THE FLAVOR IMPACT OF G-RG_1 AND G-RB_1 ON THE TASTE PERCEPTION OF GINSENG

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THESIS

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ABSTRACT

Ginseng has become a common ingredient used by makers of energy drinks who wish to infer that their product contains memory enhancing capabilities. As studies conducted on its main bioactive compounds (ginsenosides), have shown promise in producing not only medical advancements in memory, but also therapeutic benefits that current energy drink consumers expect. However, there is currently no published threshold value for ginseng, which is necessary to make accurate estimates of how much ginseng can be added to a product before imparting its bitter taste into product formulations. There are two ginsenosides present in ginseng that elicit all benefits expected from an energy drink as well as memory enhancement, ginsenoside Rb_1 and Rg_1 (G-Rg_1 and G-Rb_1). The main goal of this study was to identify the taste threshold and flavor threshold of ginseng, to identify the taste threshold of G-Rb_1 and G-Rg_1, and to identify their contribution to the overall taste of ginseng. Taste threshold tests were conducted using the R-index by the rating method on three different commercially available ginseng samples, two of which were white ginseng, and the third, red ginseng. The results of this study showed that it could be possible to add enough ginseng white ginseng or red ginseng into a single serving size beverage which contained a full day's worth of ginseng needed for therapeutic benefits. Flavor thresholds were, then, determined using the R-index by the rating method on single white ginseng sample as well as on G-Rb_1 and G-Rg_1. The flavor threshold of ginseng showed that it was not possible to add enough ginseng in adequate amounts into a single beverage to reach therapeutic benefits ,which is contrary to what was seen using the taste thresholds. However, percent contribution of G-Rb_1 and G-Rg_1 to the taste activity value and flavor activity value of ginseng was found to be below 100%. This infers that it could be possible for companies to use either G-Rb1 or G-Rg_1 in place of ginseng. Finally, data from the taste threshold and flavor threshold studies were compared, and it was found that panelists were more sensitive to ginseng

when identifying the flavor threshold. This could either be caused by panelists relying on the aromatic compounds in ginseng as a cue to identify changes in concentration or that the nose-clips used in the taste threshold made panelists more susceptible to distractions and mental fatigue.

KEY WORDS: ginseng, ginsenosides, G-Rg_1, G-Rb_1, taste activity value, flavor activity value

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CHAPTER 1- INTRODUCTION

1.1 MOTIVATION

Functional foods and beverages are one of the fastest growing markets currently in the United States. In this market the most profitable segment is energy drinks (NBJ 2010; Sloan 2010). Currently energy drinks are marketed to 18-34 year old teenagers, college students, and young adults who have physically demanding jobs (Heckman and others 2010; Mintel 2010). However, as the baby boomer market becomes one of the primary age groups in this country, companies throughout the food industry are developing products to capitalize on this critical market. Products of interest for the baby boomer market include products that promise increased energy levels, stress relief, weight loss, and memory enhancement (Mintel 2010).

While there are several different herbal supplements currently on the market, ginseng extract has shown therapeutic properties that apply to the majority of the interests of the baby boomer market; as such becoming a common ingredient in many functional beverage categories, including energy drinks. The pharmacological benefits of ginseng are due to ginseng saponins, or ginsenosides which are thought to be the main bioactive compounds in ginseng (Attele and others 1999a; Jia and Zhao 2009). There are over 100 ginsenosides present in ginseng however, there are only two ginsenosides, G-Rb1 and G-Rg1 that are both main components in ginseng and have therapeutic effects that mimic the drivers of purchase intent for the baby boomer market.

Currently functional beverage companies add *Panax ginseng* (Asian ginseng) to a product in levels ranging anywhere from 25-400 mg per bottle (Clauson and others 2008). However, therapeutic doses for ginseng begin at 100 mg/day (Clauson and others 2008). One of the reasons that ginseng is added in such small amounts is due to its inherently bitter taste that is

assumed to be created by the ginsenosides present in the extract, though very little sensory research has been done to link the two. While it is possible to purchase Asian ginseng extract that contains no ginsenosides, this effectively removes many of the therapeutic constituents from the ingredient.

In a previous study using a model energy drink solution, Tamamoto and others (2010) identified ginseng to be functional ingredient dominating the bitter sensory perception among the three most common ingredients (caffeine, taurine, and ginseng) found in energy drink energy blends. However the sample used consisted of 90% ginsenosides and it is currently unknown what percentage is used in commercial products. It stands to reason however that it may be possible to avoid a majority of the bitterness in ginseng without losing therapeutic benefits by identifying specific ginsenosides that meet the desired pharmacological benefits and adding only them into the product instead.

Therefore the main goal of this thesis is to identify the threshold of ginseng and the thresholds of G-Rb_1 and G-Rg_1, so as to make recommendations of the appropriate amounts of ginseng, G-Rb_1 and G-Rg_1 that can be added to a product without imparting undesirable flavors into the product's flavor profile.

1.2 OBJECTIVES

The two main hypotheses of this research were that 1) the taste threshold of ginseng would be higher than that of the flavor threshold and 2) the flavors of G-Rb1 and G-Rg1 would account for a large majority of the overall taste of ginseng. The first objective was to identify both the taste threshold and the flavor threshold of ginseng as previous published cited works identify aromatic compounds in ginseng that create woody and earthy flavors (Chung 2010; Kim and Sung 1985; Park and others 1999). These strong aromatic compounds most likely influence the threshold of ginseng and give panelists increase their sensitivity to ginseng. The second

objective is to identify the flavor thresholds of G-Rb_1 and G-Rg_1 and to use taste activity values (TAV) to identify their flavor contribution to the overall flavor of ginseng. Using TAVs to asses overall taste contribution has been used successfully in several different studies including identifying non-volatile taste active compounds in the meat of Chinese mitten crab (Chen 2007) and tracing the key tastants that are generated during Maillard-type reactions in food processing (Hofmann and others 2004)

The results of this study will enable companies to better assess appropriate levels of ginseng to add to current and future products on the market. It will also help determine if using ginsenosides in place of ginseng in energy drinks is a plausible option. If G-Rb_1 and G-Rg_1 have higher threshold levels than that of ginseng, or at least levels higher than the required amounts for therapeutic doses, it may be beneficial to use only the ginsenosides to avoid off-flavors present in ginseng.

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Chapter 2 – LITERATURE REVIEW

2.1 FUNCTIONAL BEVERAGES

Due to rising health costs and the current economic climate many Americans have begun to take a "do-it-yourself" approach to wellness with an increasing amount of the population turning to functional foods and beverages. Many consumers are now looking for foods and beverages that have dual purposes, satiate hunger or thirst while providing additional health benefits (Sloan 2010). In 2009, the functional food and beverage industry marked $37.4 billion in sales. The most profitable segment within the food and beverage industry is that of functional beverages which accounted for 58% of the total sales (NBJ 2010; Sloan 2010).

Functional beverages offer consumers the opportunity to either supplement an already healthy life style or the ability to counter balance an unhealthy life style (Lal 2007) . This means that many functional beverages attempt to appeal to consumers through supplementation with various vitamins and minerals and/or compounds with links to various health issues including weight loss, memory retention, and stamina enhancement (Lal 2007).

It is hard to track the specific progress of the growth of functional beverages due to the inconsistencies of categorization and segments. Mintel divides the functional beverages into 6 segments: functional fruit juice and juice drinks, energy drinks, enhanced water, functional soy, rice and almond-based, functional tea, functional yogurt drinks/smoothies (Mintel 2010). The Datamonitor Group breaks down functional beverages into only three categories, sports drinks, energy drinks, and nutraceutical drinks (Datamonitor 2008). Despite the discrepancies between the breakdown of categories published, in all studies energy drinks have been shown to be a strong leader in the functional beverage category. The term "energy drink" refers to beverages

that contain caffeine in combination with other presumed energy-enhancing ingredients such as ginseng (Heckman and others 2010).

