NC), Stone IPA (Escondido, CA), and Widmer Hefeweizen (Portland, OR)- were doctored with 10 g per 12 oz serving Soy Kefir Powder (SKP) obtained from KCLM Research, Montreal, Quebec, Canada. The beer was aseptically transferred into a beaker where the SKP was added and gently mixed to allow for homogeneity, but also to allow for minimal carbon dioxide release. The doctored beer was then again aseptically transferred back into their respective bottles. Three oz of each treatment were served in a lidded translucent plastic cup at a temperature of 11°C, the proper serving temperature of ales (BJCP, 2010). The treatments were served to each panelist in a different randomized order. The panelists rated each treatment hedonically on a 9-pt scale, 1 being extremely disliked, and 9 being extremely liked (see Appendix). Each panelist was supplied with water and unsalted crackers to clear their palate in between each sample to inhibit any carry-over effect, as well as an empty cup for expectoration of each

treatment.

A second preliminary bench study was conducted in the same manner, but six of the same panelists tested eight treatments with varying SKP concentrations: 5, 7.5, or 10 g per 12 oz of beer. Based upon the results of the first bench study, three ales were selected, Stone IPA (Escondidio, Ca), Wolander's Oatmeal Stout, and Kona Pipeline Porter (Kailua Kona, Hawaii), due to both the stout/porter and IPA categories being most accepted. The same test procedures were utilized.

b) In-house Guidance Testing/ Descriptive Analysis

Eight panelists were selected based upon them being at least 21 years of age, and the panel was considered an "in-house" panel because the majority of the panel was comprised of people from within the lab (other graduate students, laboratory technician, etc). Three sensory modalities of beer were trained upon, Beer Flavor (retronasal aroma and basic tastes), Orthonasal Aroma, and Beer Mouthfeel. For each training session[6] and test, 2-3 oz of beer were served in lidded translucent plastic cups at a serving temperature of 11°C. Each panelist was supplied with water and unsalted crackers to clear their palate in between each sample to inhibit any carry-over effect, as well as an empty cup for expectoration of each treatment. For orthonasal aroma analysis, each treatment was swirled releasing volatiles into the headspace of the cup, and while the lid was cracked just enough to stick a nose through, the panelists took small "bunny sniffs" of the treatment for accurate detection of the aromatics. Short sniffs allows for accurate detection because the olfactory bulb adjusts after approximately two seconds, therefore, a long sniff would exceed this adjustment period, and fur-

[6] This, and the following parameters, is considered the regular serving protocol to be performed on all descriptive analysis tests and training for the remainder of the research.

thermore, result in an inaccurate detection and a falsified orthonasal aroma profile. For flavor analysis, treatments were dispensed into the mouth cavity while the nose was pinched in, which closes off aromatic detection from the olfactory bulb, thus allowing for merely basic taste detection from ion channel (salty, sour) and G-protein taste receptors (sweet, bitter) that are tucked within different papillae (foliate, fungiform, and vallate) located on the tongue (Roper, 2007). Once basic taste was analyzed the panelist would un-pinch nose, releasing volatiles into nasal passage allowing for detection by the olfactory bulb. Sample was swirled within the mouth cavity while panelist breathed out of the nose allowing for optimal volatile circulation into the nasal cavity, and furthermore, an accurate retronasal aroma profile.

An extensive ale lexicon (see Appendix) was created for flavor (basic tastes + retronasal aroma), orthonasal aroma, and mouthfeel based upon the literature, but a frame of reference list was utilized to narrow down each one of these lexicons. The frame of reference (Table 4) was comprised of twenty-one different ales within the seven main ale categories: 1) Blonde/Pale/Golden, 2) Amber/Red, 3) Brown, 4) Wheat/Weizen/Weiss, 5) IPA, 6) Stout and 7) Porter. Examples from the frame of reference list were presented to the panel and were analyzed for each of the three modalities based upon their respective lexicons. Panelists at this point also had the option of writing down any additional attributes that they felt was present in any of the examples. The lexicon was reviewed as a panel, ensuring the definitions of each attribute was understood.

Table 4. Ale Frame of Reference List

Ale Category	Name	Sub-style	Brewing Company
Pale/Blonde/Golden	Carolina Blonde Ale	Blonde Ale	Carolina Brewing Co. (Holly Springs, NC)
Pale/Blonde/Golden	Sierra Nevada Pale Ale	Pale Ale	Sierra Nevada Brewing (Chico, CA)
Pale/Blonde/Golden	Magic Hat #9	Pale Ale	Magic Hat Brewing (South Burlington, VT)
Amber/Red	Fat Tire	Amber Ale	New Belgium Brewing (Fort Collins, CO)
Amber/Red	Killian's Irish Red	Irish Red	Coors Brewing Co. (Golden, CO)
Brown	Newcastle Brown Ale	English Brown Ale	Newcastle Brewery, Heineken Ltd (Tadcaster, North Yorkshire, UK)
Brown	Bad Penny	Brown Ale	Big Boss Brewing Co. (Raleigh, NC)
Wheat/Weizen/Weiss	Widmer Hefeweizen	Hefeweizen	Widmer Brothers (Portland, OR)
Wheat/Weizen/Weiss	Blue Moon Belgian White	Belgian White Wheat	Blue Moon Brewing Co (Golden, CO)
Wheat/Weizen/Weiss	Franziskaner Dunkel	Dunkelweizen	Franziskaner Brewery (Munich, Germany)
India Pale Ale	Stone IPA	IPA	Stone Brewing (Escondida, CA)
India Pale Ale	Redhook IPA	IPA	Redhook Brewing (Brooklyn, NYC, NY)
India Pale Ale	90 minute IPA	IPA	Dogfish Head Brewing (Milton, DE)
Stout	Guinness Extra Stout	Dry Stout	Guinness (Dublin, Ireland)
Stout	Samuel Smith Russian Imperial Stout	Russian Imperial Stout	Samuel Smith Brewery (Tadcaster, North Yorkshire, UK)
Stout	Samuel Adams Cream Stout	Cream/Milk Stout	Samuel Adams Brewing Co (Boston, MA)
Stout	Wolander's Oatmeal Stout	Oatmeal Stout	Wolander's Brewing
Stout	Barney Flats Oatmeal Stout	Oatmeal Stout	Anderson Valley Brewing Co (Boonville, CA)
Porter	Vanilla Java Porter	Porter	Atwater Block Brewery (Detroit, MI)
Porter	Sierra Nevada Porter	Porter	Sierra Nevada (Chico, CA)
Porter	Rogue Mocha Porter	Porter	Rogue Brewing Co (Ashland, OR)

Then, each panelist independently rated each attribute for appropriateness on a scale from 1-5, 1 being extremely inappropriate, 5 being extremely appropriate (see Appendix). Based upon these ratings and panel discussion/consensus, each lexicon was initially reduced from seventy-eight and eighty-two, orthonasal aroma and flavor respectively, to thirty-three and thirty-two, and for mouthfeel, from fifteen to nine. From these new lexicons, the references and examples for twenty-eight, for orthonasal aroma (Table 5), and twenty-two for flavor (Table 6), were prepared and presented to the panel for further narrowing down and training. The references and examples for all nine of the mouthfeel attributes (Table 7) were prepared and presented to the panel, but just for training.

Table 5. Orthonasal Aroma Ale Attributes for Further Consideration

Attribute	Chemical(s)- if applicable	Definition/description	Reference	Example(s)	Rate
Fruity Notes					
Citrus	many	Aromatic associated with the general impression of citrus fruits	Linalool (.3 mg/L beer)	1) Citrus fruits 2) Pledge furniture polish	
Fruity	Ethyl acetate	An aroma note associated with light fruity.	Ethyl acetate (100mg/L beer)	Fruit cocktail	
Isoamyl acetate	Isoamyl acetate	An aroma associated with banana.	Isoamyl acetate (10 mg/L beer)	1)Circus peanut candies 2) artificial banana flavoring	
Floral Notes					
Floral/ rose-like	2-phenylethanol, geraniol	A sweet aromatic associated with flowers; rose-like fragrance	Geraniol (500 µg/L beer)	1) Johnson's and Johnson's baby powder 2) carnation	
Spice Notes					
Spicy	Eugenol	An overall aroma term associated with pungent spices	Eugenol on perfumer's stick; 2 to 3 grains ground black pepper, 1 drop anise extract/50 ml wine	allspice	
Vanilla	vanillin	Aromatic blend of sweet, vanillin, woody, browned notes, sometimes having chocolate, tobacco, floral, or spicy components	Vanilla bean(Great Value™) in a glass jar	Vanilla extract (Great Value™)	
Sweet/syrup Notes					

Table 5. (continued) Orthonasal Aroma Ale Attributes for Further Consideration

Caramelized	many	The aromatic relating to the browning of starches and sugars.	3-hydroxy-2-methyl-4-pyrone (1 g/L beer)	1)Killian's Red Lager; Newcastle 2) Kraft's caramel candies	
Chocolate	Dimethyl pyrazine, methyl butanol	Aromatic associated with chocolate liquor, as found in roasted West Africa/Ivory Coast cocoa beans	Ivory Coast chocolate liquor	1) Lindt dark chocolate 2) Hershey's semi-sweet morsels	
Honey	many	The sweet, caramelized floral and woody aromatic associated with honey	Phenylacetic acid in sweetened water (10 ppm)	1) Clover honey	
Molasses		An aromatic associated with molasses; has a sharp, slight sulfur and/or caramelized character	Black Strap molasses (1-3 ml/25 ml beer)	1) Black Strap molasses 2) Karo Dark corn syrup	
Earthy Notes					
Piney	A-p-dimethylstyrene; β-pinene; bornyl benzoate; δ-terpinene; dihydroterpinyl acetate; α-pinene	Aromatic associated with dry, fresh cut pine wood or pine needles.	Fresh-cut pine needles in glass jar	1) Great Value rosemary 2) PineSol	
Woody	many	An aromatic associated with dry fresh cut wood; balsamic or bark-like	toothpicks	1) Bay leaves 2) Cedar, pine, popsicle sticks	
Roasted (or lack of) Notes					
Corn-like		An aroma associated with maize grits, adjuncty, canned sweet corn, etc.	Corn meal mush	1) Corn meal 2) Creamed corn	
Burnt/ Burnt toast	Octanol; indole**;** 3-methyl-1-butanol; ethyldimethylpyrazine; dimethyl sulfone; furfuryl alcohol	Aromatic associated with blackened/acrid carbohydrates; a burnt aroma note, like charred toast.	Black malt extract (20º C)	1) espresso coffee 2) Guinness 3) Burnt toast crust	
Coffee		An aroma note associated with coffee	Folger's Gourmet Supreme ground coffee (6-8 grains/25 ml red wine (Franzia table red))	Folger's Gourmet Supreme ground coffee	
Green	hexanals	The aromatic associated with unprocessed vegetation- such as grains, leaves, and grass.	Cis-3-hexen-1-ol (5 ppm in water)	1) green legumes 2) parsley	
Hay-like/straw		A grainy aromatic with some green character of air-dried grain or vegetation.	Hay (NCSU Veterinarian Medicine School)	1) hay/straw 2) dried parsley	
Malty		The aromatic reminiscent of toasted grain.	Light malt extract (American Brewmaster) (30 ml/L beer)	1) Whoppers malted milk balls halved 2) Grapenuts	
Nutty	many	Aromatic associated with nuts or nut meats	2, 6-dimethyl pyridine (2.0 ppm)	Wheat germ	
Roasted Barley		An aroma note associated with roasted barley used in the grist	Roasted Barley (American Brewmaster)	1) Guinness stout 2) same	

Table 5. (continued) Orthonasal Aroma Ale Attributes for Further Consideration

Off-putting/phenolic/sulfur Notes					
Catty		Aromatics associated with oxidized beer and cat urine.	p-Menthane-8-thiol-3-one	1) Tomato plant leaves 2) oxidized beer	
Diacetyl	Diacetyl	Aromatic associated with fermented dairy products and spoiled butter	Diacetyl (.2-.4 mg/L beer)	1) Kroger microwave buttered popcorn 2) butterscotch pudding 3) buttered popcorn Jelly Belly	
Iodoform/ Phenolic	Iodophors, phenols, chlorophenol, etc	A pharmaceutical/medicinal, hospital-like aroma- band-aid	4-methylphenol (7 mg/L beer)	1) Band-Aid bandages 2) Great Value™ regular chloraseptic sore throat spray	
Sulfur/ Mercaptan	DMS, H2S; Mercaptan	Aromatic associated with sulfur compounds (rotten egg, etc) that are reminiscent of skunk and rubber	Ethyl mercaptan (3 µg/L beer)	1) rotten eggs 2) struck match; 1) beer in a green bottle (Heineken) 2) balloons 3) coffee	
Others					
Hoppy	NA	An overall term given to fresh hop aroma.	Cascade pellets (American Brewmaster)	Same	
Solvent	Propanal, isobutanol, propyl butyrate, p-cymene, (E)-carveol	A general term used to describe the aromatics of many classes of solvents- may be reminiscent of chemical solvents, plasticizers, lighter fluid, or paint aroma notes	Acetone	1) isopropyl alcohol 2) paint 3) lighter fluid	
Yeasty	NA	Aromatics associated with fresh yeast and yeast fermentation	Active yeast (1 g yeast in 250 ml distilled water at room temperature- spike beer with 3ml)	Fresh baked yeast bread	

Table 6. Ale Flavor Attributes for Further Consideration

Attribute	Chemical(s)- if applicable	Definition/description	Reference	Example(s)	Rate
Basic Tastes					
Sweet	Sucrose, many	The taste stimulated by sucrose, and other sugars, sugar alcohols, and other sweet substances, such as Aspartame, etc.	5% sucrose in water- 5	1. Dextrose, glucose 2. Aspartame, fructose	
Sour	Acetic acid, acids	The taste stimulated by acids, such as citric, malic, and acetic.	.08% citric acid in water- 5	Lemon juice	
Bitter	many	The taste stimulated by quinine, caffeine, and hop bitters.	.08% solution of caffeine- 5	Folger's Gourmet Supreme black coffee	
Fruity Notes					

Table 6. (continued) Ale Flavor Attributes for Further Consideration

Citrus	many	Flavor associated with the general impression of citrus fruits	Linalool (.3 mg/L beer)	1) Citrus fruits 2) Pledge furniture polish (aroma only)	
Fruity	Ethyl acetate	A flavor note associated with light fruity.	10.648 ml syrup from fruit cocktail/ 12 oz MGD 64	Fruit cocktail (Great Value™)	
Isoamyl acetate	Isoamyl acetate	A flavor associated with banana.	Isoamyl acetate (10 mg/L beer)	1)Circus peanut candies 2) artificial banana flavoring	
Floral Notes					
Floral/ Rose-like	2-phenylethanol, geraniol	A sweet aroma/flavor associated with flowers/ rose-like fragrance	2 drops (20mg) Nielsen-Massey Vanillas, Inc. Rose Water/ 12 oz MGD 64	Nielsen-Massey Vanillas, Inc. Rose Water	
Spice Notes					
Spicy	many	An overall flavor term associated with pungent spices	20-25 grains ground black pepper, 1 drop anise extract/12 oz MGD 64	Allspice	
Vanilla	vanillin	Flavor note reminiscent of a blend of sweet, vanillin, woody, browned notes, sometimes having chocolate, tobacco, floral, or spicy components	3 drops (30 mg) vanilla extract/ 12 oz MGD 64	Vanilla extract (Great Value™)	
Sweet/syrup Notes					
Caramelized	many	The flavor relating to the browning of starches and sugars.	Killian's Red Lager	1)Newcastle 2) Kraft's caramel candies	
Chocolate	Dimethyl pyrazine, methyl butanol	Flavor associated with chocolate liquor, as found in roasted West Africa/Ivory Coast cocoa beans	10.648 ml Arrow chocolate liquor/ 12 oz MGD 64	1) Lindt dark chocolate 2) Hershey's semi-sweet morsels	
Honey	many	The sweet, caramelized floral and woody aromatic/flavor associated with honey	25.0 ml clover honey/12 oz MGD 64	1) Clover honey	
Molasses		A flavor note associated with molasses; has a sharp, slight sulfur and/or caramelized character	Black Strap molasses (3 ml/25 ml beer= 42.604 ml/ 12 oz)	1) Black Strap molasses 2) Karo Dark corn syrup	
Roasted (or lack of) Notes					
Burnt/ Burnt toast	Octanol; indole**;** 3-methyl-1-butanol; ethyldimethylpyrazine; dimethyl sulfone; furfuryl alcohol	Flavor associated with blackened/acrid carbohydrates; a burnt flavor note, like charred toast.	Black malt extract (American Brewmaster) (10.648 ml/12 oz MGD 64)	1) Guinness 2) Burnt toast crust	
Coffee		A flavor note associated with coffee	Folger's Gourmet Supreme ground coffee (20-25 grains/ 12 oz MGD 64)	same	

Table 6. (continued) Ale Flavor Attributes for Further Consideration

Hay-like/grassy	Hexanals	A grainy aromatic/flavor note with some green character of air-dried grain or vegetation.	.25 g dried parsley (grass)/ 12 oz MGD 64; cis-3-hexen-1-ol (5 ppm in water)	1) hay/straw 2) dried parsley	
Malty		The flavor reminiscent of toasted grain.	Malt extract (American Brewmaster) (30 ml/L beer= 10.648 ml/ 12 oz)	1) Whoppers malted milk balls halved 2) Grapenuts	
Roasted Barley		A flavor note associated with roasted barley used in the grist	3 g roasted barley/ 12 oz MGD 64 (American Brewmaster)	1) Guinness stout 2) same 3) wheat germ	
Others					
Piney	A-p-dimethylstyrene; β-pinene; bornyl benzoate; δ-terpinene; dihydroterpinyl acetate; α-pinene	Aromatic/flavor associated with dry, fresh cut pine wood and pine needles.	.50 g rosemary twig/12 oz MGD 64	1) rosemary 2) fresh cut pine needles 3) PineSol (aroma)	
Iodoform/ Phenolic	Iodophors, phenols, chlorophenol, etc	A flavor note associated with a pharmaceutical/ medicinal, hospital-like aroma- band-aid	1 spray of regular chloraseptic sore throat spray on palate (Great Value™)	1) Band-Aid bandages (aroma) 2) Great Value ™ regular chloraseptic sore throat spray	
Hoppy	NA	An overall term given to fresh hop aroma/flavor.	Cascade pellets (American Brewmaster) 1 g/12 oz MGD 64	same	
Yeasty	NA	Aromatics/flavor notes associated with fresh yeast and yeast fermentation	Active yeast (1 g yeast in 250 ml distilled water at room temperature-spike beer with 1 ml)	Fresh baked yeast bread	

Table 7. Ale Mouthfeel Lexicon

Attribute	Definition/description	Reference	Example(s)	Ref. Rating
Astringent	Puckering and constricting tactile sensation on the soft tissue on the mouth	1% Alum in water	Unripe banana; grape skins	15
Carbonation	Perceived amount of carbonation in the beer.	Soda water at 11-12°C	Diet Coke from bottle at 11-12 C	15
Velvety	Perceived impression of mouth-coating, softness, and fullness	Weyerbacher Old Heathen (Imperial Stout)	Coffeemate Fat Free Original Creamer at 10 C	11
Gritty mouthcoat	Feeling of minute, rough granules inside the mouth	Widmer Hefeweizen	1 T Great Value Applesauce	2

Table 7. (continued) Ale Mouthfeel Lexicon

Body	Perceived amount of depth inside the mouth.	Duck Rabbit Milk Stout	Reddi-whip aerosol whipped cream (1 oz)- 1.0	10
Smooth	Perceived absence of all particles	Filtered water at 22°C	MGD 64	15
Prickly	Perceived amount of prickling sensation by the oral cavity	Skittles Fizzl'd Fruits (1 on tongue)	NA	7
Viscous	Degree to which the beer resists flow under an applied force in the mouth	Highland Oatmeal Porter	Carnation evaporated milk (1 t)- 3.9	3
Warming	Perceived amount of a warming sensation across the palate	Flying Dog Horn Dog (barley wine style ale)	1-2 oz red wine swirled on tongue	5

If references had to be prepared in beer, MGD 64™ (Miller Brewing Co, Milwaukee, WI) was utilized and was prepared twenty-four hours in advance to allow for equilibration. The chemical and/or referenced agent was measured and aseptically transferred into the 12 oz bottle of MGD 64™, recapped, and stored in a refrigerated environment at approximately 3-5°C. All examples and references/referenced agents were purchased from local supermarkets, specialty food and beverage shops, or a local homebrew shop (LHBS). Chemicals were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO). All references and examples were introduced and sampled per the regular serving protocol, or for non-beer or liquids, served in their respective suitable serving measures (i.e. Reddi-whip aerosol whipped cream at 5-7°C, honey served from a plastic spoon at 22°C, etc.; the others are noted on the table). As a panel, all definitions and reference intensities, based on a scale from 0-15, 0 being non-existent, and 15 being strongest imaginable, utilizing increments of

tenths (i.e. 4.3, 8.6, etc), were all agreed upon. Individually, each panelist once again rated each remaining attribute (except for the mouthfeel attributes) for appropriateness. Based upon these ratings and panel discussion/consensus, the orthonasal aroma (Table 8) and flavor lexicons (Table 9) were both narrowed down to their final list of fifteen attributes. Again, the mouthfeel lexicon remained unchanged (Table 7).

Table 8. Orthonasal Aroma Ale Lexicon

Attribute	Chemical(s)- if applicable	Definition/description	Reference with its intensity	Example(s)
Fruity Notes/ Spice Note/ Sweet/syrup Notes				
Citrus	many	Aromatic associated with the general impression of citrus fruits	Linalool (.3 mg/L beer)- 10.0	1) Citrus fruits 2) Pledge furniture polish
Fruity	**Ethyl acetate**	An aroma note associated with light fruity.	Ethyl acetate (100mg/L beer)- 4.0	Great Value fruit cocktail
Vanilla	vanillin	Aromatic blend of sweet, vanillin, woody, browned notes, sometimes having chocolate, tobacco, floral, or spicy components	Vanilla bean in a glass jar- 15.0	Great Value vanilla extract
Caramelized	many	The aromatic relating to the browning of starches and sugars.	3-hydroxy-2-methyl-4-pyrone (1 g/L beer)	1)Killian's Red Lager; Newcastle 2) Kraft's caramel candies
Chocolate	Dimethyl pyrazine, methyl butanol	Aromatic associated with chocolate liquor, as found in roasted West Africa/Ivory Coast cocoa beans	Ivory Coast chocolate liquor- 15.0	1) Lindt dark chocolate 2) Hershey's semi-sweet morsels
Honey	many	The sweet, caramelized floral and woody aromatic associated with honey	Phenylacetic acid in sweetened water (10 ppm)- 15.0	Clover honey
Molasses		An aromatic associated with molasses; has a sharp, slight sulfur and/or caramelized character	Black Strap molasses (3 ml/25 ml beer)- 15.0	1) Black Strap molasses 2) Karo Dark corn syrup
Roasted (or lack of) Notes				
Burnt/ Burnt toast	Octanol; indole; ethyldimethylpyrazine; dimethyl sulfone; furfuryl alcohol	Aromatic associated with blackened/acrid carbohydrates; a burnt aroma note, like charred toast.	Black malt extract- American Brewmaster (20º C)- 7.5	1) Guinness 2) Burnt toast crust
Coffee		An aroma note associated with coffee	Folger's Gourmet Supreme ground coffee (6-8 grains/25 ml red wine)- 12.0	Folger's Gourmet Supreme ground coffee
Hay-like/grassy	hexanals	Grainy aromatic with some green character of air-dried grain or vegetation; Aromatic associated with unprocessed vegetation- grains, leaves, and grass.	Hay (NCSU Veterinarian Medicine School); cis-3-hexen-1-ol (5 ppm in water)- 15.0	1) hay 2) fresh parsley
Malty		The aromatic reminiscent of toasted grain.	Light malt extract- American Brewmaster (30 ml/L beer)- 15.0	1) Whoppers malted milk balls halved 2) Grapenuts

Table 8. (continued) Orthonasal Aroma Ale Lexicon

Roasted		An aroma note associated with roasted grains; nutty.	Roasted barley- American Brewmaster- 15.0	1) Guinness stout 2) same 3) wheat germ
Others				
Piney	A-p-dimethylstyrene; β-/ α-pinene; δ-terpinene; dihydroterpinyl acetate	Aromatic associated with dry, fresh cut pine wood or pine needles.	Fresh cut pine needles- 15.0	1) Great Value rosemary 2) PineSol
Hoppy	NA	An overall term given to fresh hop aroma.	Cascade pellets- American Brewmaster- 15.0	same
Yeasty	NA	Aromatics associated with fresh yeast and yeast fermentation	Active yeast (1 g yeast in 250 ml distilled water at room temperature- spike (3 ml) beer)- 7.0	Fresh baked yeast bread

Table 9. Ale Flavor Lexicon

Attribute	Chemical(s)- if applicable	Definition/description	Reference with its intensity	Example(s)
Basic Tastes				
Sweet	Sucrose, many	The taste stimulated by sucrose, and other sugars, sugar alcohols, and other sweet substances, such as Aspartame, etc.	5% sucrose in water- 5	1. Dextrose, glucose 2. Aspartame, fructose
Sour	Acetic acid, acids	The taste stimulated by acids, such as citric, malic, and acetic.	.08% citric acid in water- 5	Lemon juice
Bitter	many	The taste stimulated by quinine, caffeine, and hop bitters.	.08% solution of caffeine- 5	Folger's Gourmet Supreme black coffee
Aromatics/ Retronasal Aroma				
Fruity	Ethyl acetate, isoamyl acetate, esters, etc.	A flavor note associated with fruitiness (apple, pear, banana, etc).	10.648 ml syrup from fruit cocktail/ 12 oz MGD 64-1.0	Fruit cocktail
Vanilla	vanillin	Flavor note reminiscent of a blend of sweet, vanillin, woody, browned notes, sometimes having chocolate, tobacco, floral, or spicy components	3 drops (30 mg) vanilla extract/ 12 oz MGD 64-7.0	Vanilla extract
Caramelized	many	The flavor relating to the browning of starches and sugars.	Killian's Red Lager- 6.0	1)Newcastle 2) Kraft's caramel candies
Chocolate	Dimethyl pyrazine, methyl butanol	Flavor associated with chocolate liquor, as found in roasted West Africa/Ivory Coast cocoa beans	10.648 ml Arrow chocolate liquor/ 12 oz MGD 64- 10	1) Lindt dark chocolate 2) Hershey's semi-sweet morsels
Honey	many	The sweet, caramelized floral and woody aromatic/flavor associated with honey	25.0 ml clover honey/ 12 oz MGD 64- 15.0	1) Clover honey
Molasses		A flavor note associated with molasses; has a sharp, slight sulfur and/or caramelized character	Black Strap molasses (3 ml/25 ml beer= 42.604 ml/12 oz)- 15.0	1) Black Strap molasses 2) Karo Dark corn syrup
Burnt/ Burnt toast	Octanol; indole; 3-methyl-1-butanol; ethyldimethylpyrazine; dimethyl sulfone; furfuryl alcohol	Flavor associated with blackened/acrid carbohydrates; a burnt flavor note, like charred toast.	Black malt extract (10.648 ml/12 oz MGD 64) (20º C)- 5.0	1) Guinness 2) Burnt toast crust

Table 9. (continued) Ale Flavor Lexicon

Coffee	NA	A flavor note associated with coffee	Folger's Gourmet Supreme ground coffee (20-25 grains/12 oz MGD 64)- 9.0	Folger's Gourmet Supreme coffee
Malty	NA	The flavor reminiscent of toasted grain.	Malt extract- American Brewmaster (30 ml/L beer= 10.648 ml/ 12 oz)- 6.5	1) Whoppers malted milk balls 2) Grapenuts
Roasted	NA	A flavor note associated with roasted barley used in the grist	3 g roasted barley/ 12 oz MGD 64 (American Brewmaster)- 10.0	1) Guinness stout 2) same 3) wheat germ
Hoppy	NA	An overall term given to fresh hop flavor, which includes: floral, citrus, woody, earthy, and piney notes.	Cascade pellets (American Brewmaster)- 1 g/12 oz MGD 64- 12.0	same
Yeasty	NA	Aromatics/flavor notes associated with fresh yeast and yeast fermentation	Active yeast (1 g yeast in 250 ml distilled water at room temperature- spike beer [1 ml])- 3.0	Fresh baked yeast bread

The panel trained on all of these attributes for approximately 40 hours spanning across sixth months, by participating in two-hour sessions two to three times a month. Three to six commercial examples selected from the frame of reference list, and similar styles (Table 10), were blindly (each treatment was assigned a three-digit code) given to the panel to analyze for the each of the previously mentioned attributes. For the first two months of the training, fresh references and examples were supplied to the panel alongside the treatments to aid in development and ensure homogeneity and objectivity amongst the panel. During training, a beer was selected (R1- Sierra Nevada Pale Ale, Chico, CA) to be used as a reference during the actual descriptive analysis tests. Each of the attribute intensities of this ale was majorly in consensus across the panel; therefore, the mean intensities of each attribute were recorded for later use on the Ale Descriptive Ballot (see Appendix). Once training was complete (the panel was properly calibrated showing objectivity, with similar results), the panel was ready to participate in descriptive analysis tests and to be used as an actual instrument in

the product development phase of the research.

Table 10. Other Commercial Ale Examples Utilized for Training

Ale Category	Name	Sub-style	Brewing Company
Pale/Blonde/Golden	California Blonde Ale	Blonde Ale	Eel River Brewing (Fortuna, CA)
Pale/Blonde/Golden	Skinny Dip	Blonde Ale	New Belgium Brewing (Fort Collins, CO)
Pale/Blonde/Golden	14'er ESB	Extra Strong Bitter	Avery Brewing Co (Boulder, CO)
Pale/Blonde/Golden	Hell's Belle	Belgian Blonde Ale	Big Boss Brewing Co. (Raleigh, NC)
Pale/Blonde/Golden	Drifter Pale Ale	Pale Ale	Widmer Brothers Brewing (Portland, OR)
Amber/Red	Red Tail Ale	Amber Ale	Mendocino Brewing (Ukiah, CA)
Amber/Red	Winter's Bourbon Cask Ale	Winter Warmer	Michelob Brewing Co (St. Louis, MO
Brown	Brown Ale	Brown Ale	Duck Rabbit Craft Brewery (Farmville, NC))
Brown	Pecan Harvest Ale	American Brown Ale	Abita Brewing (Abita Springs, LA)
Wheat/Weizen/Weiss	Shiner Hefeweizen	Hefeweizen	Spoetzl Brewing Co (Shiner, TX)
Wheat/Weizen/Weiss	Pomegranate Wheat	Fruit Wheat Ale	Saranac/ Matt Brewing Co (Utica, NY)
Wheat/Weizen/Weiss	Shiner Bavarian Dark Wheat	Dunkelweizen	Spoetzl Brewing Co (Shiner, TX)
India Pale Ale	Centennial IPA	American IPA	Founders Brewing Co (Grand Rapids, MI)
India Pale Ale	Terrapin Rye PA	IPA	Terrapin Brewing Co (Athens, GA)
Stout	Dark Starr Stout	Dry Stout	Starr Hill Brewery (Charlottesville, VA)
Specialty Ale	Raison d'Etre	Belgian Strong Dark Ale	Dogfish Head Brewing (Milton, DE)

The descriptive analysis tests were done in triplicate, and each test was performed per the regular protocol. Three to six treatments were assessed for orthonasal aroma and flavor[7] each test session, but not more than six to minimize and /or prohibit palate fatigue. Ballots were collected from each panelist, however, the intensities were averaged across the panel to

[7] Even though ale mouthfeel was trained upon, it was not utilized as part of any descriptive analysis testing.

achieve one data point for each attribute. Although the mean was taken, most of the responses were very similar, which is ideal, thus deeming a well-calibrated instrument.

Diacetyl was trained upon later during the testing period. Different orthonasal aroma and flavor references were prepared at least twenty-four hours prior to any testing/training. Ten different possible diacetyl references for orthonasal aroma were prepared and five different retronasal aroma references were prepared. All of these references (2,3-butanedione, Fisher Scientific, Pittsburgh, PA; Imitation Butter Flavoring, McCormick Spices, Sparks, MD) were assigned intensities by the panel, and one reference from each modality was selected to be utilized as the reference for the remainder of the training/testing periods (*in Table 11 and 12 denotes selected reference to be utilized).

Table 11. Possible Diacetyl References for Orthonasal Aroma

Intensity	Treatment	Intensity	Treatment
12	Diacetyl at 0.10 mg/L MGD 64 = 100 ppm	2.5	Imitation Butter Flavoring at .28mg/L of MGD 64 = 280 ppm
*15	*Diacetyl at 0.20 mg/L MGD 64	4	Imitation Butter Flavoring at .56 mg/L of MGD 64
20	Diacetyl at 0.30 mg/L MGD 64	6	Imitation Butter Flavoring at .85 mg/L of MGD 64
NA	Diacetyl at 0.40 mg/L MGD 64	8	Imitation Butter Flavoring at 1.13 mg/L of MGD 64
NA	Diacetyl at 0.50 mg/L MGD 64	NA	Imitation Butter Flavoring at 1.41 mg/L of MGD 64

Table 12. Possible Diacetyl References for Retronasal Aroma

Intensity	Treatment	Intensity	Treatment
2.5	Imitation Butter Flavoring at .28mg/L of MGD 64 = 280 ppm	10	Imitation Butter Flavoring at 1.13 mg/L of MGD 64
4.5	Imitation Butter Flavoring at .56 mg/L of MGD 64	NA	Imitation Butter Flavoring at 1.41 mg/L of MGD 64
*7.5	*Imitation Butter Flavoring at .85 mg/L of MGD 64		

The chosen diacetyl references and their respective examples can be viewed through the following table.

Table 13. Diacetyl References and Examples

Attribute	Chemical(s)- if applicable	Definition/description	Reference	Example(s)
Orthonasal Aroma				
Diacetyl	2,3-butenedione	Aromatic associated with butter/dairy	Diacetyl (.20 mg/L MGD 64)- 15	Kroger microwave buttered popcorn
Retronasal Aroma				
Diacetyl	2,3-butenedione	A flavor note associated with butter/dairy	McCormick's Imitation Butter Extract (.85 mg/L MGD 64)- 7.5	1. Kroger microwave buttered popcorn 2. Buttered popcorn Jelly Belly

These references were trained upon independently for two, two-hour sessions, and they were also supplied alongside the panel test treatments for each session two months after the initial training period. Examples were supplied during the training sessions as well.

c) Blind Bench Testing

For most of the blind bench testing, the panel was utilized, or a small bench test was completed independently. For both of these, however, the regular serving protocol was utilized. For the panel tests, only two panelists were scheduled to perform the test at a time in twenty-minute sessions, and the treatments were served to each panelist in a different randomized order. All of these tests were rated hedonically on a 9-point scale. The panel tests were done in triplicate, but the bench testing was just done once. The solo bench testing was

only done to get an estimate of what concentrations to test on a larger scale or whether or not the idea/variable/ingredient would be feasible altogether. The solo bench testing included the following trials:

Soy Kefir Powder stability in beer matrix. 40 ml of still IPA beer was transferred into a 50 ml sterile centrifuge tube and .59 g of SKP was added to the beer; this was repeated seven more times to result in eight total treatments. Each sample was allowed approximately twelve hours hold time in a refrigerated environment of 4°C for the SKP to properly hydrate and mix into the beer. After the hydration period had elapsed, each treatment was given a different amount of xanthan gum (TIC Gums, Whitemarsh, MD), a pseudoplastic stabilizer, and all of the samples were shaken right before administering the xanthan gum. Xanthan gum is utilized in some salad dressings, for instance, in order to suspend particles (i.e. spices, herbs, etc) within its matrix to prevent flocculation of these items. This hydrocolloid is also thixotropic, meaning, statically it appears viscous, and allows for particle suspension, but as rate of flow, or shear, increases, its viscosity does as well. Xanthan gum was utilized in this study in order to determine if it would suspend the SKP micro-particles within the beer matrix, thus preventing flocculation, without adding a perceived amount of increased viscosity, or would not supply an undesirable amount of perceived viscosity due to these principles. Sample A was given .02g of xanthan gum, resulting in a concentration of .05%; Sample B was given .06 g, or .15%, C was given .10g (.25%), D was given .14g (.35%), E was given .18g (.45%), F was given .22g (.55%), G was given .26g (.65%), and Sample H was given .30g, resulting in a concentration of .75%. The xanthan gum was mixed into the solution by shaking, and all of the samples were kept cool at about 4°C for twenty-four hours and then

suspension/flocculation levels were checked.

Strawberry fruit flavoring usage trial. Strawberry fruit flavoring (LD Carlson, Kent, OH) was purchased from American Brewmaster, a LHBS (Raleigh, NC). Its recommended usage level is 2 oz per 18.9 liters of beer. Eight different treatments were tested with varying concentrations of the strawberry flavoring ranging from 1.5-5.0 oz per 18.9 liters of beer. 29.57 ml of the kefir beer was added to lidded translucent plastic cups, and the following was added to each different cup:

Table 14. Amounts of Strawberry Fruit Flavoring Added for Bench Trial

Sample	Target Volume of Strawberry Flavoring/ 18.9 liters of Beer	Amount of Strawberry Flavoring Added (µl)
A	1.5 oz	69.0
B	2.0 oz	92.0
C	2.5 oz	115.5
D	3.0 oz	139.0
E	3.5 oz	162.0
F	4.0 oz	185.0
G	4.5 oz	208.0
H	5.0 oz	231.0

Once the flavoring was added, the samples were stirred slightly to allow for homogeneity of the flavoring, and then placed in a refrigerated environment of about 4°C for one hour to allow for further dispersion. The treatments were then sensorally tested utilizing the regular serving protocol.

B. Laboratory Analysis

a) Total Solids Analysis of Soy Kefir

In triplicate, approximately 10 ml of the liquid kefir, pre-clarification, was weighed in aluminum weigh boats, and while wearing gloves to prohibit a weight

change by the addition of skin oils, these boats were transferred into a Fisher Scientific Isotemp Oven (Pittsburgh, PA) set at 85°C. These samples were allowed to dry, removing all the liquid weight, for a period no longer than twenty-four hours. After the samples were sufficiently dried (the samples appeared dark brown in color, granular, and appeared as if all free and un-bound water had been evaporated), the boats were once again removed by gloved hands, and weighed. From the two weights for each sample, an average total solids concentration could be calculated.

b) Diacetyl Analysis

Standards. A stock solution of 2,3-butanedione (Sigma-Aldrich, St. Louis, MO)- were made in HPLC grade water and stored in a temperature of -20°C. This standard was analyzed using a reverse-phase High Pressure Liquid Chromatography (HPLC) instrument in HPLC grade water.

Reagents/Solvents. 0.05mM of sulfuric acid (Fisher Scientific, Pittsburgh, PA) was used as the mobile phase

Sample Preparation. KK samples from the diacetyl rest- 0 hour diacetyl rest, 12 hour, 24 hour diacetyl rest, 36 hour, and 48 hour diacetyl rest (T=0, T=12, T=24, T=36, T=48)- were tested for the compound diacetyl, 2,3-butanedione. 15 ml of each sample were centrifuged in 50 ml centrifuge tubes and centrifuged at 15000 g for 15 minutes. The supernatant was collected. Due to quenching issues, sensitivity was low; however, each of the sample's supernatants were spiked with 2,3-butanidione (diacetyl) at 0.0097, 0.097, 0.97, and 9.7% to obtain detectable results, and then was filtered through a .20 micron syringe filter before being loaded into the HPLC.