2.2 ENERGY DRINKS

Originally energy drinks were considered to be part of a niche market and only was marketed toward athletes. From there, the market expanded into those who wished to live more active lifestyles, and then finally into the mainstream population (Ballard and others 2010). Now energy drinks typically cater to 18-34 year olds, strongly focusing on young adults with physically demanding jobs, teenagers, and college students (Heckman and others 2010; Mintel 2010). Marketing for energy drinks has placed them as a commonplace consumption item throughout the entire day and evening. Uses can widely range from replacing one's early morning coffee or tea to being used as a mixer for alcoholic beverages at bars (Higgins and others 2010). The first energy drink introduced into the United States was Red bull in 1997 (Higgins and others 2010). Originally introduced in Austria in 1987 (Higgins and others 2010), Red Bull now accounts for 42% of the overall market share of energy drinks in the U.S. (Beverage Spectrum 2008) and is a common mixer in bars to serve such drinks as a Red Bull and Vodka.

The "energy" in energy drinks usually comes from similar sources no matter what brand is purchased. Sugar is a common additive in energy drinks because it is a rapid source of energy; however, due to calorie concerns many brands offer sugar-free varieties. Other common ingredients that energy drinks list as part of their "energy blend" include taurine, caffeine, and ginseng (Clauson and others 2008; Heckman and others 2010).

TAURINE

Taurine (2-aminoethyl sulfonic acid) is an essential nutrient that is the most prevalent intracellular amino acid in the human body (Seidl 2000). Taurine has numerous biological and physiological functions, including bile acid conjugation and cholestasis prevention, antiarrhythmic, inotropic, and chronotropic effects, central nervous system neuromodulation, retinal development and function, endocrine or metabolic effects, and antioxidant and anti-inflammatory properties(Louren 2002). Taurine has also been shown to enhance endurance performance and to aid in the reduction of lactic acid build-up after exercise (Matsuzaki and others 2002; Imagawa and others 2009). However, amounts currently found in energy drinks are below any recommended dosage for therapeutic benefits, which range from a daily intake of 1-3 grams (Clauson and others 2008).

CAFFEINE

Caffeine, a methylxanthine, is the most common ingredient in energy blends. Energy drinks have been shown to contain between 70 and 200 mg of caffeine per 16-oz serving in comparison to an 8-oz cup of drip coffee which contains 110 to 150 mg (Clauson and others 2008). Caffeine has many physiological effects, including stimulation of the central nervous system, heart, and skeletal muscles (Clauson and others 2008).

Caffeine is listed as GRAS (Generally Recognized As Safe) by the US Food and Drug Administration's, but is limited to no more than 0.02% by volume in cola-type products (Food and Drug Administration 2006). There are no current limits on caffeine content currently imposed on energy drinks (Food and Drug Administration 2006). Adverse side effects from caffeine can occur at doses of 10 to 15 grams per day or at even smaller doses depending on factors such as smoker status, cardiac health and prior caffeine use (Clauson and others 2008) .

Guarana, a plant that produces small-berry like fruit is also usually grouped in the same category as caffeine. The fruit of the guarana plant contains 1 to 3 dark seeds which contain large amounts of caffeine. Every 1 g of guarana contains 40 mg of caffeine (Finnegan 2003).

2.3. GINSENG

The word ginseng means "the essence of man", which refers to the man like appearance of the picked root of the plant (full plant that consists of a root, stem, leaves, flowers, and berries) (Matsuda and Doty 1995). There are over 30 different species of ginseng that are grown throughout the world; however, the three most common varities are *Panax ginseng* C.A. Meyer (Asian ginseng), *Panax quinquefolius* (American ginseng), and *Panax japonicas* (Japanese ginseng) (Attele and others 1999b; Mahady and others 2000).

There are two main methods of preservation post-harvest for the ginseng root, which is the most common part used. The method chosen for preservation is often a deciding factor in what the ginseng will be used for after processing. The first method is when the root is peeled and then air dried to the point of dehydration (water content must be reduced to 12% or less), which results in white ginseng. The second method is when the root is not peeled and is steamed for 2-4 hours to create red ginseng. White ginseng is the form most commonly used as an added ingredient in functional foods and beverages as well as supplements. Red ginseng is most often used as the base of a tonic (Choi 2008; Jia and Zhao 2009).

Ginseng has been used for at least 2000 years in forms of Asian holistic medicine and is widely heralded as a cure all in oriental cultures (Mahady and others 2000). The first written account of the therapeutic uses of ginseng can be traced back to the first century, A.D., where ginseng is describes as having the ability of "repairing the five viscera, quieting the spirit,

curbing the emotion, stopping agitation, removing noxious influence, brightening the eyes, enlightening the mind, and increasing the wisdom (Hu 1977).

While ginseng has been used for thousands of years in Asian cultures, it was not until the last century that Western civiliazations have begun to take note of its pharmacological and chemical properties (Choi 2008). Now, research spans hundreds of different applications, and in 2005, there were over 4600 listings for ginseng in The Chemical Abstacts (Coates 2010). However, there is no standard ginseng sample. It is under the discretion of each scientist which variety of ginseng to use as well as the ginsenoside content their sample contains.

Ginseng samples are sold ranging from 0% ginsenosides up to 90% ginsenoside content. Ginseng research also encompases all parts of the plant, though most research is done on the root as it is the most commonly used, as well as all three different preparations of the ginseng root, freshly harvested or preserved as white or red ginseng. The wide variety of options becomes highly problematic when trying to cross compare results from previous studies (Kitts and Hu 2000). This problem is not only seen when compairing ginseng as a whole entity but difficulties also arise when compairing studies foucsing on individual ginsenosides, which account for the majority of ginseng research. While there are some ginsenoside standards, due to the large number of ginsenosides identified and the limited amount that some are found in ginseng samples, several ginsenosides to date have no available standards for purchase (Harkey and others 2001).

2.3.1 GINSENOSIDES

There are over 200 different bioactive and non-bioactive compounds found in ginseng (Chu and Zhang 2009). Out of these, 100 are ginseng saponins, or ginsenosides, which are largely considered to be the main bioactive compounds of ginseng (Jia and Zhao 2009). The

ginsenosides present in a plant as well as their quanitity can depend on several different variables including variety, age, location of cultivation, and season of harvest (LIU and XIAO 1992; Li and others 1996).

Ginsenosides all consist of a simiar backbone structure, a gonane steroid nucleus with 17 carbon atoms arranged in four rings . Each ginsenoside has defining characteristics steming from the type, position, and number of sugar moieties attached by the glycosidic bond at C-3, C-6, and C-20 (Figure 1.1) (Coates 2010). Ginsenosides are grouped into four categories based on the glyco-chain connection on the aglycone backbone: protopanaxadiol-type, protopanaxatriol-type, ocotillo type, and oleanolic acid type (Mazza and Oomah 2000; Jia and Zhao 2009).

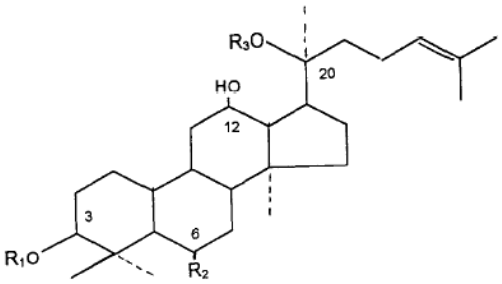

Figure 2.1: Backbone structure of a ginsenoside

While the content and quantity of ginsenosides can vary from plant to plant, six ginsenosides have been chosen as reference standards for ginseng products: Rb_1, Re, Rc, Rd, Rb_2 and Rg_1 (Ma and others 1996). All six of these ginsenosides appear in every variety of ginseng. Also Rf, while not used as a standard, is used as a marker to differentiate between American ginseng and Asian Ginseng as it only appears in Asian ginseng samples (Jia and Zhao 2009).

As each ginesnoside has a different chemical structure, each ginsenoside has the potential to provide different health benefits. The benefit of researching ginseng's individual constituents helps bypass the problem of the large varying presence and quantity of ginsenosides found from species to species and plant to plant. Ginsenosides have shown promise in all of following, but is not limited to areas of increasing immunization functions, improving liver functions, adjusting blood pressure, releaving stress and fatigue, improving female climacteric disorder and male

sexual dysfunctions, diminishing pain, improving cerebral functions, preventing cancer and certain types of tumors , inhibiting AIDS virus (HIV) growth, and slowing the aging process (Choi 2008).

2.3.2 GINSENOSIDES AND DRIVERS OF PURCHASE INTENT FOR ENERGY DRINKS

Mintel reported drivers of purchase intent for energy drinks to be incentives of decreased stress and fatigue, improved memory enhancement, and increased energy or stamina (Mintel 2010). Specific investigations of ginseng has elucidate several different uses of individual ginsenosidesthese areas of interest..

G-Rb_1 has been shown to block stress responses at an early age, prevent the stress-induced impairments of reproductive fucntions, prevent stress-induced brain degeneration rat models (Zhang and others 2006), and improve central nervous system dysfunctions (Hao and others 2011). G-Rg_3 has shown to reduce stress levels in restraint-stressed rats (Kim and others 2010a). G-Rb_1, G-Rg_1, and G-Rh_2 were shown to improve memory deficiency induced by scopolamine (Yang and others 2009; Wang and others 2010), and spatial learning and reasoning in rats (Liu and others 2010; Liu and others 2011). G-Rg_2 has been shown to protect memory impairment in rats with vascular dementia (Zhang and others 2008). G-Rb_1 and G-Re decrease cardiac contraction in rats (Scott and others 2001). G-Rg1 and G-Rb1 have also shown promise in enhancing aerobic performance (Wang and others 1998).

2.3.3 SENSORY STUDIES ON GINSENG

Despite ginseng's up and coming status in the functional food and beverage category, seemingly little sensory research has been published on the subject. Terms such as earthy, woody, molasses, astringent, bitter, and sweet flavors have been used to describe products

containing ginseng (Kim and Sung 1985; Park and others 1999). It is usually assumed that the bitter notes of ginseng are caused by the ginsenosides, as saponins are traditionally known to be bitter; however, very little research has been done to directly compare the two. Tamamoto and others (2010) identified cyclodextrins as possible bitter masking agents in an energy drink model solution; however, an optimal ratio of ginsenosides to cyclodextrins was not identified.

While in Western culture ginseng is currently only an ingredient in functional beverages, some research has been done into the possible expansion in to other consumer market segments. Chung and others (2010) identified dark chocolate and products that are predominantly sweet and fruity that contain ginseng as possible product development options. Other research has been done on the impact of the addition of ginseng on sensory characteristics of foods with the addition of ginseng ingredients including studies on tofu with ginseng extract (Kim and others 1996), pumpkin cookies with ginseng extract (Song and others 2007), ginseng-whey beverages (Kee and Hong 1993), pork cutlet containing ginseng saponins (Cho and others 2003), ginseng-yogurt (Lee and Paek 2003), kiwi-ginseng beverages (Park and others 1994), alcohol beverages containing ginseng (Yoon and others 2007), and ginseng coated coffee beans (Kim and others 2010b).

There are currently no published threshold levels for any variety of ginseng. There is a published threshold for ginsenosides G-Rb1, G-Rc, G-Rg1 G-Re; however, the thresholds reported lack any methodology or description of medium used in the testing process.

2.4. CONCLUDING REMARKS

As the popularity of functional beverages, specifically energy drinks grows it is possible that a greater number of food companies will become interested in adding ginseng to their products. The problem with the limited amount of sensory research published is that little is

known about the threshold of ginseng. Due to ginseng's inherent bitter characteristics, companies are forced to either add minimal amounts of ginseng so that the flavor goes unnoticed by consumers or add a secondary ingredient in order to mask ginseng's undesirable flavors.

Descriptive panels surrounding ginseng and ginseng-related food products (Chung 2010, Kim and Sung 1985; Park and others 1999) have identified several different terms (i.e. earthy and woody) which infer strong aromatic compounds present in ginseng. At this time, however, is it unclear how much influence the aromatic compounds have on the threshold of ginseng. This information could be used in further product development, as most beverage companies add some form of flavorings which often contain strong aromatic compounds of their own. It may also be possible to choose a flavorant which has the ability to mask or dominate the aromatic profile created by multiple supplements including ginseng.

Another possible option for companies that utilize ginseng in their products could be to target individual health benefits for their product by adding only specific ginsenosides. This could enable companies to create more specialized products that are tailored toward their consumers. However, like ginseng, the majority of the threshold values for ginsenosides are unknown. Without first determining the threshold values of ginseng and ginsenosides, it is impossible to determine whether appropriate amounts could be added without destroying the existing flavor profile of a product.

Therefore future ginseng studies should identify not only the threshold of ginseng but also identify the thresholds of key ginsenosides. This will allow commercial entities the ability to make better informed decisions during formulation or reformulation.

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Chapter 3: IDENTIFICATION OF THE TASTE EMPIRICAL THRESHOLD OF GINSENG

3.1 ABSTRACT

Ginseng has a wide range of pharmacological benefits and has been used in Asian holistic medicine for thousands of years (Chu and Zhang 2009). Current recommended therapeutic doses of ginseng begin at 100-200 mg/day. In Western societies ginseng has become a popular ingredient in functional beverages, specifically energy drinks; however, due to ginseng's inherently bitter taste most beverages limit the amount of ginseng in their products to 25-50 mg per bottle, whose volume can range anywhere from 240 to 600 mL. In this study the empirical taste threshold of ginseng was identified for three commercially available ginseng extracts (two white ginseng extracts and one red ginseng extract), with ranging ginsenoside content levels (Sample A contains 90% ginsenosides, Sample B contains 70% ginsenosides, and Sample C contains 1.7% ginsenosides). This will identify the amount of ginseng that can be added to a beverage without interfering with its current flavor profile. Threshold levels were calculated using the R-index by the rating method.

Individual ginseng threshold values ranged from 0.014 to 0.0213 g/100 mL for Sample A (n=31), 0.023 to 0.450 g/100 mL for Sample B (n=30), and 0.009 to 2.176 g/100 mL (n=28) for Sample C. Average threshold values were found to be 0.053 to 0.005 g/100 mL for Sample A, 0.090 ± 0.010 g/100 mL for Sample B, and 0.787 ± 0.085 g/100 mL for Sample C at $\alpha=0.05$. Group threshold values were identified by combining data for all panelists to identify a single threshold for each sample. These values were found to be 0.050 g/100 mL, 0.092 g/100 mL, and 1.07 g/100 mL, for Samples A, B, and C, respectively. Strong distinctions among the ranges of Samples A and B compared to Sample C infer that the overall ginsenoside content could have

implications on the concentration of the taste threshold. If assumed that an energy drink contains at least 240 mL, according to the results from this study, a company could add either Sample A, B, or C at the lowest recommended intake level for pharmacological benefits (100 mg/day) into a single serving, without reaching the taste threshold level.

3.2 INTRODUCTION

While Asian cultures have trusted ginseng to cure a variety of ails for thousands of years (Chu and Zhang 2009), it has not been until the last few decades that Western cultures have begun to take notice of its possible medicinal applications. Current scientific research on ginseng is now focused on hundreds of different pharmacological benefits including but not limited to antifatigue, antidiabetes, antidepresessant, and anti-inflammatory, and memory stimulation activity (Ligor and others 2005; Wahid and others 2010).