HPLC Analysis. Analysis and separation of the diacetyl was performed on a Rezex RHM-Monsaccharide H+(8%) (Phenomenex, Torrance, CA) with a size of 300 x 7.8 mm. The high-pressure chromatography system (Waters, Milford, MA) was equipped with a carousel autosampler (Waters 717 plus), binary HPLC pump (Waters 1525), Refractive Index Detector (Waters 2414), and *Breeze* software (Waters). The analysis was done at a flow rate of 0.50 ml/min, with a column temperature of 65°C, an injection volume of 200 µl, and a detection wavelength of 210 and 290 nm. The reverse-phase high-pressure chromatography was also performed with a Carbo-H 4x3.0mm guard column (Phenomenex, Torrance, CA), a run time of 30 min, with an isocratic mobile phase gradient system.

Calculation. The responses for the diacetyl were determined by calculating the slope (*m)* and intercept (*b),* using linear regression analysis of area counts versus the response for the diacetyl standard.

c) Major Fermentation By-Product Analysis and Composition of Soy Kefir via HPLC

Standards. All standards were run using a reverse-phase High Pressure Liquid Chromatography (HPLC) instrument (exact apparatus and methods to follow) in the following concentrations in HPLC grade water:

Table 15. Concentrations of Fermentation Compound Standards for HPLC

Standard	g/100ml	Standard	g/100ml	Standard	g/100ml	Standard	g/100ml
Dextrin	0.01953125	Citric Acid	1.25000000	Mannose	5.00000000	Lactic Acid	0.07812500
Dextrin	0.03906250	Citric Acid	0.62500000	Mannose	2.50000000	Lactic Acid	0.03906250
Dextrin	0.07812500	Citric Acid	0.31250000	Mannose	1.25000000	Lactic Acid	0.01953125
Dextrin	0.15625000	Citric Acid	0.15625000	Mannose	0.62500000	Lactic Acid	0.009765625
Dextrin	0.31250000	Citric Acid	0.07812500	Mannose	0.31250000	Glycerol	20.00000000
Dextrin	0.62500000	Citric Acid	0.03906250	Mannose	0.15625000	Glycerol	10.00000000

Table 15. (continued) Concentrations of Fermentation Compound Standards for HPLC

Dextrin	1.25000000	Citric Acid	0.01953125	Mannose	0.07812500	Glycerol	5.00000000
Dextrin	2.50000000	Tartaric Acid	5.00000000	Mannose	0.03906250	Glycerol	2.50000000
Dextrin	5.00000000	Tartaric Acid	2.50000000	Galactose	10.00000000	Glycerol	1.25000000
Dextrin	10.00000000	Tartaric Acid	1.25000000	Galactose	5.00000000	Glycerol	0.62500000
Maltotriose	0.01953125	Tartaric Acid	0.62500000	Galactose	2.50000000	Glycerol	0.31250000
Maltotriose	0.03906250	Tartaric Acid	0.31250000	Galactose	1.25000000	Glycerol	0.15625000
Maltotriose	0.07812500	Tartaric Acid	0.15625000	Galactose	0.62500000	Glycerol	0.07812500
Maltotriose	0.15625000	Tartaric Acid	0.07812500	Galactose	0.31250000	Glycerol	0.03906250
Maltotriose	0.31250000	Tartaric Acid	0.03906250	Galactose	0.15625000	Glycerol	0.01953125
Maltotriose	0.62500000	Glucose	30.00000000	Galactose	0.07812500	Acetic Acid	20.00000000
Maltotriose	1.25000000	Glucose	15.00000000	Galactose	0.03906250	Acetic Acid	10.00000000
Maltotriose	2.50000000	Glucose	7.50000000	Galactose	0.01953125	Acetic Acid	5.00000000
Maltotriose	5.00000000	Glucose	3.75000000	Fructose	20.00000000	Acetic Acid	2.50000000
Maltotriose	10.00000000	Glucose	1.87500000	Fructose	10.00000000	Acetic Acid	1.25000000
Maltotriose	20.00000000	Glucose	0.93750000	Fructose	5.00000000	Acetic Acid	0.62500000
Maltose	20.00000000	Glucose	0.46875000	Fructose	2.50000000	Acetic Acid	0.31250000
Maltose	10.00000000	Glucose	0.93750000	Fructose	1.25000000	Acetic Acid	0.15625000
Maltose	5.00000000	Glucose	0.46875000	Fructose	0.62500000	Acetic Acid	0.07812500
Maltose	2.50000000	Glucose	0.23437500	Fructose	0.31250000	Acetic Acid	0.03906250
Maltose	1.25000000	Glucose	0.11718750	Fructose	0.15625000	Acetic Acid	0.019531250
Maltose	0.62500000	Glucose	0.05859375	Fructose	0.07812500	Propionic Acid	5.00000000
Maltose	0.31250000	Glucose	0.029296875	Fructose	0.03906250	Propionic Acid	2.50000000
Maltose	0.15625000	Glucose	0.014648438	Fructose	0.01953125	Propionic Acid	1.25000000
Maltose	0.07812500	Pyruvic Acid	5.00000000	Mannitol	5.00000000	Propionic Acid	0.62500000
Maltose	0.03906250	Pyruvic Acid	2.50000000	Mannitol	2.50000000	Propionic Acid	0.31250000
Lactose	10.00000000	Pyruvic Acid	1.25000000	Mannitol	1.25000000	Propionic Acid	0.15625000
Lactose	5.00000000	Pyruvic Acid	0.62500000	Mannitol	0.62500000	Propionic Acid	0.07812500
Lactose	2.50000000	Pyruvic Acid	0.31250000	Mannitol	0.31250000	Propionic Acid	0.03906250
Lactose	1.25000000	Pyruvic Acid	0.15625000	Mannitol	0.15625000	Ethanol	20.00000000
Lactose	0.62500000	Pyruvic Acid	0.07812500	Mannitol	0.07812500	Ethanol	10.00000000

Table 15. (continued) Concentrations of Fermentation Compound Standards for HPLC

Lactose	0.31250000	Pyruvic Acid	0.03906250	Mannitol	0.03906250	Ethanol	5.00000000
Lactose	0.15625000	Malic Acid	5.00000000	Lactic Acid	20.00000000	Ethanol	2.50000000
Lactose	0.07812500	Malic Acid	2.50000000	Lactic Acid	10.00000000	Ethanol	1.25000000
Lactose	0.03906250	Malic Acid	1.25000000	Lactic Acid	5.00000000	Ethanol	0.62500000
Lactose	0.01953125	Malic Acid	0.62500000	Lactic Acid	2.50000000	Ethanol	0.31250000
Lactose	0.009765625	Malic Acid	0.31250000	Lactic Acid	1.25000000	Ethanol	0.15625000
Citric Acid	10.00000000	Malic Acid	0.15625000	Lactic Acid	0.62500000	Ethanol	0.07812500
Citric Acid	5.00000000	Malic Acid	0.07812500	Lactic Acid	0.31250000	Ethanol	0.03906250
Citric Acid	2.50000000	Malic Acid	0.03906250	Lactic Acid	0.15625000	Ethanol	0.019531250
						Ethanol	0.009765625

Reagents/Solvents. Mobile Phase- 5mM sulfuric acid, 200 ml 50mM sulfuric acid (Fisher Scientific, Pittsburgh, PA) in 1800 ml HPLC grade water.

Sample Preparation. All samples were filtered into 1 ml glass HPLC vials using a HPLC .45 micron filter syringe (VWR, Radnor, PA), and then placed in the HPLC for analysis.

HPLC Analysis. Analysis and separation of the fermentation compounds was performed on a *Rezex RHM-Monosaccharide H+(8%)* column (Phenomenex, Torrance, CA), with a size of 300 x 7.8 mm. The high-pressure chromatography system (Waters, Milford, MA) was equipped with a carousel autosampler (Waters 717 plus), binary HPLC pump (Waters 1525), Refractive Index Detector (Waters 2414), and *Breeze* software (Waters). The analysis was done at a flow rate of 0.50 ml/min, with a column temperature of 65°C, and an injection volume of 10 µl. The reversed phase high-pressure chromatography was also performed with a *SecurityGuard* guard

column (Phenomenex, Torrance, CA), and a run time of 30 min with an isocratic mobile phase system of 100% 5 mM sulfuric acid.

Calculation. The response for each compound was determined by calculating the slope (*m)* and intercept (*b)*, using linear regression analysis of area counts versus the response for each of the standards.

d) Major Soy Isoflavone Analysis via HPLC

Standards. Stock solutions of all of the standards- Genistin, Genistein, Daidzin, Daidzein (Sigma-Aldrich , St. Louis, MO)- were made with a set concentration in DMSO (Fisher Scientific, Pittsburgh, PA) , and stored in a temperature of -20°C. These isoflavones were analyzed using a reverse-phase High Pressure Liquid Chromatography (HPLC) instrument in the following concentrations in 50:50, DMSO:HPLC grade water:

Table 16. Concentrations of Standard Isoflavones Ran in HPLC

Sample Name	% of isoflavone	Retention Time (min)	Sample	% of isoflavone	Retention Time (min)
Daidzein	0.0625	18.613	Genistein	0.0625	25.942
Daidzein	0.03125	18.661	Genistein	0.03125	26.007
Daidzein	0.015625	18.669	Genistein	0.015625	25.974
Daidzein	0.0078125	18.655	Genistein	0.0078125	26.007
Daidzein	0.00390625	18.687	Genistein	0.00390625	26.021
Daidzein	0.00195313	18.689	Genistein	0.00195313	26.005
Daidzein	0.00097656	18.711	Genistein	0.00097656	26.023
Daidzein	0.00048828	18.714	Genistein	0.00048828	26.035
Daidzein	0.00024414	18.722	Genistein	0.00024414	26.043
Daidzein	0.00012207	18.729	Genistein	0.00012207	26.054
Daidzein	6.1035E-05	18.727	Genistein	6.1035E-05	26.068
Daidzein	3.0518E-05	18.729	Genistein	3.0518E-05	26.071
Daidzin	0.0078125	6.155	Genistin	0.015625	11.878
Daidzin	0.00390625	6.16	Genistin	0.0078125	11.851
Daidzin	0.00195313	6.128	Genistin	0.00390625	11.81
Daidzin	0.00097656	6.14	Genistin	0.00195313	11.794

Table 16. (continued) Concentrations of Standard Isoflavones Ran in HPLC

Daidzin	0.00048828	6.137	Genistin	0.00097656	11.776
Daidzin	0.00024414	6.135	Genistin	0.00048828	11.793
Daidzin	0.00012207	6.141	Genistin	0.00024414	11.789
Daidzin	6.1035E-05	6.176	Genistin	0.00012207	11.792
Daidzin	3.0518E-05	6.174	Genistin	6.1035E-05	11.794
			Genistin	3.0518E-05	11.787
			Genistin	1.5259E-05	11.784

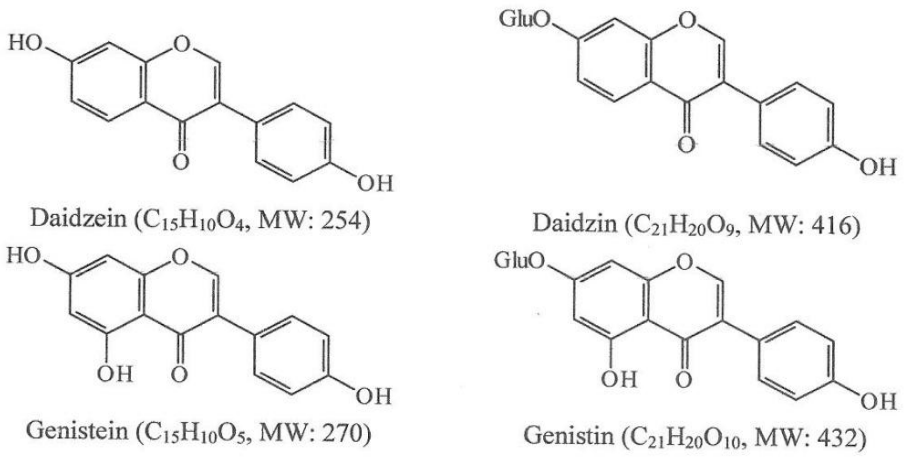

Figure 7. Chemical Structures of the Standard Isoflavone Compounds (Botanical Center for Age-related Diseases, 2001)

An internal standard was also run. This standard consisted of spiking beer with a known amount of each of the isoflavones (.0005% for all), and then subjecting the treatment to the same preparation procedures (solvent extraction and saponification). Once the concentrations for each of the isoflavones were calculated (using the equation to be noted later), the loss was calculated, and a "loss factor" was created to account for this loss.

Table 17. Internal Standard and Loss Constant Used for HPLC Soy Isoflavone Analysis

Sample Name	Isoflavone	% of Isoflavone in Full Sample	Actual % of Isoflavone in Full Sample	Loss Constant
003iosfl	daidzin	5.81888E-11	.0005	8592722.764
003iosfl	genistin	2.27752E-10	.0005	2195373.792
003iosfl	daidzein	1.83119E-11	.0005	27304667.443
003iosfl	genistein	1.29198E-10	.0005	3870041.816

Reagents/Solvents. Acetic Acid, glacial (Fisher Scientific, Pittsburgh, PA); Acetonitrile, HPLC grade (Fisher Scientific, Pittsburgh, PA); Extraction Stock Solution- 80% methanol solution , 800 ml of HPLC grade methanol (Fisher Scientific, Pittsburgh, PA) was added to a 1000 ml glass Corning Bottle, and to it, 200 ml of HPLC grade water was added. This solution was then mixed by inversion, and stored in an environment of 22°C until its use; Methanol, HPLC grade (Fisher Scientific, Pittsburgh, PA); Mobile Phase A- 0.1% Acetic acid (2 ml), 5.0% Acetonitrile (100 ml) (Fisher Scientific, Pittsburgh, PA) in HPLC grade water (2000 ml); Mobile Phase B- 0.1% Acetic Acid in Acetonitrile (2000 ml); Sodium Hydroxide (2 M) Stock Solution- 50 ml of HPLC grade water was added to a 150 ml glass beaker with a magnetic stir bar. The beaker was placed on a stir plate and set at a medium speed. Then, 8g of sodium hydroxide (Fisher Scientific, Pittsburgh, PA) were slowly added to the water, and left to stir until the sodium hydroxide was in solution. After this, first the stir bar was carefully removed, and the beaker was removed from the stir plate and the solution was slowly poured into a graduated cylinder. The beaker was rinsed with more HPLC grade water and poured into the

graduated cylinder, bringing the total volume of the solution to 100 ml. This solution was then transferred into a 250 ml glass Corning Bottle and then was stored in an environment of 22°C until its use; Water, HPLC grade.

Sample Preparation. (adapted from the *AOAC Official Method 2001.10*, 2002*)* 5 ml of the beer/sample under investigation was added to a 50 ml centrifuge tube and 40 ml of the extraction solution was added to the sample. The tube was securely capped and inverted several times. The tube was then placed in the incubating/cooling Micro Plate Shaker (VWR, Radnor, PA) and left to shake at a speed of 430 (medium-high) for two hours at 65°C. Once cool, 3 ml of the 2M sodium hydroxide solution was added to the tube, and then it was placed back in the shaker and shook for another 10 min. After the ten minutes had elapsed, 1 ml of acetic acid was added and this new solution was mixed by inversion. Then, 12.25 ml of this solution was transferred into a 15 ml centrifuge tube and 1.75 ml of the extraction solution was added to it. 5 ml of this solution was then transferred into another 15 ml centrifuge tube, where 4 ml of HPLC grade water was added, and then an aliquot of methanol was added to bring the entire volume up to 10 ml. This tube was inverted several times, and then 1 ml of it was transferred into 1.5 ml microcentrifuge tubes. This step was repeated three times to allow for a total volume of 3 ml for each sample. This final solution was then centrifuged for 5 minutes at 7000 RPM (the total number of vials depended upon the number of samples analyzed for each “run”). The clear supernatant of each treatment was transferred into 2 ml

HPLC glass vials (VWR, Radnor, PA). 500 µl from each 1 ml sample was transferred into the vial to allow for a total sample volume of 1.5 ml. The vials were then placed into a 2 ml HPLC vial rack, and then inserted into the HPLC autosampler for isoflavone analysis.

HPLC Analysis. (adapted from the method specified in Lin and Giusti, 2005) Analysis and separation of the isoflavone compounds was performed on a *Kinetex C18*, 2.6u 100Å column (Phenomenex, Torrance, CA), with a size of 150 x 4.6 mm. The high-pressure chromatography system (Waters, Milford, MA) was equipped with an autosampler (Waters 2707), binary HPLC pump (Waters 1525), Photodiode Array Detector (DAD) (Waters 2998), and *Breeze 2* software (Waters). The analysis was done at a flow rate of 1.00 ml/min, with a column temperature of 30°C, an injection volume of 200 µl, and a detection wavelength of 254 nm. The reverse-phase high pressure chromatography was also performed with a *SecurityGuard* guard column (Phenomenex, Torrance, CA), a run time of 40 min, with a 10 min wash period, and a mobile phase gradient system of the following:

Table 18. Mobile Phase Gradient System for HPLC Isoflavone Analysis

Time (min)	Concentration of A (%)	Concentration of B (%)
0.01	90	10
0.10	90	10
10.00	86	14
12.00	80	20
20.00	80	20
30.00	30	70
33.00	30	70
34.10	90	10
40.00	Stop	Stop

Calculation. The response for each isoflavone was determined by calculating the slope (*m)* and intercept (*b)*, using linear regression analysis of area counts versus the response for each of the isoflavone standards. The concentration of each of the isoflavones in the test sample can be calculated using the following equation:

$$\text{Isoflavones µg per ml} = \frac{[((As \times m) \times b) \times 50 \times 10]}{Vs \times 5}$$

Where *As*= peak area of isoflavone in test solution; *m*=slope of linear regression for standard response; *b*= intercept from linear regression standard response; *Vs*= volume of test sample (ml); 50= first dilution volume; 10= second dilution volume; and 5= aliquot of sample. This result was then calculated to represent % concentration, and then was also multiplied by its isoflavone "loss constant" that was specified earlier, and accounted for losses due to the sample preparation procedure.

e) Microbiological Analysis

Kristalkefir (KK) Pasteurization and Thermal Death Trials. KK post diacetyl rest was subjected to thermal inactivation and filter pasteurization trials. For the thermal inactivation study, three autoclaved glass bioreactor spinner flasks (Bellco Glass, Vineland, NJ) were each filled with 200 ml of KK. Three different sanitized thermometers were placed within one of the side arms of each of the bioreactors, in a manner that allowed for the tip of it to be suspended within the KK matrix. The thermometers were secured with label tape and Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL), which was stretched atop the opening of

arm. Meanwhile, a heating stir plate was set to 120°C in order to be hot upon arrival of the bioreactor flasks. The bioreactors were then placed upon the plate spinning at a medium speed, with a starting KK temperature of 10°C. After 50 minutes, the KK reached the target temperature of 60°C. The temperature on the hot plate was then set to 110°C, ensuring a constant liquid kefir temperature of 60°C throughout the duration of the thermal inactivation trial. Ten ml samples were aseptically taken out of the heated KK into 15 ml sterile centrifuge tubes at five-minute intervals immediately and placed in a test tube rack that was submerged in an ice water bath (0°C) for rapid cool-down of the samples. Then, each of these samples were aseptically serially diluted appropriately, and were plated in triplicate on *Difco WL* (Wallerstein Laboratories) *nutrient* agar (BD Biosciences, Sparks, MD), and *Difco Lactobacilli MRS* (de Man, Rogosa and Sharpe) agar (BD Biosciences, Sparks, MD) + cycloheximide at 10µg/ml (Sigma-Aldrich, St. Louis, MO) in square, gridded plates. For t=0, the sample at 10^{-5} and 10^{-6} was plated; at t=5, 10^{0}, 10^{-1}, 10^{-2}, and 10^{-3} was plated; at t=10, 10^{0}, 10^{-1}, 10^{-2}, and 10^{-3} was utilized; at t=15, 10^{0} and 10^{-1} was plated; and for t=20, 10^{0}, 10^{-1} was plated. 10 µl of each previously mentioned dilution (times three) were aseptically pipetted onto the solidified media at the top of each grid column. The plate was then covered and turned vertically to allow for the sample liquid to drip downwards on the media along its respective column. The plates were sealed with a strip of Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL) and then incubated (Fisher Scientific, Pittsburgh, PA) at 37°C for 48 hours. After the incubation period, the

colonies, if any, were counted, and the cell count (cfu/ml) of each sample was calculated.

For the filter pasteurization trial, an aliquot of KK was aseptically transferred into a Millipore disposable vacuum-driven .45 micron filter bottle attachment unit (Billerica, MA) that was secured to an autoclaved 250 ml Corning Bottle. The unit was then attached to the vacuum source and filtered. This procedure was repeated with a .22-micron filter unit (Billerica, MA). After the filtration was complete, the bottles were recapped, and then 10µl of each treatment were plated in triplicate also on *Difco WL* (Wallerstein Laboratories) *nutrient* agar (BD Biosciences, Sparks, MD), and *Difco Lactobacilli MRS* (de Man, Rogosa and Sharpe) agar (BD Biosciences, Sparks, MD) + cycloheximide at 10µg/ml (Sigma-Aldrich, St. Louis, MO) in square, gridded plates. Again, the 10 µl were aseptically pipetted onto the solidified media at the top of each grid column. The plate was then covered and turned vertically to allow for the sample liquid to drip downwards on the media along its respective column. The plates were sealed with a strip of Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL) and then incubated (Fisher Scientific, Pittsburgh, PA) at 37°C for 48 hours. After the incubation period, the colonies, if any, were counted, and the cell count (cfu/ml) of each sample was calculated.

The thermal inactivation trial was repeated a second time, but samples were aseptically taken out at t=0, t=15, t=17, t=19, and t=21, and only plated on MRS+cylcoheximide. The same plating protocol was performed using the following

dilutions: for t=0, 10^{-5} and 10^{-6} and for t=15, 17, 19, and 21, 10^{0} and 10^{-1}.

Accelerated Shelf-life Microbiological Analysis. Treatments for the Accelerated Shelf-life Study, 003- Aardbeien Lambic Control, 512- Aardbeien Lambic with .45 micron filtered KK, and 920- Aardbeien Lambic with heat pasteurized KK, were microbiologically tested at t=0, and t= 4 months at target term shelf-life. The heat pasteurized Kristalkefir and filter pasteurized KK were also tested at t=0. The treatments were plated in triplicate on *Difco Lactobacilli MRS* (de Man, Rogosa and Sharpe) agar (BD Biosciences, Sparks, MD) + cycloheximide at 10µg/ml (Sigma-Aldrich, St. Louis, MO) in square, gridded plates, and on LMDA (Lee's Multi Differential Agar) (Brewing Science Institute, Woodland Park, CO) in small round plates with a diameter of approximately 60mm. 10 µl of each previously mentioned sample, non-diluted, (times three) were aseptically pipetted onto the solidified media (MRS) at the top of each grid column. The plate was then covered and turned vertically to allow for the sample liquid to drip downwards on the media along its respective column. The LMDA plates were partitioned into three columns by drawing two vertical lines on the backs of the plates. 3.5 µl of each previously mentioned sample, non-diluted, (times three) were aseptically pipetted onto the solidified LMDA media at the top of each column. The plate was then covered and turned vertically to allow for the sample liquid to drip downwards on the media along its respective column. The plates were sealed with a strip of Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL) and then incubated (Fisher Scientific, Pittsburgh, PA) at 37°C for 24 hours (LMDA) and 48 hours (MRS+cycloheximide).

After the incubation period, the colonies, if any, were counted, and the cell count (cfu/ml) of each sample was calculated.

C. Production of Treatments/Development Process

Based on the baseline formula (see Appendix), which was created considering the results of the preliminary bench testing, two commercial examples were chosen most similar to the base formula (i.e. wheat ales, honey notes, low enough IBU to be manipulated with the administration of the tetra iso-alpha acid extract, some fruitiness/esters) to act as a "canvas" for other development to produce an analogue of the intended product at current development. These two examples were Leinenkugel's Honeyweiss (Chippewa Falls, WI) and Atwater Block Brewery's Dirty Blonde (Detroit, MI). Both of these examples were purchased from a local beer and wine specialty store. For each treatment production as part of the development process, the methods were very similar. The basic procedure included aseptically transferring the commercial beer into sanitized glass 4000 ml Corning Bottles, 5 gallon bottling buckets, 2.5 gallon bottling buckets, or one gallon bottling buckets. A priming solution was made with a concentration of one-part sucrose to three-part water. The exact concentrations for these ingredients are 144 g of sucrose into 16 oz (472.5 ml) of water per five gallons (2419.2 L) of beer to be charged. This solution was boiled for fifteen minutes to allow for sterilization. Once the priming solution was cooled (27°C or cooler), it was transferred into the bottling bucket. Then the specified adjuncts were added (i.e. Hopsteiner Tetra Iso-

Alpha Extract, strawberry fruit flavoring), and the SKP or Kristalkefir (KK)[8] was added at this point as well. Control treatments were aseptically transferred into separate bottling vessels prior to the addition of SKP of KK. Safale US-05 American Ale dry yeast (Fermentis, Marcq-en-Baroeul, France) was added to this beer solution at a concentration of 1.5 g of dry yeast per 3.78 L to allow for natural carbonation to take place. The beer solution was aseptically mixed with a long-handled sanitized slotted stainless steel spoon to allow for homogeneity prior to bottling. The solution was then bottled into 12 oz sanitized glass amber beer bottles (donated by Big Boss Brewing Co., Raleigh, NC) and capped with sanitized oxygen barrier crowns (LD Carlson, Kent, OH) purchased from a LHBS. The treatments were labeled and stored in a dark, cool environment of approximately 21°C for a period of five to seven days to allow for the yeast to produce carbon dioxide, thus creating a carbonated beer treatment.

IBU Variance Study with SKP. Hopsteiner Tetra Iso-Alpha Hop extract (Yakima, Washington) was utilized in these treatments in order to create varying IBUs. The Tetra Iso-Alpha extract was prepared by transferring it into a 250 ml Corning Bottle and incubating it in a 45°C water bath for twenty-four hours. The extract was added in the beer stream to allow for optimal dispersion. There were two main five gallon sanitized bottling buckets to begin with, a SKP bucket and a control bucket. As different IBU treatments were produced, the SKP beer or control beer was transferred into smaller one gallon sanitized bottling buckets to receive its specified amount of Tetra Iso-Alpha extract. Then, the treatments were bottled out of this final vessel. The SKP was added in a

[8] Krstalkefir is clarified liquid soy kefir defined in more detail in the Materials and Methods section *D. Production of Soy Kefir*.

concentration of 5.25g/ 355 ml beer serving (Leinenkugel's Honeyweiss (Chippewa Falls, WI)). These treatments were analyzed with the in-house guidance panel hedonically as blind bench testing as well as descriptively.

Table 19. IBU Variance Study with SKP

Treatment #	Treatment Size (bbl)	Treatment Size (hL)	Amount of Tetra Iso-Alpha Hop Extract (ml)	IBU contribution	IBU from beer	Total IBU	Amount of Soy Kefir (g)
167	0.012	0.014	0.832	36	13.5	49.5	28
186	0.012	0.014	0.832	36	13.5	49.5	0
316	0.012	0.014	1.063	46	13.5	59.5	28
362	0.012	0.014	1.063	46	13.5	59.5	0
512	0.012	0.014	1.294	56	13.5	69.5	28
523	0.012	0.014	1.294	56	13.5	69.5	0
783	0.012	0.014	1.525	66	13.5	79.5	28
799	0.012	0.014	1.525	66	13.5	79.5	0
Totals:	0.168	0.19656	16.493	NA	13.5	NA	196

IBU Variance Study with KK. The KK was produced on-site, in the laboratory, and this process will be explained in the Materials and Methods section *D. Production of Soy Kefir*. The same procedures from the IBU Variance Study with SKP was utilized here, but instead of a powder added, a liquid was added. The amount of liquid added equates to 5.25g of SKP/12 oz beer serving[9] (Leinenkugel's Honeyweiss (Chippewa Falls, WI)), and it will remain at this concentration for the remainder of the trials. These treatments were

[9] Values calculated from the total solids analysis of liquid soy kefir.

analyzed with the in-house guidance panel hedonically as blind bench testing as well as descriptively.

Table 20. IBU Variance Study with KK

Treatment #	Treatment Size (bbl)	Treatment Size (hL)	Amount of Tetra -Iso Hop Extract (ml)	IBU contri-bution	IBU from beer	Total IBU	Amount of Soy Kefir (ml)
120	0.012	0.014	0.832	36	13.5	49.5	466.375
193	0.012	0.014	0.832	36	13.5	49.5	0
300	0.012	0.014	1.063	46	13.5	59.5	466.375
377	0.012	0.014	1.063	46	13.5	59.5	0
536	0.012	0.014	1.294	56	13.5	69.5	466.375
587	0.012	0.014	1.294	56	13.5	69.5	0
712	0.012	0.014	1.525	66	13.5	79.5	466.375
745	0.012	0.014	1.525	66	13.5	79.5	
Totals:	0.096	0.11232	9.425		13.5		1865.5

KK Diacetyl Rest Study. Beer treatments (Atwater Block Brewery's Dirty Blonde (Detroit, MI))[10] were created with KK in which had varying diacetyl rests as part of its production, as well as a control treatment without any added KK. These treatments were analyzed by means of descriptive analysis with the in-house guidance panel.

[10] This commercial beer example was used for the remainder of the project.

Table 21. KK Diacetyl Rest Trial Treatment Breakdown

Treatment #	Treatment Volume (L)	Amount of Tetra -Iso Hop Extract (ml)	IBU contribution	IBU from beer	Total IBU	Amount of KK (ml)	Diacetyl Rest Duration of the KK(hrs)
600	1.42	.719	41.5	8	49.5	0	NA
126	1.42	.719	41.5	8	49.5	349.8	0
362	1.42	.719	41.5	8	49.5	349.8	24
912	1.42	.719	41.5	8	49.5	349.8	48
Total	5.68	2.877 ml	NA	NA	NA	1049.4	NA

IPA and Aardbeien Lambic Analogue Trials. 7.44 L of commercial beer was aseptically transferred into a sanitized 2.5 gallon (9.45 L) plastic fermentation vessel and allowed to dry hop for a period of 48 hours at 22°C with 5.67 g of Amarillo and 17.00 g of Centennial hop pellets (Hop Union, Yakima, Washington); these values are based off the initial baseline formula (see Appendix). Once the dry hop period was over, this beer, with other beer treatments, were bottled using the previously mentioned protocol. An aardbeien[11] lambic style was chosen to immolate because previous consumer studies on kefir found that kefir with a strawberry flavor additive was most accepted (Cole and Marshall, 1985). All of the KK added was produced with a twenty-four hour diacetyl rest. KK-A was produced by fermenting it with just glucose (Fisher Scientific, Pittsburgh, PA) added, and KK-B was produced by fermenting it with glucose and fructose (Spectrum Laboratories, Rancho Dominguez, CA) added. Further description can be found in the Materials and Methods section *D. Production of Soy Kefir*. Sucralose (Splenda brand, McNeil Nutritionals, Fort Washington, PA) was added to the priming

[11] Aardbei is Dutch for strawberry, and when referring to strawberry-flavored lambic ale, it is called aardbeien lambic.

solution for the aardbeien lambic treatments in order to back-sweeten[12] these treatments. These treatments were analyzed with the in-house guidance panel hedonically as well as descriptively.

Table 22. IPA and Aardbeien Lambic Analogue Trial Treatment Breakdown

Treatment #	Treatment Volume (L)	IBU from beer	Amount of Tetra -Iso Hop Extract (ml)	Total IBU	Dry-hopped (y/n)	Volume of KK-A (ml)	Volume of KK-B (ml)	Straw-berry flavor (ml)	Sucralose (g)
136	2.48	8	1.26	50	Y	612	0	0	0
214	2.48	8	1.26	50	Y	0	612	0	0
389	2.48	8	1.26	50	Y	0	0	0	0
416	2.48	8	0	8	N	612	0	17.73	2.4
562	2.48	8	0	8	N	0	612	17.73	2.4
690	2.48	8	0	8	N	0	0	17.73	2.4
Total	14.88	NA	3.78	NA	NA	1224	1224	53.2	7.2

Aardbeien Lambic Trials with Different KK Pasteurization Methods. 3.19 L of commercial beer were aseptically transferred into a sanitized 5-gallon plastic bottling bucket at a time (three times for each different treatment, totaling 9.56 L), and the beer treatments were bottled per the previously described protocol. Sucralose (Splenda brand, McNeil

[12] Back-sweetening is a technique used to add sweetness to the final beer after fermentation is complete. Sucralose was utilized here because it not fermented by *S. cerevisiae*, and therefore it would not be utilized during bottle conditioning- thus resulting in a slight sweetness in the final product.

Nutritionals, Fort Washington, PA) was added to the priming solution for the aardbeien lambic treatments in order to back-sweeten these treatments. The heat pasteurized KK was pasteurized at 60°C for 20 minutes and the filter pasteurized KK was filtered with a Millipore disposable vacuum-driven .45 micron filter bottle attachment unit (Billerica, MA) (see Materials and Methods section *D. Production of Soy Kefir,* sub-section *c) Pasteurization of Soy Kefir*). These treatments were also used in the accelerated shelf-life study. The treatment breakdown is as follows:

Table 23. Aardbeien Lambic with Different KK Pasteurization Methods Treatment Breakdown

Treatment #	Treatment Volume (L)	Total IBU	Volume of KK heat pas-teurized (ml)	Volume of KK filter pas-teurized (ml)	Strawberry flavor (ml)	Sucralose (g)
003	3.19	8	0	0	22.45	3.36
512	3.19	8	0	786.86	22.45	3.36
920	3.19	8	786.86	0	22.45	3.36
Total	9.57	NA	786.86	786.86	67.35	10.08

D. Production of Soy Kefir

a) Without Diacetyl Rest

A particular strain of kefir grains obtained from KCLM Research in Nutrition Inc. was utilized for the soy kefir production. Skim milk and unsweetened soy milk (Silk brand, WhiteWave Foods, Broomfield, CO) was purchased from local supermarkets. All equipment was sanitized prior to its utilization in the process. The grains were stored in airtight plastic freezer bags with an equal volume of skim milk in the freezer (-20°C);

therefore, first the grains had to be thawed by leaving the freezer bag at room temperature (22°C) for a period of approximately 18 hours. Once completely thawed, the grains were aseptically transferred onto a fine sieve and gently rinsed with distilled water. After the grains were drained, they were weighed. Meanwhile, a five gallon food grade bucket was sanitized and prepared with the following ingredients per 100 grams of grains:

- 1.0 L of skim milk
- 10 g of dextrose (Fisher Scientific, Pittsburgh, PA)
- 2 g of potassium citrate (Fisher Scientific, Pittsburgh, PA)

The grains were added to this milk solution and the bucket was lightly covered with its sanitized lid. The mixture was left to ferment in an environment with an ambient temperature of 22°C for a period of twenty-four hours to allow for re-activation of the kefir grains. Once the fermentation was complete, the grains were collected off the top of the kefir, the kefir was carefully mixed with sanitized stainless steel slotted spoon, and the pH of this solution was taken and recorded (fully activated grains should be able to drop the pH of the milk below 4.5 within a twenty-four hour period). This kefir was discarded. Some grains may be at the bottom of the fermentation vessel, but they were not collected because they were not properly re-activated; active grains will not flocculate out of the matrix and because carbon dioxide is being produced, the active grains are shuttled upwards. These steps were usually performed two-three times before inoculating the grains into unsweetened soymilk.

Once grains were fully activated and ready to be inoculated into the soymilk, the grains were collected off the top of the kefir per the usual, but then gently rinsed with

distilled water. After the grains were drained, they were weighed. Meanwhile, one, or two, five gallon food grade buckets were sanitized and prepared with the following ingredients per 100 grams of grains:

- 4.0 L of unsweetened, unflavored pasteurized soy milk (Silk brand, White Wave Foods, Broomfield, CO)
- KK-A (just glucose) and all other soy kefir productions except KK-B→ 40 g of dextrose (glucose)
- KK-B (glucose and fructose[13])→ 20 g of glucose and 20 g of fructose (Spectrum Laboratories, Rancho Dominguez, CA)

The grains were added to this milk solution and the bucket was lightly covered with its sanitized lid. The mixture was left to ferment in an environment with an ambient temperature of 22°C for a period of twenty-four hours to allow for re-activation of the kefir grains. Once the fermentation was complete, the grains were collected off the top of the soy kefir, the kefir was carefully mixed with a sanitized stainless steel slotted spoon, and the pH of this solution was taken and recorded. This kefir was discarded. These steps were usually performed two-three times before grains were fully activated and adjusted to the soymilk matrix (the grains are able to drop the pH of the soy milk below 4.5 and protein coagulation is observed). Then these steps were repeated once more after the grains were fully activated for the actual production of the soy kefir. After the twenty-four hour fermentation period, the kefir grains were once again aseptically collected off the top

[13] Fructose was also added here as a substrate because Athanasiadis, et al found that the utilization of fructose as substrate in kefir production increased the amounts of desired volatile aromatics, for example, ethyl acetate (fruity) and amyl alcohols- more specifically, 2-methyl-1-butanol (isoamyl alcohol, or banana) and 3-methyl-1-butanol (fusel/solvent-like note) (2001).

of the kefir, the kefir was gently mixed, and the pH of the kefir was taken. The collected grains were placed in an airtight plastic freezer bag with an equal volume of skim milk and stored in the freezer (-20°C). The lid was placed back on top of the fermentation vessel, and the vessel was moved to a cold environment of approximately 4°C for a forty-eight hour maturation period. Once matured, an aliquot of the liquid soy kefir was aseptically collected into sanitized 64 oz glass amber growlers (donated by Big Boss Brewing Co., Raleigh, NC). The kefir was then centrifuged 1L at a time in autoclaved 250 ml plastic centrifuge vessels at 3500 RPM for 20 min with a spin temperature of 22°C. The clarified kefir, or Kristalkefir (KK), was aseptically transferred back into new sanitized 64 oz glass amber growler bottles, sealed with an oxygen barrier growler cap, and stored in a refrigerated environment of 4°C until further use.

b) With Diacetyl Rest

For KK production with a diacetyl rest, the previously mentioned production protocol was performed, but after the kefir was clarified, it underwent the diacetyl rest prior to "crash-cooling" it to 4°C. The diacetyl rest procedure consisted of inoculating the KK with krausened *Saccharomyces cerevisiae* yeast to result in a yeast count of 10^6 cfu/ml, followed by incubation in a New Brunswick incubator/shaker (New Brunswick, NJ) at 28°C for a period of twenty-four hours. During the production of beer, this rest period allows for active yeast to convert all potential diacetyl to free diacetyl, uptake all diacetyl within the matrix, and then reduce it into the non-offensive flavor-active volatile, 2,3-butanediol. The yeast utilized for this rest was Wyeast Bavarian Wheat (3638) (Odell, OR), which was obtained from a LHBS. In order to krausen, a starter with a target

original gravity (OG) of 1.040 was made from Light Dried Malt Extract (DME) wort (light DME (LD Carlson, Kent, OH) also purchased from a LHBS). 208.65 g of light DME was added to 7.6 L of hot water; this solution was mixed thoroughly and boiled for fifteen minutes to sterilize. After boiling was complete, the boiling vessel was placed in an ice bath to allow for a rapid cool-down. Once the wort was cooled to 26°C, it was aseptically transferred into a sanitized 2.5 gallon plastic fermentation bucket and inoculated with 62.5 ml of the Bavarian Wheat yeast smack pack. The vessel was sealed with its sanitized lid equipped with air lock. The wort was shook periodically across ten minutes to allow for proper aeration of the wort, allowing for an appropriate concentration of dissolved oxygen, a growth requirement for the yeast. The vessel was then placed in a dark, moderately cool environment of 20°C, and left to ferment. After approximately forty-eight hours, krausened yeast was collected from the fermentation vessel, and counted for yeast concentration. An appropriate amount of yeast/beer solution was then transferred from the beer matrix and inoculated into the KK, again to obtain a yeast concentration of 10^6 cfu/ml. After the rest was completed, the KK was stored at a temperature of 4°C.

c) Pasteurization of Soy Kefir

Two different pasteurization methods were utilized for the pasteurization of KK: heat pasteurization and filter pasteurization. For heat pasteurization, after the diacetyl rest has been completed and the KK has been "crash cooled" and held in the 4°C environment for at least forty-eight hours, the KK was aseptically transferred into an autoclaved 500 ml glass Corning Bottle. A sanitized magnetic stirrer was added to the bottle, a thermometer

was attached to the mouth of the bottle in a manner that allowed for the tip of it to be suspended within the KK matrix, and secured with label tape and Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL), which was stretched atop the opening of the bottle. The bottle was then placed onto a pre-heated stir/hot plate, and heated to 60°C, which took about 30 minutes. The KK was stirred the entire time at a moderate speed to allow for heat homogeneity within the matrix. The KK was then held at 60°C for 20 minutes to allow for thermal inactivation of microorganisms, and furthermore, adequate heat pasteurization of the KK[14]. After the 20 minutes had elapsed, the bottle was immediately transferred into an ice bath atop another stir plate. This mixture was stirred at a moderate speed to allow for a quick cool-down period, and once at a temperature of 22°C, which took approximately 15 minutes, the thermometer was removed. Then, the bottle was aseptically re-capped, and then stored at a temperature of 4°C.