It is generally assumed that ginsenosides (ginseng saponins) found in ginseng are the main bioactive compounds responsible for ginseng's medicinal benefits (Attele and others 1999; Jia and Zhao 2009). There are currently about 100 ginsenosides known (Jia and Zhao 2009); however, the ginsenoside content found in an individual plant can vary widely depending on several different factors including: species, maturity of plant at the time of harvest, season of harvest, part of the plant used, location of growth, and method of preservation post-harvest (Liu and Xiao 1992; Li and others 1996).

There are two main methods of preservation post-harvest for the ginseng root, which is the most common part of the plant used. The first method is when the root is peeled and air dried to the point of dehydration (water content must be reduced to 12% or less), which results in white ginseng. The second method is when the root is not peeled and is steamed for 2-4 hours to create red ginseng. White ginseng is the form most commonly used as an ingredient in

functional foods and beverages. Red ginseng is most often used as the base of a tonic (Choi 2008; Jia and Zhao 2009).

Due to ginseng's well-known pharmacological benefits, ginseng extract has become a common additive in many different functional beverages including energy drinks. Ginseng is typically marketed in energy drinks as having the ability to improve physical performance as well as improving cognitive function, concentration, and memory (Clauson and others 2008; Kennedy and Scholey 2003). However, ginseng is well known for its bitter flavor which requires the addition of bitter blockers or sweeteners (either natural or artificial) when ginseng is used in excess (Eckert and Riker 2007). The current recommended therapeutic dose for ginseng can range anywhere between 100-200 mg/day (Clauson and others 2008), but because ginseng is classified as a dietary supplement, the amount used in a product does not need to be identified on the label (Duyff and American Dietetic Association 2006), and the amount of ginseng added to energy drinks can be minimal without having to remove the ginseng name from the ingredient list.

To date, there has been no reported threshold for ginseng. The goal of this research is to identify the taste threshold of Asian ginseng in a baseline solution. Asian ginseng was chosen as it is the most common form currently used in Western energy drinks (Heckman and others 2010). Three commercially available extracts were chosen to be tested to allow us to investigate the impact that ginsenoside content has on the threshold value. We also deemed it an area of interest as to the difference in threshold levels between red ginseng and white ginseng; therefore, two of the extracts purchased were white ginseng and the third was red ginseng. Thresholds were identified using R-index by the rating method which has been shown to be as effective as the

current ASTM recommended method of ascending limits (Robinson and others 2005; Kappes and others 2006).

3.3 MATERIALS AND METHODS

Pilot Study to Determine Test Concentration Range

Sample Preparation

For this study, three different commercially available ginseng extracts with varying ginsenoside concentrations were used: two white ginseng samples, Sample A (90% ginsenosides, AmaxNutra Source, Eugene, OH) and Sample B (70% ginsenosides, Xi'an TonKing Biotech, Xi'an, China), and a red ginseng sample, Sample C (1.7% ginsenosides, Cheong Kwan Jang, Daejun, Korea). Solutions for each sample were prepared at an assumed extreme high and low concentration based off of literature reviews, with the intent of encompassing the 75% R-index level. Sample A was presented as 0.675 g/100 mL and 0.0009 g/100 mL. Sample B was presented as 0.562 g/100 mL and 0.0008 g/100 mL. Sample C was presented as 2.7 g/100 mL and 0.0037 g/100 mL.

Amounts of ginseng required to make 1.5 L of two times the highest concentration tested for each sample were weighed in to 2 L beakers. Spring water (Absopure, Urbana, IL) in the amount of 1.5 L was added to the beakers using a 1.0 L graduated cylinder, and were stirred for 5 minutes. Solutions were then dispensed and diluted into appropriate concentrations using a graduated cylinder, spring water, and 12.6 mL of propylene glycol (PG) (Fisher Scientific, Philadelphia, PA) and placed into 2.0L beakers. Propylene glycol was added to each solution so that the results from this study could be compared to later studies in which pure ginsenosides are used, as ginsenosides are not water soluble.

Solutions were again stirred for 5 minutes, after which they were poured into individual dispensing pipettes (Cole Parmer, Veron Hills, IL). The pipettes were used to dispense 10 mL of solution into 30 mL plastic cups with lids (Solo Cup Company, Urbana, IL) for the experimental samples. The noise reference (50 mL of a 0.7% propylene glycol and spring water solution) was available during the experiment in 163 mL plastic cups with lids. All samples were prepared the day before and were stored and served at room temperature (22ºC). The containers were coded with three-digit random numbers. The reference for noise was labeled as ‘‘noise.’’

Rinse Preparation

A 0.55% solution of carboxymethlycellose (CMC) was prepared the day before testing to be utilized as a rinse in-between samples. Eleven grams of CMC was weighed out and poured into a 2000 mL beaker. Using a 1000 mL graduated cylinder, 2000 mL of hot water (65ºC) was added to the beaker. The solution was then stirred until all CMC had dissolved. Solution was stored at room temperature until use.

Panel Selection

Nine panelists, 4M and 5F, ages 21-40, which were familiar with the R-index by the rating method, were recruited to participate in the pilot study. There were no requirements for participation aside from familiarity with the R-index by the rating method.

Sample Presentation

A randomized complete block design using 3 replicates was used for two different signal samples and one noise sample per ginseng extract. The randomization was done using Compusense *Five* 5.0 (Guelph, Canada). Three replicates of the noise and 3 replicates of the two concentrations of the three ginseng samples consisted of a complete set for the session.

Panelists were instructed to take the whole sample into the mouth, swirl it around for 2–3 seconds, expectorate, and complete the task given during the experiment. Samples were presented monadically and panelists were asked if the sample was a signal sure, signal unsure, noise sure, or noise unsure, where the 0.7% PG solution represented the noise and the ginseng solutions represented the signal. Panelists rinsed twice in-between samples, first using room temperature CMC, followed by warm water to prevent residual bitterness carryover. After every 8th sample, panelists were required to wait 2 minutes before starting with the next group of samples. At any time during the experiment, panelists were allowed to retaste the noise reference in order to refamiliarize themselves with the noise sample. For Samples A and B, panelists evaluated samples at 27 ºC in individual booths with incandescent lighting.. For Sample C, to minimize color bias, samples were served on a tray using purple paper and red light.

Data Analysis

For each ginseng sample an R-index response matrix was constructed for all samples (O'Mahony 1992). R-index calculations were done on the group overall and not on individual panelists due to the number of replications.

Main Study

Sample Preparation

For this study solutions for each sample were prepared at seven concentrations, in threefold increments that were predetermined based on the results from the preliminary study. Sample A was presented in the following concentrations: $5.74x10^{-1}$, $1.91x10^{-1}$, $6.38x10^{-2}$, $2.13x10^{-2}$, $7.09x10^{-3}$, $2.36x10^{-3}$, and $7.87x10^{-4}$ g/ 100 mL. Sample B was presented in the following concentrations: $4.50x10^{-1}$, $1.50x10^{-1}$, $5.00x10^{-2}$, $1.67x10^{-2}$, $5.56x10^{-3}$, $1.85x10^{-3}$, and

$6.17x10^{-4}$ g/100 mL. Sample C was presented in the following concentrations: 2.16, $7.20x10^{-1}$, $2.40x10^{-1}$, $8.00x10^{-2}$, $2.67x10^{-2}$, $8.89x10^{-3}$, and $2.96x10^{-2}$ g/100 mL. Samples were prepared according to the methodology used in the pilot study.