For filter pasteurization of the KK, after the diacetyl rest was completed and the KK "crash cooled" and held in the 4°C environment for at least forty-eight hours, the KK was aseptically transferred into a Millipore disposable vacuum-driven .45 micron filter bottle attachment unit (Billerica, MA) that was secured to an autoclaved 1000 ml Corning Bottle. The unit was then attached to the vacuum source and filtered. This procedure was repeated numerous times in order to filter the required amount. After the filtration was complete, the bottle was aseptically re-capped, and stored at a temperature of 4°C.

E. Accelerated Shelf-Life Test

The aardbeien lambic treatments with different KK pasteurization methods were

[14] These parameters were based upon the results form the *Pasteurization and Thermal Inactivation Study*.

utilized for this test. After treatments were done bottle conditioning, they were all placed in a dark environment with an ambient temperature of 22°C. The acceptable temperature that craft beer should be stored at is around 5°C- refrigerator temperature, with a shelf-life expectancy of four months, or 122 days; therefore, by storing the treatments in an elevated temperature, the reactions that take place over its shelf-life are sped-up allowing for a quicker shelf-life- hence accelerated shelf-life testing. Samples were tested at day 0 microbiologically, descriptively, hedonically (blind bench with panel), and also for major isoflavones. Samples were set aside to be tested at various stages in the shelf-life for isoflavone analysis as well as descriptive analysis. Alongside month 0, treatments were tested at day 45 (1.5 months), day 90 (3 months), day 136 (approximately 4 months), and day 160 (approximately 5 months). These projections are based off the Arrhenius and Q_{10} equations, with a Q value of 2 (which is usually set at a constant value of 2, 3, or 4 with a value of 2 predicting a more conservative shelf-life (Magari, 2003)):

$$t_{predicted} = (t_{actual}) \times (Q^{n}); where\ n = \frac{(T_2 - T_1)}{10°C}$$

Figure 8. Accelerated Shelf-life Prediction Equation

$t_{predicted}$ = predicted time (in days) for shelf life; t_{actual} = time in days of real-time shelf storage; Q (a constant) = 2; = accelerated shelf storage temperature; and T = appropriate storage temperature. The month four beer sample, a term shelf-life beer, was also tested microbiologically.

CHAPTER 3. RESULTS

Preliminary Bench Testing

The results from the first preliminary bench test that was performed in order to choose an ale style that is best suited for the Soy Kefir Powder (SKP) can be viewed in Table 25. Of the three highest scoring treatments (Samuel Smith Russian Imperial Stout, Widmer Hefeweizen, and Stone IPA), treatment 689 was not significantly scored higher (with a p-value of .05) than 123, but 387 was rated significantly higher than 123 and 689. Treatment 387 was also selected as the preferred sample the most often.

Table 24. Preliminary Bench Study #1

Sample #	Beer +5.0 g SKP/ 355 ml beer	Average Score
475	Guinness Extra Stout	1.4
264	New Belgium Fat Tire	2.6
123	Samuel Smith's Russian Imperial Stout	2.8
689	Widmer Hefeweizen	3.6
387	Stone IPA	5.3
032	Carolina Blonde Ale	2.4

From these highest-ranking treatments, a second study was done with different concentrations of SKP- 5.0 g, 7.5g/ 355 ml beer. The results from this preliminary bench test can be viewed in Table 25.

Table 25. Preliminary Bench Study #2

Sample #	Beer	Amount of SKP/ 355 ml beer	Average Score
127	Stone IPA	5	5.3[ab]
263	Stone IPA	7.5	5.83 [cd]
349	Kona Pipeline Porter	5	3.5 [ac]
412	Kona Pipeline Porter	7.5	3.8 [bd]
671	Wolander's Oatmeal Stout	5	4.3
733	Wolander's Oatmeal Stout	7.5	4.8

Overall, the IPA treatments were ranked higher than the other ale style treatments, and they were significantly higher than the Porter treatments. Although the IPA treatment with 7.5 g of SKP/ 355 ml beer serving was scored slightly higher, it was not significant. Treatment 127 was selected the preferred sample the most often. After each test, the treatments were discussed with the panel, and it was noted that the bitterness supplied by the IPA style helped to mask some of the SKP offensiveness (beany, grassy, waxy notes, for example). Withstanding this, the ale style of IPA was selected to be the intended ale style of this product because it was the most acceptable and compatible with the SKP addition.

Design Specifications

From the preliminary bench tests, design specifications for the nutraceutical beer via biological isolates of soy kefir could be made. As mentioned previously, a style of IPA showed to be the best choice, and while an SKP concentration of 7.5 g/ 355 ml beer serving was ranked slightly higher than with a concentration of 5.0 g/ 355 ml beer serving, this was not significant and therefore a concentration of 5.25 g/ 355 ml beer serving was selected (slightly higher than 5.0 g, thus allowing for a slightly higher intake of SKP per serving). In continuation it was noted that the SKP contributed quite a turbid appearance to the beer treatments, which increased as the

SKP concentration increased, therefore, a lesser concentration of SKP logically seemed more appropriate considering high turbidity in beer is not typically desired. However, an ale style that is accepted with a fair amount of turbidity is the wheat/weizen/weiss category. This phenomenon is largely due to the particular yeast strain utilized for these fermentations (lower flocculating yeast), as well as the higher protein concentration, which is contributed by the larger concentrations of wheat utilized (40-70% of the grain bill). Withstanding this, a hybrid style was selected- an IWA, or India Wheat Ale; therefore, labeling it as a wheat ale, consumers will accept the product more considering its cloudy appearance. In conclusion, product specifications were made based off this data, perceptions, and ideas, and a baseline formula was created for this nutraceutical beer. This baseline formula can be viewed in the Appendix. These specifications are: an IWA nutraceutical beer, made with a grain bill comprised of 54% 2-row pale base malt, 29% white wheat malt, 6.25% caramel malt at 40°L, 4.8% honey malt, and 2.4% Carapils; with an IBU of 85 supplied by the Magnum, Centennial, and Amarillo hop cultivars; inoculated with Wyeast Bavarian Wheat (3638) (Odell, OR) *Saccharomyces cerevisiae* strain; fermented at a temperature of 21°C; and with added Soy Kefir Powder downstream at a concentration of 5.25 g/ 355 ml beer serving (1.5%) prior to carbonation.

In-house Guidance Testing/ Descriptive Analysis

Training

As mentioned previously, the panel trained over a period of six months for a total of 40 hours. The training period resulted in a panel that could be utilized as an

instrument for ale orthonasal aroma and flavor detection and profiling. The results from an earlier session can be viewed in Figures 9 and 10 and the results from a later session can be viewed in Figures 11 and 12. These results show that while at first the panel was not able to objectively analyze ales, through an extensive training period they demonstrated that they were a well-calibrated instrument.

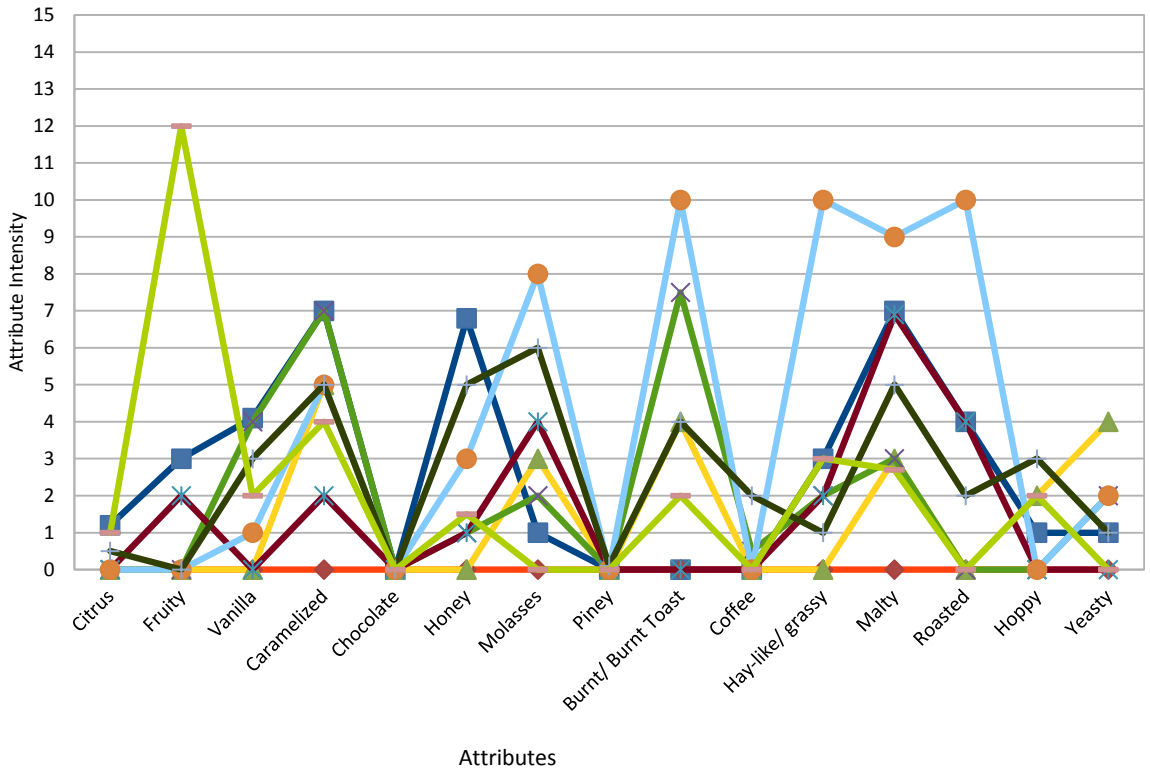

Figure 9. Orthonasal Aroma Profile for an Ale Treatment at the Beginning of Training

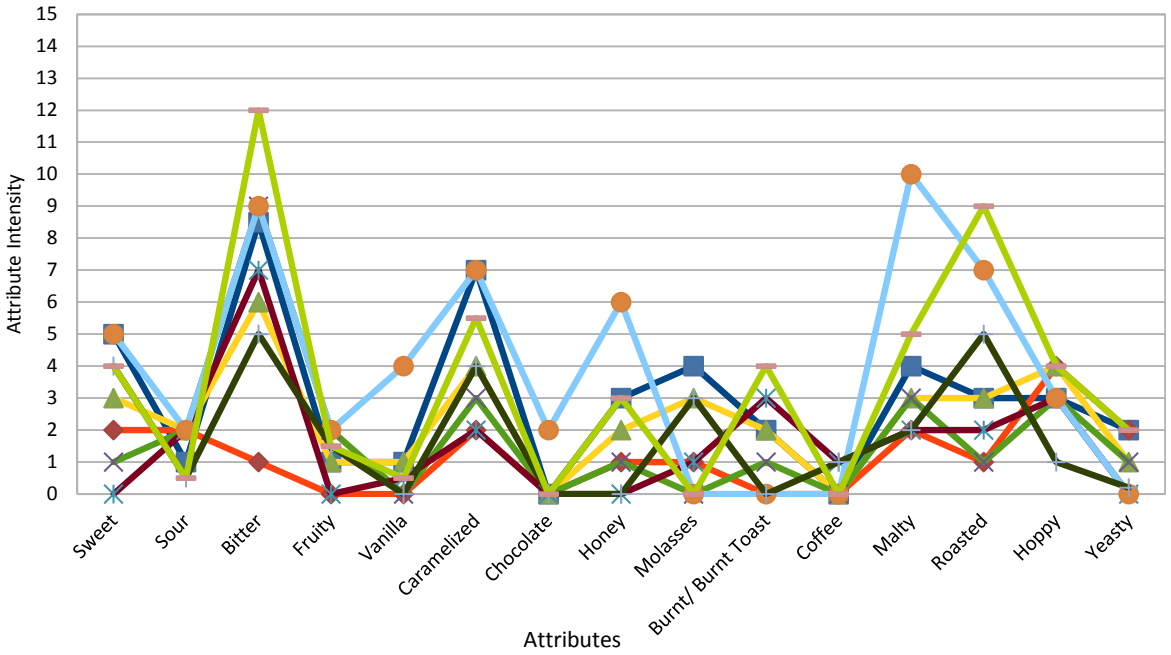

Figure 10. Flavor Profile for an Ale Treatment at the Beginning of Training

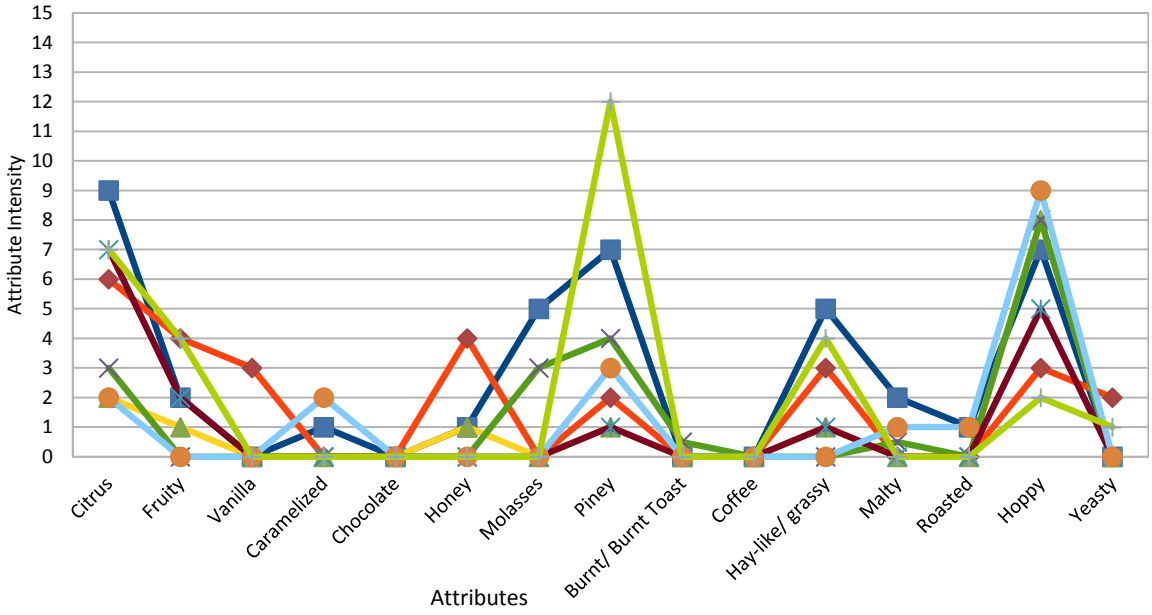

Figure 11. Orthonasal Aroma Profile for an Ale Treatment at the End of Training

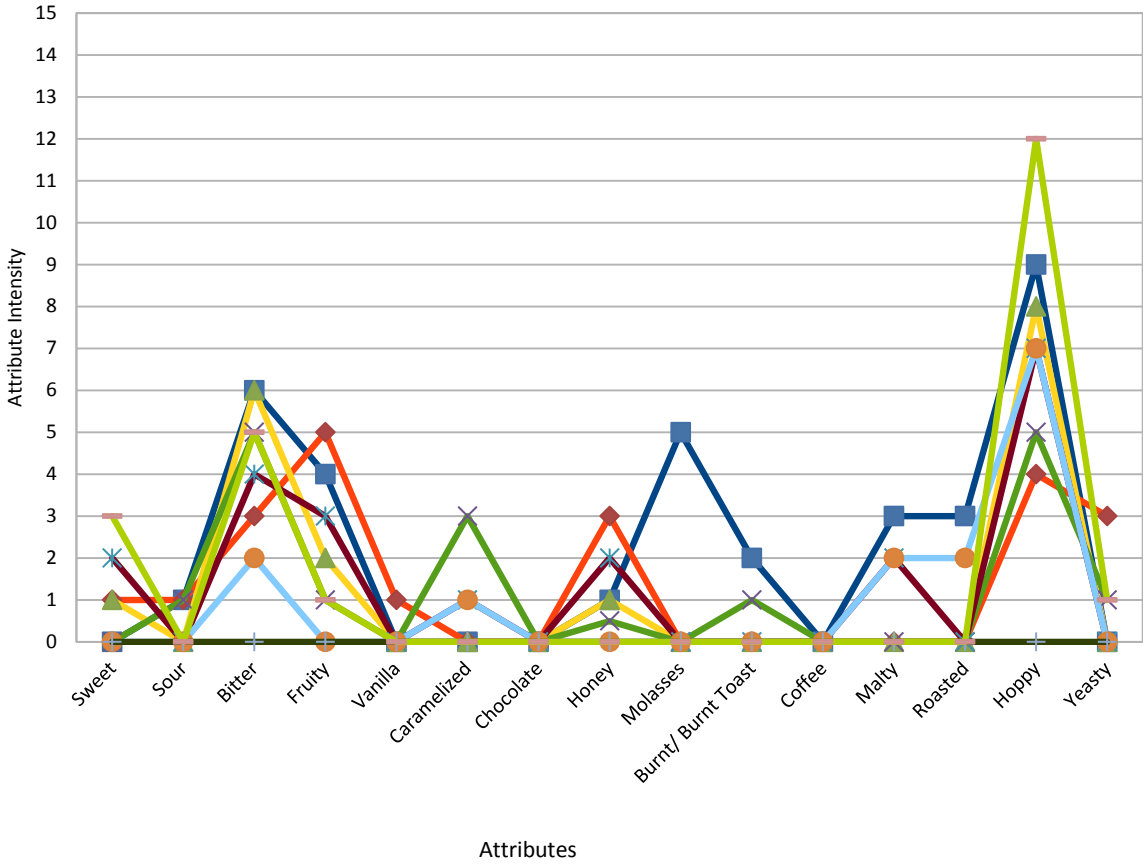

Figure 12. Flavor Profile for an Ale Treatment at the End of Training

Soy Kefir Powder Stability in Beer Matrix

Flocculation was noted substantially in the treatments unless xanthan gum was added at a concentration of at least 0.25%; however, at this high of a concentration, it was unacceptably turbid throughout the entire sample (figure 13). Withstanding this, it can be inferred that SKP is not stable in a beer matrix in terms of appearance, resulting in flocculation (figure 14), or an unacceptable turbid appearance where xanthan gum is added.

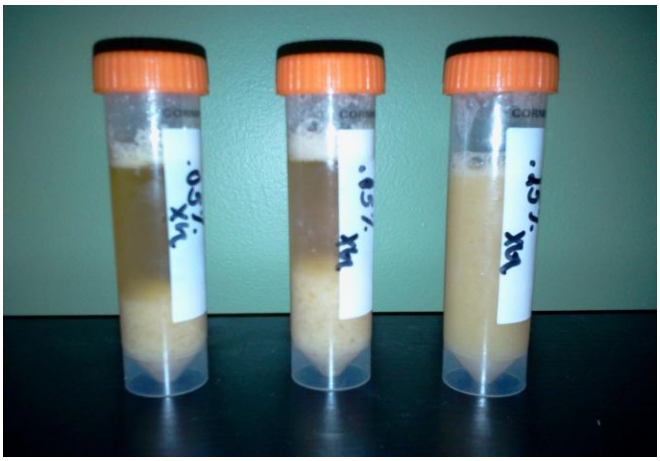

Figure 13. Stability of SKP in Beer Treatments with Xanthan Gum

Figure 14. Flocculation of SKP in Beer Matrix

IBU Variance Study with Soy Kefir Powder and IBU Variance Study with Kristalkefir

Blind Bench-top

Both of these studies were done separately, however, the results will be combined due to the need to compare and see the importance of the kefir variable. The results (the mean hedonic scores) can be viewed in the following table. Figures

15 and 16 also show the variances in treatments as well.

Table 26. Comparison of the Acceptance of Varying IBU with SKP and KK and their Control Beer Treatments

IBU	SKP Beer Treatments	KK IBU Treatments	Control Beer
50	2.75[ab]	3.5 [ag]	5.42 [b]
60	2.81	3.25 [cg]	5.04 [c]
70	2.5 [d]	2.88 [eg]	5.42 [de]
80	2.44	2.25 [fg]	4.71 [f]

* treatments with the same letter denotes significant difference with a p-value of .05 or less, this is true for all result tables, unless otherwise noted

From Table 26, it can be known that SKP beer treatments at 50 and 70 IBU are significantly less accepted than their beer controls, and KK beer treatments at 60, 70, and 80 IBU accepted significantly less than their beer controls. While change in IBU does not significantly affect the acceptance of the controls, the change does have significant affect in the kefir treatments. In the Kristalkefir treatments, as IBU increases, acceptability decreases significantly; therefore, this leaves KK 50 IBU as the most accepted of the KK treatments, which is also the only KK treatment not significantly less accepted than its control, and it is also significantly accepted more than its SKP counterpart (SKP at 50 IBU). Withstanding this, it would appear that a 50 IBU KK Beer treatment would be the best option, and therefore, the most accepted

(the 50 IBU control was tied with the 70 IBU for the most accepted control treatment).

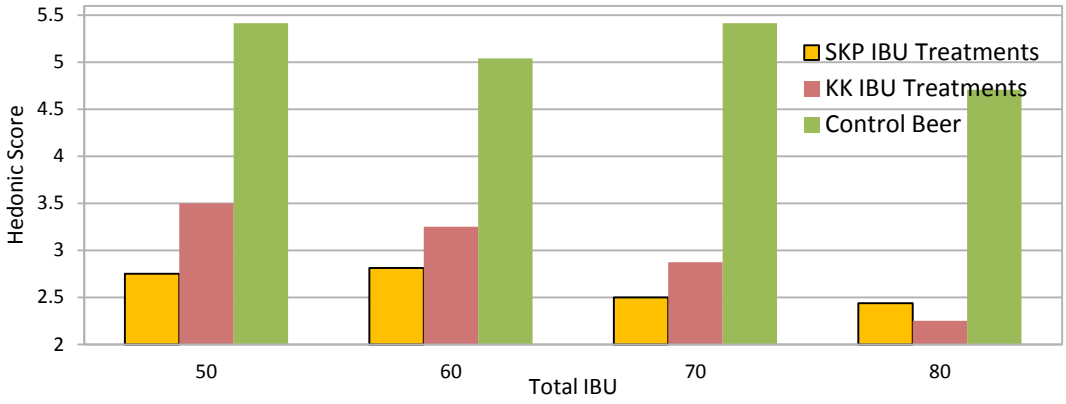

Figure 15. Acceptability of Varying IBUs of Kefir and Beer Treatments

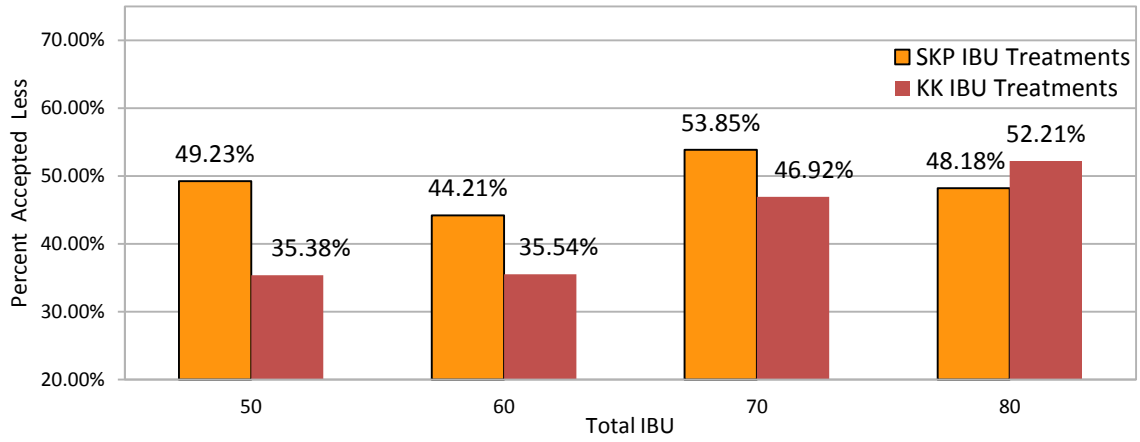

Figure 16. Acceptability Percent of Control for Varying IBUs of SKP and KK Beer Treatments

Descriptive Analysis

The descriptive analysis results can be viewed through Tables 27 and 28, as well as Figures 17 and 18.

Table 27. Flavor Profile of Different SKP and KK Treatments at Varying IBUs

Attribute	50 IBU+SKP	50 IBU+KK	50 IBU	60 IBU+SKP	60 IBU+KK	60 IBU
Sweet	2.4 [ac]	1.4[ab]	2.0 [bd]	2.4 [e]	1.3 [cde]	1.9
Sour	2.3	2.2	1.4	1.8	1.7	1.1
Bitter	2.0 [acde]	3.5 [ab]	3.5 [cf]	2.5 [bgh]	3.9[dg]	4.4 [efh]
Fruity	2.5[ac]	1.3[ab]	2.2[d]	2.9[be]	1.0[cde]	1.9
Vanilla	0.3	0.3	0.5	0.4	0.3	0.2
Caramelized	0.4[a]	0.4	0.8 [a]	0.5	0.4	0.5
Chocolate	0.0	0.1	0.0	0.0	0.0	0.0
Honey	0.6	0.6	0.5	0.7	0.3	0.6
Molasses	0.0	0.0	0.2	0.1	0.0	0.3
Burnt/ Burnt Toast	0.0	0.1	0.1	0.0	0.0	0.3
Coffee	0.0	0.3	0.0	0.0	0.0	0.0
Malty	0.9	0.5	1.3	0.8	0.6	1.1
Roasted	0.1	0.4	0.0	0.0	0.3	0.2
Hoppy	1.8 [cd]	2.1 [ab]	4.2 [acef]	1.9 [fg]	2.1 [eh]	4.0 [bdgh]
Yeasty	1.3	1.5	1.3	1.4	1.4	1.4
Waxy/beany	1.9 [abc]	0.8 [ag]	0.0 [bde]	2.1 [efgh]	0.9 [dh]	0.1 [cf]
Diacetyl	1.4 [ch]	2.4 [ab]	0.1 [acde]	1.8 [ef]	3.6 [dg]	0.3 [bfgh]

Table 28. Orthonasal Aroma Comparison of Different SKP and KK Treatments at Varying IBUs

Attribute	50 IBU+SKP	50 IBU+KK	50 IBU	60 IBU+SKP	60 IBU+KK	60 IBU
Citrus	2.1 [a]	1.0 [abc]	2.3 [bd]	1.8	1.0 [de]	2.6 [ce]
Fruity	3.0 [c]	1.7 [ab]	3.7 [ad]	3.0 [e]	1.0 [cdef]	3.5 [bf]
Vanilla	0.5	0.8	1.0	0.6	0.9	1.2
Caramelized	0.2	0.3	0.5	0.4	0.3	0.7
Chocolate	0.0	0.0	0.0	0.0	0.0	0.0
Honey	0.7	0.5 [a]	1.6 [b]	0.6	0.6 [b]	1.3 [a]
Molasses	0.0	0.0	0.3	0.3	0.0	0.2
Piney	0.5	0.4	0.8	0.4 [b]	0.3 [a]	1.1 [ab]
Burnt/ Burnt Toast	0.0	0.0	0.0	0.0	0.0	0.0
Coffee	0.3	0.0	0.0	0.0	0.0	0.0
Hay-like/ grassy	1.0	0.6	0.5	1.1	0.6	0.6
Malty	0.4 [bd]	0.5 [ac]	1.9 [abef]	0.4 [e]	0.4 [f]	1.4 [cd]
Roasted	0.0	0.1	0.3	0.0	0.0	0.4
Hoppy	1.0 [b]	1.3 [a]	2.3	1.3 [d]	1.1 [c]	2.5 [abcd]
Yeasty	1.5	1.3	1.3	1.2	1.0	1.2
Waxy/beany	1.9 [acde]	0.4 [ab]	0.0 [cf]	1.5 [bfgh]	0.4 [dg]	0.0 [eh]
Diacetyl	2.2 [abd]	4.3 [acd]	0.3 [ae]	2.4 [cef]	4.9 [bef]	0.4 [df]

It is evident that there is a great deal of information to gather from this study showing differences between all the treatments, however, only a fraction of these show significant differences. Bitterness was the only real difference between 50 and 60 IBU treatments, however only the 60 IBU control was significantly more bitter than its 50 IBU counterpart, whereas both of the 60 IBU kefir treatments were not

significantly more bitter than their 50 IBU counterparts.

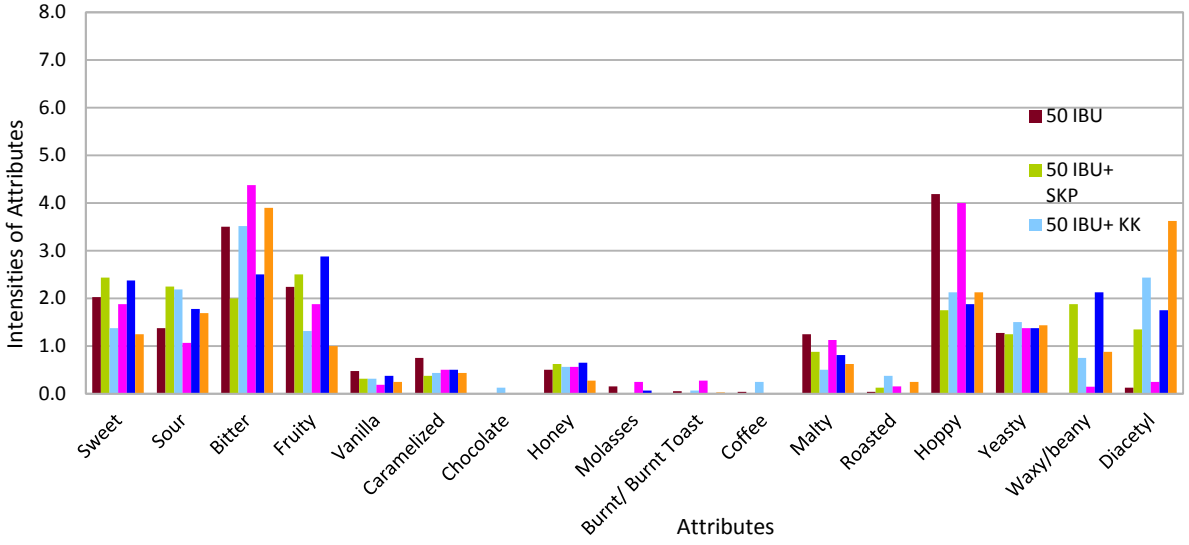

Figure 17. Flavor Profile Comparison of Varying IBU SKP and KK Treatments

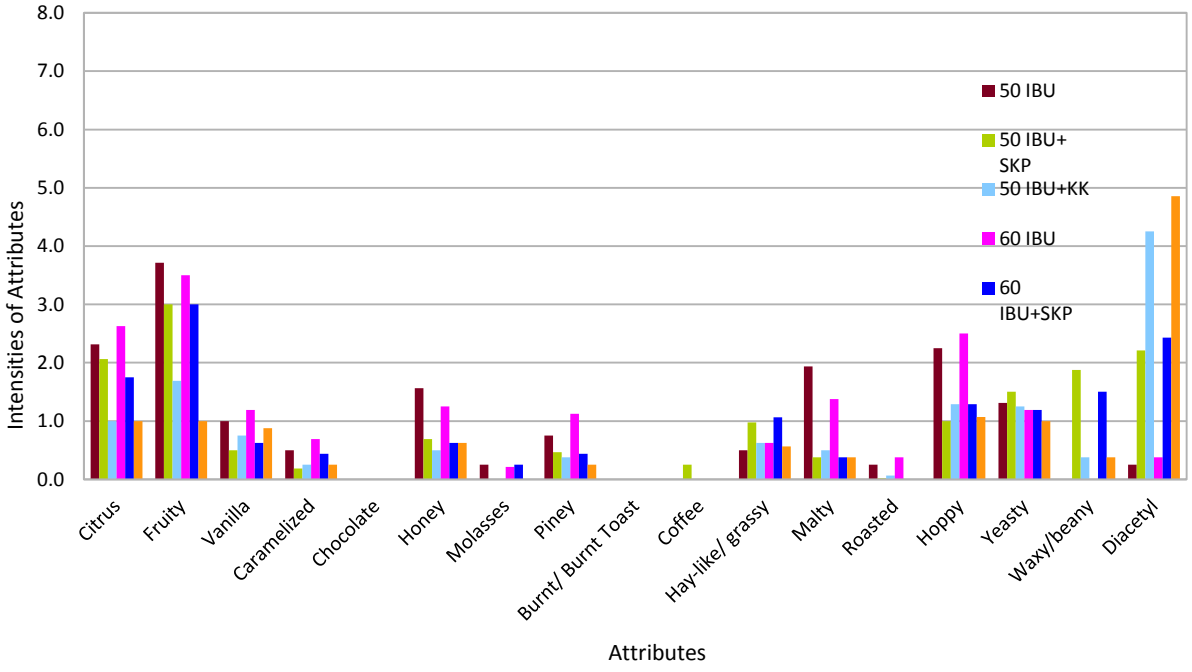

Figure 18. Orthonasal Aroma Profile Comparison of Varying IBU SKP and KK Treatments

The SKP treatments were significantly less bitter than both the KK and control treatments, which could be due to these treatments being perceived as significantly sweeter than the KK treatments (the SKP treatments were perceived as sweeter than the controls as well, but this difference is not statistically significant). The 50 IBU KK treatment was also perceived as significantly less sweet than its control as well. The SKP treatments were also significantly fruitier than the KK treatments for both 50 and 60 IBU for RA, and just the 60 IBU treatments for OA, and the SKP treatments were also significantly perceived with more waxy/beany notes than the KK treatments (OA and RA). Diacetyl in KK treatments was only significantly perceived more than the SKP treatments orthonasally. The controls had significant differences with the kefir treatments as well; they were perceived as significantly more bitter than the SKP treatments, for example. The controls were all perceived as hoppier than their kefir counterparts for both 50 and 60 IBU in RA, and just 60 IBU in OA. The SKP treatments significantly showed more waxy/beany notes than the controls for both OA and RA, and diacetyl was significantly much lower in the control treatments than the kefir treatments, and was virtually undetectable.

Production of Soy Kefir

Liquid soy kefir was utilized in the treatments throughout the rest of the project; compositional analysis was performed and can be viewed in the following two sections.

Total Solids Analysis of Soy Kefir

The results from the total solids analysis of the liquid soy kefir can be viewed in the following table.

Table 29. Total Solids Analysis of Liquid Soy Kefir

Sample	Total Weight (g)	Weight of Solids (g)	Total Liquid %	Total Solids %
1	10.87	0.7	93.56%	6.44%
2	12.87	0.82	93.63%	6.37%
3	10.50	.6	94.29%	5.71%
AVERAGE	**12.78**	**0.87**	**93.83%**	**6.17%**

Based upon the results, liquid soy kefir is 6.17% solids and 93.83% liquid, therefore, a solid powder concentration of 5.25 g/12 oz (355 ml) serving equates to a liquid soy kefir, kristalkefir, concentration of 89 ml/12 oz (355 ml) serving at approximately 6% total solids.

Major Fermentation By-Product Analysis and Composition of Soy Kefir via HPLC

The results from the HPLC analysis of fermentation by-products can be viewed through the following table and figure. It should be noted here that the ABV of the beer without added kefir was 5.2%.

Table 30. Major Fermentation By-Products and Constituents in Kristalkefir and Beer Treatments

Constituent Name	KK pre-pasteurized (g/100 ml)	KK pasteurized for 20 min at 60°C (g/100 ml)	KK filter pasteu-rized at .45µ (g/100 ml)	Aardbeien Lambic with KK heat pasteu-rized (g/100 ml)	Aardbeien Lambic with KK filter pasteurized (g/100 ml)
Dextrin	0.17	0.12	0.12	2.52	2.54
Maltotriose	0.11	0.12	0.12	0.00	0.00
Lactose	0.07	0.07	0.08	0.05	0.05
Citric Acid	0.00	0.00	0.00	0.01	0.01
Pyruvic Acid	0.12	0.12	0.12	0.04	0.04
Mannitol	0.10	0.10	0.10	0.01	0.01
Lactic Acid	1.12	1.13	1.13	0.20	0.19
Glycerol	0.00	0.00	0.00	0.06	0.06
Acetic Acid	0.00	0.00	0.00	0.00	0.00
Ethanol = ABV%	0.21	0.23	0.22	4.22	4.23

Pasteurization and type of pasteurization appears to have no effect on these constituents/compounds, with the exception of a small difference in dextrin level, which is slightly higher in the un-pasteurized treatment. Outside of this difference, all of the kristalkefir treatments were virtually the same, and so were the beer+KK treatments.

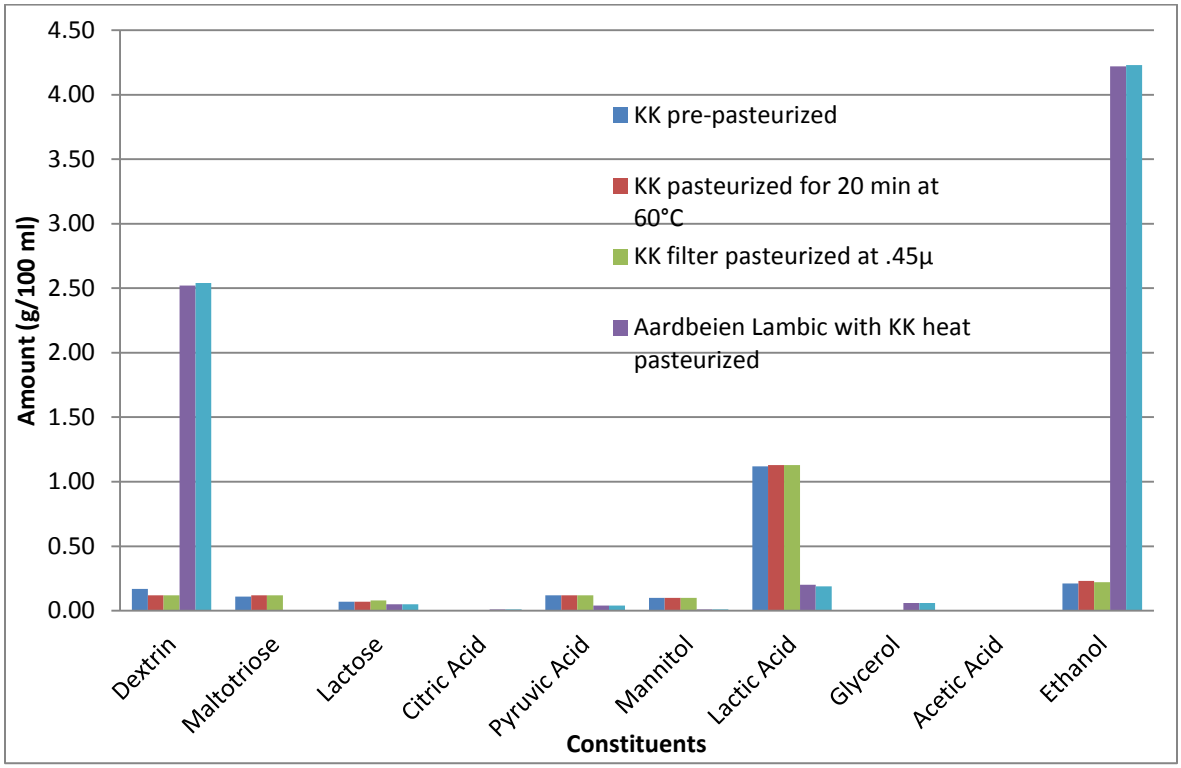

Figure 19. Major Fermentation By-Products and Constituents in Kristalkefir and Beer Treatments

Elimination of Aromatic compound diacetyl

Descriptive Analysis

The results from the different diacetyl rest KK beer treatments can be viewed in Tables 31 and 32, and Figures 20 and 21.