Panel Selection

The panelist recruitment and selection process consisted of two questionnaires, the first of which included items concerning demographic information, allergies, smoker status, frequency of consumption of products containing ginseng, and schedule availability (See Appendix A). Based off of their responses from the original screener, a second screener was sent out to panelists who identified themselves as either "somewhat familiar" or "very familiar" with ginseng (See Appendix B) to identify which panelists were familiar with traditional forms of ginseng (i.e., red ginseng tonic, ginseng snacks, or ginseng tea) and those that were familiar with Western forms (i.e., energy drinks, teas, etc.). Thirty-one panelists, 5M and 26F, ages 21–60 years old, were recruited to participate. Of this 31, 23 panelists self-identified as either somewhat or very familiar of ginseng and fourteen self-identified as being at least somewhat familiar with traditional sources of ginseng for greater than one year's time.

Prior to testing, panelists were given a presentation on the basics of sensory evaluation and discrimination testing, specifically the R-index method, in order to familiarize them with the type of test being used. Panelists were advised not to drink or eat one hour prior to the sessions.

Sample Presentation

A randomized complete block design using ten replicates was used for seven different signal samples and one noise sample per ginseng extract. The randomization was done using Compusense *Five* 5.0 (Guelph, Canada). Five replicates of the noise and five replicates of the seven concentrations of one ginseng sample consisted of a complete set for the session. Testing

was split across 6 days so that panelists saw only 1 ginseng extract a day and saw all 10 replications over the course of 2 consecutive days. Panelists were instructed to take the whole sample into their mouth, swirl it around for 2–3 seconds, expectorate, and complete the task given during the experiment. Samples were presented monadically and panelists were asked if the sample was a signal sure, signal unsure, noise sure, or noise unsure, where the 0.7% PG solution represented the noise and the ginseng solutions represented the signal. Panelists rinsed twice in-between samples, first using room temperature CMC, followed by warm water to prevent residual bitterness carryover. After every 8th sample, panelists were required to wait 2 minutes before starting with the next group of samples. At any time during the experiment, panelists were allowed to retaste the noise reference in order to refamilarize themselves with the noise sample. Panelists evaluated samples at 27ºC in individual booths with incandescent lighting used for Samples A and B. For Sample C to minimize color bias, samples were served on a tray using purple paper and red light. Panelists were required to wear nose-clips throughout the duration of the study so that the results would reflect the taste threshold and not the flavor threshold of ginseng.

Data Analysis

For each ginseng sample thresholds were found using the R-index response matrix method (O'Mahony 1992). The ginseng concentration (g/100 mL) for each sample was plotted as a function of R-index percentage. The two points found directly above and below an R-index of 75% were identified and a linear trend line was created between these two points to identify the ginseng concentration at an R-index of 75%. Matrices were constructed for each individual panelists and all responses were combined together to create a single matrix for the group,

allowing thresholds to be identified for each panelist and the group as a whole (group threshold). Average threshold was determined by taking the arithmetic mean of all the individual thresholds.

3.4 RESULTS AND DISCUSSION

Pilot Study

Calculated R-index values were 90% for 0.675 g/100 mL and 62% for 0.0009 g/100 mL for Sample A, 94% for 0.562 g/100 mL and 81% for 0.0008 g/100 mL for Sample B, 96% for 2.7 g/100 mL and 67% for 0.0037 g/100 mL for Sample C. For all samples, except Sample B, the tested ranges encompassed an R-index of 75%. Based on the results of this study, it was concluded that all concentrations would be appropriately adjusted to ensure that the concentrations tested for the main study would fall above and below an R-index of 75% while covering the smallest range possible.

Main Study

Individual ginseng threshold values ranged from 0.014 to 0.213 g/100 mL for Sample A (n=31) with an average value of 0.053 ± 0.005 g/100 mL; 0.023 to 0.450 g/100 mL for Sample B (n=30) with an average value of 0.090 ± 0.010; and 0.009 to 2.176 g/100 mL (n=28) with an average value of 0.787 ± 0.085 for Sample C for an α of 0.05 (Table 3.1). Three of the original 31 panelists did not cross an R-index of 75% during the testing for Sample C, and one for Sample B, so there are not threshold values for those samples. Group thresholds were identified to be 0.050 g/100 mL for Sample A, 0.092 g/100 mL for Sample B, and 1.07 g/100 mL for Sample C.

Figure 1 shows the distribution of the detection thresholds obtained for the two white ginseng samples, Samples A and B. As is seen in the graph, while the ranges of the two distributions are very similar, the general shapes of the distributions are different. Sample A's

distribution is skewed to the left while the distribution of the threshold for Sample B is more evenly distributed. The overall range of the distribution for Sample C is considerably wider than that of Samples A and B, as is shown in Figure 3.2. The threshold of the red ginseng sample (Sample C) had a much larger range than that of the other two samples. This is most likely due to the overall ginsenoside content present in the sample. While Samples A and B are relatively close in ginsenoside content, Sample C contained only 1.7% ginsenosides. All samples were found to have threshold levels, calculated both as a group and on an individual basis, in amounts that would allow for the addition of ginseng at the lowest recommended intake level for pharmacological benefits (100 mg/day) to a single serving size beverage (240 mL).

Further research based on this topic should focus on using samples such as Samples A and B, as they are both white ginseng samples and red ginseng is not used in Western industrial applications. According to Chung (2010), several of the bitter notes of ginseng are capable of being masked by the bitterness of caffeine; therefore, it may be possible to increase the amount of ginseng past the lowest threshold level if appropriate amounts of caffeine are also added to the product. Tamamoto and others (2010), also showed cyclodextrins as a possible bitter masking agent in an energy drink model solution, which could also be a solution should a company be concerned with possible supertasters in their consumer base.

Post analysis, participants were divided into two groups, either familiar or unfamiliar with ginseng prior to this study based on their responses to the second questionnaire. The results for Samples A, B, and C, are presented in Figure 3.3 and Table 3.2, Figure 3.4 and Table 3.3, and Figure 3.5 and Table 3.4, respectively. There is no definite trend shown from the comparison analysis between the two panelist groups who were familiar or unfamiliar with ginseng prior to this study to infer that either group is more or less sensitive to any of the 3 samples. In Figures

3.3, 3.4, and 3.5, both groups have similar distributions. Mean values calculated for each sample according to familiarity show no significant differences between ranges within a 95% confidence interval (See Table 3.2, 3.3, and 3.4). What discrepancies exist are most likely due to the unfamiliar group having a segment that appears larger than those of the familiar group. This can be due to the unfamiliar group having a larger sample size. For Sample A, in the unfamiliar group n=18 and for the familiar group n=13. For Sample B, in the unfamiliar group n=18 and for the familiar group n=12. For Sample C in the unfamiliar group n=16 and for the familiar group n=12. The n value for each group varies depending on which panelists met the threshold level for each sample, as it was not always the same panelists who failed to reach the threshold level for Sample B and Sample C.

3.5 CONCLUSIONS

Thresholds were identified for three commercially available ginseng extracts. Taste thresholds for Samples A, B, and C were found to range from 0.014 to 0.213 g/100 mL, 0.023 to 0.450 g/100 mL, and 0.009 to 0.218 g/100 mL, respectively. Mean threshold values for were calculated to be 0.053 ± 0.005 for Sample A, 0.090 ± 0.010 for Sample B, and 0.787 ± 0.085 for Sample C, for an α=0.05. Group threshold values for Samples A, B, and C were identified as 0.050 g/100 mL, 0.092 g/100 mL, and 1.07 g/100 mL for Sample C, respectively.

Strong distinctions among the ranges of Samples A and B compared to Sample C infer that the overall ginsenoside content could have implications on the concentration of the taste threshold. However, Samples A and B are both white ginseng samples, whereas Sample C is red ginseng. Group threshold values were shown to differ significantly from individual threshold mean values only for Sample C. This is most likely due to the wide range of thresholds identified when thresholds were calculated on an individual basis as Sample C encompassed a

much larger range than Samples A and B. No conclusive trend was found between panelists who were familiar with ginseng prior to the study versus those who were unfamiliar prior to the study.