Table 31. Flavor Profile of Different Diacetyl Rest KK+Beer Treatments[15]

	126.0	**362.0**	**912.0**	**600**
Treatment Description	**50 IBU + 0 D-rest KK**	**50 IBU + 24 hr**	**50 IBU + 48 hr**	**50 IBU**
Sweet	2.01	2.20	1.82 [a]	2.21[a]
Sour	3.08	3.43 [n]	3.67 [a]	2.13 [an]
Bitter	3.57 [o]	3.19 [n]	3.20 [a]	4.38 [ano]
Fruity	2.46 [a]	3.18 [n]	2.28	2.17 [an]
Vanilla	0.28 [a]	0.21	0.17 [a]	0.38
Caramelized	0.46 [a]	0.33	0.30 [a]	0.49
Chocolate	0.00	0.00	0.00	0.00
Honey	0.64	0.67 [n]	0.42 [n]	0.75
Molasses	0.00 [b]	0.00 [a]	0.10	0.12 [ab]
Burnt/ Burnt Toast	0.07 [c]	0.05 [b]	0.04 [a]	0.30 [abc]
Coffee	0.00	0.00	0.04	0.04
Malty	0.77 [bn]	1.05 [n]	0.89 [a]	1.41 [ab]
Roasted	0.02 [a]	0.05 [b]	0.17	0.30 [ab]
Hoppy	1.66 [ab]	2.71 [acn]	1.87 [no]	3.17 [bco]
Yeasty	1.33	0.98 [an]	1.29 [a]	1.40 [n]
Waxy/beany	0.0	0.0	0.0	0.0
Diacetyl	3.53[np]	2.79[no]	4.28[oq]	2.77 [pq]

[15] intensities with the same letter are significantly different: letters *a* through *m* are significant with a p-value of .05 or less, and *n* through *z* with a p-value of .10 or less

Table 32. Orthonasal Aroma Profile of Different Diacetyl Rest KK+Beer Treatments[16]

	126.0	**362.0**	**912.0**	**600**
Treatment Description	**50 IBU + 0 D-rest KK**	**50 IBU + 24 hr**	**50 IBU + 48 hr**	**50 IBU**
Citrus	2.70 [n]	2.35	2.01 [n]	2.14
Fruity	3.74 [a]	3.24	3.22 [an]	3.78 [n]
Vanilla	0.53	0.63	0.60	0.71
Caramelized	0.39	0.39	0.37 [n]	0.62 [n]
Chocolate	0.10	0.00	0.06	0.10
Honey	0.77	0.66	0.79	0.76
Molasses	0.05	0.14 [n]	0.02 [n]	0.24
Piney	0.63 [a]	0.43 [an]	0.61 [n]	0.62
Burnt/ Burnt Toast	0.00	0.17	0.00	0.00
Coffee	0.00	0.00	0.00	0.00
Hay-like/ grassy	0.58 [a]	0.57 [b]	0.58 [c]	0.74 [abc]
Malty	0.63	0.90	0.94	1.78
Roasted	0.09 [n]	0.00 [an]	0.08 [b]	0.09 [ab]
Hoppy	1.36 [a]	1.85 [bn]	1.01 [n]	1.71 [ab]
Yeasty	1.62	1.39 [n]	1.67 [n]	1.46
Waxy/beany	0.0	0.0	0.0	0.0
Diacetyl	3.92 [np]	3.40 [nqo]	4.91 [ao]	3.11 [apq]

[16] intensities with the same letter are significantly different: letters *a* through *m* are significant with a p-value of .05 or less, and *n* through *z* with a p-value of .10 or less, this is the case for the remainder of the data

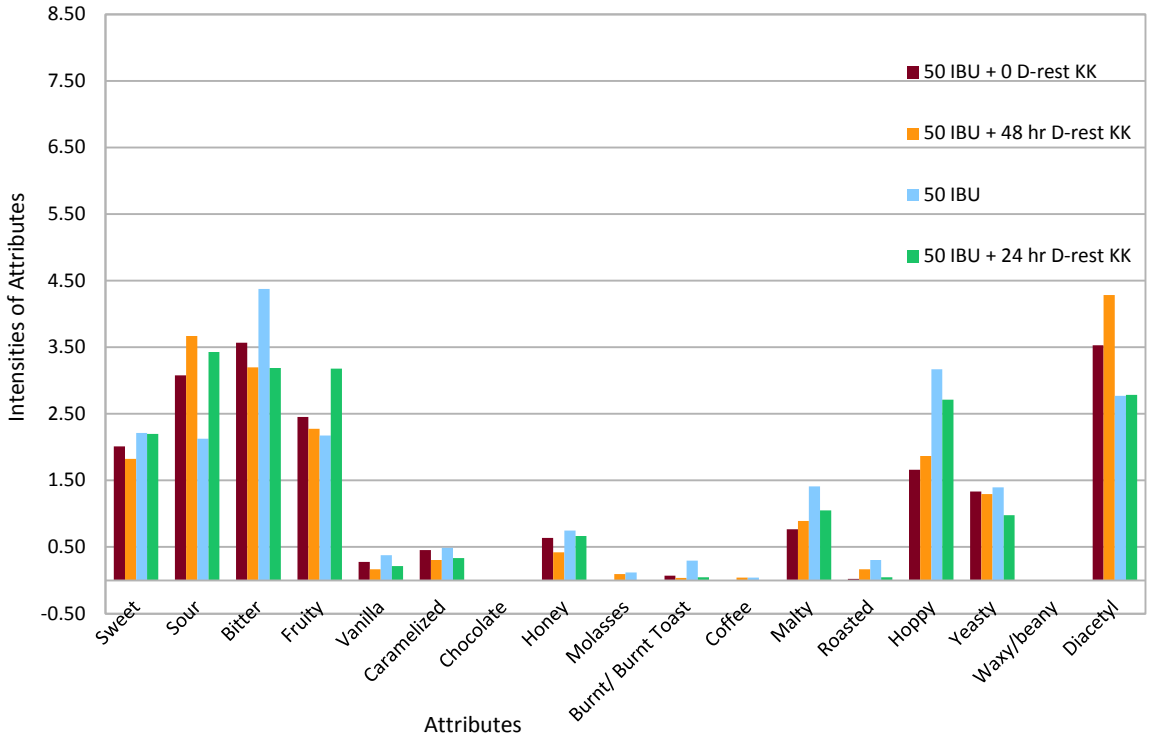

Figure 20. Flavor Comparison for Different KK Diacetyl Rest+Beer Treatments

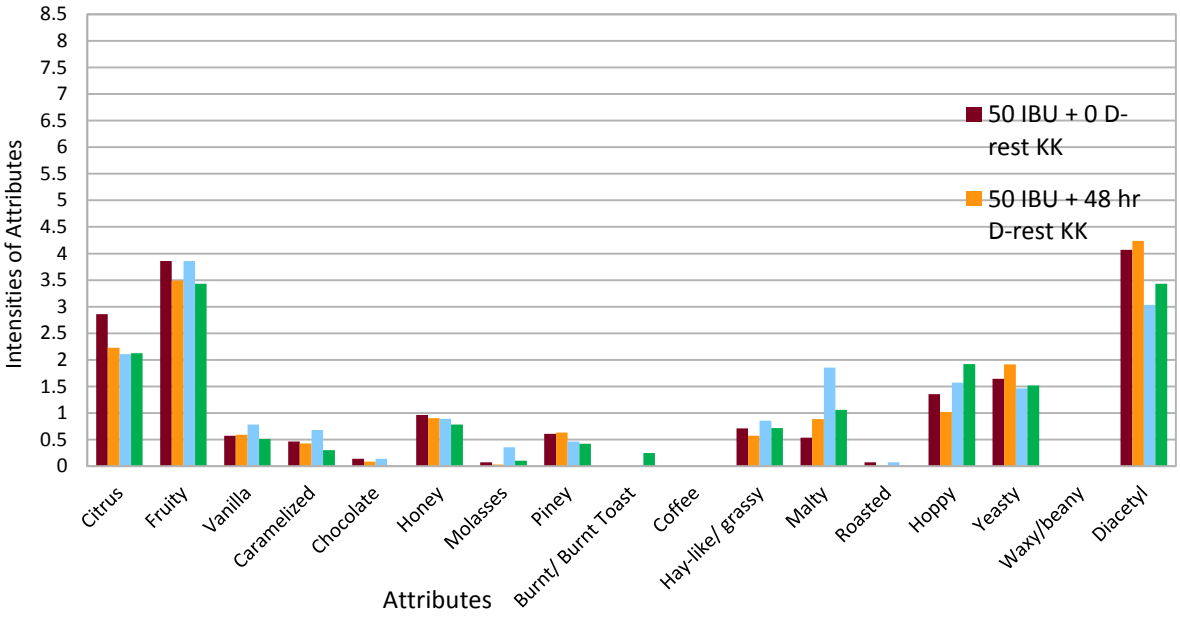

Figure 21. Orthonasal Aroma Comparison for Different KK Diacetyl Rest+Beer Treatments

There is a great deal of information to gather from this study showing differences between all the treatments, however, a handful of these show significant differences. The control is only significantly sweeter than the beer with KK48, less sour than both the beer treatments with KK24 and KK48, and more bitter than all the treatments with KK. The control is also significantly retronasally less fruity than the beer treatments with KK0 and KK24, and orthonasally more fruity than the beer treatment with KK48. Other significant differences of the control includes more caramelized than beer with KK48 (OA), more burnt/burnt toast than all of the KK treatments (RA), maltier than beer with KK0 and KK48 (RA), more hoppy retronasally than all KK treatments and orthonasally for beer with KK0, and less hoppy orthonasally than beer with KK24, more yeasty than beer with KK24 (RA), and less diacetyl than beer treatments with KK0 and KK48. The beer treatment with KK without a diacetyl rest has significantly more vanilla and caramelized notes (RA) and more fruity and citrus notes (OA) than the beer treatment with KK48, less malty and hoppy notes (RA), and more perceived pineyness and diacetyl (OA and RA) than the beer treatment with KK24. The beer treatment with KK24 has significantly more honey (RA) and hoppy notes (OA and RA), less yeastiness (OA and RA), molasses notes (OA), pineyness (OA), and diacetyl (OA and RA) than the beer treatment with KK48. There was no significant difference for diacetyl between the control and the beer treatment with KK at a 24-hour diacetyl rest, which this alone would allow for a more desirable product than the KK without a diacetyl rest, and with a rest of 48 hours. Another advantage of a 24-hour diacetyl rest would be the creation of a

perceived profile possessing desirable ale characteristics (sweeter, more fruity (RA), slightly more honey (RA), maltier, and hoppier).

Diacetyl Analysis via HPLC

The results from the analysis can be viewed in the following Figures (22 and 23).

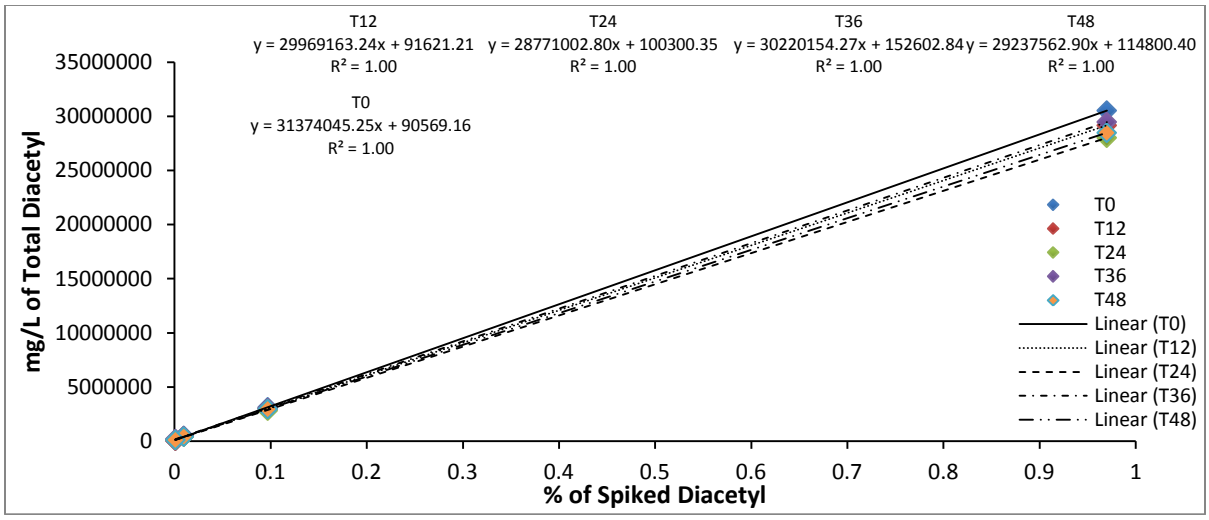

Figure 22. Diacetyl Analysis of Kefir Throughout the Diacetyl Rest Process

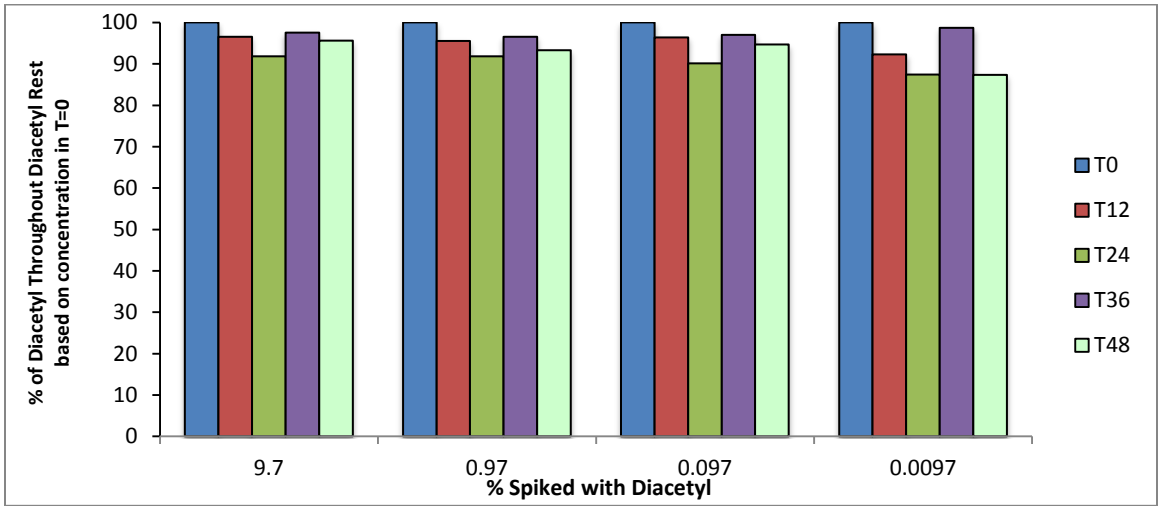

Figure 23. Percent of Diacetyl Change Throuhgout Diacetyl Rest Based on T=0

From the results, one can see that the general trends are that the kefir samples without the diacetyl rest (T=0) have the highest amount of diacetyl, and at 24 hours of rest period, the diacetyl is the lowest, but then the diacetyl increases after the 24-hour period. From the results, it can be known that at 36 hours of rest-period the diacetyl increases a fair amount, but then is on the decline at hour 48. It should be noted, however, that these changes in diacetyl levels are fairly small.

Strawberry Fruit Flavoring Usage Trial

Strawberry note was not apparent in a masking capacity until its concentration reached 4 oz/ 5 gallons of kefir beer, or 185µl/29.57 ml of kefir beer (treatment F). The next higher concentration, 208µl/29.57 ml of KK beer (treatment G), had a more desirable strawberry flavor and appeared to have a better masking effect; however, at the next highest concentration, and the highest at 231µl/29.57 ml (treatment H), the strawberry flavor was almost nauseating, and was deemed inappropriate. Withstanding this, treatment G's strawberry flavoring concentration was deemed most appropriate and selected to be utilized in the aardbeien lambic treatments.

Soy Kefir IPA and Soy Kefir in Aardbeien analogue Study

Both of these sets of treatments were analyzed by the panel altogether, therefore their data and results will be showed and discussed altogether.

Blind Bench-top, Panel

The results from this test can be viewed through the following table. It was found that the controls were accepted more than their kefir treatment counterparts, and the KK A treatments were accepted more than the KK B treatments.

Table 33. Comparison of the Acceptance of KK IPA and Aardbeien Treatments

	KK A	KK B	Control
IPA	5.09	4.65[ab]	5.91 [a]
Aardbeien	4.86	4.39	5.39 [b]

Overall, it was found that the IPA treatments were accepted more than the aardbeien treatments; however, the only significant difference found was between the KK B IPA treatment and both of the controls- it was accepted less than the controls. It was also found that despite the IPA treatments being accepted more overall than the aardbeien treatments, they, however, had a greater accepted less percentage when compared to their control than the aardbeien treatments did when compared to their control (Figure 24).

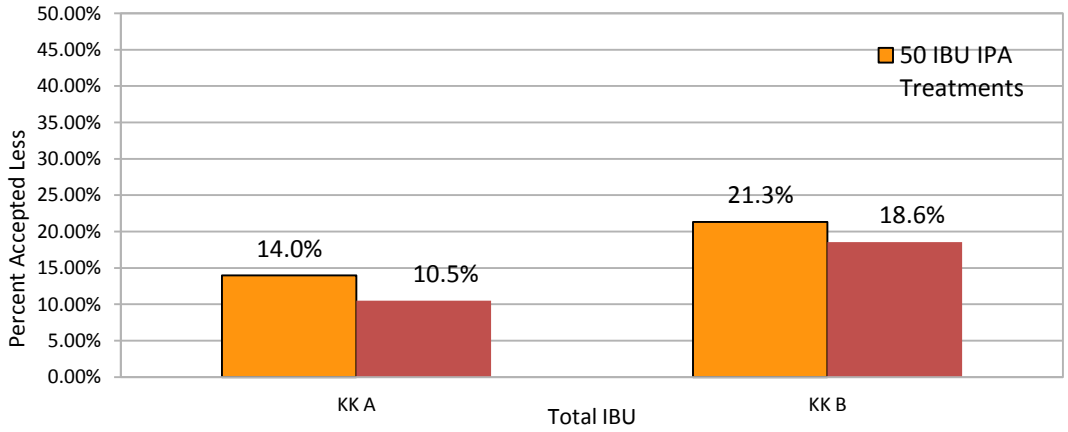

Figure 24. Affect of KK Fermented with Fructose vs. 100% Glucose on IPA and Aardbeien KK Treatments on Acceptability with their Controls

Descriptive Analysis

The results from this test can be viewed through the following tables and figures (Table 34 and 35, Figure 25 and 26).

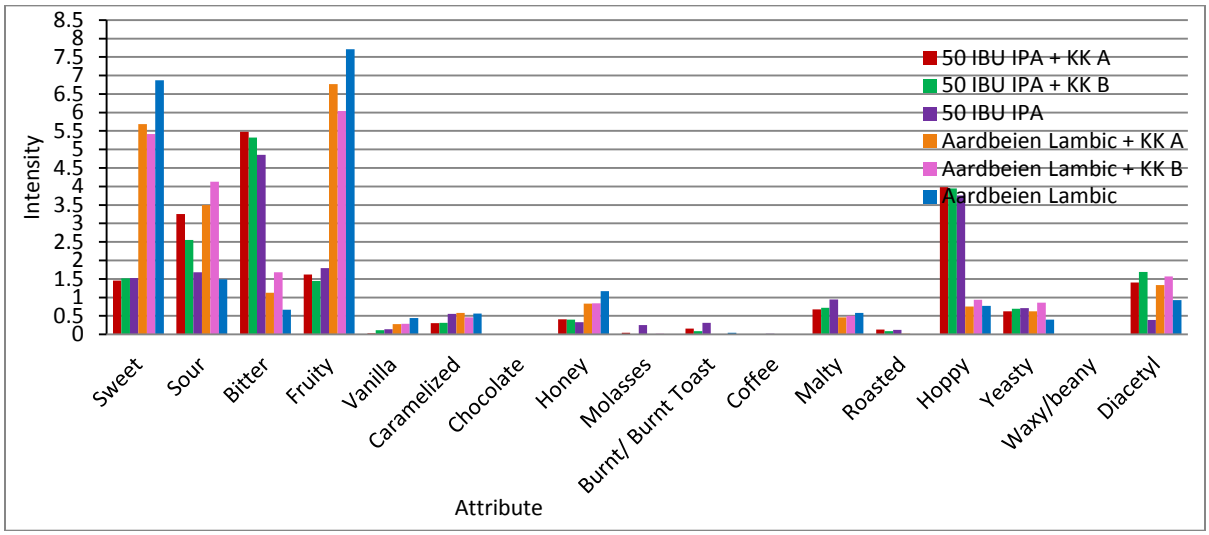

Figure 25. Flavor Comparison of KK IPA and Aardbeien Treatments

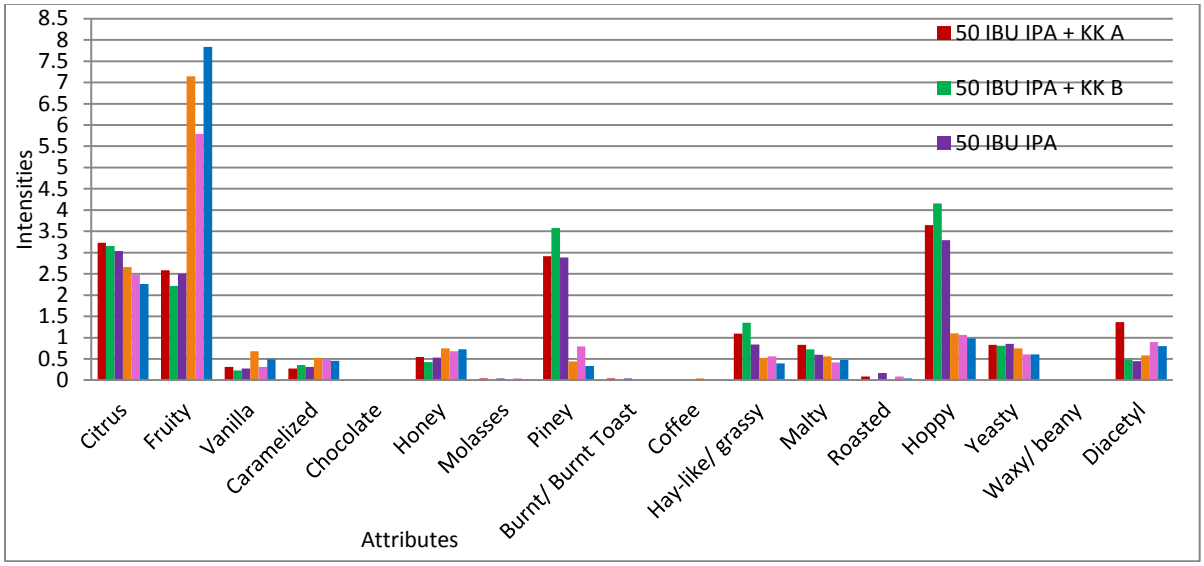

Figure 26. Orthonasal Aroma Comparison of KK IPA and Aardbeien Treatments

It is evident that there is much to gather from this data pertaining to differences in treatments, however, only some of these differences were significant. The IPA treatments were significantly perceived as less sweet, more bitter, less fruity, possessing less honey notes (RA), more hay-like/grassy (OA), and more hoppy. The controls were significantly perceived as less sour and less diacetyl (RA, with the exception of KK A aardbeien and its control).

Table 34. Flavor Comparison of KK IPA and Aardbeien Treatments

	136.0	**214.0**	**389.0**	**416.0**	**562.0**	**690.0**
Treatment Description	**50 IBU IPA + KK A**	**50 IBU IPA + KK B**	**50 IBU IPA**	**Aardbeien Lambic + KK A**	**Aardbeien Lambic + KK B**	**Aardbeien Lambic**
Sweet	1.45^{ab}	1.52^{cd}	1.52^{ef}	5.69^{ace}	5.42^{bdf}	6.88^{bdf}
Sour	3.25^{ab}	2.55^{c}	1.68^{ad}	3.5^{e}	4.13^{cd}	1.49^{bce}
Bitter	5.48^{a}	5.32^{b}	4.85^{c}	1.13^{abc}	1.68^{abc}	0.67^{abc}
Fruity	1.62^{abc}	1.45^{def}	1.80^{abc}	6.77^{ad}	6.04^{be}	7.71^{cf}
Vanilla	0.02^{ab}	0.11^{cde}	0.14	0.28^{c}	0.28^{ad}	0.45^{be}
Caramelized	0.31^{a}	0.31^{b}	0.55^{a}	0.58^{b}	0.46^{a}	0.56
Chocolate	0.0	0.0	0.0	0.0	0.0	0.0
Honey	0.41^{a}	0.40^{bcd}	0.33^{efg}	0.83^{be}	0.84^{acf}	1.17^{dg}
Molasses	0.05	0.0	0.25	0.0	0.02	0.0
Burnt/ burnt toast	0.16	0.09	0.31	0.0	0.0	0.04
Coffee	0.0	0.0	0.02	0.0	0.0	0.0
Malty	0.68	0.72^{a}	0.94^{a}	0.46	0.5	0.58
Roasted	0.13	0.08	0.13	0.02	0.0	0.0
Hoppy	3.98^{abc}	3.95^{def}	3.75^{ghi}	0.75^{adg}	0.93^{beh}	0.77^{cfi}
Yeasty	0.63^{a}	0.70	0.71	0.63	0.85^{a}	0.40
Waxy/ Beany	0.0	0.0	0.0	0.0	0.0	0.0
Diacetyl	1.40^{a}	1.69^{b}	0.39^{abc}	1.33	1.58^{c}	0.93^{c}

Table 35. Orthonasal Aroma Comparison of KK IPA and Aardbeien Treatments

	136.0	214.0	389.0	416.0	562.0	690.0
Treatment Description	**50 IBU IPA + KK A**	**50 IBU IPA + KK B**	**50 IBU IPA**	**Aardbeien Lambic + KK A**	**Aardbeien Lambic + KK B**	**Aardbeien Lambic**
Citrus	3.23[ab]	3.16	3.03	2.66	2.48[a]	2.26[b]
Fruity	2.58[ab]	2.21[cd]	2.50[ef]	7.15[ace]	5.79[bdf]	7.83[bdf]
Vanilla	0.31[a]	0.23	0.27[b]	0.68[b]	0.31	0.49[a]
Caramelized	0.27	0.36	0.31	0.52	0.48	0.46
Chocolate	0.0	0.02	0.0	0.0	0.0	0.0
Honey	0.54	0.43[a]	0.53	0.75	0.68	0.73[a]
Molasses	0.04	0.0	0.04	0.0	0.04	0.0
Piney	2.92[ab]	3.58	2.88	0.44[a]	0.79	0.33[b]
Burnt/ burnt toast	0.04	0.0	0.04	0.0	0.0	0.0
Coffee	0.0	0.0	0.0	0.04	0.0	0.0
Hay like/grassy	1.10[ab]	1.35[c]	0.84	0.52[c]	0.56[a]	0.40[b]
Malty	0.83[ab]	0.73	0.60	0.56[a]	0.42[b]	0.48
Roasted	0.08	0.0	0.17	0.0	0.08	0.04
Hoppy	3.64[abc]	4.15[def]	3.29[ghi]	1.10[adg]	1.06[beh]	0.98[cfi]
Yeasty	0.83	0.81	0.85[a]	0.75	0.60	0.60[a]
Waxy/ beany	0.0	0.0	0.0	0.0	0.0	0.0
Diacetyl	1.36	0.49	0.45	0.58	0.90	0.80

The IPA control was significantly perceived as less sour, more fruity (RA), more caramelized (RA), and less diacetyl (RA) than the KK A IPA, and it also had significantly less diacetyl (RA) than the KK B IPA treatment. The aardbeien control was significantly perceived as less sour and less bitter than the KK A aardbeien, and it was significantly perceived as more sweet, less sour, less bitter, more fruity (OA), and less diacetyl than the KK B aardbeien treatment. The IPA KK A treatment was not significantly different from the IPA KK B treatment, whereas the Aardbeien KK A treatment was only significantly perceived as less bitter than the Aardbeien KK B

treatment. The Aardbeien KK A treatment was the only treatment not possessing a perceived significant difference for diacetyl (RA) with its control treatment. Beer treatments with KK fermented with fructose appeared to have no perceived significant affect on fruitiness compared to the treatments with KK fermented with 100% glucose.

Pasteurization and Thermal Inactivation Study

The results from the thermal inactivation study can be viewed through the following tables:

Table 36. Thermal Inactivation Cell Count Data for Kristalkefir for 5 minute Increments

Plate	Time (min)	Temperature C	Cell Count (cfu/ml)
MRS + cyclo	0	60	$3.97x10^{8}$
MRS + cyclo	5	60	$1.50x10^{4}$
MRS + cyclo	10	60	70
MRS + cyclo	15	60	30
MRS + cyclo	20	60	0.00
WL	0	60	$4.67x10^{8}$
WL	5	60	$1.80x10^{3}$
WL	10	60	470
WL	15	60	100
WL	20	60	0.00

Table 37. Thermal Inactivation Cell Count Data for Kristalkefir for 2 minute Increments

Plate	Time (min)	Temperature C	Cell Count (cfu/ml)
MRS + cyclo	0	60	$2.67x10^{7}$
MRS + cyclo	15	60	300
MRS + cyclo	17	60	200
MRS + cyclo	19	60	100
MRS + cyclo	21	60	0.000

The thermal inactivation study was repeated with 2-minute increments as opposed to five to see if there was complete cell death before minute 20. It was found that this was not the case, and at indeed at minute 20 there was complete thermal inactivation. The thermal death curves of both of these studies can be viewed in Figures 27 and 28. For both of the filter-pasteurized treatments, filtered at .22 micron and .45 micron, there was found to be no growth, therefore making both filter options sufficient for complete inactivation. Withstanding this, a 20-minute heat pasteurization cycle at 60°C was selected for the heat pasteurization technique, and the KK was selected to be filter pasteurized with the .45-micron vacuum filter, due to it a) making the filtering process easier and b) it still eliminating all contaminating microorganisms.

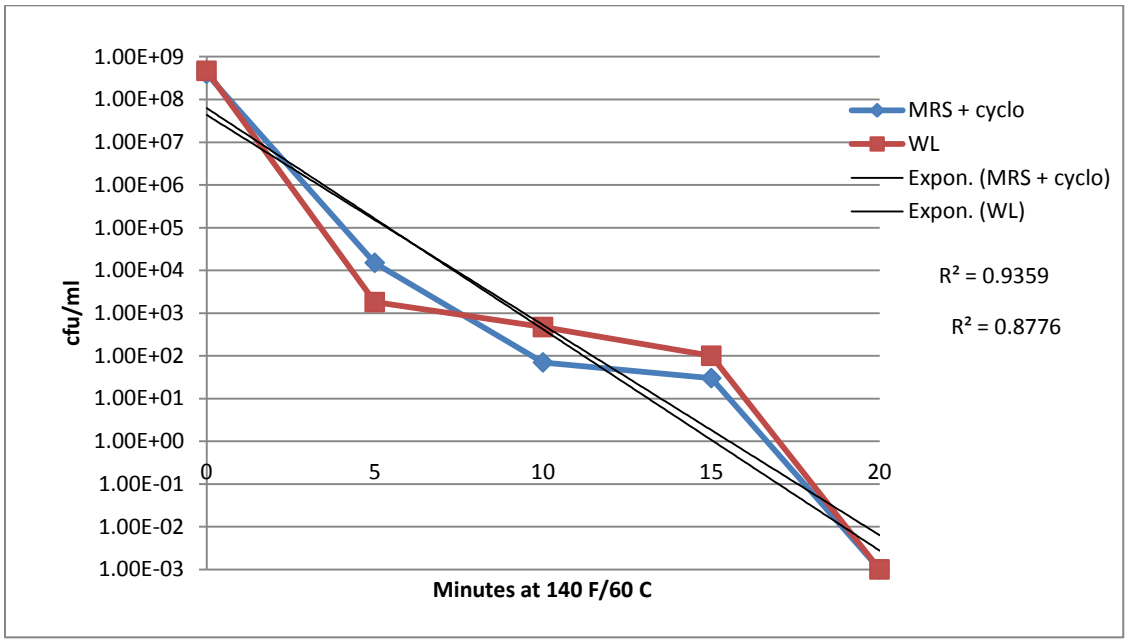

Figure 27. Thermal Death Curve of Kefir at 60°C with 5 minute Increments

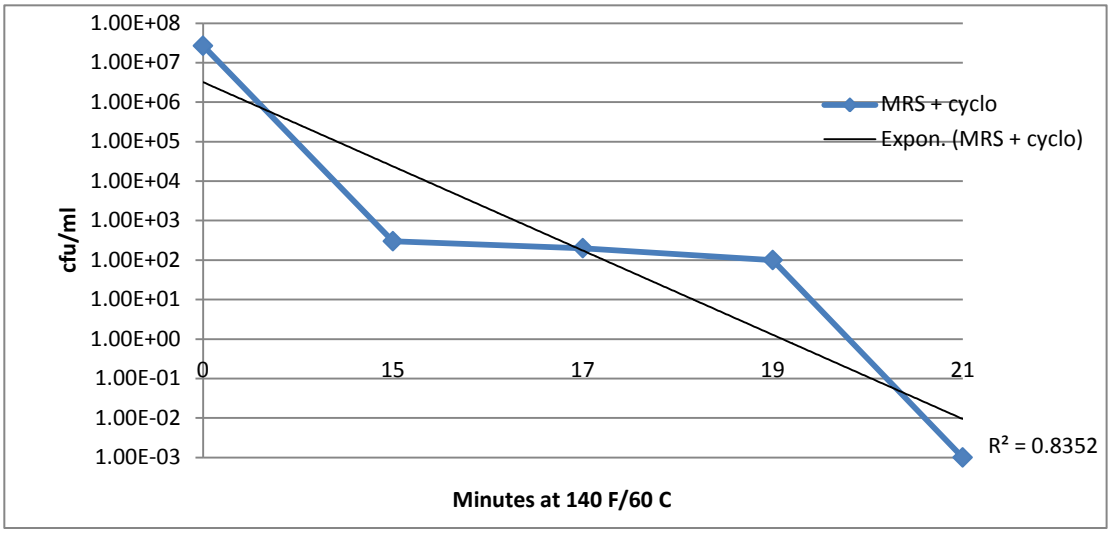

Figure 28. Thermal Death Curve of Kefir at 60°C with 2 minute Increments

Pasteurized Soy Kefir Treatments in Aardbeien Lambic Study

Blind Bench-top

The results from this test can be viewed through the following figures:

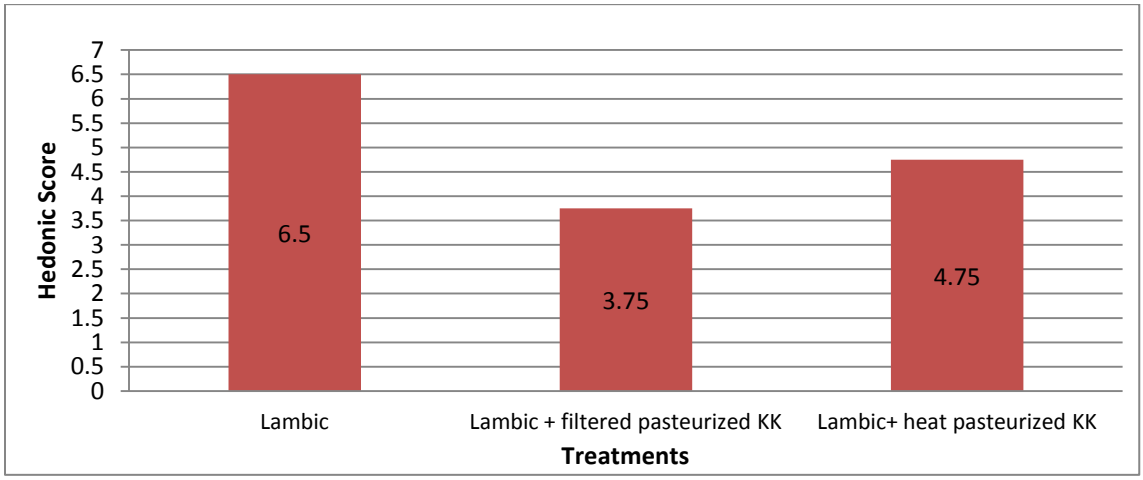

Figure 29. Acceptability of Aardbeien Lambic with Different Pasteurized KK Treatments

It was found that the control treatment was accepted the most, followed by the lambic treatment with the heat-pasteurized kristalkefir, and then lastly, the aardbeien treatment with the filter pasteurized kristalkefir was accepted the least. The control was significantly accepted more than both of the KK treatments, however, the heat-pasteurized treatment was not significantly accepted more than its filtered counterpart.

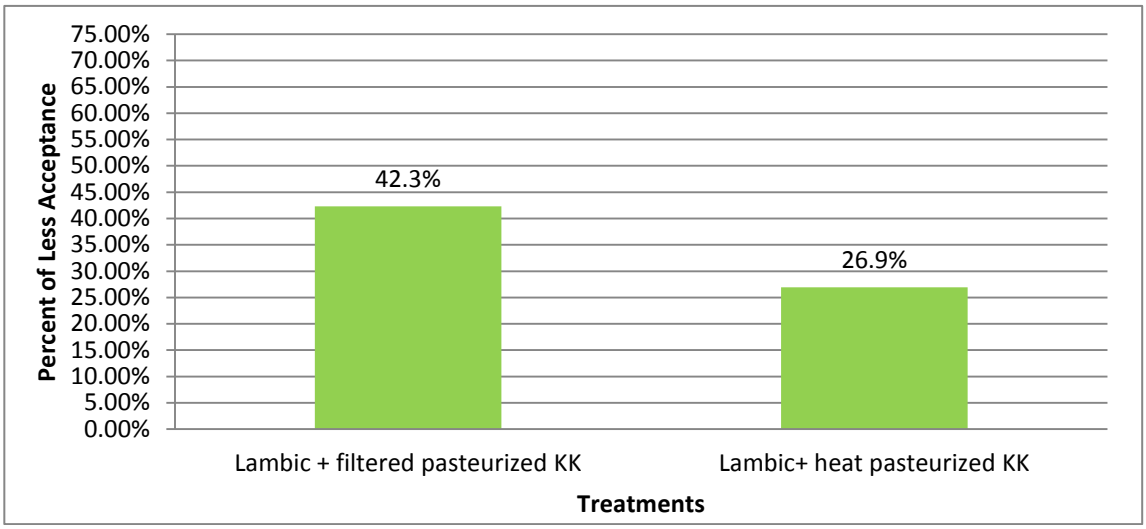

Figure 30. KK Pasteurized Aardbeien Lambic Treatments Percent Accepted Less Than Control

It was also found that the filter-pasteurized treatment was accepted, however, 42.3% less than the control, while the heat-pasteurized treatment was only accepted 26.9% less than the control.