To further investigate the influence of ginsenosides on the taste threshold of ginseng, several white ginseng extracts could be tested with varying ginsenoside content levels (in this study Sample A contained 90% ginsenosides while Sample B contained 70%).

Based on the results from Samples A and B, it could be possible for industrial companies to elevate the level of white ginseng present in their product without modifying their present flavor profile. However, this study is meant to be a baseline study to investigate the taste threshold of ginseng only, which is why nose-clips were used. To verify whether adding ginseng levels of 100 mg per serving or greater is appropriate, a similar threshold test study should be done without nose-clips as consumers utilize all components of flavor including aroma and aroma-by-mouth when determining overall acceptance of a product.

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3.7 TABLES AND FIGURES

Panelist	Sample A (g/100 mL)	Sample B (g/100 mL)	Sample C (g/100 mL)
Panelist 1	0.014	0.048	2.167
Panelist 2	0.015	0.040	1.500
Panelist 3	0.017	0.045	0.560
Panelist 4	0.018	0.025	0.234
Panelist 5	0.021	0.123	0.537
Panelist 6	0.028	0.139	0.187
Panelist 7	0.029	0.023	0.226
Panelist 8	0.034	0.150	0.171
Panelist 9	0.034	0.111	1.360
Panelist 10	0.036	0.026	0.673
Panelist 11	0.037	0.124	1.528
Panelist 12	0.038	0.084	NA
Panelist 13	0.040	0.124	2.174
Panelist 14	0.040	0.042	0.446
Panelist 15	0.043	0.050	0.007
Panelist 16	0.045	0.030	0.278
Panelist 17	0.046	0.067	NA
Panelist 18	0.046	0.067	0.472
Panelist 19	0.046	0.126	1.282
Panelist 20	0.048	0.034	0.590
Panelist 21	0.051	0.082	0.555
Panelist 22	0.054	0.045	0.177
Panelist 23	0.055	0.067	NA
Panelist 24	0.056	0.081	0.524
Panelist 25	0.058	0.098	0.206
Panelist 26	0.061	0.145	0.145
Panelist 27	0.073	0.058	2.043
Panelist 28	0.094	0.03	0.640
Panelist 29	0.128	NA	0.216
Panelist 30	0.138	0.104	1.325
Panelist 31	0.213	0.116	1.827
Average	0.053 ± 0.005	0.090 ± 0.010	0.787 ± 0.085

Table 3.1: Detection thresholds in g/100 mL for Sample A, B, and C shown for all panelists. Panelists who did not reach a threshold level for a sample are identified by a "NA." Confidence intervals are calculated for an α=0.05 for all tables.

Unfamiliar with Ginseng	Detection Threshold (g/100 mL)		Familiar with Ginseng	Detection Threshold (g/100 mL)
Panelist 2	0.015		Panelist 1	0.014
Panelist 3	0.017		Panelist 6	0.028
Panelist 4	0.018		Panelist 7	0.029
Panelist 5	0.021		Panelist 9	0.034
Panelist 8	0.034		Panelist12	0.038
Panelist 10	0.036		Panelist 16	0.045
Panelist 11	0.037		Panelist 20	0.048
Panelist 13	0.040		Panelist 21	0.051
Panelist 14	0.040		Panelist 24	0.056
Panelist 15	0.043		Panelist 25	0.058
Panelist 17	0.046		Panelist 26	0.061
Panelist 18	0.046		Panelist 27	0.073
Panelist 19	0.046		Panelist 29	0.128
Panelist 22	0.054			
Panelist 23	0.055			
Panelist 28	0.094			
Panelist 30	0.138			
Panelist 31	0.213			
Average	***0.055 ± 0.022***		***Average***	***0.051 ± 0.015***

Table 3.2: Detection thresholds in g/100 mL for Sample A are shown for all panelists separated into two groups, those who were familiar with ginseng prior to the study and those who were not. Panelists who did not reach a threshold level for the sample are identified by a "NA." Confidence intervals are calculated for an α=0.05 for all tables.

Unfamiliar with Ginseng	Detection Threshold (g/100 mL)		Familiar with Ginseng	Detection Threshold (g/100 mL)
Panelist 4	0.025		Panelist 7	0.023
Panelist 10	0.026		Panelist 16	0.030
Panelist 16	0.030		Panelist 20	0.034
Panelist 2	0.041		Panelist 1	0.048
Panelist 14	0.042		Panelist 27	0.058
Panelist 22	0.045		Panelist 24	0.081
Panelist 15	0.050		Panelist 21	0.082
Panelist 17	0.067		Panelist 12	0.084
Panelist 18	0.067		Panelist 25	0.098
Panelist 23	0.067		Panelist 9	0.111
Panelist 30	0.104		Panelist 6	0.139
Panelist 31	0.116		Panelist 26	0.145
Panelist 5	0.123		Panelist 29	NA
Panelist 11	0.124			
Panelist 13	0.124			
Panelist 19	0.126			
Panelist 8	0.150			
Panelist 22	0.450			
Average	0.099 ± 0.044		***Average***	0.078 ± 0.022

Table 3.3: Detection thresholds in g/100 mL for Sample B shown for all panelists separated into two groups, those who were familiar with ginseng prior to the study and those who were not. Panelists who did not reach a threshold level for the sample are identified by a "NA." Confidence intervals are calculated for an α=0.05 for all tables.

Unfamiliar with Ginseng	Detection Threshold (g/100 mL)		Familiar with Ginseng	Detection Threshold (g/100 mL)
Panelist 15	0.007		Panelist 26	0.145
Panelist 8	0.171		Panelist 6	0.187
Panelist 22	0.177		Panelist 25	0.206
Panelist 4	0.234		Panelist 29	0.216
Panelist 14	0.446		Panelist 7	0.226
Panelist 18	0.472		Panelist 16	0.278
Panelist 5	0.537		Panelist 24	0.524
Panelist 3	0.560		Panelist 21	0.555
Panelist 28	0.640		Panelist 20	0.590
Panelist 10	0.673		Panelist 9	1.360
Panelist 19	1.282		Panelist 27	2.043
Panelist 30	1.325		Panelist 1	2.167
Panelist 2	1.500		Panelist 12	NA
Panelist 11	1.528			
Panelist 31	1.827			
Panelist 13	2.174			
Panelist 17	NA			
Panelist 23	NA			
Average	0.847 ± 0.314		***Average***	0.708 ± 0.400

Table 3.4: Detection thresholds in g/ 100 mL for Sample C shown for all panelists separated into two groups, those who were familiar with ginseng prior to the study and those who were not. Panelists who did not reach a threshold level for the sample are identified by a "NA." Confidence intervals are calculated for an α=0.05 for all tables.