Descriptive Analysis

The results from this test can be viewed through the following tables and figures (Figures 31 and 32, and Tables 38 and 39):

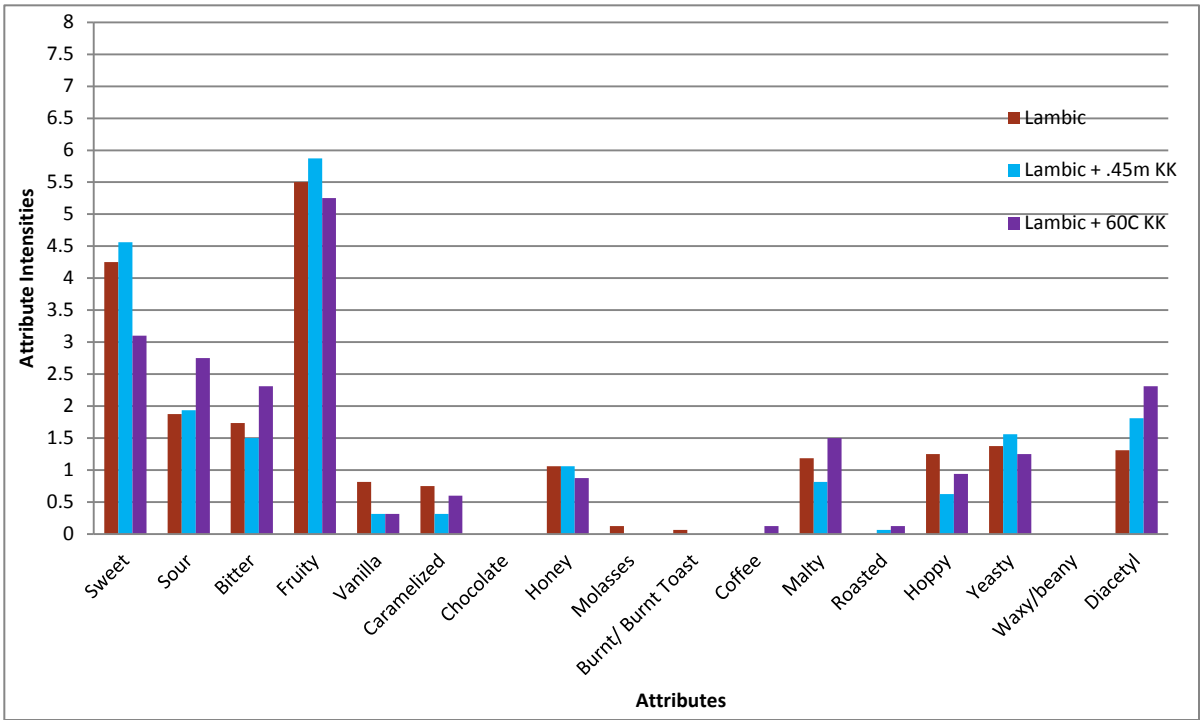

Figure 31. Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments

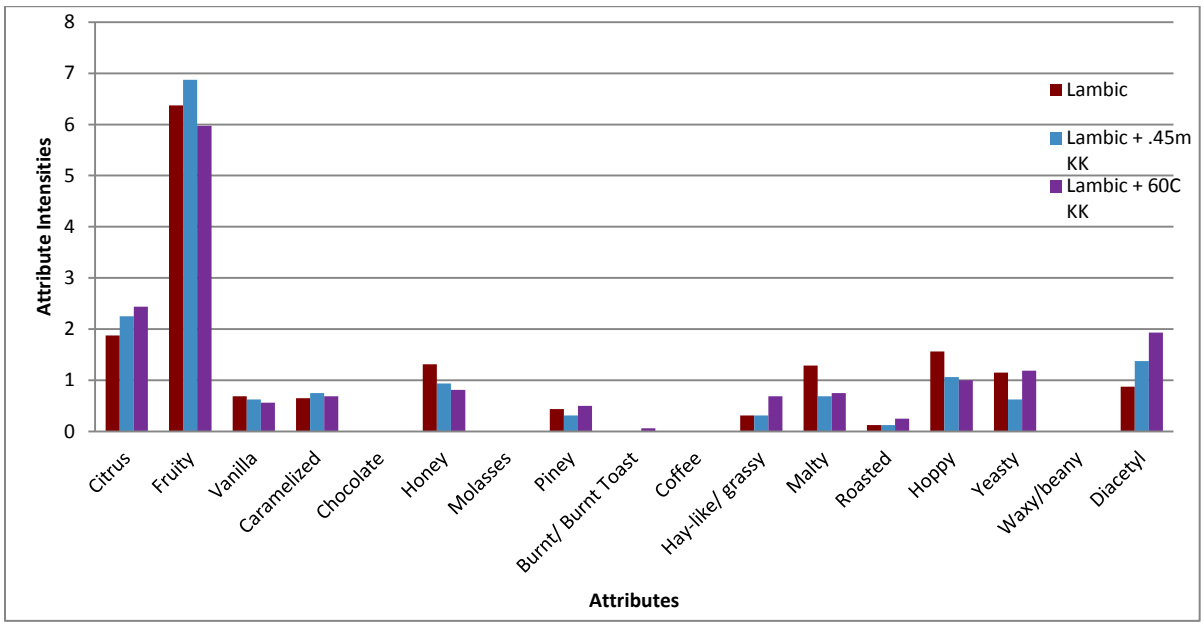

Figure 32. Orthonasal Aroma of Aardbeien Lambic with Different KK Pasteurization Treatments

As one can see, there is a great deal of information to gather from this study and furthermore, some general inferences can be made from the data: overall, the control treatment is perceived as less sour, having more honey notes, more hoppy, and having less diacetyl than the KK lambic treatments. The filter-pasteurized lambic treatment (512) was found to be perceived as sweeter, less bitter, fruitier, less caramelized (RA), less piney (OA), and less malty than all of the treatments, and less sour, more honey, less hoppy (RA), and less diacetyl than the heat pasteurized treatment (920). Although many differences were found, only a few were found to be significant.

Table 38. Flavor Comparison of Pasteurized KK in Aardbeien Treatments

	003	**512**	**920**
Treatment Description	**Aardbeien Lambic**	**Aardbeien Lambic+ .45µ KK**	**Aardbeien Lambic+60°CKK**
Sweet	4.25[a]	4.56[b]	3.1[ab]
Sour	1.88[a]	1.94	2.75[a]
Bitter	1.74	1.5	2.31
Fruity	5.5	5.88	5.25
Vanilla	0.81	0.31	0.31
Caramelized	0.75	0.31	0.6
Chocolate	0	0	0
Honey	1.06	1.06	0.88
Molasses	0.13	0	0
Burnt/Burnt toast	0.06	0	0
Coffee	0	0	0.13
Malty	1.19[a]	0.81[ab]	1.5[b]
Roasted	0	0.06	0.13
Hoppy	1.25	0.63	0.94
Yeasty	1.38	1.56	1.25
Waxy/ beany	0.0	0.0	0.0
Diacetyl	1.31	1.81	2.31

Table 39. Orthonasal Aroma Comparison of Pasteurized KK in Aardbeien Treatments

	003	**512**	**920**
Treatment Description	**Aardbeien Lambic**	**Aardbeien Lambic+ .45µ KK**	**Aardbeien Lambic+60°CKK**
Citrus	1.88	2.25	2.44
Fruity	6.38	6.88	5.98
Vanilla	0.69	0.63	0.56
Caramelized	0.65	0.75	0.69
Chocolate	0	0	0
Honey	1.31	0.94	0.81
Molasses	0	0	0
Piney	0.44	0.31	0.5
Burnt/Burnt toast	0	0	0.06
Coffee	0	0	0
Hay-like/grassy	0.31^{a}	0.31^{b}	0.69^{ab}
Malty	1.29^{ab}	0.69^{a}	0.75^{b}
Roasted	0.13	0.13	0.25
Hoppy	1.56	1.06	1
Yeasty	1.15	0.63	1.19
Waxy/ beany	0.0	0.0	0.0
Diacetyl	.88	1.38	1.93

Treatment 920 was significantly perceived as less sweet than all of the treatments, more sour than the control, more malty retronasally than treatment 512 and less malty orthonasally than the control, and more hay-like/ grassy (OA) than all of the other treatments. Treatment 512 was significantly perceived as less malty than all the other treatments retronasally, and orthonasally, only less malty than the control. Despite diacetyl being slightly different in all of the treatments, this difference, however, was not significant.

Major Soy Isoflavone Analysis via HPLC

SKP and KK and their Beer Treatments

The isoflavone results for the SKP and SKP+Beer treatments can be viewed in the following table (Table 40); the results for the initial KK and KK+beer treatments can be viewed in Table 41, as the treatments are the same as the *KK0* and *126* treatments. The isoflavone data for the soymilk used for the production of the liquid kefir is also included in Table 40.

Table 40. Isoflavone Data for SKP and its Beer Treatment

Treatment	Treatment Description	Daidzin %	Genistin %	Total Glycoside %	Total Glycoside % vs. SKP published data[17] (.09-.37*%)	Daidzein %	Daidzein % vs. SKP data (0.006-.020*%)	Genistein %	Genistein % vs. SKP data (0.003-0.01*%)	Total Alglycone %	Total Alglycone % vs. SKP data (0.01-.03*%)
SKP 1.0%	1.0% SKP in HPLC water	0.0036	0.00057	0.00417	1.1%*	0.00038	1.900%*	0.00002	0.20%	0.0004	1.3%*
SKP +Beer	5.25 g SKP in 355 ml beer; 1.5%	0.0052	0.00088	0.00608	1.6%*	0.0006	3.00%*	0.000091	0.91%	0.000691	2.3%*
Soymilk[18]	Silk unsweetened soymilk @ 100%	0.0036	0.0134	0.017	4.6%*	0.00015	.75%*	0.000039	.39%	0.000189	.6%*

This data also validates the *loss constant*, considering the SKP tested is at 1.0%, its isoflavone concentration should be at approximately 1.0% as well, and the SKP in the SKP+Beer treatment is at 1.5%, therefore its isoflavones should also be at a concentration of around 1.5%. This was found to be correct, thus validating the

17 Kubow, Stan, Sheppard, Dr. John, "USE OF SOY KEFIR POWDER FOR REDUCING PAIN, BLOOD PRESSURE AND INFLAMMATION," United States Patent Application 20090221469, 09-03-2009.

[18] Isoflavone values were also personally obtained from White Wave Foods (Broomfield, CO), and the total isoflavone concentration was listed as approximately 35 μg/8 oz soymilk serving. This value validates what was found through this analysis, due to it equating to a value of 0.013%, similar to what was found here in this analysis.

aforementioned *loss constant*. It should be noted that the genistein concentration was found to be quite a bit lower than expected; this could be due to oxidation of this isoflavone compound during storage of the powder. The soymilk showed a greatly larger portion of isoflavones being of the glycosidic nature, which is expected due to the conversion of these said isoflavones into their respective conjugates, the alglycones (which are of greater importance here), during fermentation. Overall, the concentrations of all isoflavones are lower in the soymilk, due to the concentrated nature of the SKP.

Diacetyl Rest KK and their Beer Treatments

The isoflavone results from the different diacetyl rest kristalkefirs and their beer treatments can be viewed in the following table.

Table 41. Isoflavone Data for KK at Different Diacetyl Rests and their IPA Treatments

Treatment	Treatment Description	Daidzin %	Genistin %	Total Glycoside %	Total Glycoside% vs. SKP published data (.09*-.37%)	Daidzein %	Daidzein% vs. SKP data (0.006*-.020%)	Genistein %	Genistein % vs. SKP data (0.003*-0.01%)	Total Alglycone %	Total Alglycone % vs. SKP data (0.01*-.03%)
KK0	KK without diacetyl rest; 100%	0.0172	0.0277	.0449	49.9%	.0075	125.0%	.000933	31.0%	.00843	84.3%
KK24	KK with 24 hr diacetyl rest; 100%	0.0101	0.0068	0.0169	18.8%	0.0094	156.7%	0.0035	116.7%	0.0129	129.0%
KK48	KK with 48 hr diacetyl rest; 100%	0.0049	0.0066	0.0115	12.8%	0.017	283.3%	0.0053	176.7%	0.0223	223.0%
126	KK0 + beer; 25%	0.0018	0.00349	.00529	5.9%	0.0023	38.3%	0.0005	16.7%	0.0028	28.0%
362	KK24 + beer; 25%	0.00055	0.002	0.00255	2.8%	0.0033	55.0%	0.0008	26.7%	0.0041	41.0%
912	KK48 + beer; 25%	0.00059	0.0007	0.00129	1.4%	0.0045	75.0%	0.0012	40.0%	0.0057	57.0%

It was found that the kristalkefir without a diacetyl rest has the most glycoside concentration, at 49.9% of the published data, however, has the least concentration of alglycones, daidzein and genistein, at 84.3%. It was also found that as diacetyl rest increased, hydrolysis from glycosides to alglycones increased; KK24 had a lesser concentration of glycosides than KK0, at 18.8%, and had a higher concentration of alglycones, at 129.0%, whereas KK48 had an even lower amount of glycosides, 12.8%, and the most amount of alglycones, at 223.0%. The beer treatments showcased a similar pattern with their respective KK diacetyl rest treatments, validating this discovery.

Glucose and Glucose+Fructose KK and their IPA and Aardbeien Analogue Treatments

The isoflavone results from the different substrate fermented kristalkefirs and their IPA and aardbeien lambic beer treatments can be viewed through the following table.

Table 42. Isoflavone Data for KK Fermented with Different Sugar Substrates and their IPA and Aardbeien Treatments

Treatment	Treatment Description	Daidzin%	Genistin %	Total Glycoside %	Total Glycoside% vs. SKP published data (.09*-.37%)	Daidzein %	Daidzein% vs. SKP published (0.006*-.020%)	Genistein %	Genistein % vs. SKP published data (0.003*-0.01%)	Total Alglycone %	Total Alglycone % vs. SKP published data (0.01*-.03%)
KK a	KK with just glucose; 100%	0.0057	0.0094	0.0151	16.7%	0.0094	156.67%	0.0035	116.7%	0.0129	129.0%
KK b	KK with 50/50 glucose/ fructose; 100%	0.0024	0.006	0.0084	9.3%	0.013	216.67%	0.0035	116.7%	0.0165	165.0%
136	KK a + IPA; 25%	0.00014	0.002	0.00214	2.4%	0.005	83.33%	0.0014	46.7%	0.0064	64.0%
214	KK b + IPA; 25%	0.0001	0.0017	0.0018	2.0%	0.0035	58.33%	0.0008	26.7%	0.0043	43.0%

Table 42. (continued) Isoflavone Data for KK Fermented with Different Sugar Substrates and their IPA and Aardbeien Treatments

416	KK a + Aardbeien; 25%	0.00094	0.0004	0.00134	1.5%	0.0034	56.67%	0.0009	30.0%	0.0043	43.0%
562	KK b + Aardbeien; 25%	0.0014	0.00045	0.00185	2.1%	0.003	50.00%	0.0005	16.7%	0.0035	35.0%

It was found that KK B had a lower amount of glycosidic, at 9.3% of the published data, and a higher amount of alglycosidic isoflavones, at 165.0%, than KK A, at 16.7 and 129.0%, respectively. The beer treatments, however, did not demonstrate this same trend. One would expect the beer treatments to possess isoflavone concentrations at 25% of the KK concentrations, due to the KK being at a 25% concentration in the beer treatments. Withstanding this, the KK A beer treatments would have an alglycone concentration of 32.3% and the KK B beer treatments would have a concentration of 41.3%; in continuation, this difference, though, does not appear to be that great. This may be due to human error from not ensuring proper homogeneity of the kristalkefir and beer prior to bottling. Regardless of this possible causative agent, all of the glycosides were around the same concentration throughout all of the treatments, and the alglycone concentrations appeared to be lower in the aardbeien treatments versus the IPA treatments. It is important to note while KK B does have a higher alglycone concentration, KK A's concentration is at a concentration greater than that of the published data, at nearly 30% more.

Pasteurized KK and their Aardbeien Lambic Beer Treatments

The isoflavone data for different pasteurized kristalkefir and their aardbeien lambic treatments can be viewed through the following table.

Table 43. Isoflavone Data for KK with Different Pasteurizations and Aardbeien Treatments

Treatment	Treatment Description	Daidzin %	Genistin %	Total Glycoside %	Total Glycoside% vs. SKP data (.09*-.37%)	Daidzein %	Daidzein% vs. SKP data (0.006*-.020%)	Genistein %	Genistein % vs. SKP data (0.003*-0.01%)	Total Alglycone %	Total Alglycone % vs. SKP data (0.01*-.03%)
KK pre	KK before pasteurization; 100%	0.0055	0.0085	0.014	15.6%	0.0086	143.3%	0.004	133.3%	0.0126	126.0%
KK 60°C	KK pasteurized at 60°C; 100%	0.0011	0.009	0.0101	11.2%	0.0004	6.7%	0.003	100.0%	0.0034	34.0%
KK .45µ	KK .45µ filter pasteurized; 100%	0.0013	0.008	0.0093	10.3%	0.0004	6.7%	0.0008	26.7%	0.0012	12.0%
512	.45µ pasteurized KK+aardbeien; 25%	0.0003	0.0003	0.0006	0.7%	0.00012	2.0%	0.0004	13.3%	0.00052	5.2%
920	pasteurized at 60°C KK + aardbeien; 25%	0.00025	0.0025	0.00275	3.1%	0.00011	1.8%	0.00075	25.0%	0.00086	8.6%

It was found that both pasteurization treatments were deleterious to the isoflavone compounds seeing how there was over a 70% loss of the alglycones, and an approximate 30% loss of the glycosides due to the process. The heat pasteurization technique, at 34.0% and 11.2% respectively, however, was found to be less deleterious to the isoflavone compounds than the filter variety, at 12.0% and 10.3% respectively. This may be due to the isoflavones being bound to other compounds, or not fully in solution, making them larger in particle size, thus not allowing them to pass through a pore size of .45 micron. The beer treatments showcased a similar pattern with their respective KK pasteurization treatments, validating this discovery.

Comparison of all Treatments up to the Accelerated Shelf-life Study

Kristalkefir was at 25% concentration in all of the beer treatments, thus making the isoflavones at 25% of what they are in the KK. Table 44 shows all of the isoflavone data for KK and KK beer treatments, in which the majority of the treatments validate the previously mentioned statement. Kristalkefir treatments KK24, KK A and KK pre are essentially the same treatment (same ingredients and processing), therefore their isoflavone breakdowns should be essentially the same as well; this notion can be viewed in Figure 33.

Table 44. Isoflavone Data of all Kristalkefir and its Beer Treatments

Treatment	Treatment Description	Daidzin %	Genistin %	Total Glycoside %	Total Glycoside % (SKP published (.09-.37%)	Daidzein %	Daidzein% SKP published (0.006-.020%)	Genistein %	Genistein % SKP published (0.003-0.01%)	Total Alglycone %	Total Alglycone % SKP published (0.01-.03%)	Total Isoflavone %
KK0	KK without diacetyl rest; 100%	0.0172	0.0277	0.0449	49.89%	0.0075	125.00%	0.00093	31.00%	0.00843	84.30%	0.0533
KK0 x .25		0.0043	0.006925	0.011225	12.47%	0.001875	31.25%	0.0002325	7.75%	0.0021075	21.08%	0.0133
126	KK0 + beer; 25%	0.0018	0.00349	0.00529	5.88%	0.0023	38.33%	0.0005	16.67%	0.0028	28.00%	0.0081
KK24	KK with 24 hour diacetyl rest; 100%	0.0101	0.0068	0.0169	18.78%	0.0094	156.67%	0.0035	116.67%	0.0129	129.00%	0.0298
KK24 x .25		0.002525	0.0017	0.004225	4.69%	0.00235	39.17%	0.000875	29.17%	0.003225	32.25%	0.0075
362	KK24 + beer; 25%	0.00055	0.002	0.00255	2.83%	0.0033	55.00%	0.0008	26.67%	0.0041	41.00%	0.0067
KK48	KK with 48 hour diacetyl rest; 100%	0.0049	0.0066	0.0115	12.78%	0.017	283.33%	0.0053	176.67%	0.0223	223.00%	0.0338
KK48 x .25		0.001225	0.00165	0.002875	3.19%	0.00425	70.83%	0.001325	44.17%	0.005575	55.75%	0.0085
912	KK48 + beer; 25%	0.00059	0.0007	0.00129	1.43%	0.0045	75.00%	0.0012	40.00%	0.0057	57.00%	0.0070
KK a	KK with just glucose; 100%	0.0057	0.0094	0.0151	16.78%	0.0094	156.67%	0.0035	116.67%	0.0129	129.00%	0.0280
KK a x .25		0.001425	0.00235	0.003775	4.19%	0.00235	39.17%	0.000875	29.17%	0.003225	32.25%	0.0070
136	KK a + IPA; 25%	0.00014	0.002	0.00214	2.38%	0.005	83.33%	0.0014	46.67%	0.0064	64.00%	0.0085
416	KK a + Aardbeien; 25%	0.00094	0.0004	0.00134	1.49%	0.0034	56.67%	0.0009	30.00%	0.0043	43.00%	0.0056

Table 44. (continued) Isoflavone Data of all Kristalkefir and its Beer Treatments

KK b	KK with 50/50 glucose/fructose; 100%	0.0024	0.006	0.0084	9.33%	0.013	216.67%	0.0035	116.67%	0.0165	165.00%	0.0249
KK b x .25		0.0006	0.0015	0.0021	2.33%	0.00325	54.17%	0.000875	29.17%	0.004125	41.25%	0.0062
214	KK b + IPA; 25%	0.0001	0.0017	0.0018	2.00%	0.0035	58.33%	0.0008	26.67%	0.0043	43.00%	0.0061
562	KK b + Aardbeien; 25%	0.0014	0.00045	0.00185	2.06%	0.003	50.00%	0.0005	16.67%	0.0035	35.00%	0.0054
KK 60°C	KK heat pasteurized at 60°C; 100%	0.0011	0.009	0.0101	11.22%	0.0004	6.67%	0.003	100.00%	0.0034	34.00%	0.0135
KK 60°C x .25		0.000275	0.00225	0.002525	2.81%	0.0001	1.67%	0.00075	25.00%	0.00085	8.50%	0.0034
920	heat pasteurized at 60°C KK + aardbeien; 25%	0.00025	0.0025	0.00275	3.06%	0.00011	1.83%	0.00075	25.00%	0.00086	8.60%	0.0036
KK .45µ	KK .45µ filter pasteurized; 100%	0.0013	0.008	0.0093	10.33%	0.0004	6.67%	0.0008	26.67%	0.0012	12.00%	0.0105
KK .45µ x .25		0.000325	0.002	0.002325	2.58%	0.0001	1.67%	0.0002	6.67%	0.0003	3.00%	0.0026
512	.45µ filter pasteurized KK+aardbeien; 25%	0.0003	0.0003	0.0006	0.67%	0.00012	2.00%	0.0004	13.33%	0.00052	5.20%	0.0011

The isoflavone data for all of the KK+beer treatments can be viewed in graphical form in Figure 34. Overall, the isoflavone potency of the liquid kristalkefir was found to be higher than that of the Soy Kefir Powder. Although the KK glycoside concentrations were much lower than the SKP's, the alglycone concentration is of more importance because they have an increased efficacy as therapeutic compounds. As Figure 33 shows, all of the KK treatments except the pasteurized ones have a larger concentration of daidzein, and all of the KK treatments except KK0 and the filter pasteurized KK have a genistein concentration that is equal to or greater than that of the SKP.

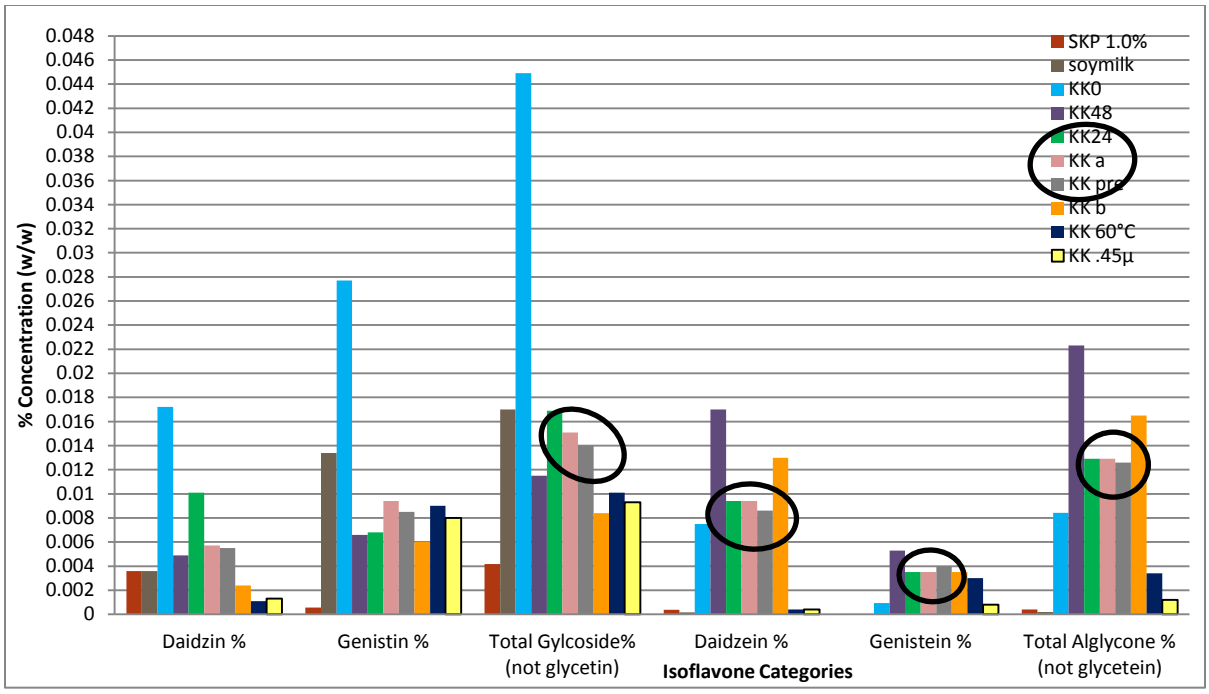

Figure 33. Isoflavone Concentrations of all Kefir Treatments

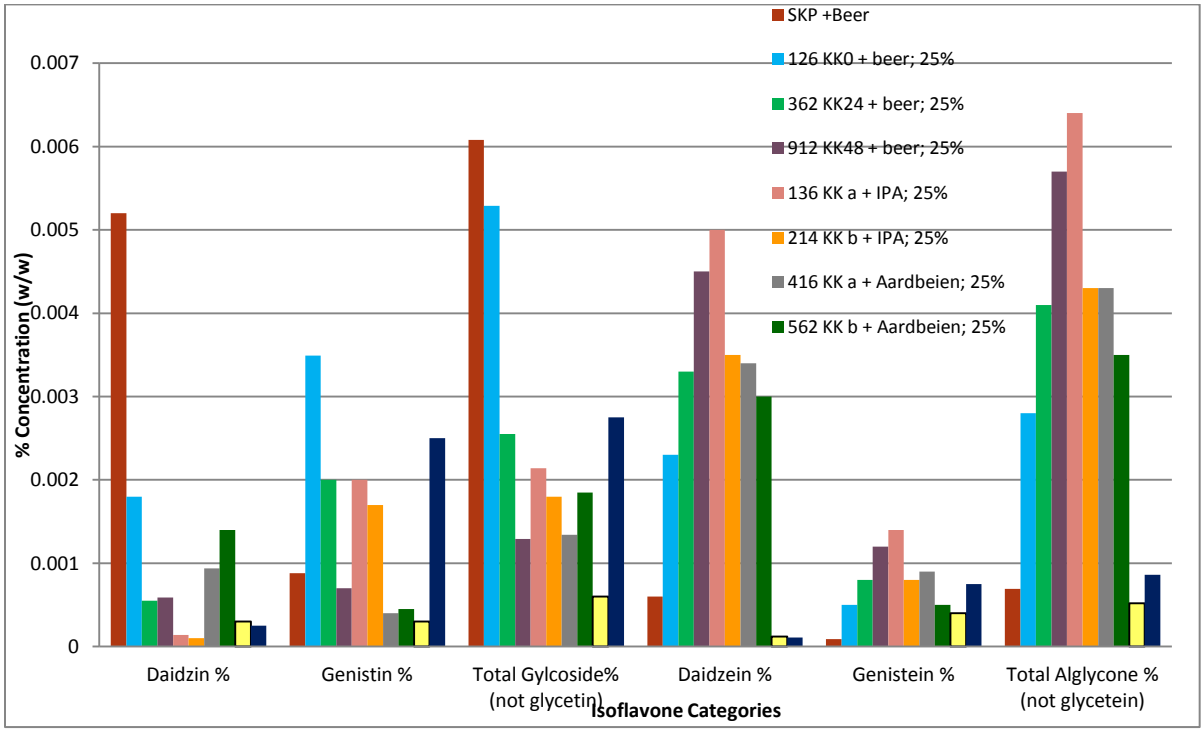

Figure 34. Isoflavone Concentrations of all Kefir + Beer Treatments

Figure 36 also illustrates the increased potency of the KK, as it is still shown to be more potent downstream in the beer matrix. Again, all of the KK+beer treatments, except for the pasteurized treatments, were found to have a higher concentration of daidzein than the SKP as well, and all of the KK+beer treatments, except the KK0+beer, 562, and the filter pasteurized KK+beer treatment, were found to have a genistein concentration equal to or greater than that of the SKP's.

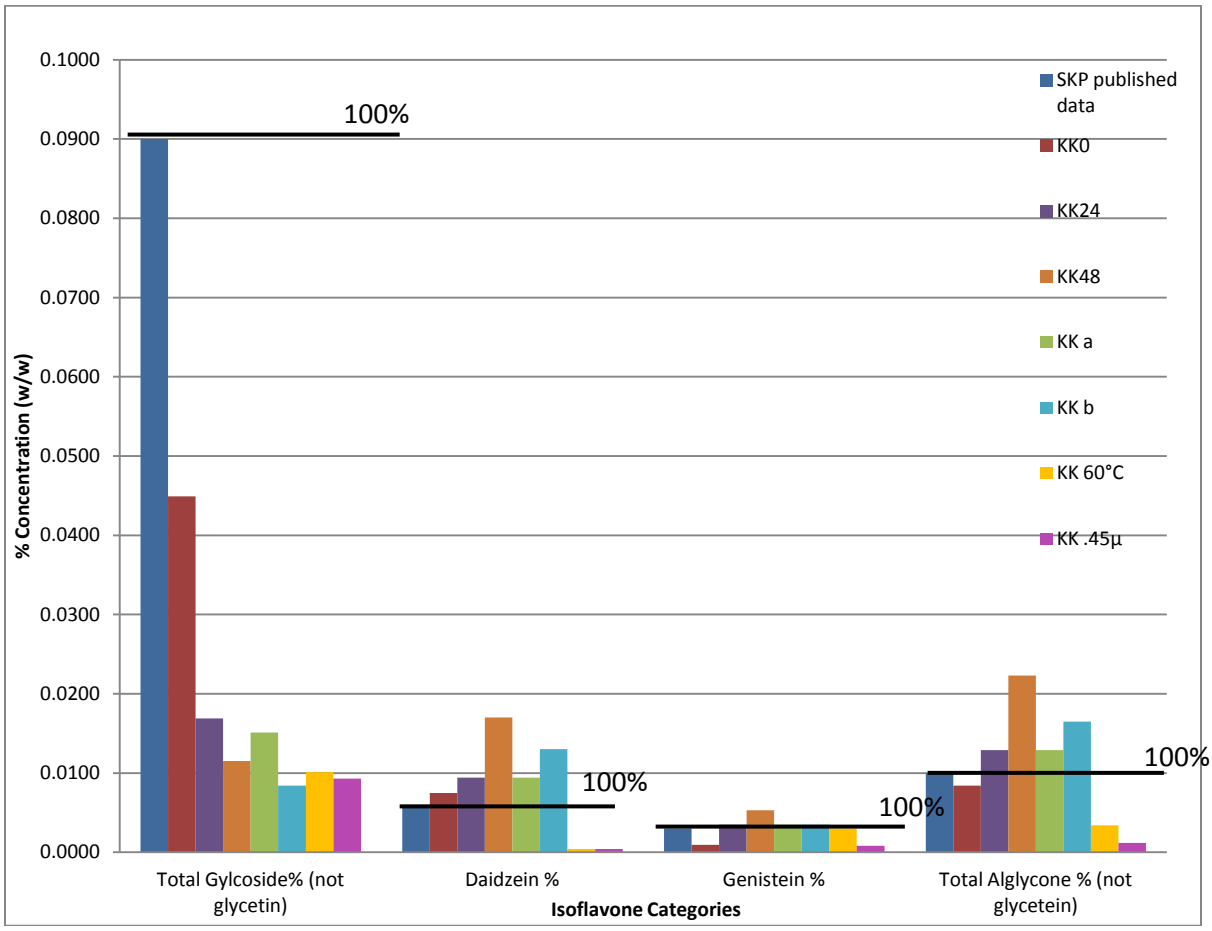

Figure 35. KK Treatments Isoflavone Concentration Compared to SKP's

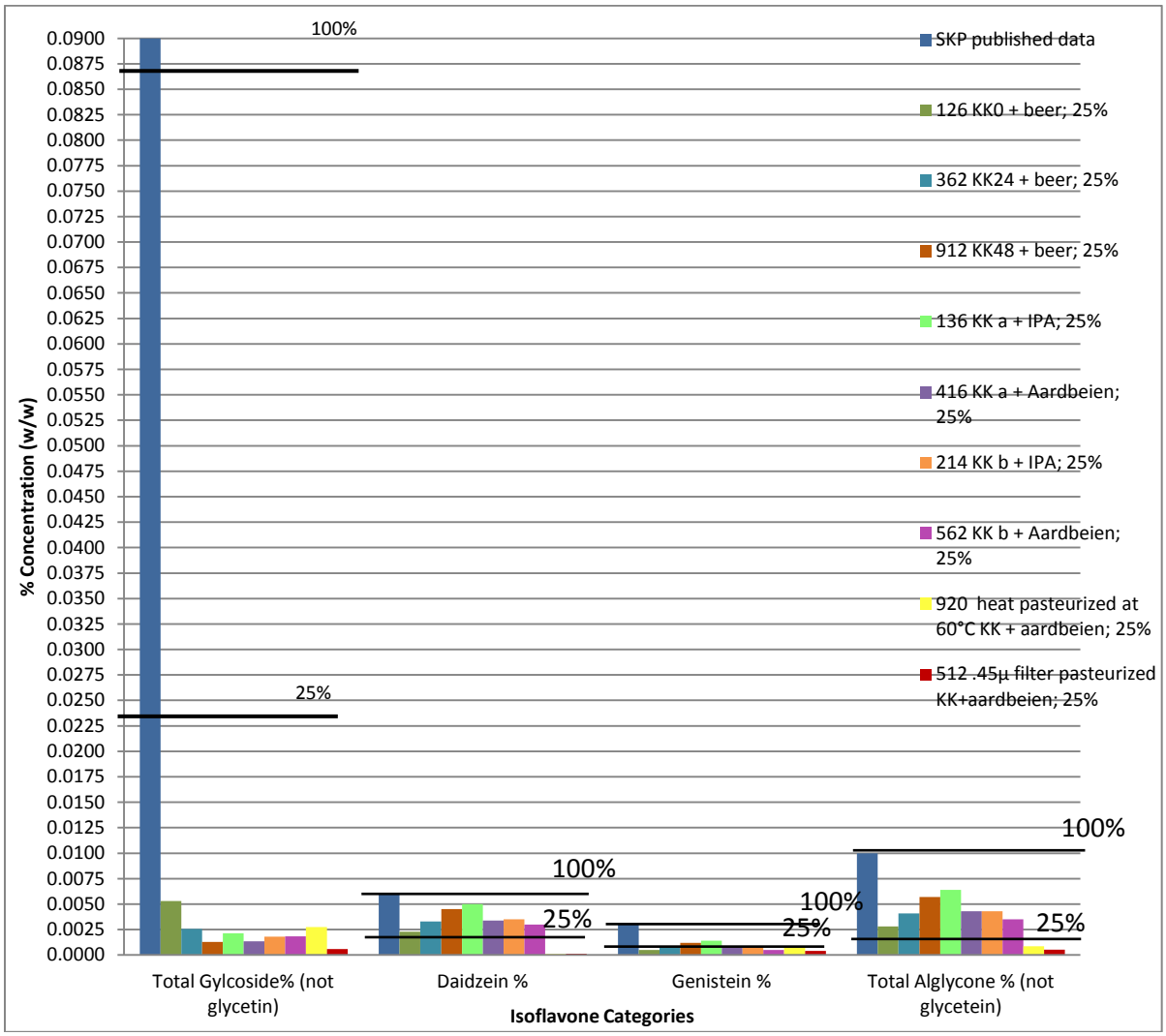

Figure 36. KK + Beer Treatments Isoflavone Concentration Compared to SKP's

Accelerated Shelf-life Testing

Sensory Quality

The descriptive analysis results from the accelerated shelf-life study can be viewed in the following figures and tables:

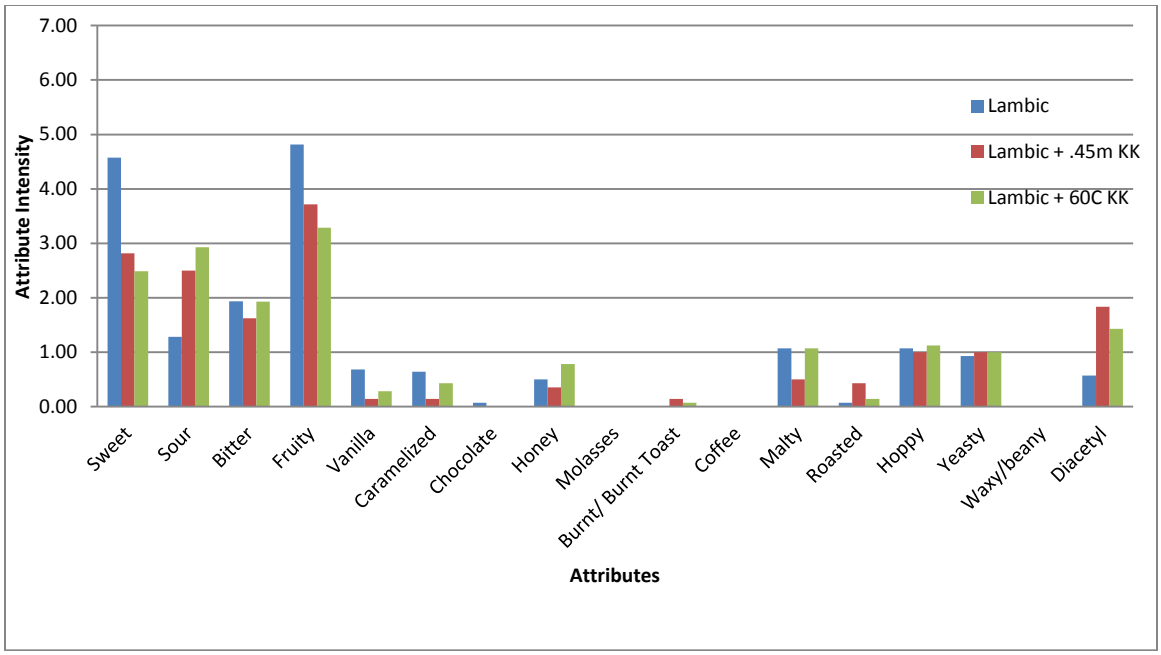

Figure 37. Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments Month 1.5

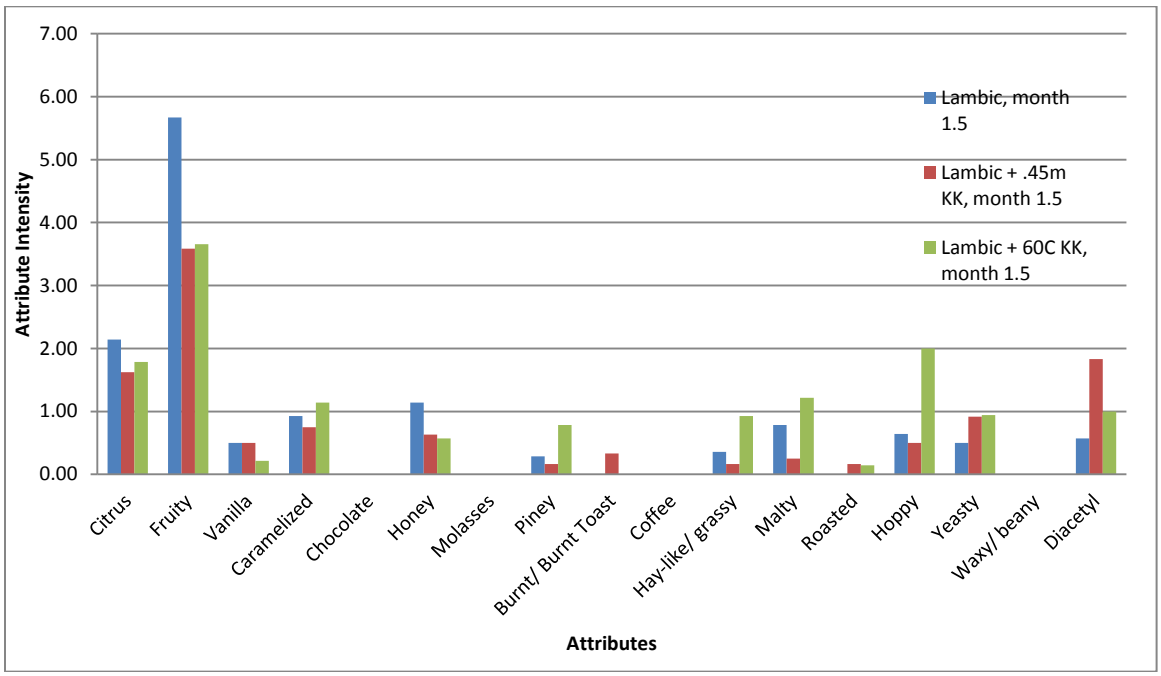

Figure 38. Orthonasal Aroma of Aardbeien Lambic with Different KK Pasteurization Treatments, month 1.5

As one can see, there are several differences between the samples, but only a few of them are significant (Table 45 and 46); the control treatment was significantly perceived as sweeter and more vanilla (RA) than both of the other treatments, more sour, more honey (OA), and less hoppy (OA) than the heat pasteurized treatment, and more fruity (OA) than the filter-pasteurized treatment. The filter-pasteurized treatment was significantly perceived as less malty (OA) and hoppy (OA) than the heat-pasteurized treatment.