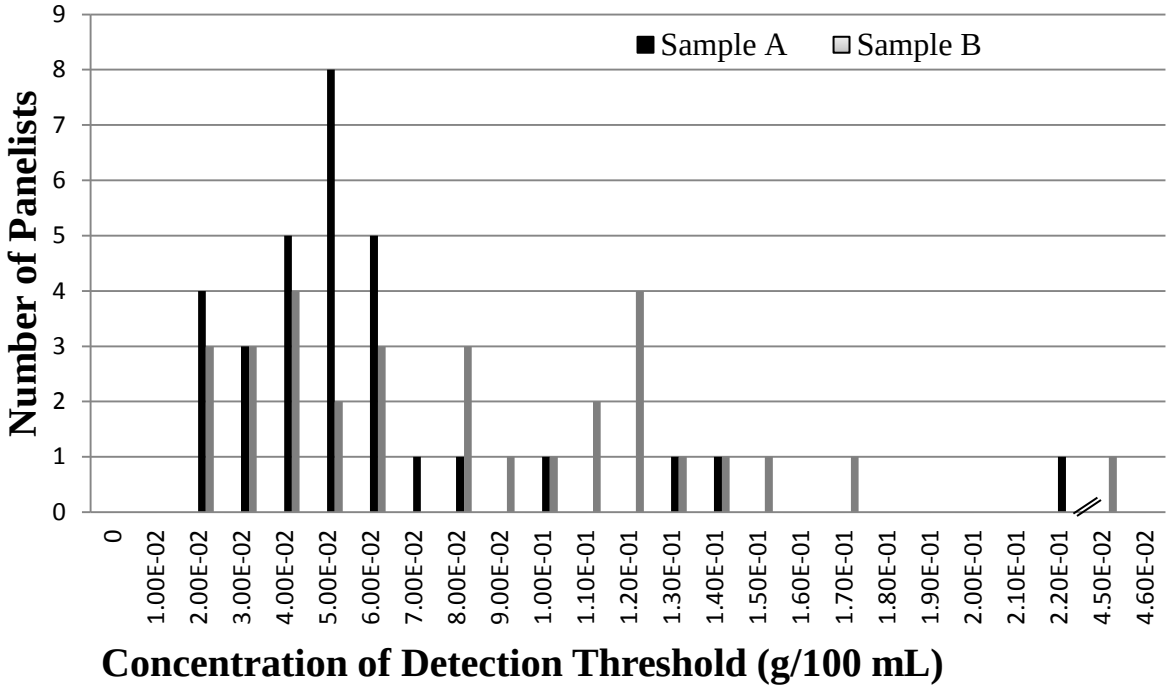

Figure 3.1: Distribution of detection thresholds (g/100 mL) for both white ginseng samples, Sample A (AmaxNutra Source, n=31) and Sample B (Xi'an Biotech Company, n=30).

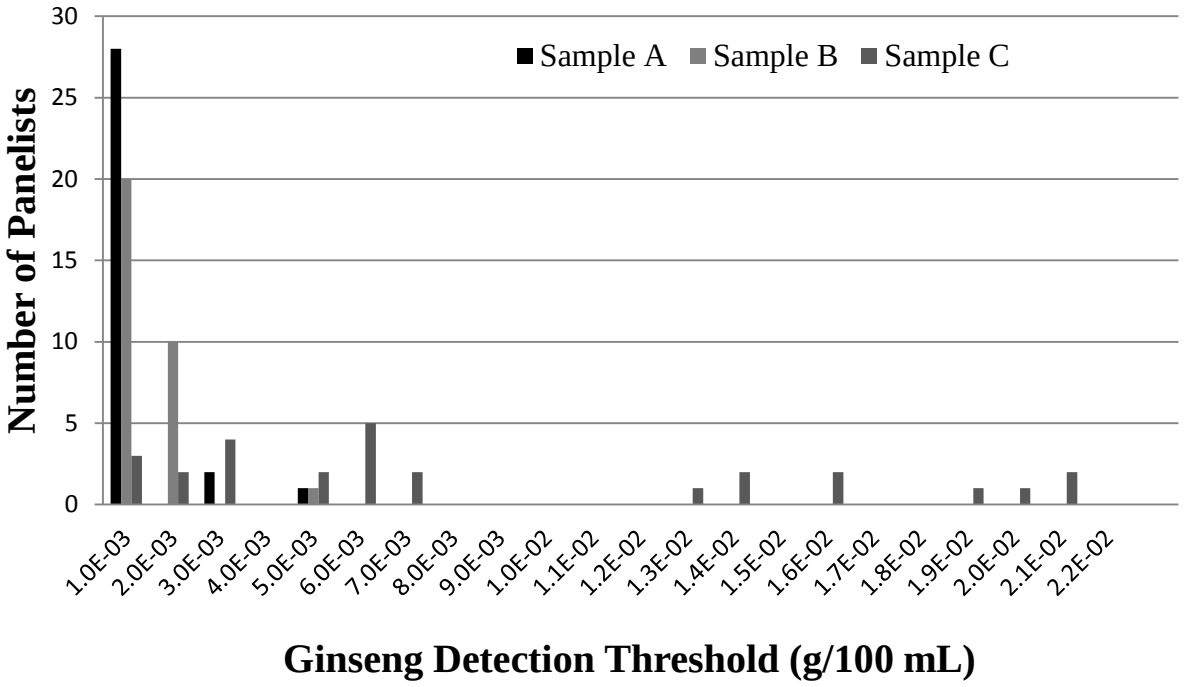

Figure 3.2: Distribution of detection thresholds (g/100 mL) for all three samples. Sample C (n= 28) has a much wider range than that of Sample A (n=31) or Sample B (n=30).

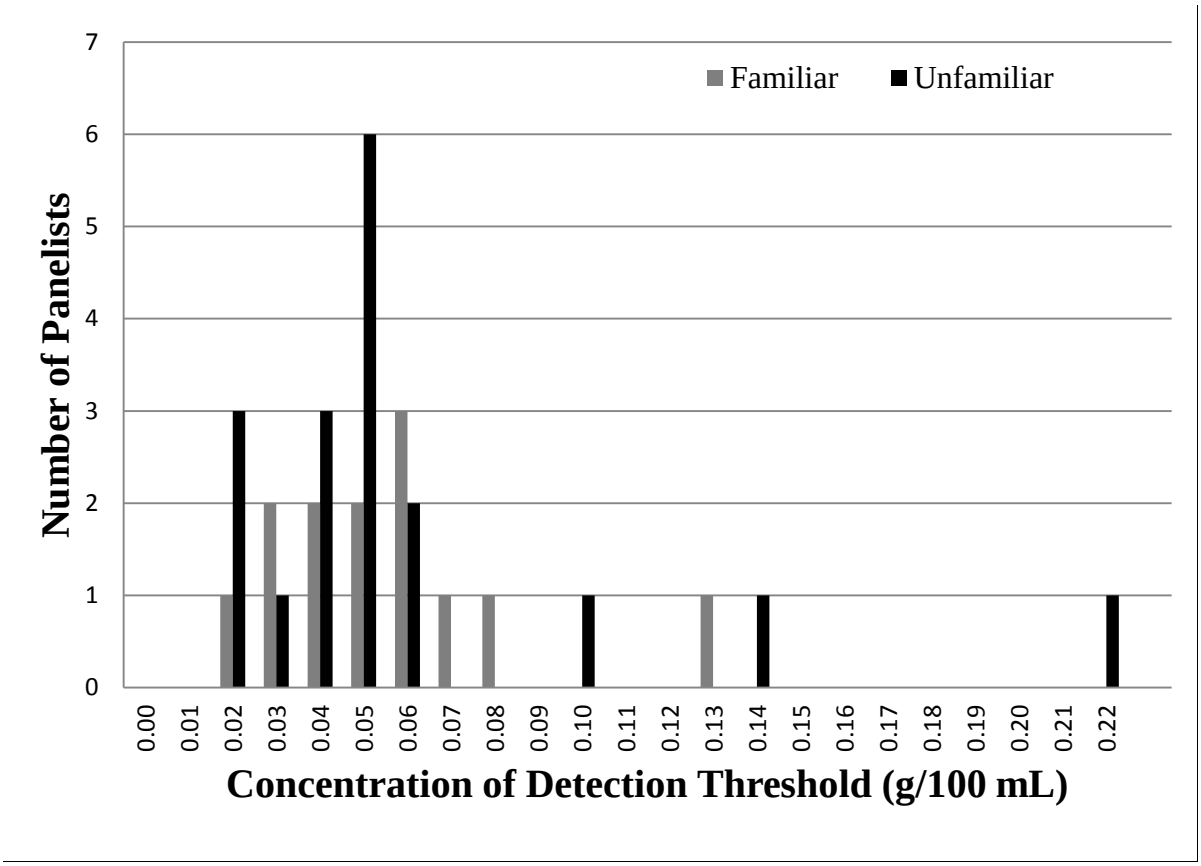

Figure 3.3: Comparison of distributions of detection thresholds (g/100 mL) for panelists who self-identified as familiar with traditional forms of ginseng (n=18) versus those who claimed to be unfamiliar with traditional sources of ginseng (n=13) prior to this study for Sample A.