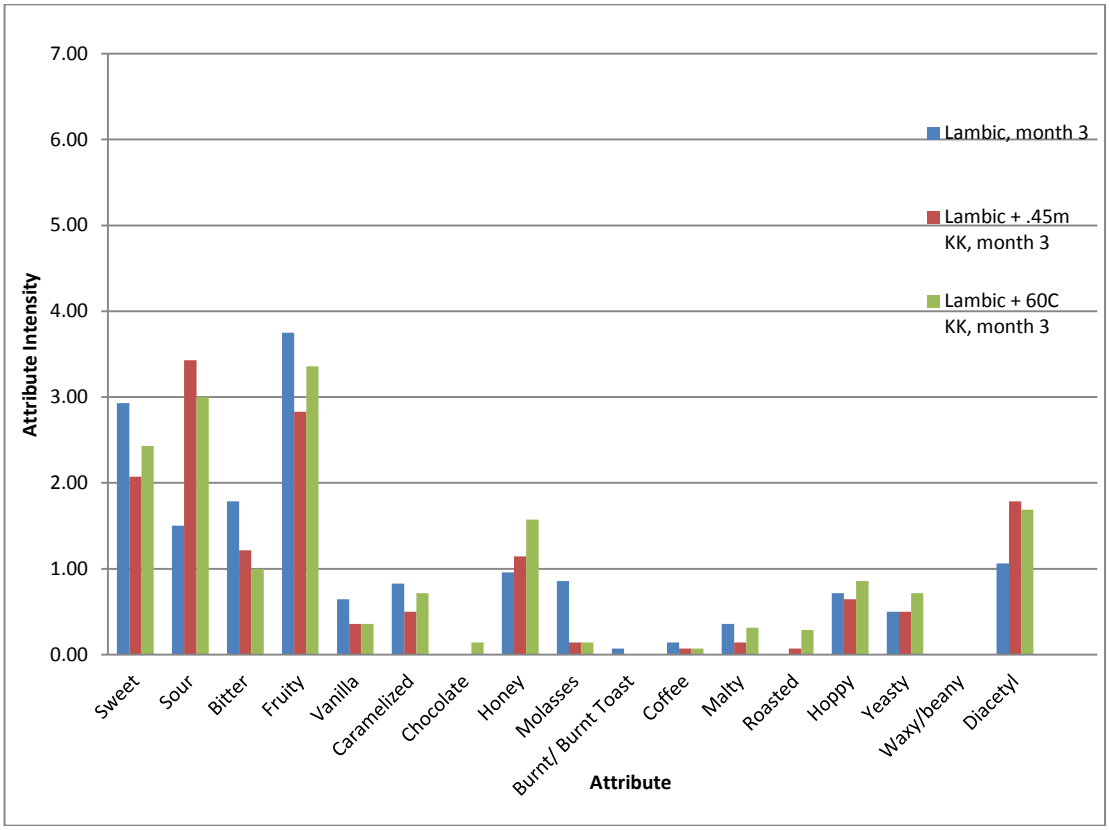

Figure 39. Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments Month 3

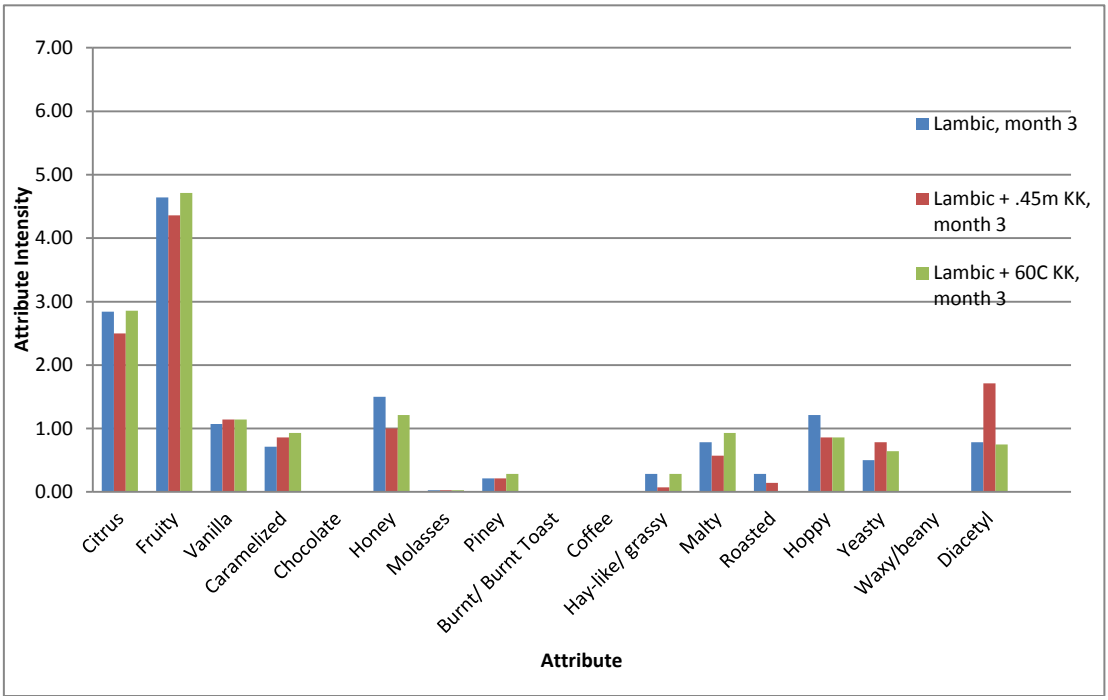

Figure 40. Orthonasal Aroma of Aardbeien Lambic with Different KK Pasteurization Treatments, month 3

Overall, for month 3, the control treatment was perceived as sweeter, less sour, more bitter, more fruity, more vanilla (RA), more caramelized (RA), more hoppy (OA), less yeasty, and less diacetyl (RA) than all of the other treatments, and more malty (OA) and less diacetyl (OA) than the filter-pasteurized treatment. The filter-pasteurized treatment was perceived as less sweet, more sour, less fruity, less citrus (OA), less caramelized (RA), less honey (OA), less malty (RA), an more diacetyl than all of the other treatments, and more bitter, and less honey than the heat-pasteurized treatment. Again, only a handful of these differences are significant; the control is significantly perceived as less sour than both of the other treatments, and more honey

(OA) and less diacetyl (OA) than the filter-pasteurized treatment. The filter-pasteurized treatment was significantly perceived as less caramelized (RA) and honeyness (RA) than the heat-pasteurized treatment.

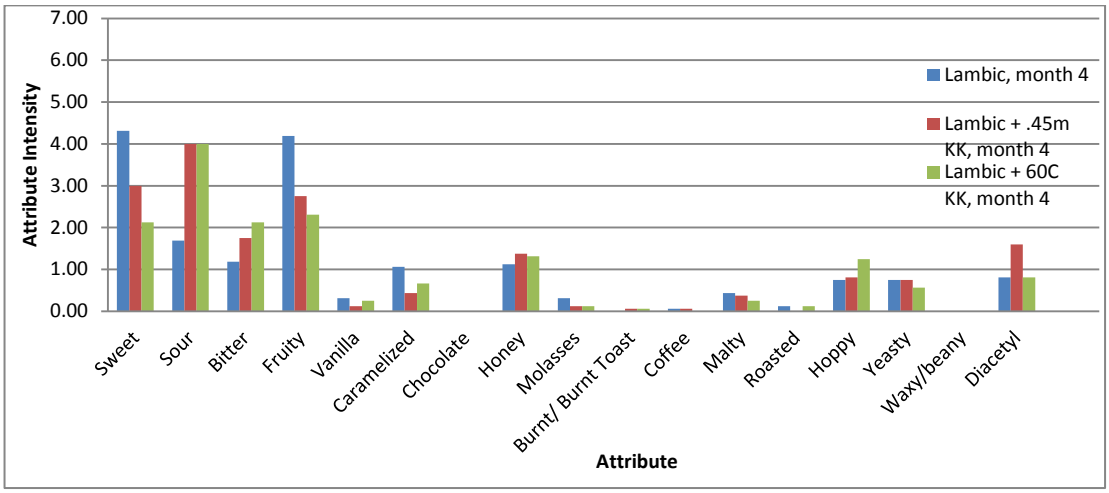

Figure 41. Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments Month 4

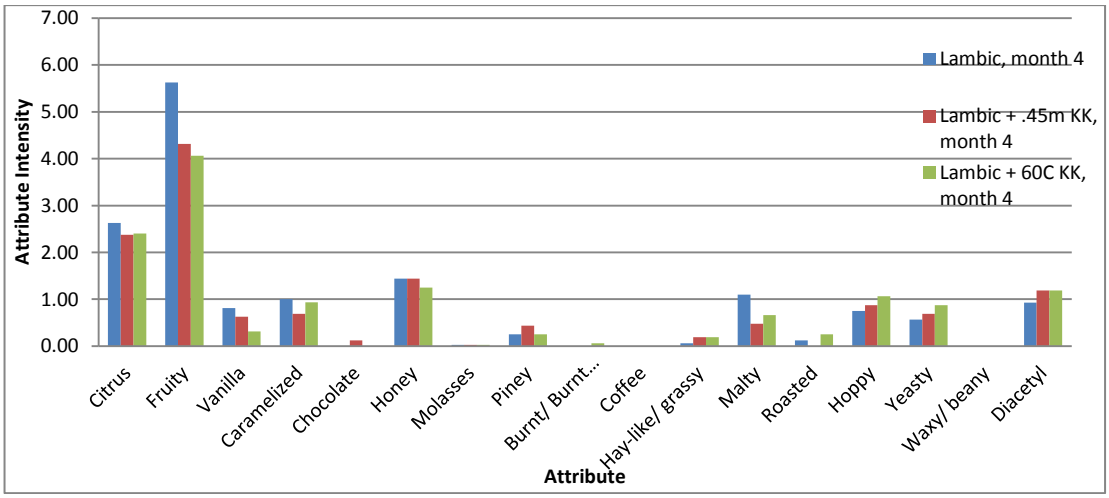

Figure 42. Orthonasal Aroma of Aardbeien Lambic with Different KK Pasteurization Treatments, month 4

Overall, for month 4, the control treatment was perceived as sweeter, less sour, less bitter, more citrusy (OA), more fruity, more vanilla, more caramelized, more malty, less hoppy, and less diacetyl (OA) than all of the other treatments, and less diacetyl (RA) than the filter-pasteurized KK treatment. The heat-pasteurized treatment was perceived as less sweet, more bitter, less fruity, less vanilla, and more hoppy than all of the treatments, and more caramelized, less honey, and less diacetyl (RA) than the filter-pasteurized KK treatment. Although these differences were noted, only some of them are significant; the control treatment was significantly perceived as more sweet, less sour, more fruity (RA), more vanilla (OA), and less yeasty (OA) than the heat-pasteurized KK treatment.

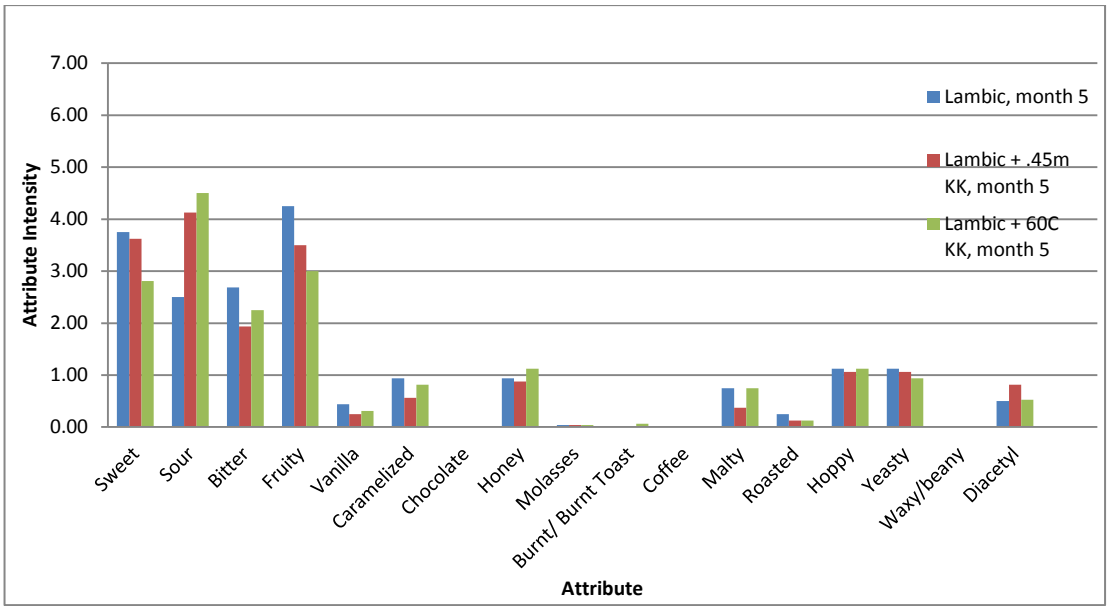

Figure 43. Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments Month 5

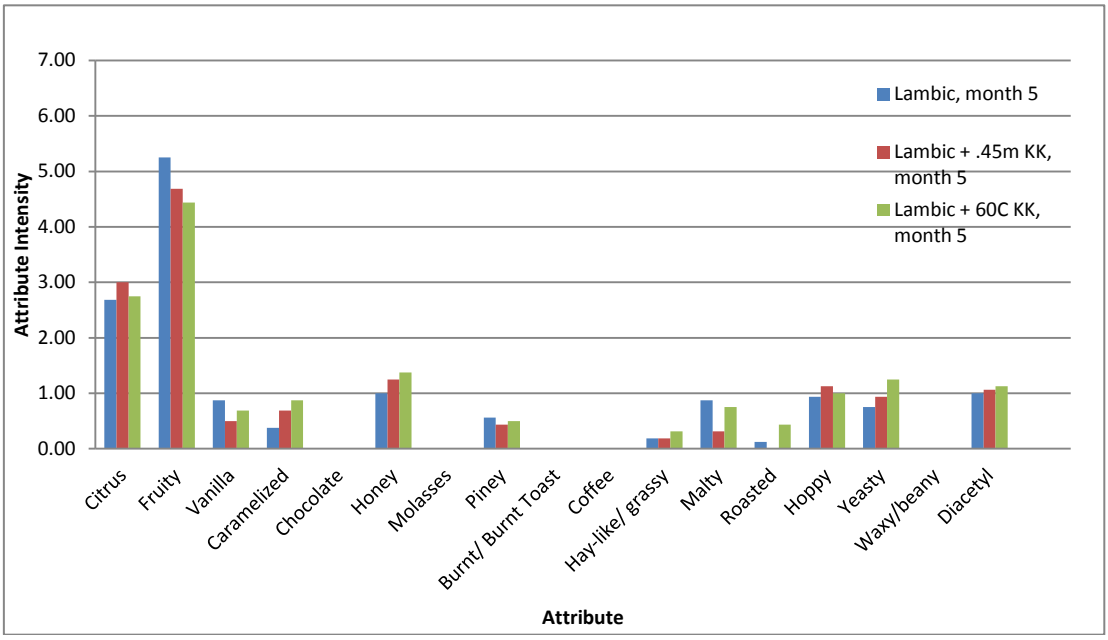

Figure 44. Orthonasal Aroma of Aardbeien Lambic with Different KK Pasteurization Treatments, month 5

Overall, in month 5, the control treatment was perceived as sweeter, less sour, more bitter, more fruity, more vanilla, less caramelized (OA), and less honey than all of the other treatments, and more malty and less diacetyl than the filter-pasteurized KK treatment. The heat pasteurized treatment was perceived as less sweet, more sour, less fruity, more honey, and more caramelized (OA) than both of the treatments, and more bitter, more caramelized (RA), more malty, and less diacetyl (RA) than the filter-pasteurized treatment. However, only a portion of these differences are significant; the control treatments is significantly perceived as more fruity (RA) than all of the other treatments, more sweet and less sour than the heat-pasteurized treatment, and more malty (OA) than the filter-pasteurized treatment. The heat-

pasteurized treatment was significantly perceived as less sweet than all of the other treatments and more honey (RA) than the filter-pasteurized treatment.

Table 45. Flavor Comparison of Aardbeien Lambic with Different KK Pasteurization Treatments Over Time

	003, t=1.5	**512, t=1.5**	**920, t=1.5**	**003, t=3**	**512, t=3**	**920, t=3**	**003, t=4**	**512, t=4**	**920, t=4**	**003, t=5**	**512, t=5**	**920, t=5**
Sweet	4.57 [ab]	2.81 [a]	2.49 [b]	2.93	2.07	2.43	4.31 [c]	3	2.13 [c]	3.75 [e]	3.63 [d]	2.81 [de]
Sour	1.29 [a]	2.5	2.93 [a]	1.5 [bc]	3.43 [b]	3 [c]	1.69 [d]	4	4 [d]	2.5 [e]	4.13	4.5 [e]
Bitter	1.94	1.63	1.93	1.79	1.21	1	1.19	1.75	2.13	2.69	1.94	2.25
Fruity	4.81	3.71	3.29	3.75	2.83	3.36	4.19 [a]	2.75	2.31 [a]	4.25 [bc]	3.5 [b]	3 [c]
Vanilla	0.69 [ab]	0.14 [a]	0.29 [b]	0.64	0.36	0.36	0.31	0.13	0.25	0.44	0.25	0.31
Caramelized	0.64	0.14	0.43	0.83	0.5 [a]	0.71 [a]	1.06	0.44	0.66	0.94	0.56	0.81
Chocolate	0.07	0	0	0	0	0.14	0	0	0	0	0	0
Honey	0.5	0.36	0.79	0.96	1.14 [a]	1.57 [a]	1.13	1.38	1.31	0.94	0.88 [b]	1.13 [b]
Molasses	0	0	0	0.86	0.14	0.14	0.31	0.13	0.13	0.04	0.04	0.04
Burnt/ Burnt Toast	0	0.14	0.07	0.07	0	0	0	0.06	0.06	0	0	0.06
Coffee	0	0	0	0.14	0.07	0.07	0.06	0.06	0	0	0	0
Malty	1.07	0.5	1.07	0.36	0.14	0.31	0.44	0.38	0.25	0.75	0.38	0.75
Roasted	0.07	0.43	0.14	0	0.07	0.29	0.13	0	0.13	0.25	0.13	0.13
Hoppy	1.07	1	1.13	0.71	0.64	0.86	0.75	0.81	1.25	1.13	1.06	1.13
Yeasty	0.93	1	1	0.5	0.5	0.71	0.75	0.75	0.56	1.13	1.06	0.94
Waxy/beany	0	0	0	0	0	0	0	0	0	0	0	0
Diacetyl	0.57	1.83	1.43	1.06	1.79	1.69	0.81	1.6	0.81	0.5	0.81	0.53

Table 46. Orthonasal Aroma Comparison of Aardbeien Lambic with Different KK Pasteurization Treatments Over Time

	003, t=1.5	512, t=1.5	920, t=1.5	003, t=3	512, t=3	920, t=3	003, t=4	512, t=4	920, t=4	003, t=5	512, t=5	920, t=5
Citrus	2.14	1.63	1.79	2.84	2.50	2.86	2.63	2.38	2.40	2.69	3.00	2.75
Fruity	5.67[a]	3.58 [a]	3.66	4.64	4.36	4.71	5.63	4.31	4.06	5.25	4.69	4.44
Vanilla	0.50	0.50	0.21	1.07	1.14	1.14	0.81[a]	0.63	0.31[a]	0.88	0.50	0.69
Caramelized	0.93	0.75	1.14	0.71	0.86	0.93	1.00	0.69	0.94	0.38	0.69	0.88
Chocolate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00
Honey	1.14 [a]	0.63	0.57 [a]	1.50 [b]	1.00 [b]	1.21	1.44	1.44	1.25	1.00	1.25	1.38
Molasses	0.00	0.00	0.00	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00	0.00
Piney	0.29	0.17	0.79	0.21	0.21	0.29	0.25	0.44	0.25	0.56	0.44	0.50
Burnt/ Burnt Toast	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Coffee	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hay-like/ grassy	0.36	0.17	0.93	0.29	0.07	0.29	0.06	0.19	0.19	0.19	0.19	0.31
Malty	0.79	0.25 [a]	1.21 [a]	0.79	0.57	0.93	1.10	0.48	0.66	0.88 [b]	0.31 [b]	0.75
Roasted	0.00	0.17	0.14	0.29	0.14	0.00	0.13	0.00	0.25	0.13	0.00	0.44
Hoppy	0.64 [a]	0.50 [b]	2.00 [ab]	1.21	0.86	0.86	0.75	0.88	1.06	0.94	1.13	1.00
Yeasty	0.50	0.92	0.94	0.50	0.79	0.64	0.56 [a]	0.69	0.88 [a]	0.75	0.94	1.25
Waxy/ beany	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diacetyl	0.57	1.83	1.00	0.79 [a]	1.71 [a]	0.75	0.93	1.19	1.19	1.00	1.06	1.13

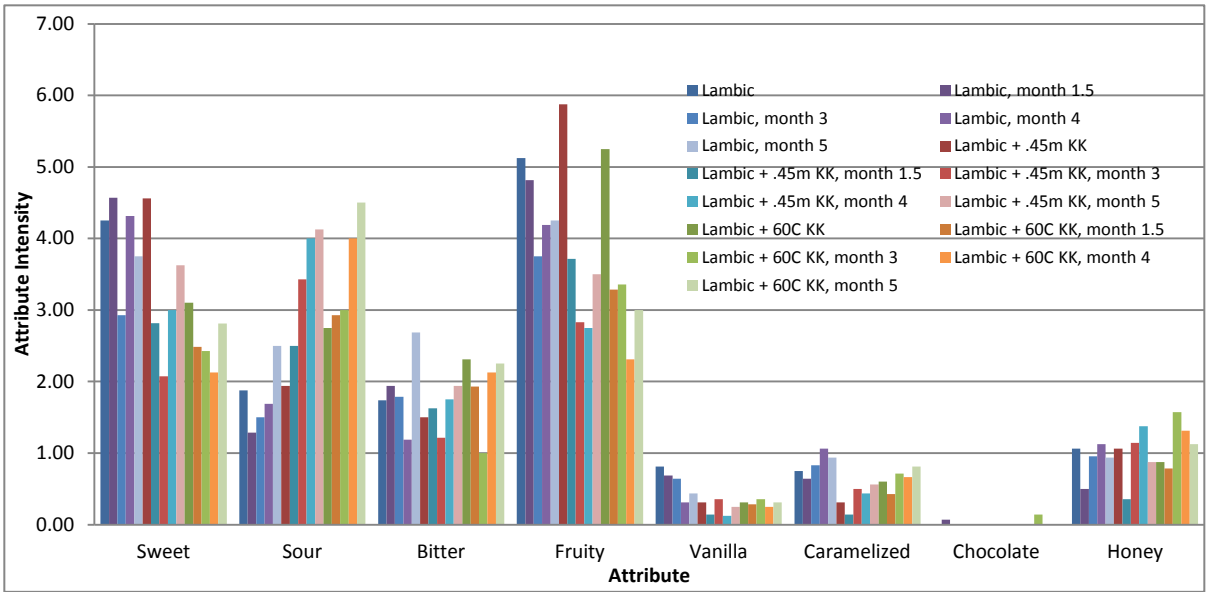

Figure 45. Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments Over Time, pt. 1

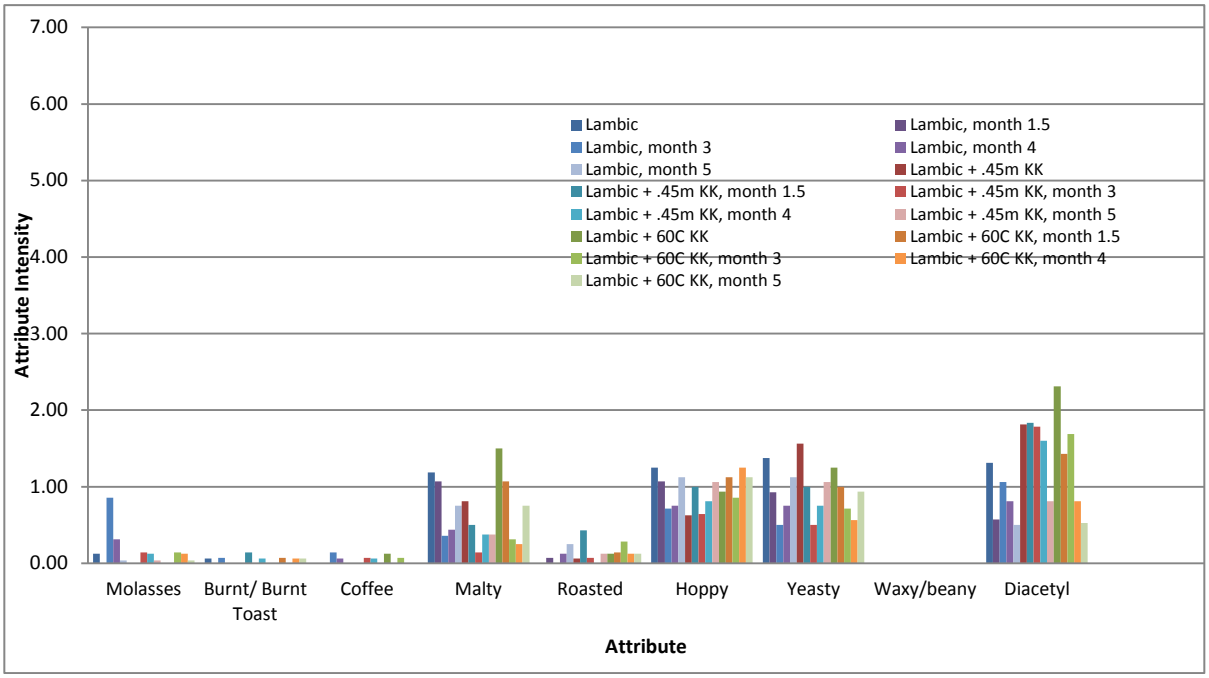

Figure 46. Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments Over Time, pt. 2

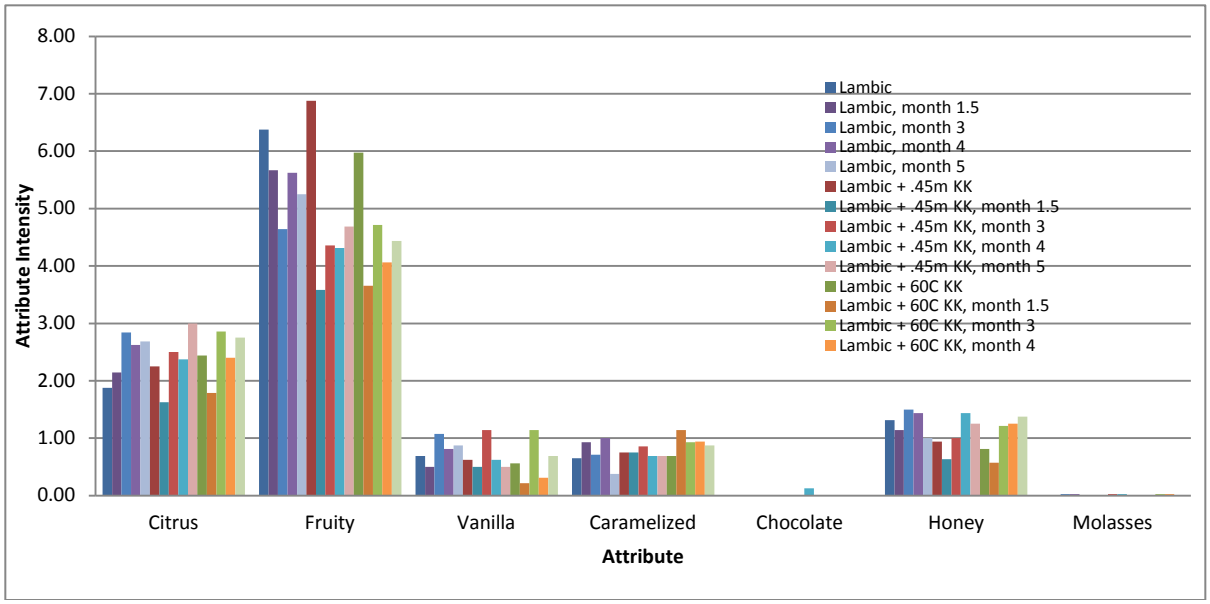

Figure 47. Orthonasal Aroma of Aardbeien Lambic with Different KK Pasteurization Treatments Over Time, pt. 1

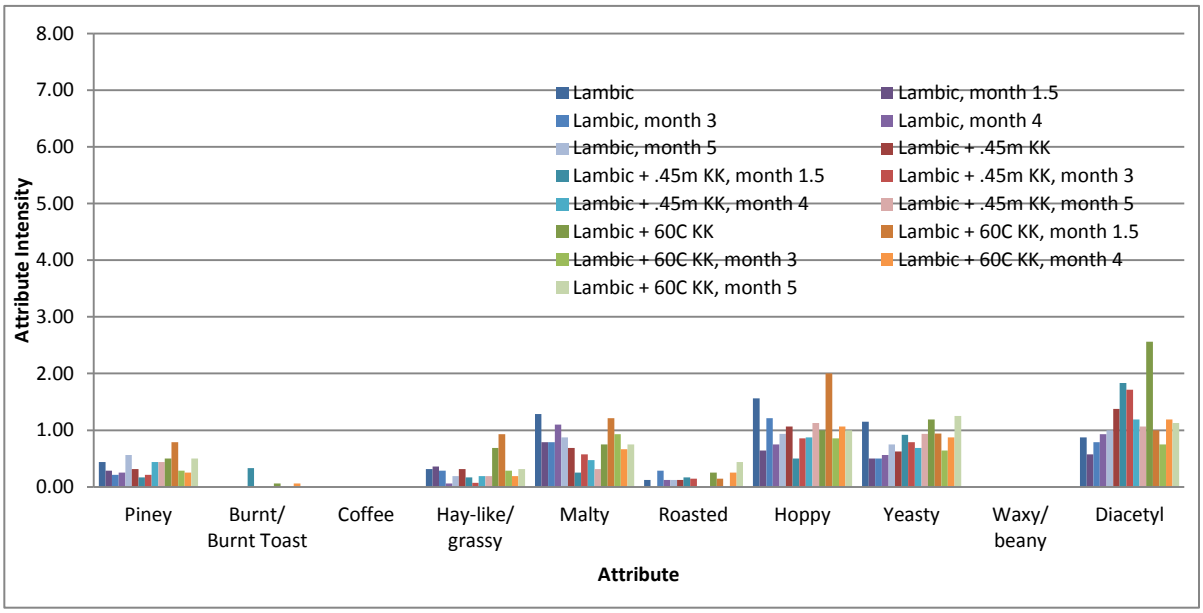

Figure 48. Orthonasal Aroma of Aardbeien Lambic with Different KK Pasteurization Treatments Over Time, pt. 2

It is evident that there is a great deal of information to gather from this study, especially when comparing all of the treatments over storage time. Furthermore, there are some general inferences that can be made from the data: in all of the treatments, at the end of storage time, sweetness is decreased, sourness is increased, bitterness increases, fruitiness decreases, caramelized (RA) increases slightly, maltiness (RA) decreases, and diacetyl decreases, except for the OA control, it remains about the same. It was specified earlier that a typical ale has a shelf-life of about 122 days, or four months; therefore, it is important to see how this product compares to this shelf-time. In order to assess the situation more thoroughly, a comparison was made between the t=0 treatments and their t=4 and t=5 counterparts (Table 47. and 48.). In other words, it was necessary to see if the treatments at month 4 and month 5 were overall significantly different than their t=0 counterparts- thus making them a different a product and furthermore, reaching the end of shelf-life.

Table 47. Effect of Storage Time on Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments

	Lambic, month 0	**Lambic+.45µ KK, month 0**	**Lambic+60 °CKK, month 0**	**003, t=4**	**512, t=4**	**920, t=4**	**003, t=5**	**512, t=5**	**920, t=5**
Sweet	4.25^{an}	4.56^{bo}	3.1^{ab}	4.31^{c}	3	2.13^{c}	3.75^{en}	3.63^{do}	2.81^{de}
Sour	1.88^{a}	1.94^{de}	2.75^{afg}	1.69^{b}	4^{d}	4^{bf}	2.5^{c}	4.13^{e}	4.5^{cg}
Bitter	1.74	1.5	2.31	1.19	1.75	2.13	2.69	1.94	2.25
Fruity	5.5^{de}	5.88^{fg}	5.25^{hi}	4.19^{ad}	2.75^{f}	2.31^{ah}	4.25^{bce}	3.5^{bg}	3^{ci}
Vanilla	0.81	0.31	0.31	0.31	0.13	0.25	0.44	0.25	0.31
Caramelized	0.75^{a}	0.31	0.6	1.06	0.44	0.66	0.94^{a}	0.56	0.81
Chocolate	0	0	0	0	0	0	0	0	0
Honey	1.06	1.06	0.88	1.13	1.38	1.31	0.94	0.88^{a}	1.13^{a}
Molasses	0.13	0	0	0.31	0.13	0.13	0.04	0.04	0.04
Burnt/ Burnt Toast	0.06	0	0	0	0.06	0.06	0	0	0.06

Table 47. (continued) Effect of Storage Time on Flavor of Aardbeien Lambic with Different KK Pasteurization Treatments

Coffee	0	0	0.13	0.06	0.06	0	0	0	0
Malty	1.19[a]	0.81[ab]	1.5[bcn]	0.44	0.38	0.25[c]	0.75	0.38	0.75[n]
Roasted	0	0.06	0.13	0.13	0	0.13	0.25	0.13	0.13
Hoppy	1.25	0.63	0.94	0.75	0.81	1.25	1.13	1.06	1.13
Yeasty	1.38[a]	1.56[b]	1.25[n]	0.75[a]	0.75[b]	0.56[n]	1.13	1.06	0.94
Waxy/beany	0.0	0.0	0.0	0	0	0	0	0	0
Diacetyl	1.31	1.81	2.31[an]	0.81	1.6	0.81[n]	0.5	0.81	0.53[a]

Table 48. Effect of Storage Time on Orthonasal Aroma of Aardbeien Lambic with Different KK Pasteurization Treatments

	003, t=0 Lambic, month 0	**512, t=0 Lambic + .45 micron KK, month 0**	**920, t=0 Lambic + 60C KK, month 0**	**003, t=4**	**512, t=4**	**920, t=4**	**003, t=5**	**512, t=5**	**920, t=5**
Citrus	1.88	2.25	2.44	2.63	2.38	2.40	2.69	3.00	2.75
Fruity	6.38[o]	6.88[ab]	5.98[n]	5.63	4.31[a]	4.06[n]	5.25[o]	4.69[a]	4.44
Vanilla	0.69	0.63	0.56	0.81[a]	0.63	0.31[a]	0.88	0.50	0.69
Caramelized	0.65	0.75	0.69	1.00	0.69	0.94	0.38	0.69	0.88
Chocolate	0	0	0	0.00	0.13	0.00	0.00	0.00	0.00
Honey	1.31	0.94	0.81[no]	1.44	1.44	1.25[n]	1.00	1.25	1.38[o]
Molasses	0	0	0	0.03	0.03	0.03	0.00	0.00	0.00
Piney	0.44	0.31	0.5	0.25	0.44	0.25	0.56	0.44	0.50
Burnt/ Burnt Toast	0	0	0.06	0.00	0.00	0.06	0.00	0.00	0.00
Coffee	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
Hay-like/ grassy	0.31[a]	0.31[b]	0.69[abcn]	0.06	0.19	0.19[c]	0.19	0.19	0.31[n]
Malty	1.29[ab]	0.69[ad]	0.75[b]	1.10	0.48	0.66	0.88[c]	0.31[cd]	0.75
Roasted	0.13	0.13	0.25	0.13	0.00	0.25	0.13	0.00	0.44
Hoppy	1.56	1.06	1.00	0.75	0.88	1.06	0.94	1.13	1.00
Yeasty	1.15[n]	0.63[b]	1.19	0.56[a]	0.69	0.88[a]	0.75[n]	0.94[b]	1.25
Waxy/ beany	0.0	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00
Diacetyl	.88	1.38	1.93[an]	0.93	1.19	1.19[a]	1.00	1.06	1.13[n]

At month 4, the control is perceived as significantly less fruity (RA) and less yeasty (OA and RA), 512 is perceived as significantly more sour, less fruity (OA and RA), and less yeasty (RA), and 920 is significantly perceived as more sour, less fruity (OA and RA), less malty (RA), less yeasty (RA), less diacetyl (OA and RA), more

honey (OA), and less hay-like/ grassy (OA). At month 5, the control is significantly perceived as less sweet, less fruity (OA and RA), more caramelized (RA), less malty (OA), and less yeasty (OA), 512 is perceived as significantly less sweet, more sour, less fruity (OA and RA), less malty (OA), and more yeasty (OA), and 920 is significantly perceived as more sour, less fruity (RA), less malty (RA), less diacetyl (OA and RA), more honey (OA), and less hay-like/ grassy (OA).

It can be noted that at month 4, the control treatment has less significant change in profile than the kristalkefir treatments, and both of the kefir treatments did experience a substantial amount of significant change by month 4; however, it appeared as if treatment 920 experienced the most amount of significant change at month 4. At month 5, the control treatment showed a greater amount of significant change than it did at month 4, and it appeared as if the KK treatments did as well. It still seemed as if the KK treatments still showed a greater significant change at month 5 from their respective month 0 treatments than the control treatment did, and form the KK treatments, 920, again, appeared to have experienced the most significant change at month 5. Overall, at month four, it appears as if there is a substantial amount of significant change, and furthermore, implicating a shelf-life of less than four months in terms of sensory quality.

Therapeutic Quality

The isoflavone results from the accelerated shelf-life aardbeien treatments can be viewed in the following table:

Table 49. Shelf Stability and Isoflavone Breakdown in Pasteurized Aardbeien Lambic Treatments

Treatment	Treatment Description	Daidzin %	Genistin %	Total Glycoside %	Total Glycoside % vs. SKP published (.09*-.37%)	Daidzein %	Daidzein% vs. SKP published (0.006*-.020%)	Genistein %	Genistein % vs. SKP data (0.003*-0.01%)	Total Alglycone %	Total Alglycone % vs. SKP published (0.01*-.03%)
512 t=0	.45µ filter pasteurized KK+aardbeien; 25%	0.0003	0.0003	0.0006	0.7%	0.00012	2.0%	0.0004	13.3%	0.00052	5.2%
920 t=0	heat pasteurized at 60°C KK + aardbeien; 25%	0.00025	0.0025	0.00275	3.1%	0.00011	1.8%	0.00075	25.0%	0.00086	8.6%
512, t=1.5	.45µ filter pasteurized KK+aardbeien; 25%, month 1.5	0.0002	0.0002	0.0004	0.4%	0.00016	2.7%	0.00012	4.0%	0.00028	2.8%
920, t=1.5	heat pasteurized at 60°C KK + aardbeien; 25%, month 1.5	0.0002	0.0021	0.0023	2.6%	0.00013	2.2%	0.00072	24.0%	0.00085	8.5%
512, t=3	.45µ filter pasteurized KK+aardbeien; 25%, month 3	0.0002	0.000057	0.000257	0.3%	0.00027	4.5%	0.000042	1.4%	0.000312	3.1%
920, t=3	heat pasteurized at 60°C KK + aardbeien; 25%, month 3	0.00039	0.00007	0.00046	0.5%	0.00011	1.8%	0.00012	4.0%	0.00023	2.3%
512, t=4	.45µ filter pasteurized KK+aardbeien; 25%, month 4	0.00037	0.00005	0.00042	0.5%	0.00009	1.5%	0.000059	2.0%	0.000149	1.5%
920, t=4	heat pasteurized at 60°C KK + aardbeien; 25%, month 4	0.00027	0.00005	0.00032	0.4%	0.00035	5.8%	0.00011	3.7%	0.00046	4.6%
512, t=5	.45µ filter pasteurized KK+aardbeien; 25%, month 5	0.00022	0.00004	0.00026	0.3%	0.00012	2.0%	0.000036	1.2%	0.000156	1.6%
920, t=5	heat pasteurized at 60°C KK + aardbeien; 25%, month 5	0.00021	0.000041	0.000251	0.3%	0.0002	3.3%	0.000045	1.5%	0.000245	2.5%

It was found that overall throughout the shelf-life period, the aardbeien treatments that contained the heat pasteurized KK had a higher alglycone concentration than its

filter-pasteurized counterpart, and also had a higher glycoside concentration until month 4, where both treatments' concentration remained relatively equal throughout the rest of the shelf study. While total alglycone and glycoside concentrations in both treatments appeared to decrease over time fairly linearly, treatment 920 (heat pasteurized) did not see a great change in, and furthermore, decrease in isoflavone concentrations until month 3. The individual isoflavone categories, however, were found to be slightly less straightforward.

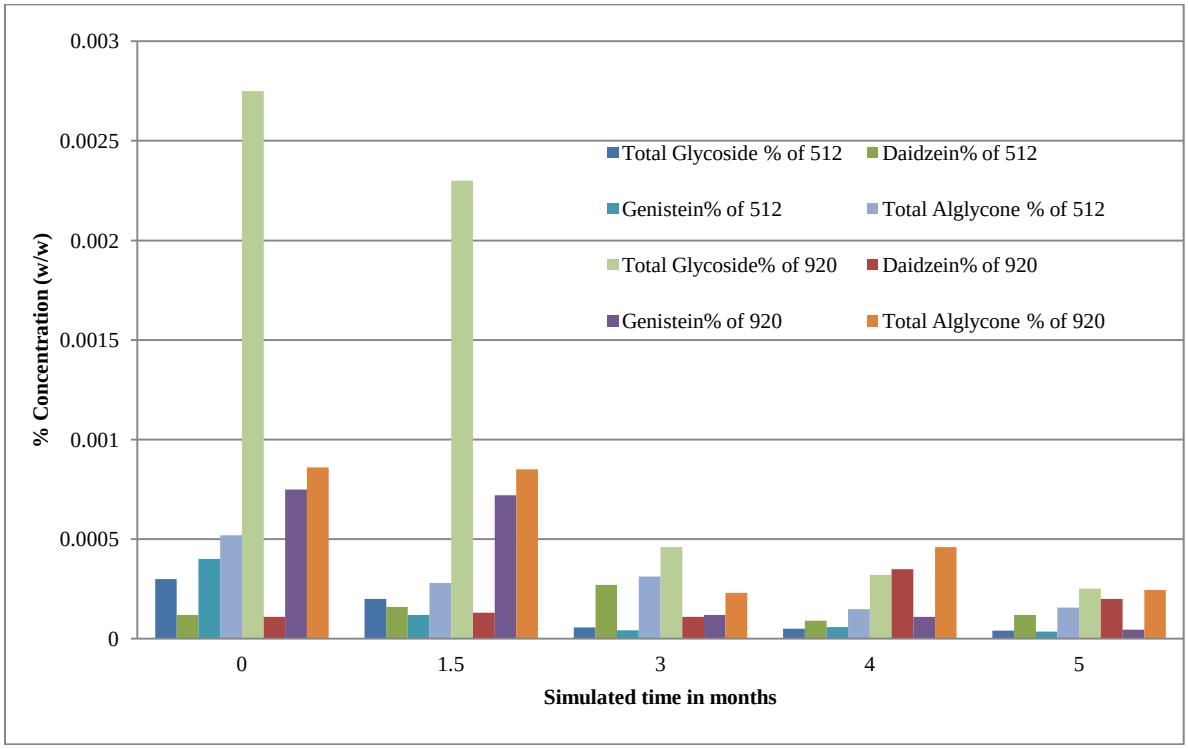

Figure 49. Isoflavone Concentrations of Pasteurized KK Aardbeien Treatments Over Shelf-life

As Figure 50 shows, genistein and genistin in both treatments do show a decrease in concentrations over time, with again, treatment 920 not showing a great decrease until month 3; however the daidzein and daidzin concentrations of both treatments do not show the same trend (Figure 51). Both treatments' daidzin concentrations begin higher than their daidzein concentrations and then start to decrease, whereas their daidzein concentrations start to increase.

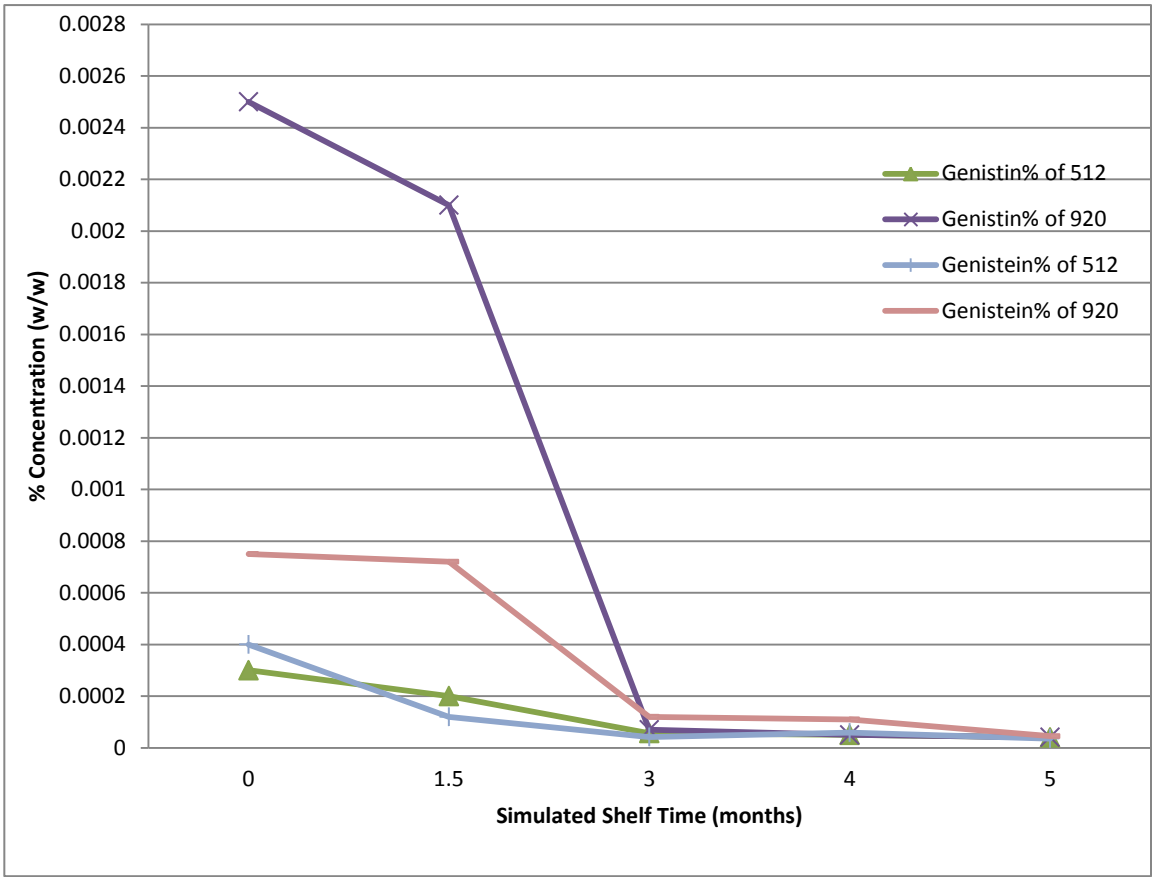

Figure 50. Shelf Stability of Genistin/ Genistein in Aardbeien Lambic

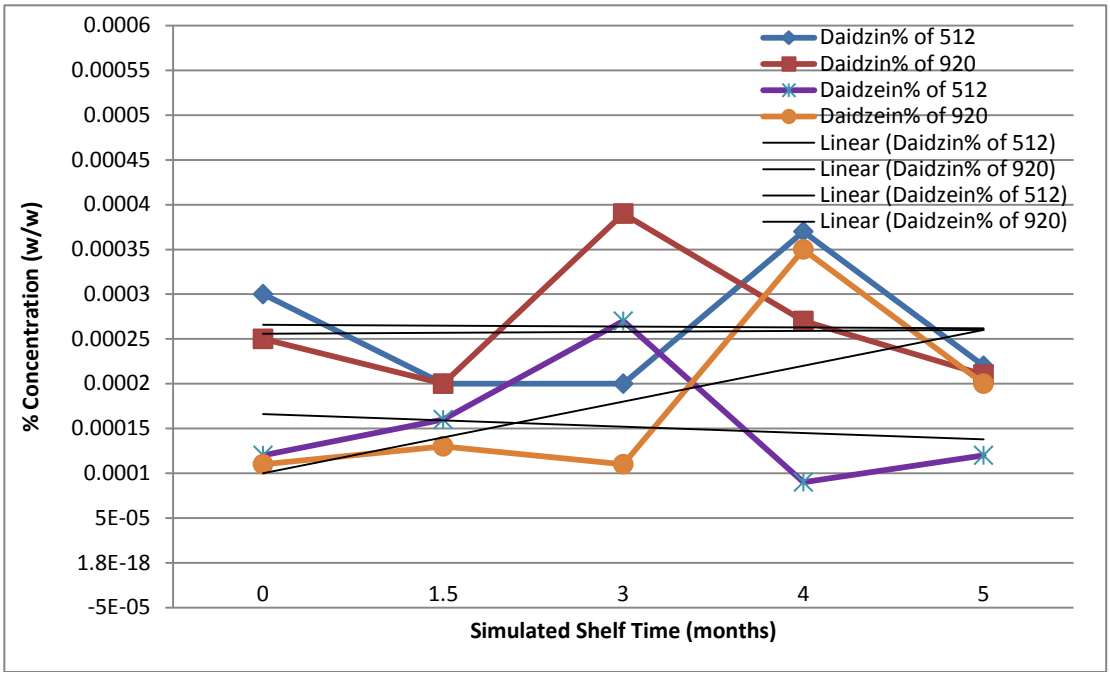

Figure 51. Shelf Stability of Daidzin/ Daidzein in Pasteurized Aardbeien Lambic Treatments

Then, in month 3, treatments 920 has a spike in daidzin concentration, while its daidzein decreases; this may be true due to a possible conversion taking place within the matrix, however, treatment 512 experienced the opposite effect at month 3- spike in daidzein and a decrease in daidzin. Then, at month four, treatment 920's daidzein concentration increases while its daidzin concentration decreases, whereas treatment 512 showed the opposite effect. At month five, both treatments showed a decrease in both compounds, with the exception of a slight increase in daidzein in treatment 512. During the shelf-life, however, both treatments showed an overall decrease in daidzin, and treatment 512 showed a slight decrease in daidzein, where treatment 920 showed an increase in daidzein.

Microbiological Status

The results from the microbiological analysis can be viewed through the following table:

Table 50. Microbiological Analysis of Accelerated Shelf-life Treatments at t=0, and t=5

	003- Aardbeien control, t=0 (cfu/ml)	512- filter-pasteurized KK lambic, t=0 (cfu/ml)	920- heat-pasteurized KK lambic, t=0 (cfu/ml)	003- control, t=5 (cfu/ml)	512- filter-pasteurized KK lambic, t=5 (cfu/ml)	920- heat-pasteurized KK lambic, t=5 (cfu/ml)
MRS+cyclo.	0	0	0	$2.0x10^4$	$1.6x10^4$	$1.9x10^4$
LMDA	0	0	0	$1.6x10^4$	$1.3x10^4$	$1.1x10^4$

All treatments at t=0 had no growth, while at t=5, all of the treatments appeared to have the same amount of growth at 10^4 cfu/ml, with little substantial difference between the growth on the LMDA versus the MRS+cycloheximide plates, therefore indicating there was a) most likely some contamination during the bottling process and b) the microorganisms were most likely of the lactobacillus category. In addition, the colonies on the LMDA plates showed zone clearing, indicating acid production, and were smooth and white, thus ultimately characterizing them as lactobacilli.

CHAPTER 4. DISCUSSION and CONCLUSION

4.1 Discussion

All of the aforementioned studies were done in order to develop a feasible beer or beer-like product with added biological isolates of soy, that would allow for some added health benefit to the consumer, in which feasibility is defined as:

a) Sensorally pleasing as, and/or contains a sensory profile similar to that of ales

b) Biological isolates of soy, and more specifically soy isoflavones, are present within the product at sufficient amounts to result in some therapeutic effect

c) Similar shelf life/conditions to that of an ale

d) The potential to be a successful product in the current marketplace, withstanding its price point and intended target audience

It was found that the results from these studies did assist in the development, and furthermore, mostly satisfied these objectives. To further understand the development process and the importance of each of the studies' outcomes, the results will be discussed in more detail.

Design Specifications and Early Development

As mentioned in the *Design Specifications*, the ale style of IPA was selected to be the intended ale style of this product because it was the most acceptable and compatible with the Soy Kefir Powder addition, and due to the beer's turbid appearance, a hybrid ale style, an IWA, with a grain bill consisting of 29% white wheat, was finally selected considering the consumer acceptability of natural turbidity in wheat ales. Because an IPA IBU typically ranges from 40-70 IBU (BJCP, 2010), the IBU Variance Study was done to assess which IBU

would be better suited for the product; again, this study was done with the SKP as well as the Kristalkefir. From the IBU Variance Study with Soy Kefir Powder and IBU Variance Study with Kristalkefir, and the Soy Kefir Powder Stability in Beer Matrix study, it was found that: the SKP is not stable in a beer matrix in terms of appearance, resulting in flocculation (Figure 14), or an unacceptable turbid appearance when xanthan gum is added; KK 50 IBU was the most accepted of the KK treatments, which is also the only KK treatment not significantly less accepted than its control, and it is also significantly accepted more than its SKP counterpart (SKP at 50 IBU). The SKP treatments were significantly less bitter than both the KK and control treatments, which could be due to these treatments being perceived as significantly sweeter than the KK treatments (the SKP treatments were perceived as sweeter than the controls as well, but this difference was not statistically significant). The 50 IBU KK treatment was also perceived as significantly less sweet than its control. The SKP treatments were also significantly fruitier than the KK treatments for both 50 and 60 IBU for RA (retronasal aroma), and just the 60 IBU treatments for OA (orthonasal aroma), and the SKP treatments were also significantly perceived with more waxy/beany notes than the KK treatments (OA and RA). Diacetyl in KK treatments was only significantly perceived more than the SKP treatments orthonasally. The controls had significant differences with the kefir treatments as well; they were perceived as significantly more bitter than the SKP treatments, for example. The controls were all perceived as hoppier than their kefir counterparts for both 50 and 60 IBU in RA, and just 60 IBU in OA. The SKP treatments significantly showed more waxy/beany notes than the controls for both OA and RA, and diacetyl was significantly much lower in the control treatments than the kefir treatments, and was virtually

undetectable. Withstanding this, it would appear that a 50 IBU KK Beer treatment would be the best option, due to it being the most accepted of the kefir treatments, being perceived as significantly less waxy/beany than the SKP treatments, and not significantly more waxy/beany than the controls, only perceived as possessing more diacetyl than the SKP treatments orthonasally, while all kefir treatments, even the SKP treatments, were all perceived as possessing more diacetyl than the control treatments, and it only supplies the ale matrix with very little turbidity and flocculation, thus making it more desirable in the case of appearance as well. Therefore, the 50 IBU KK treatment was selected for further study and development.

Another reason, not discussed previously, factored into this decision as well- cost. The Soy Kefir Powder is markedly more costly to purchase or produce than the Kristalkefir, due to the extra cost of spray-drying (as mentioned previously, SKP is essentially spray-dried liquid kefir). The SKP production cost is approximately $20.00/kg, whereas the KK costs approximately $0.50/L; Table 51 compares the characteristics of these two kefir products.

Table 51. SKP versus KK Added Cost Comparison in Ale Production

	Cost (USD)	Amount/12 oz (355 ml) Serving	Added Cost to Beer Production/Serving (USD)	Average Cost for Craft Beer Production/ Serving (USD)	Approx. Cost for Product/ Serving (USD)
Soy Kefir Powder	20.00/kg	5.25 g	0.11	0.50	0.61
Kristalkefir	0.50/L	88 ml	0.04		0.54

In addition, the SKP production would require an up-front cost for the purchase of a spray-dryer, as well as an increased production time, and furthermore, an increase in labor costs. As mentioned previously, the KK was clarified initially by means of centrifugation, which is also a technique utilized in the brewing industry, therefore, this would not equate to an extra cost burden onto its production due to the brewery already having a centrifuge in use. Desludger disc stack centrifuges are the typical type of centrifuge utilized in the brewing industry for removal of yeast from green beer. While this is not the type of centrifuge utilized previously used for the clarification of the Kristalkefir, the process could be modified slightly to allow for the utilization of this particular "industry standard," centrifugation instrument. $0.61 versus $0.54 does not seem like a huge difference, but this is per 12 oz or 355 ml bottle, and most beer is typically sold in packs of six, not individually; therefore, that brings the cost up to $3.66 and $3.24, respectively, with an SKP cost of nearly 13% more than that of the KK. Withstanding this, and the previously mentioned reasons, it was clear the 50 IBU KK treatment was also the most cost effective treatment to develop further.

Diacetyl Rest Study

The next major study performed in the development process was elimination of diacetyl, which was done to primarily assess whether or not a diacetyl rest would help to eliminate the perception of the aromatic compound, diacetyl, and how the rest affected the isoflavones. The conclusion from this study was that while both the beer treatments with KK0 (KK without a diacetyl rest) and KK48 (KK with a 48 hour diacetyl rest) were perceived as having significantly more diacetyl than the control, there was no significant difference for diacetyl between the control and the beer treatment with KK at a 24-hour

diacetyl rest. This discovery may be due to while at 24 hours there was an ample amount of diacetyl, or 2,3-butanedione, being taken up by the *Saccharomyces cerevisiae* and then dissimilated into 2,3-butanediol, perhaps after 24 hours, there had been more diacetyl being made or assimilated by the gram-positive lactobacillus bacteria still within the kefir matrix, thus resulting in a greater perception of diacetyl in the KK after 48 hours than after 24 hours. The analytical results from the base kefir confirm this discovery (Figure 23). The trend that was found was that over the rest period, diacetyl decreased (it was dissimilated into 2,3-butanediol), but then increased after 24 hours (more was being produced), where it appeared to be on the decline once again at hour 48. As noted previously, these changes analytically (detection via HPLC) were fairly small, but the sensory data supported that these changes were significantly different based on detection/perception via olfactory bulb. This decreased perception of diacetyl was found to allow for other important ale aromatics to be perceived, for example, the beer treatment with KK without a diacetyl rest was perceived as significantly less malty and hoppy (RA) than the beer treatment with KK24, and KK24 was also significantly perceived as having more honey (RA) and hoppy notes (OA and RA) than the beer treatment with KK48. In terms of the isoflavone data, it was found that the kristalkefir without a diacetyl rest had the most glycoside concentration, at 49.9% of the published data, however, had the least concentration of alglycones, daidzein and genistein, at 84.3%. It was also found that as the diacetyl period increased, hydrolysis from glycosides to alglycones increased; KK24 had a lesser concentration of glycosides than KK0, at 18.8%, and had a higher concentration of alglycones, at 129.0%, whereas KK48 had an even lower amount of glycosides, 12.8%, and the most amount of alglycones, at 223.0%. The beer

treatments showcased a similar pattern with their respective KK diacetyl rest treatments, validating this discovery.

As noted previously, during beer production, the increase in temperature re-activates the *S. cerevisiae* during the diacetyl rest, and in the case of the KK diacetyl rest, krausened yeast was actually inoculated into the kefir matrix prior to the start of the rest. In continuation, diacetyl uptake and dissimilation appears to not be the only biochemical activity occurring during the diacetyl rest, or "activation" period, and it can be realized from the data that perhaps an increase in β-glucosidase activity is occurring as well. This activity would allow for hydrolysis of the glycosides to occur, converting them into their respective conjugates, alglycones, thus explaining the parallel between alglycone concentration and diacetyl rest duration (as the rest duration increases, so does the alglycone concentration). Withstanding the aforementioned results, despite KK48 and its beer treatment possessing the greatest amount of alglycones within each of their categories, at 223.0% and 57.0% of the SKP published data, respectively, the KK24 beer treatment was selected to continue on in the development process. This was due to it being perceived as having the least amount of diacetyl, which is not significantly greater than the control treatment, and being perceived as also having a greater concentration of a few desirable ale attributes, when compared to the other KK treatments, such as maltiness, honey, and hoppy. In continuation, even though the KK48 treatments had a greater amount of alglycones, the KK24 treatments still had a greater alglycone concentration than the SKP published data, at 129.0% in the KK treatment, and 41.0% in the KK24 beer treatment (versus a value of 25.0%), which still makes it an exceptionally potent product.

IPA versus Lambic Study

The next major study performed in the development process was the Soy Kefir IPA and Soy Kefir in Aardbeien analogue Study, which was done in order to a) asses whether or not KK fermented with fructose produced more higher alcohol and other desired volatile aromatics, for example, ethyl acetate (fruity) and amyl alcohols- more specifically, 2-methyl-1-butanol (isoamyl alcohol, or banana) and 3-methyl-1-butanol (fusel/solvent-like note) aromatics, in a perceivable amount in the beer matrix that would increase its acceptability, to b) determine whether or not an aardbeien lambic style would be a better suited style for the addition of the Kristalkefir due to their similarities in flavor profile, for example, lactic acid production/ sourness, fruity/apple-like (acetaldehyde), as well as some sour styles of ales (Flanders Red) even possessing low diacetyl notes (BJCP, 2010), and furthermore creating a more balanced and desirable product, and to c) asses whether or not these variables have an effect on isoflavone concentrations. In the blind-bench portion, it was found that overall, the IPA treatments were accepted more than the aardbeien treatments; however, the only significant difference found was between the KK B IPA treatment and both of the controls- it was accepted less than the controls. In addition, the KK A treatments were accepted more than the KK B treatments, and despite the IPA treatments being accepted more overall than the aardbeien treatments, the aardbeien treatments, however, were not accepted as less as the IPA treatments were when compared to their controls, at 14.0% and 21.4% accepted less, for KKA and KKB IPA respectively, versus 10.5% and 18.6%. Through descriptive analysis, it was found that the IPA treatments were significantly perceived as less sweet, more bitter, less fruity, possessing less honey notes (RA), and more hoppy, which are all obvious and typical

differences between any IPA and lambic ale style. The controls overall were significantly perceived as less sour and less diacetyl than their KK treatments (RA, with the exception of KK A aardbeien and its control), which shows that sourness and diacetyl are the main impacts of the KK addition. Other impacts of the KK addition were discovered, however, it was dependent upon the exact matrix (treatment); for example, the KK addition in the IPA matrix with KK A (100% glucose) significantly decreased the amount of perceived fruity (RA) and caramelized (RA) notes. The KK addition in the aardbeien matrix with KK B (50:50 glucose and fructose), however, significantly decreased the amount of perceived sweetness and fruitiness (OA), and increased the amount of perceived bitterness. In continuation, the different KK treatments appear to behave differently in the IPA and the lambic treatments, and while it was hypothesized that the KK B treatments would be perceived as more fruity, and thus not allowing for a significant difference in between them and their control treatments, this theory was proven wrong. Additionally, the IPA KK A treatment was not significantly different from the IPA KK B treatment, whereas the Aardbeien KK A treatment was only significantly perceived as less bitter than the Aardbeien KK B treatment. Beer treatments with KK fermented with fructose appeared to have no perceived significant affect on fruitiness compared to the treatments with KK fermented with 100% glucose. Lastly, the Aardbeien KK A treatment was the only treatment not possessing a perceived significant difference for diacetyl (RA) with its control treatment.

From the isoflavone analysis, it was found that KK B had a lower amount of glycosidic isoflavones, at 9.3% of the published data, and a higher amount of alglycosidic isoflavones, at 165.0%, than KK A, at 16.7 and 129.0%, respectively. The beer treatments,

however, did not demonstrate this same trend. One would expect the beer treatments to possess isoflavone concentrations at 25% of the KK concentrations, due to the KK being at a 25% concentration in the beer treatments. Withstanding this, the KK A beer treatments would have an alglycone concentration of 32.3% and the KK B beer treatments would have a concentration of 41.3%, however, this difference, though, does not appear to be that great. Again, this may be due to human error from not ensuring proper homogeneity of the kristalkefir and beer prior to bottling. Regardless of this possible causative agent, all of the glycosides were around the same concentration throughout all of the treatments, and the alglycone concentrations appeared to be lower in the aardbeien treatments versus the IPA treatments, which are 43.0 and 35.0% (KKA and KKB, respectively) versus 64.0 and 43.0%. Despite KK B possessing higher alglycone amounts, KK A was selected to continue because it is the traditional method for soy kefir production, and because the KK B appeared to have no perceived significant affect on fruitiness, while it was significantly perceived as having more diacetyl than its control treatments. In addition, the KKA treatments were more accepted than the KKB treatments, and KK A still had nearly 30% more alglycones than the SKP published data. The KKA Aardbeien Lambic treatment was ultimately selected to continue on in the development process due to the IPA treatments not being significantly accepted more than the aardbeien treatments and because the KKA aardbeien treatment had a smaller accepted less percentage versus its control than the KKA IPA treatment did versus its control, which was 10.5% versus 14.0%, respectively. Also significant, the KKA Aardbeien Lambic treatment was not perceived as possessing more diacetyl than its control treatment, whereas the IPA treatments did. The KK A Aardbeien Lambic treatment still would be an

exceptionally potent product, with an alglycone concentration of 43.0% of the SKP published data, versus an expected concentration of 25.0%.

Pasteurization of Kristalkefir

The next major study performed in the development process was to determine the effects of pasteurization treatments in the Aardbeien Lambic Study. This study was done in order to a) determine which pasteurization methods were capable of a full inactivation of beer spoilage microorganisms, b) assess whether or not different pasteurization methods of the Kristalkefir would have an effect on the ale product sensorally, and then c) determine how the heat or filter pasteurization techniques affected the isoflavone concentrations and breakdown within the product. Two pasteurization methods were found to allow for a full inactivation of beer spoilage microorganisms, which were a thermal inactivation of 20 minutes at 60°C, and a filter inactivation, with a .45-micron pore size filter. From the blind bench-top study, it was found that the control treatment (aardbeien lambic without KK), was the most accepted, followed by the lambic treatment with the heat-pasteurized kristalkefir, and then lastly, the aardbeien treatment with the filter pasteurized kristalkefir was accepted the least. The control was significantly more acceptable than both of the KK treatments, however, the heat-pasteurized treatment was not significantly more acceptable than its filtered counterpart. It was also found that the filter-pasteurized treatment was accepted, however, 42.3% less than the control, while the heat-pasteurized treatment was only accepted 26.9% less than the control. From the descriptive analysis, it was found that the control overall was only significantly perceived as more malty than the KK treatments, showing that maltiness was the main impact of the pasteurized KK addition. It was found that the type of

pasteurized KK addition, however, did make more of an impact; for example, the heat pasteurized KK addition (920), significantly decreased the amount of perceived sweetness, increased the amount of perceived sourness, and while the addition did affect its amount of perceived maltiness (OA), it did not affect its maltiness as much as the filter pasteurized KK addition did (RA). Withstanding this, the filter pasteurized KK addition (treatment 512) significantly decreased the amount of perceived maltiness (OA and RA). Diacetyl, however, was not perceived as significantly different across all of the treatments, therefore, showing that adding pasteurized KK to an aardbeien lambic does not have an affect on perceived diacetyl.

From the isoflavone analysis, it was found that both pasteurization treatments were deleterious to the isoflavone compounds based on an over 70% loss of the alglycones, and an approximate 30% loss of the glycosides due to the process. The heat pasteurization technique, at 34.0% and 11.2% respectively, however, was found to be less deleterious to the isoflavone compounds than the filtration, at 12.0% and 10.3% respectively. This may be due to the isoflavones being bound to other compounds, or not fully in solution. Agglomerated particles may not have been able to pass through a pore size of .45 micron. The beer treatments demonstrated a similar pattern with their respective KK pasteurization treatments. Withstanding all of the aforementioned results, the heat-pasteurized KK aardbeien lambic treatment appeared to be the best treatment for the product due to it being accepted more than the filter-pasteurized KK treatment, and it being only accepted 26.9% less than the control. In addition, diacetyl was not significantly perceived more than the control, and the heat-pasteurized KK treatment was significantly perceived as less sweet than all of the treatments,

and more sour than the control, which are desired attributes in the lambic ale style, and more malty retronasally than the filter pasteurized treatment, which is also a desired attribute in ales. The heat pasteurization method would also be more cost effective, and furthermore, substantially less costly than the filter pasteurization method due to the exceptionally high cost of the filters, especially at that small of a pore size. In addition, because of the small pore size, many filters would most likely need to be used to filter one batch, again, making the filter-pasteurization method substantially more costly than the heat-pasteurization method. In addition, pasteurization and type of pasteurization appears to have no effect on the major fermentation by-products. Lastly, it appears to be the best treatment for the product due to the heat-pasteurization treatment not being as deleterious to the isoflavones as filtering was discovered to be, and while there was still a great deal of loss, the heat pasteurized aardbeien lambic treatment still showed a fair amount of alglycones, which would still have the potential to allow for some therapeutic effect.

Shelf-life

All three of these aardbeien lambic treatments, 003- the control, 512- filter-pasteurized KK, and 920- heat-pasteurized KK, were then used in the Accelerated Self-life Study. The study parameters were based off the previously mentioned equation ("Q_{10}"), which is a standard equation and practice used within the food industry. Over storage time of a food product there are numerous of biochemical reactions taking place, such as enzymatic degradation, oxidation, and perhaps bacterial growth, and further more, spoilage, and the principal behind this practice is that the use of heat, or an increase in the product's normal temperature (by 10°C), rather, speeds-up these reactions. Therefore, this study was done to

try and determine an appropriate shelf-life for the Aardbeien Lambic product, based upon both sensory and isoflavone analytical data; how quickly are these reactions taking place within each of the matrices that would cause a significant change in quality and the product profile? Over storage time, it was found that in all of the treatments, sweetness is decreased, sourness is increased, bitterness increases, fruitiness decreases, caramelized (RA) increases slightly, maltiness (RA) decreases, and diacetyl decreases, except for the OA control, it remains about the same. One would expect some fruitiness in an ale to diminish over time due to ester degradation, such as the ester, isoamyl acetate (banana) (Delvaux et al, 1997). A sweet/caramelized flavor, or sherry-like aromatic, increase is also expected as beer ages due to oxidation, and its intensity would correlate to the amount of headspace, dissolved oxygen, and reactive oxygen species within the beer bottle, and furthermore, ale matrix.

"[There is an] initial acceleration of sweet aroma development, [such as] the formation of caramel, burnt-sugar and toffee-like aromas (also called leathery), [which] coincides with the sweet taste increase" (Derdelinckx et al, 2006). Again, overall sweetness did decrease in all of the treatments, however, it was also found that at month 4, for 003 and 512, and month 5, for 920, there was a slight increase in sweetness. The decrease can be explained by the bacterial, and furthermore lactobacilli, growth, which then increased the concentration of organic acids- most likely, lactic acid- thus creating an increase in sourness, which would warrant a decrease in perceived sweetness. There was also an overall increase in bitterness, but it should be noted that there appeared to be an initial decrease in bitterness, which would be caused by degradation of the iso-alpha acids, which include both the trans- and cis-isomers of the isomerized alpha acid compounds. In continuation, the early detection of the

decrease in bitterness is due to the trans-isomers being quite sensitive to degradation (Derdelinckx et al, 2006). Then, after the initial decrease, bitterness began to increase, at month 4 for both of the KK treatments, and at month 5 for the control, which is due to the increase in, or perhaps reformation/changes in, polyphenols/polyphenolic content.

> After a lag period of about 5 weeks, the levels of tannoids began to increase and the changes in the polyphenol contents were associated with the appearance of harsh, [after-bitter, and] astringent tastes (Derdelinckx et al, 2006).

The KK treatments most likely experienced an earlier onset due to these treatments possessing more polyphenolic content than the control, contributed by the soy (i.e. isoflavones).

Although not an attribute on the ballot, it was noted that many panelists expressed detection of, or wrote-in, a solvent-like attribute for treatments 512 and 920 starting at month 4, and even the control treatment, but not until month 5. This solvent-like note is also considered a common beer-staling flavor attribute, which is brought on by a few mechanisms and end products. It is noted by Coghe et al (2003), that as storage time increases, there is an increase in the volatile compound furanic ethyl ether above its flavor threshold, of 6 μg/L, which imparts a solvent-like flavor. The Strecker degradation of amino acids is another mechanism that contributes to a solvent-like attribute as well. This mechanism is initiated with the amino acids leucine or valine, and oxygen, and the reaction is catalyzed by iron and copper ions. The end products are the higher alcohol compounds, 2-methyl-propanal and 3-methyl-butanal, which, again, will impart a solvent-like aromatic (Blockmans et al, 1975). The KK treatments most likely experienced an earlier formation, or an earlier detection,

rather, due to there probably being a higher amount of amino acids in the ale matrix contributed by the soy kefir.

Through the Accelerated Shelf-life Study, it was also found that at month 4, the control was perceived as significantly less fruity (RA) and less yeasty (OA and RA) than its initial profile at month 0, 512 was perceived as significantly more sour, less fruity (OA and RA), and less yeasty (RA), and 920 was significantly perceived as more sour, less fruity (OA and RA), less malty (RA), less yeasty (RA), less diacetyl (OA and RA), more honey (OA), and less hay-like/ grassy (OA). At month 5, the control was significantly perceived as less sweet, less fruity (OA and RA), more caramelized (RA), less malty (OA), and less yeasty (OA), 512 was perceived as significantly less sweet, more sour, less fruity (OA and RA), less malty (OA), and more yeasty (OA), and 920 was significantly perceived as more sour, less fruity (RA), less malty (RA), less diacetyl (OA and RA), more honey (OA), and less hay-like/ grassy (OA). It appears as at month four, both of the KK treatments experienced a substantial amount of change, with the heat-pasteurized KK treatment experiencing the most amount of significant change from its initial month 0 profile. The control treatment did not have an ample amount of significant change until month 5.

While this equation and practice based off "Q_{10}" was utilized to "speed-up" and simulate the "aging" process, it is possible that this technique or exact technical parameters are too general of a practice for the food industry in its entirety. In other words, the same practice utilized for a can of ham and bean soup may be ill fitted for a cream-filled snack cake and, an aardbeien lambic. It was found through the study, however, that the same staling/aged ale attributes were perceived in the treatments in a time-line comparable to other

beers- four months or 122 days. Due to the contamination during the bottling process, however, there was bacterial, and moreover, most-likely lactobacillus, growth, which affected the flavor profile over time, by means of increased sourness, and decreased sweetness, which could potentially affect the shelf-life results in terms of shelf duration. On the other hand, in lambic/sour ale styles, increased sourness and lesser sweetness are both typically desired attribute intensities; despite this known fact, from a sensory standpoint, a definitive shelf-life cannot be determined at this point, but the data suggests that up to a four month shelf-life, comparable to similar products on the market, would be the best shelf-life duration for this nutraceutical beer.

From the isoflavone data, it was found that overall throughout the shelf-life period, the aardbeien treatments that contained the heat pasteurized KK had a higher alglycone concentration than its filter-pasteurized counterpart, and also had a higher glycoside concentration until month 4, where both treatments' concentration remained relatively equal throughout the rest of the shelf study. While total alglycone and glycoside concentrations in both treatments appeared to decrease over time fairly linearly, treatment 920 (heat pasteurized) did not see a great change in, and furthermore, decrease in isoflavone concentrations until month 3. Genistein and genistin was found to have a similar trend in both treatments- a decrease in concentrations over time, with again, treatment 920 not showing a great decrease until month 3; however the daidzein and daidzin concentrations of both treatments did not show the same trend. Both treatments' daidzin concentrations begin higher than their daidzein concentrations and then start to decrease, whereas their daidzein concentrations start to increase. Then, in month 3, treatment 920 has a spike in daidzin

concentration, while its daidzein decreases; this may be true due to a possible conversion taking place within the matrix, however, treatment 512 experienced the opposite effect at month 3- a spike in daidzein and a decrease in daidzin. Then, at month four, treatment 920's daidzein concentration increases while its daidzin concentration decreases, whereas treatment 512 showed the opposite effect. At month five, both treatments showed a decrease in both compounds, with the exception of a slight increase in daidzein in treatment 512. During the shelf-life, however, both treatments showed an overall decrease in daidzin, and treatment 512 showed a slight decrease in daidzein, where treatment 920 showed an increase in daidzein. In continuation, this shelf-life study validates the initial decision to select 920, the heat-pasteurized KK aardbeien lambic treatment, as the best treatment for the product, due to it mainly possessing a higher alglycone concentration throughout the storage duration; however, again, it was noted that at month 3, there was a drastic decrease in alglycone concentration in this treatment- what is the reasoning behind this discovery?

As mentioned previously in this paper, isoflavones posses a great deal of antioxidant capabilities, however, some antioxidant-capable compounds, also can be pro-oxidant in different matrices, or when exposed to certain compounds. Just as oxygen present within a bottle of beer can have a detrimental effect on its sensory profile, as was demonstrated through the aardbeien treatments, it is possible that it can also have a detrimental effect on this products isoflavone breakdown/ concentrations? This phenomenon again is noticed at month 3, where similarly, oxidation of volatile and non-volatile compounds are beginning to show a change in the ale's sensory profile, or if the oxidative products are not at a perceivable level yet, at least the reactions are taking place within the matrix at this point in

storage. Cao et al (1997) states that "flavonoids, including flavones, isoflavones, and flavanones, serve as prooxidants in the presence of Cu^{+2}." It is known that this copper ion is present within the ale matrix, due to it also instigating/ catalyzing the Strecker degradation of amino acid reaction, resulting in the formation of solvent-like, higher aromatic volatile compounds, which was perceived in the product, again in both of the KK treatments at month 4. Therefore, it is a fair assumption to be made that the reasoning behind the drastic decrease in alglycone concentration at month 3, is due to oxidation of the alglycone compounds, genistein and daidzein, and primarily, genistein.

Withstanding all of the previously mentioned information from this study, the heat-pasteurized KK aardbeien lambic treatment with a shelf-life of 3 months would appear to be the best option for the nutraceutical ale via biological isolates of soy kefir due to it at month 3 being significantly perceived as more honey (RA) than the filter-pasteurized treatment, and not significantly perceived as more diacetyl than the control, where the filter-pasteurized KK treatment was. In addition, at month 4, both of the KK treatments appeared to have a substantial amount of significant differences from their respective initial treatment profiles, therefore, this would suggest a shelf-life up to four months; however, 3 months was ultimately deemed most appropriate do to the isoflavone/ therapeutic quality. The therapeutic quality, by means of alglycone concentration remained stable at approximately 8.6% of the SKP published data until month 3, where it showed a great decrease, and this treatment was also substantially greater in alglycone concentration than the filter-pasteurized treatment. In continuation, despite the pasteurized KK treatments possessing lower amounts of alglycones, the heat-pasteurized KK aardbeien treatment still has a greater % of Recommended Daily

Intake of SKP[19] than the SKP+beer treatment due to the increased isoflavone potency of the Kristalkefir, at 18.74% versus 17.50%.

Table 52. Isoflavone and Serving Breakdown in Kefir Beer Treatments

Treatment	Total % Solids of Kefir Utilized in Treatment	Kefir amount/ 355 ml serving	Kefir Concentration in Treatment	Compared Total Alglycone %	% More Alglycone %	SKP equivalent (g)	% of Recommended Daily Intake of SKP
SKP + Beer	100.00%	5.25 g	1.50%	1.50%	0.00%	5.25	17.50%
KK + Beer	6.00%	88 ml	25.00%	49.33%	47.83%	7.76	25.87%
920 (KK 60°C + Beer)	6.00%	88 ml	25.00%	8.60%	7.10%	5.62	18.74%

This recommendation of 30 g/day, is for those individuals experiencing moderately severe conditions; however, for those individuals with less severe conditions, they may not need to intake as much to relieve their pain, fatigue, inflammation, etc., therefore, supplying the product with an even higher % of Recommended Daily Intake for these individuals. In continuation, it appears as if this nutraceutical aardbeien lambic via biological isolates of soy kefir meets the feasibility objectives a, b, and c: a) it is sensorally pleasing as, or contains a sensory profile similar to that of ales (satisfying the latter); b) biological isolates of soy, and

[19] Based off a 30 g/day recommendation (Kubow and Sheppard, 2009)

more specifically soy isoflavones, are present within the product at sufficient amounts that may allow for some therapeutic effect; and c) the product has some shelf stability and similar shelf life/conditions to that of an ale; however, does it satisfy objective d- has the potential to be a successful product in the current marketplace, withstanding its price point and intended target audience?

Viability in the Current Marketplace

As mentioned previously in this paper, this product would appear to be a very viable product in the current market. There is both a trend in the market for nutraceutical beverages, and craft beer, and furthermore, an opportunity for a large market share. In addition, this particular nutraceutical beverage would fill, or have the potential to fill a consumer need, with a target audience of "baby boomers," nearly 30% of the US population, a population that is knee-deep in, or just beginning to experience the physiological factors of getting older, by offering added health benefits such as relief from pain, fatigue, hypertension, and inflammation. In continuation, according to a report from the Boston Beer Company, "aging and more affluent baby boomers do not appear to be satisfied with the run-of-the-mill beers. They want unique items, and as a result, are more inclined to sample what the market has to offer" (Bride et al, 2007). Therefore, this shows that baby boomers may be more apt to try this product, despite it being a new ale product, and moreover, a new product category altogether- thus making it quite a unique item indeed. While this may be true, how does price affect this "sampling of the market?" What are "baby boomers" willing to pay for these types of products, how does the price of this lambic compare to other lambics currently on the market, and how does the price of this nutraceutical beverage compare to other similar

nutraceutical beverages currently on the market?

As stated earlier in this paper, the cost to produce a basic beer with added Kristalkefir is $0.54/ 12 oz or 355 ml serving. The final product, the Aardbeien Lambic with heat-pasteurized Kristalkefir, would approximately be another $0.05 per serving on top of this (the "aardbeien" part of the aardbeien lambic costs about $0.05/ serving, and the pasteurization cost per serving would be negligible due to minor cost of the heat required for this procedure, and due to most breweries already having the necessary equipment for this procedure as well- i.e. steam-jacketed and pressurized stainless steel tank and a heat-exchanger), bringing the total cost to produce to $0.59/12 oz or 355 ml serving, or $3.54/6-pack. The selling price for other lambics and sour ales currently on the market range from about $8.00/ 6-pack, Samuel Adams Cranberry Lambic (Boston Beer Co, Boston, MA), to about $20.00/ 12 oz or 355 ml beer bottle, Hanssens Experimental Raspberry (Hanssens Artisinaal, Dworp, Belgium), and this category has an average price of about $5.00/ 12 oz or 355 ml beer bottle. Withstanding this information, it is easy to see how this product would be well within the lambic/sour ale style price point, and moreover, substantially lower than the average price point. In Ogle's book, *Ambitious Brew: the Story of American Brew*, she notes that the craft brew scene and the invention of the "microbrewery" was initiated and created as the "baby boomers" came of legal drinking age. She notes that:

> in the 1960s and 1970s, young Americans backpacked through Europe and there discovered "real" ales and stouts. They returned eager to try their hand at making those beers at home. In the 1980s, some of the homebrewers opened microbreweries and brewpubs [which instigated the initial rise in the craft beer scene] (2006).

Withstanding this, and the previously mentioned statement about baby boomers being not satisfied with run-of-the-mill beers, it can be inferred that "baby boomers" are willing to pay for craft beer, thus sacrificing quantity for quality. In continuation, the average price of craft beer is approximately $9.00/six-pack, therefore, it would appear as if this price is an acceptable price point for this target audience of "baby boomers." This price would be a reasonable price for the product at current development, therefore, making it within a reasonable price range for purchase by the target audience of "baby boomers." In addition, "baby boomers" are identified as the drivers of the nutraceutical market and are more inclined to purchase functional foods (Kastenholz, 2008). In continuation, functional and nutraceutical foods are still largely accepted by consumers with a greater price, with an average mark-up margin of 30% (PricewaterhouseCoopers, 2009), therefore, this information implies that "baby boomers" are more apt to pay a premium price for a added health benefit in a convenient package- such as the Aardbeien Lambic. In conclusion, it would appear as if this nutraceutical Aardbeien Lambic via biological isolates of soy kefir would satisfy objective d- has the potential to be a successful product in the current marketplace, withstanding its price point and intended target audience- as well.

Despite this product seemingly satisfying all of the objectives, labeling and regulatory issues exist, mainly due to it being an alcoholic beverage. The federal organization that controls the majority of the alcoholic beverages in the United States is the Alcohol and Tobacco Tax and Trade Bureau (TTB), while most of the other part of the food industry, including nutraceutical beverages, is regulated by the Food and Drug Administration (FDA). The TTB clearly states in the Code of Federal Regulations, 27CFR7.29, "Prohibited

Practices," in regards to labeling regulations for malt beverages:

> (i) *Health-related statements.* In general, labels may not contain any health-related statement that is untrue in any particular or tends to create a misleading impression as to the effects on health of alcohol consumption. TTB will evaluate such statements on a case-by-case basis and may require as part of the health-related statement a disclaimer or some other qualifying statement to dispel any misleading impression conveyed by the health-related statement.
>
> (ii) *Specific health claims.* (A) TTB will consult with the Food and Drug Administration (FDA), as needed, on the use of a specific health claim on a malt beverage label. If FDA determines that the use of such a labeling claim is a drug claim that is not in compliance with the requirements of the Federal Food, Drug, and Cosmetic Act, TTB will not approve the use of that specific health claim on a malt beverage label.
>
> (B) TTB will approve the use of a specific health claim on a malt beverage label only if the claim is truthful and adequately substantiated by scientific or medical evidence; sufficiently detailed and qualified with respect to the categories of individuals to whom the claim applies; adequately discloses the health risks associated with both moderate and heavier levels of alcohol consumption; and outlines the categories of individuals for whom any levels of alcohol consumption may cause health risks. This information must appear as part of the specific health claim (Alcohol and Tobacco Tax and Trade Bureau, 2010).

Withstanding this, it appears as if there is ambiguity in their regulation, and does not supply a definitive answer to whether or not a health, or therapeutic effect, claim could be made on

the label, and is left up to the discretion of the TTB. The TTB legally defines a malt beverage as:

> A beverage made by the alcoholic fermentation of an infusion or decoction, or combination of both, in potable brewing water, of malted barley with hops, or their parts, or their products, and with or without other malted cereals, and with or without the addition of unmalted or prepared cereals, other carbohydrates or products prepared therefrom, and with or without the addition of carbon dioxide, and with or without other wholesome products suitable for human food consumption (Alcohol and Tobacco Tax and Trade Bureau, 2010).

Any other malt beverage that does not satisfy this definition, is exempt from these rulings due to it being out of the TTB's jurisdiction, and falls within the jurisdiction of the FDA. This product in its final formula (see Appendix for Aardbeien Lambic Style Final Formula) only has an IBU of 4.5, and has a minute hop addition, at 1.24 oz/bbl, which is typical of the lambic ale style; therefore, removing the hop addition in order to fall within the FDA's jurisdiction, would be a feasible amendment, without having a severe change in the product's profile. The FDA has more clear regulations for labeling, and although health claim specifications for this particular therapeutic/health benefit (soy isoflavones and relief from pain, fatigue, and inflammation) has not yet been addressed, and furthermore approved, a health claim, or therapeutic claim, may be easier to be accepted by the FDA versus the TTB. In addition, the FDA states that if a product contains a nutrient that in which does not have a legal Recommended Daily Intake (RDI) or Daily Recommended Value (DRV), information about the nutrient can still be listed on the label.

> [This information can be listed] provided that the information is truthful and not misleading and is provided outside the Nutrition Facts label. Such information is limited to statements of amount or percent of a nutrient (eg. 300 mg omega 3) and may not characterize the level of the nutrient (you may not state "High in Omega-3").
>
> 21 CFR 101.13(i)(3) (U.S. Department of Health and Human Services, FDA, 2009)

In conclusion, while the current formula for the Aardbeien Lambic with Kristalkefir, has the possibility to be approved for the inclusion of a health or therapeutic claim within the jurisdiction of the TTB, a claim, or inclusion of isoflavone content, on the product label would be more than likely be easier to be approved by the FDA, pending an amendment to the product formula- removal of the hops.

4.2 Conclusion

In conclusion, a feasible beer or beer-like product with added biological isolates of soy kefir was developed; in which feasibility is defined as:

a) It is sensorally pleasing as, or contains a sensory profile similar to that of certain ales (satisfying the latter).

b) Biological isolates of soy, and more specifically soy isoflavones, are present within the product at sufficient amounts that may result in some therapeutic effect.

c) Based on the use of pasteurization, the product has similar shelf life/conditions to that of an ale.

d) Has the potential to be a successful product in the current marketplace, withstanding its price point and intended target audience.

The Aardbeien Lambic ale containing liquid soy kefir at a concentration of 25%, which was previously heat-pasteurized at 60° C for 20 minutes, proved to satisfy the feasibility objectives. While this product was only accepted with a blind bench-top score of 4.75, neither liked nor disliked, it was only accepted 26.9% less than the lambic control, at a hedonic score of 6.5, moderately liked. In addition, diacetyl was not significantly perceived more than the control, and it was significantly perceived as less sweet than all of the treatments, and more sour than the control, which are desired attributes in the lambic ale style, and more malty retronasally than the filter pasteurized KK treatment, which is also a desired attribute in ales. All of the other attributes of this product were not significantly different than the lambic ale control, thus showing that it does have a similar sensory profile to that of ales, or its particular ale style. Despite heat-pasteurization showing to be deleterious to the soy isoflavones, they were still within the matrix at a moderate amount, allowing the alglycones, to be present within the product in a sufficient amount, at 8.60% of the Soy Kefir Powder published data. Withstanding this, one 12 oz or 355 ml serving of this product provides the consumer with 18.74% of the Recommended Daily Intake (RDI) of SKP, at 30 g/day, thus allowing the product to have the potential to supply some therapeutic effect, based on the previous SKP clinical trials (Kubow and Sheppard, 2009). The data from this product implicates that it would have a shelf-life of up to three months, based upon the sensory and isoflavone data.

At month 3, the product was significantly perceived as more honey (RA) than the filter-pasteurized KK treatment, and again, it was not significantly perceived as more diacetyl than the control, but was significantly perceived as more sour (again, a desired attribute for lambic and *sour* ales). All of the other attributes of this product at month 3 were not signifi-

cantly different than the lambic ale control, thus showing that it does have a similar sensory profile to that of ales, or its particular ale style. In addition, at month 4, both of the KK treatments appeared to have a substantial amount of significant differences from their respective initial treatment profiles, therefore, this would suggest a shelf-life up to four months; however, 3 months was ultimately deemed most appropriate do to the isoflavone/ therapeutic quality. The therapeutic quality, by means of alglycone concentration remained stable at approximately 8.6% of the SKP published data until month 3, where it showed a great decrease. The above data was based off a storage temperature of 5°C, which is the recommended storage temperature for beer, thus making this product possess some shelf stability and similar shelf life/conditions to that of an ale, considering the average shelf-life for ales is 122 days, or 4 months, which is not substantially longer than this product's, at 3 months. In continuation, this product would appear to be a very viable product in the current market, because there is both a trend in the market for nutraceutical beverages, and craft beer, and furthermore, an opportunity for a large market share, and, this particular nutraceutical beverage would fill, or have the potential to fill a consumer need, with a target audience of "baby boomers," nearly 30% of the US population, a population that is knee-deep in, or just beginning to experience the physiological factors of getting older, by potentially offering added health benefits such as relief from pain, fatigue, hypertension, and inflammation. In addition, its price point is within a range that "baby boomers" will most likely feel is acceptable, in terms of either a) craft beer, and moreover, lambic and sour ale styles, and b) nutraceutical beverages. Lastly, it has real potential to be marketed as a "nutraceutical beer," legally making some sort of notation on its label about either its isoflavone/ alglycone content or % RDI

of SKP, and/or its therapeutic effect, in which provides relief from pain, fatigue, inflammation, and hypertension.

Future Work

Despite the previous findings, and evidence of met objectives, there is still some future work that the product either calls for, or would benefit from. For example, while there was some hedonic data collected on this product, it was only in a blind bench-top capacity. In order to further substantiate acceptance of this product, and furthermore, potential product launch, a consumer test must be performed on this product. This would be done with moderate ale consumers, consumes once at least every other week, who are at least twenty-one years of age, with a sample size of 150 participants. Each participant would come at his or her designated time, and each 15-minute time slot would allow for five panelists, therefore, allowing for optimum test parameter control (i.e. serving temperature of 11°C, minimal carbon dioxide escape, randomized order, etc.). The panelists would analyze four total samples- this product, and three commercial lambic or sour ale examples- for acceptance, again, on a 9-pt hedonic scale. The product would also benefit from improvements to the product to allow for increased stability of the isoflavones within its matrix.

As noted previously in this paper, the isoflavone standards were all stored in dimethyl sulfoxide, or DMSO, and were ran in the HPLC in a 50:50 solution of DMSO and HPLC grade water. This was done because these isoflavones are highly stable/ highly soluble in the presence of this compound. It was also noted earlier that DMSO is moderately present within the wort, which is supplied by the malted barley

during the mashing process; however, it is not present within the beer downstream, due to it a) being driven off in the kettle by heat, or by b) it being converted into dimethyl sulfide (DMS) by the *Saccharomyces cerevisiae*, which again, is considered an off-flavor, as it gives off a cooked corn flavor note. As stated in the literature review, the gene found to be responsible for this mechanism is the MXR1 gene.

> Saccharomyces yeasts contain an enzymatic activity that reduces DMSO to DMS in an NADPH-dependent manner, and a so-called MetSO (methionine sulfoxide) reductase isolated from yeast was suggested to be identical to the DMSO reductase (Bech et al, 2002).

Due to this by-product, DMS, being an off-flavor, and ultimately, its presence in the beer downstream being an issue as far as consumer acceptance, research has recently been conducted to genetically modify yeast in such a way that does not allow the microorganism to be able to metabolize DMSO and convert it into DMS, thus resulting in the absence of a non-desired cooked corn flavor. Bech et al (2002), found that 80% of the DMS present in the final product is due to the over-expression of the MXR1 gene in *S. carlsbergensis* (also known as *S. pastorianus*) and *S. cerevisiae*, and by inactivating MXR1 gene activity, these yeasts were not able to reduce DMSO into DMS, thus preserving the DMSO and allowing for it to remain present downstream in the beer matrix. While this is a great discovery for beer in terms of sensory acceptance, this is also is a great discovery in terms of this particular product at current invention- the Aardbeien Lambic with Kristalkefir. With the use of this particular modified yeast strain, DMSO would, again, remain in the beer downstream,

thus allowing for an increase in isoflavone stability, which could potentially increase shelf-life, or preserve the isoflavone compounds during pasteurization. Without known commercialization of this particular yeast strain, it would be challenging to replicate and utilize in a commercial brewery setting; however, the utilization of this strain could prove to be an eminent benefit to this product, and future work could be done to create this strain and furthermore, utilize it in the production of the nutraceutical Aardbeien Lambic via biological isolates of soy kefir, through the addition of Kristalkefir.

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APPENDIX

Appendix A. Beer Test Screener

Name (Print) : _____ Date: _____

1. Are you at least 21 years of age? (Circle correct response) Yes No
2. How often do you consume beer? (Circle correct response)

   a) at least once a week

   b) at least once every other week

   c) at least once a month

   d) less than once a month

   e) I do not consume beer

Appendix B. Beer Style Ballot→ used for preliminary bench testing

Name: _____ Date: _____

→ Circle the number that correlates with overall liking of each sample. Number 1 correlates with "dislike extremely" and number 9 correlates with "like extremely."

Sample #: _____	Sample #: _____
1 2 3 4 5 6 7 8 9 dislike extremely like extremely	1 2 3 4 5 6 7 8 9 dislike extremely like extremely
Sample #: _____	Sample #: _____
1 2 3 4 5 6 7 8 9 dislike extremely like extremely	1 2 3 4 5 6 7 8 9 dislike extremely like extremely
Sample #: _____	Sample #: _____
1 2 3 4 5 6 7 8 9 dislike extremely like extremely	1 2 3 4 5 6 7 8 9 dislike extremely like extremely

- Which sample did you prefer? _____

Appendix C. Initial Ale Lexicon for Orthonasal Aroma and Flavor→ initial orthonasal aroma lexicon includes all except the Basic Tastes

* Please rate in the designated column from 1-5: 1 corresponding with extremely inappropriate and 5 corresponding to extremely appropriate based upon the class of "ales" as a whole.

Attribute	Chemical(s)- if applicable	Definition/description	Reference	Example(s)	Rate
Basic Tastes					
Sweet	Sucrose, many	The taste stimulated by sucrose, and other sugars, sugar alcohols, and other sweet substances, such as Aspartame, etc.	5% sucrose in water- 5	1. Dextrose, glucose 2. Aspartame, fructose	
Sour	Acetic acid, acids	The taste stimulated by acids, such as citric, malic, and acetic.	.08% citric acid in water- 5		
Bitter	many	The taste stimulated by quinine, caffeine, and hop bitters.	.08% solution of caffeine- 5		
Salty	Sodium Chloride	The taste stimulated by sodium salts, and in part by other salts (KCl)	.35% solution of sodium chloride in distilled water- 5		
Fruity Notes					
Acetaldehyde	Acetaldehyde; ethyl hexanoate	A flavor note reminiscent of green apples.	Acetaldehyde (75 mg/L beer)	Harvey's Bristol Cream Sherry	
Black Currant		Fruity flavor characteristic of black currants	Black currant fruit	Same	
Citrus	many	Flavor associated with the general impression of citrus fruits	Linalool (.3 mg/L beer)	1) Citrus fruits 2) Pledge furniture polish	
Coconut		Flavor associated with coconut meat or milk	Fresh coconut	1)Unsweetened dried coconut 2) Macaroons	
Ethyl hexanoate	Ethyl hexanoate	An apple like flavor note with a note of aniseed	ethyl hexanoate (100 mg/L beer)	Apple sauce or juice with a slight amount of anise seed	
Fruity	Ethyl acetate	A flavor note associated with light fruity.	Ethyl acetate (100mg/L beer)	Fruit cocktail	
Isoamyl acetate	Isoamyl acetate	A flavor associated with banana.	Isoamyl acetate (10 mg/L beer)	1)Circus peanut candies 2) artificial banana flavoring	

Appendix C. (continued) Initial Ale Lexicon for Orthonasal Aroma and Flavor

Jam-like		A sweet flavor reminiscent of fruit jam	Berry jam	Same	
Melon	2,6-dimethyl-5-hepten-1-al	A fruit-like flavor associated with cantaloupe, watermelon, etc.	Melonal (3 µg/L beer)	1) Sour watermelon candy 2) ripe cantaloupe	
Pear		A flavor note associated with fresh pears	Ethyl 2,4 decadienoate (.5 mg/L beer)	Pear cocktail juice	
Raisiny	NA	A browned, sweet, fruity flavor, reminiscent of raisins	Re-hydrated raisins in beer	1) Raisins 2) Dr. Pepper	
Raspberry		Flavor associated with fresh raspberries	Crushed fresh raspberries	Raspberry jam or liquor	
Strawberry	Lactones	Strawberry or tropical fruit flavor associated with lactones	Dodecalactone	Strawberries	
Floral Notes					
Floral		A sweet aroma/flavor associated with flowers	Rose oil (1 drop/100 mL beer)	1) Johnson's and Johnson's baby powder 2) carnation	
Indole	2,3-Benzopyrrole	An aroma/flavor note reminiscent of floral jasmine, burnt, and earthy			
Perfumey		Having a light fragrant aromatic/flavor note characteristic of perfumes	Linalool	Jujube candies	
Rose-like	2-phenylethanol, geraniol	A flavor note associated with a rose-like fragrance	Geraniol (500 µg/L beer)	American Beauty Rose	
Spice Notes					
Garlic	Many	Flavor associated with garlic.	Fresh crushed garlic	Garlic powder	
Licorice	Estragole, dehydro-ar-ionene	Fruity flavor associated with licorice or anise	1 drop anise extract/50 ml white wine	1) Guinness stout 2) Licorice	
Spicy	many	An overall flavor term associated with pungent spices	Eugenol on perfumer's stick;2 to 3 grains ground black pepper, 1 drop anise extract/50 ml wine	allspice	
Vanilla	vanillin	Flavor note reminiscent of a blend of sweet, vanillin, woody, browned notes, sometimes having chocolate, tobacco, floral, or spicy components	Vanilla bean in a glass jar	Vanilla extract	

Appendix C. (continued) Initial Ale Lexicon for Orthonasal Aroma and Flavor

Vinylguaiacol	Vinylguaiacol	Flavor reminiscent of cloves and curry			
Sweet/syrup Notes					
Caramelized	many	The flavor relating to the browning of starches and sugars.	Caramelized sugar; 3-hydroxy-2-methyl-4-pyrone (1 g/L beer)	1)Killian's Red Lager; Newcastle 2) Kraft's caramel candies	
Chocolate	Dimethyl pyrazine, methyl butanol	Flavor associated with chocolate liquor, as found in roasted West Africa/Ivory Coast cocoa beans	Ivory Coast chocolate liquor	1) Lindt dark chocolate 2) Hershey's semi-sweet morsels	
Honey	many	The sweet, caramelized floral and woody aromatic/flavor associated with honey	Phenylacetic acid in sweetened water (10 ppm)	1) Clover honey	
Molasses		A flavor note associated with molasses; has a sharp, slight sulfur and/or caramelized character	Black Strap molasses (¼ t in 1 c water; 1-3 ml/25 ml beer)	1) Black Strap molasses 2) Karo Dark corn syrup	
Primings		The sweet taste of sugar primings			
Syrupy		A flavor note associated with clear (golden) syrup	Corn syrup (light)	Same	
Earthy Notes					
Earthy	many	Flavor note associated with an aromatic characteristic of damp soil, wet foliage, or slightly undercooked boiled potato.	Geosmin (.3 µg/l beer); 2-fenchyl alcohol (15 µg/L beer)	1) raw mushrooms 2) damp potting soil	
Moldy	Dimethylthiazol	Aromatic/flavor note characteristic of mold growth or wet damp soil	2-ethyl-1-hexanol (10,000 ppm in glycol)	1) moldy cheese 2) moldy bread	
Musty		Aromatic/flavor note associated with closed air spaces such as attics, closets, and basements.	2,4,6, tri-chloroanisole (30 ppb)	1) Old books 2) White pepper	
Piney	A-p-dimethylstyrene; β-pinene; bornyl benzoate; δ-terpinene; dihydroterpinyl acetate; α-pinene	Aromatic/flavor associated with dry, fresh cut pine wood and pine needles.	Canadian fir oil (.5%); pine sap	1) sage, rosemary 2) fresh cut pine needles 3) PineSol	
Resinous		A medicinal woody character of products that often contain woody notes	Retsina wine	1. Cedar wood 2. Sawdust	

Appendix C. (continued) Initial Ale Lexicon for Orthonasal Aroma and Flavor

Woody	many	An aromatic/flavor note associated with dry fresh cut wood; balsamic or bark-like	Wood chips, toothpicks	1) Bay leaves 2) Cedar, pine, popsicle sticks	
Vegetable Notes					
Beany		Flavor characteristic of soybeans and other legumes	Hexanal (60 ppm in safflower oil)	1) Raw soybeans (22g soaked in 75 ml evaporated milk) 2) Canned pinto beans	
Cabbage		A flavor note associated with overcooked green vegetables	Boiled cabbage (20 min)	Temperature abused beer	
Cooked Onion		A flavor note associated with cooked/boiled onion	Ethyl mercaptane (> 2.0 ppb)	1) Boiled onion 2) Onion powder	
Cooked tomato		flavor associated with cooked tomato	Canned tomato puree (in glass container)	1) Contadina tomato paste 2) Hunt's all natural tomato juice	
Corn grits		Flavor associated with maize grits, adjunct	Corn meal mush	Corn meal	
Methional	Methional	The flavor that refers to the internal portion of a baked potato.	Ore-Ida prebaked microwave potato	Ore-Ida prebaked microwave potato (Internal temperature 65-71°)	
Parsnip/celery		A flavor note associated with the effect of wort infection	Raw parsnip	Same	
Sweet corn		A flavor note associated with canned sweet corn.	Dimethyl sulfide (>50 ppb)	Creamed corn	
Roasted (or lack of) Notes					
Biscuit	4-heptenal				
Burnt	Octanol; indole**;** 3-methyl-1-butanol; ethyldimethylpyrazine; dimethyl sulfone; furfuryl alcohol	Flavor associated with blackened/acrid carbohydrates.	Burnt sugar (200% sucrose solution)	1) espresso coffee 2) Guinness	
Burnt toast		A burnt flavor note, like charred toast.	Black malt extract (20º C)	1) Guinness Extra Stout 2) Burnt toast crust	
Coffee		A flavor note associated with coffee	Ground coffee (6-8 grains/25 ml red wine)	same	

Appendix C. (continued) Initial Ale Lexicon for Orthonasal Aroma and Flavor

Green	hexanals	Flavor reminiscent of an aromatic relating to unprocessed vegetation, such as grains, leaves, and grass.	cis-3-hexen-1-ol (5 ppm in water)	1) green legumes 2) parsley	
Hay-like/straw		A grainy aromatic/flavor note with some green character of air-dried grain or vegetation.	Hay	1) hay/straw 2) dried parsley	
Husky		Aroma/flavor note associated with malt husks, chaff, Glattwasser.	Malt husks	Same	
Malty		The flavor reminiscent of toasted grain.	Malt extract (30 ml/L beer)	1) malted milk balls 2) Grapenuts	
Nutty	many	Flavor associated with nuts or nut meats	2, 6-dimethyl pyridine (2.0 ppm)	1) wheat germ 2) roasted coffee beans	
Roasted Barley		A flavor note associated with roasted barley used in the grist	Malted barley	1) Guinness stout 2) same	
Smokey	Many				
Wheaty		A flavor associated with flour.	Wheat flour	Wheat bread	
Fatty Notes					
Caprylic	Caprylic acid, octanoic acid	The aromatics/flavor associated with soapy, goaty, fatty, and tallow	Octanoic acid (30 mg/L beer)		
Fatty	Caproic acid	An overall term for all fatty flavor notes.	Caproic acid	Vegetable oil	
Oily	Many	An overall term for the aroma and flavor notes reminiscent of vegetable oil or mineral oil products.	Vegetable oil	1) Soybean oil 2) Mineral oil	
Waxy	Many	An aromatic/flavor associated with very long chain fatty acids, such as lauric, myristic, or stearic acids- reminiscent of waxes	Stearic acid	1) Candle wax 2) Crayola crayons	
Off-putting/phenolic/sulfur Notes					
Cardboardy	Malonaldehyde	Flavor associated with slightly oxidized fats and oils, reminiscent of wet cardboard	Malonaldehyde	1) wet cardboard 2) nonfat dry milk	
Catty		Flavors associated with oxidized beer and cat urine.	p-Menthane-8-thiol-3-one	1) Tomato plant leaves 2) oxidized beer	

Appendix C. (continued) Initial Ale Lexicon for Orthonasal Aroma and Flavor

Diacetyl	Diacetyl	Flavor associated with fermented dairy products and spoiled butter	Diacetyl (.2-.4 mg/L beer)	1) movie popcorn 2) butterscotch pudding 3) buttered popcorn Jelly Belly	
Iodoform/ Phenolic	Iodophors, phenols, chlorophenol, etc	A flavor note associated with a pharmaceutical/ medicinal, hospital-like aroma- band-aid	Capidyne; 4-methylphenol (7 mg/L beer)	1) Band-Aid bandages 2) chloraseptic sore throat spray	
Isovaleric/butyric	Isovaleric acid, butyric acid	Aroma/flavor associated with dry, stale cheese, old hops, and rancidity; sweaty	Isovaleric acid (5 mg/L beer); butyric acid (3 mg/L beer)		
Mercaptan	Mercaptan	Aromatic/flavor associated with sulfur compounds that are reminiscent of skunk and rubber	Ethyl mercaptan (3 µg/L beer)	1) beer in a green bottle 2) balloons 3) coffee	
Plastic		Aromatic/flavor note associated with plastic polyethylene containers or food stored in plastic; waxy, musty, pungent, smokey, or phenolic	1) Food stored in zippered bag 2) Plastic cup taste/smell water		
Sulfur	DMS, H2S	Aromatic associated with hydrogen sulfide, rotten egg	Hydrogen sulfide	1) rotten eggs 2) struck match	
Others					
Alcoholic	Ethanol	flavor/aromatic characteristic of the chemical class of compounds known as alcohols- ethanol	Ethanol (50g/L beer)	vodka	
Almond, nutty	Benzaldehyde	Aromatic of roasted almonds that is not cherry-like	Benzaldehyde (6 mg/L beer)	1) Marzipan 2) Almonds	
Hoppy	many	An overall term given to fresh hop aroma/flavor.	Mt. Hood; Cascade	same	
Leathery	Na	Aromatic/flavor note associated with tanned animal hides	Leathered water (soak leather in water overnight at room temperature)	1) Leather goods 2) Extremely stale beer	
Shrimp-like	Na	An aroma/flavor note associate with water in which shrimp have been cooked	Boiled shrimp water	Same	

Appendix C. (continued) Initial Ale Lexicon for Orthonasal Aroma and Flavor

Solvent	Propanal, isobutanol, propyl butyrate, p-cymene, (E)-carveol	A general term used to describe the aromatics of many classes of solvents- may be reminiscent of chemical solvents, plasticizers, lighter fluid, or paint aroma notes; fusely	Acetone	1) Isopropyl alcohol 2)Paint 3)Lighter fluid	
Tar		An aroma/flavor associated with hot asphalt		1)Tar 2)Liquid hickory smoke flavoring	
Vinous		An aroma/flavor note associated with bouquet, fusely, and wine-like character	Beer spiked with white wine	White wine	
Walnut		A flavor note associated with fresh (not rancid) walnuts	Freshly chopped walnuts	Same	
Worty	Na	An overall term associated with fresh wort aroma	Fresh warm wort (spike beer)	Wort collected from an ale	
Yeasty	NA	Aromatics/flavor notes associated with fresh yeast and yeast fermentation	Active yeast (1 g yeast in 250 ml distilled water at room temperature-spike beer)	Fresh baked yeast bread	

Appendix D. Initial Ale Mouthfeel Lexicon

Texture/Mouthfeel Attributes:

Attribute	Definition/description	Reference	Example(s)
Astringent	Puckering and constricting tactile sensation on the soft tissue on the mouth	1% Alum in water	Unripe banana; grape skins
Carbonation	Perceived amount of carbonation in the beer.	Soda water at 11-12 C	Diet Coke from bottle at 11-12 C
Creamy	Perceived impression of mouth-coating, softness, and fullness		
Denseness	Perceived density or weight of the beer in the mouth	Philadelphia light cream cheese (1/2 in cube)- 13.0	Reddi-whip aerosol whipped cream (1 oz)- 1.0
Velvety		Weyerbacher Old Heathen (Imperial Stout)	Coffeemate Fat Free Original Creamer at 10 C
Gritty mouthcoat	Feeling of minute, rough granules inside the mouth	Widmer Hefeweizen	1 T Great Value Applesauce
Metallic	A flat chemical feeling factor stimulated on the tongue by metal coins		
Mouthfullness/ Body	Perceived amount of depth inside the mouth.	Duck Rabbit Milk Stout	
Oily mouthcoat	Slippery, oil-like film inside the mouth		
Smooth	Perceived absence of all particles	Filtered water at 22 C	MGD 64
Sting	Intensity of initial sharp pain associated with carbon dioxide		
Tingly	Perceived amount of prickling sensation by the oral cavity		
Viscous	Degree to which the beer resists flow under an applied force in the mouth	Highland Oatmeal Porter	Carnation evaporated milk (1 t)- 3.9
Warming	Perceived amount of a warming sensation across the palate	Flying Dog Horn Dog (barley wine style ale)	1-2 oz red wine swirled on tongue

Appendix E. Ale Descriptive Ballot→ Ale Flavor used as example

Ale Flavor

Name:_____ Panelist #:_____
Date: _____

After sampling the product, please rate it on each attribute corresponding to the scale from 0-15 (can use tenths- 4.3, 4.4, etc.): 0- non-existent, and 15- strongest imaginable

	R1	920	003	512		
Sweet	2.1					
Sour	2.4					
Bitter	6.4					
Fruity	2.1					
Vanilla	0.3					
Caramelized	1.8					
Chocolate	0					
Honey	0.9					
Molasses	0.2					
Burnt/ Burnt toast	0.4					
Coffee	0.3					
Malty	2.4					
Roasted	0.3					
Hoppy	7.3					
Yeasty	2.5					
Diacetyl						

Others:

Appendix F. India Wheat Ale (IWA) Baseline Formula

Yield --> 25 gal	IWA									
GRAIN BILL										
Ingredient	Amount(#)	Amount(g)	% of tot Grain	% of tot weight	Color in L	EP	IG	I %	ME 100%	OG w/75% ME
2-Row Pale Malt	28	12700.52	54.11%	26.63%	3.4	37	41.44	0.827	1	
Caramel Malt	5	2267.95	6.25%	4.75%	40	34	6.80	0.077	1	
White Wheat	15	6803.85	28.99%	14.26%	2.8	39	23.40	0.038	1	
Honey Malt (Gambrinus)	2.5	1133.98	4.83%	2.38%	22.5	30	3.00	0.038	1	
Carapils (Briess)	1.25	566.99	2.42%	1.19%	1.3	33	1.65	0.019	1	
Total Malt Weight	51.75	23473.28	100.00%	49.21%			76.290	1.00	76.290	57.2175
Water	41.25	18710.59	na	39.23%						
HOPS (oz)			% of tot Hops		AA%	U%	IBU	GC	Gb	
Magnum (60 min)	5	141.75	33.33%	0.30%	0.13	0.3	61.63	1.055	61.000	
Centennial (10 min)	5	141.75	33.33%	0.30%	0.1	0.1	15.80	1.055	61.000	
Amarillo (5 min)	2.5	70.88	16.67%	0.15%	0.08	0.05	3.16	1.055	61.000	
Centennial (0 min)	2.5	70.88	16.67%	0.15%	0.1	0.05	3.95	1.055	61.000	
Total Hops :	15	425.25	100.00%	0.89%			84.54			
EXTRAS						EP				
Cane Sugar	1.59	720.00	na	1.51%		n/a				
Water for prime (oz)	80	2365.88	na	4.96%		n/a		ABV	est. FG	
Yeast- S. cerevisiae (oz)	21.25	602.44	na	1.26%				6.19	10	
Soy Kefir Powder	3.09	1400.00		2.94%						
TOTAL WEIGHT		47697.44		100.00%		MAXIMUM OG			76.290	57.2175

Appendix G. Aardbeien Lambic Style Final Formula

Yield --> 25 gal	**Aardbeien Lambic**									
GRAIN BILL										
Ingredient	Amount (#)	Amount (g)	% of tot Grain	% of tot weight	Color in L	EP	IG	I %	ME 100%	OG w/75% ME
2-Row Pale Malt	28	12700.52	54.11%	22.10%	3.4	37	41.44	0.827	1	
Caramel Malt	5	2267.95	6.25%	3.95%	40	34	6.80	0.077	1	
White Wheat	15	6803.85	28.99%	11.84%	2.8	39	23.40	0.038	1	
Honey Malt (Gambrinus)	2.5	1133.98	4.83%	1.97%	22.5	30	3.00	0.038	1	
Carapils (Briess)	1.25	566.99	2.42%	0.99%	1.3	33	1.65	0.019	1	
Total Malt Weight	51.75	23473.28	100.00%	40.84%			76.290	1.00	76.290	57.2175
Water	41.25	18710.59	na	32.55%						
HOPS (oz)			% of tot Hops		AA%	U%	IBU	GC		
Cascade	1	28.35	100.00%	0.05%	0.05	0.3	4.49	1		
Total Hops :	0.5	28.35	100.00%	0.05%			4.49			
EXTRAS						EP				
Strawberry Flavoring (oz)	112.5	3189.38	na	5.55%						
Cane Sugar	1.59	720.00	na	1.25%		n/a				
Water for prime (oz)	80	2365.88	na	4.12%		n/a	Total ABV	ABV	est. FG	
Yeast- S. cerevisiae (oz)	21.25	602.44	na	1.05%			4.86	6.19	10	
Kristalkefir	8386.89	8386.89		14.59%						
TOTAL WEIGHT		57476.81		100.00%		MAXIMUM OG			76.290	57.2175