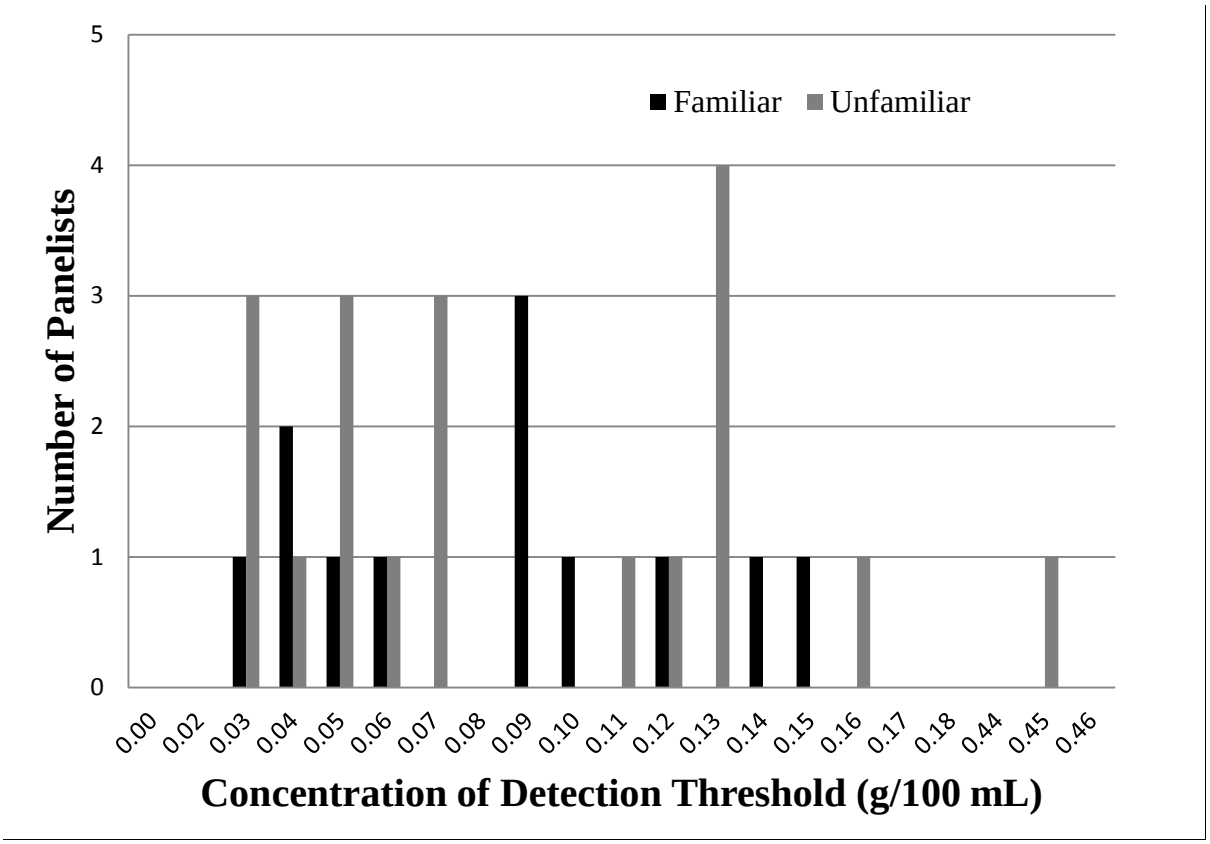

Figure 3.4: Comparison of distributions of detection thresholds (g/100 mL) for panelists who self-identified as familiar with traditional forms of ginseng (n=18) versus those who claimed to be unfamiliar with traditional sources of ginseng (n=12) prior to this study for Sample B.

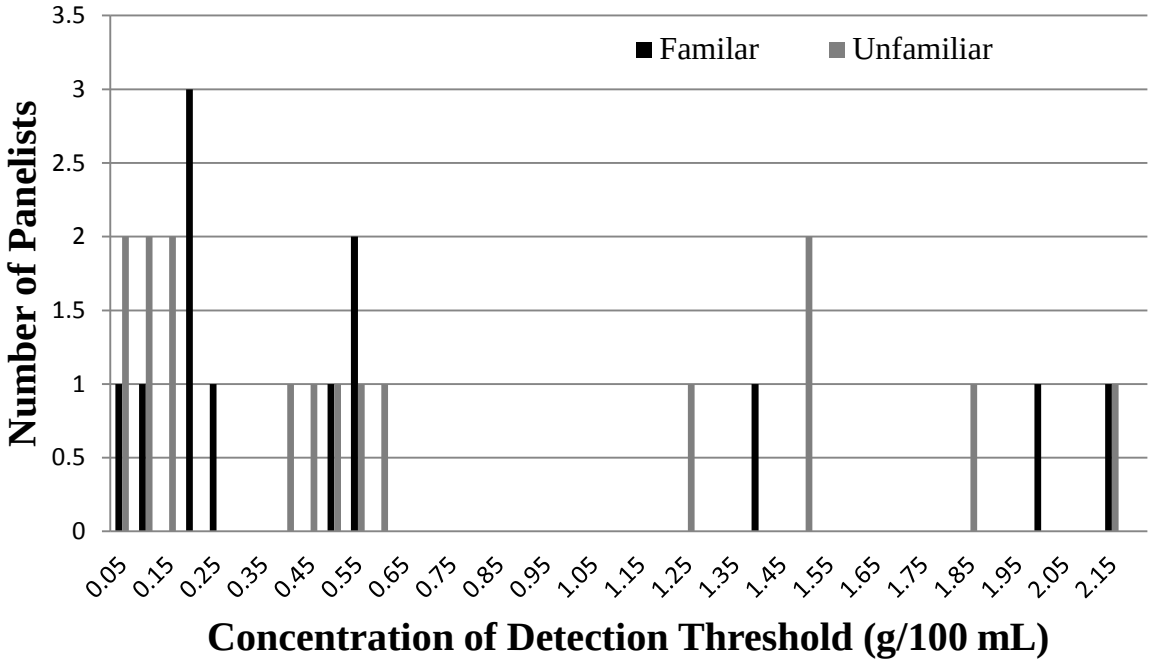

Figure 3.5: Comparison of distributions of detection thresholds (g/100 mL) for panelists who self-identified as familiar with traditional forms of ginseng (n=16) versus those who claimed to be unfamiliar with traditional sources of ginseng (n=12) prior to this study for Sample C.

Chapter 4: PERCENT CONTRIBUTION OF GINSENOSIDE RB_1 AND RG_1 TO THE OVERALL FLAVOR OF GINSENG

4.1 ABSTRACT

Due to the fast growth of the functional beverage market, the industry is constantly looking for different consumer groups to cater towards. Energy drinks, the most profitable segment of functional beverages, are currently marketed to 18-34 year olds; however, to gain traction with an older demographic, memory enhancing supplements have become commonplace ingredients in beverage formulations. One of the most common additives is ginseng, which has been used for thousands of years in forms of Asian holistic medicine (Chu and Zhang 2009). Yet, due to ginseng's inherently bitter taste most companies choose to add sub-therapeutic levels in to a single serving size, which begins at 100-200 mg/day.

The pharmacological benefits of ginseng are produced by ginsenosides. Of the 100 ginsenosides known only two, ginsenoside Rb_1 and Rg_1 (G-Rb_1 and G-Rg_1), have therapeutic benefits that match those currently associated with energy drinks (energy enhancement, stress relief, and increased stamina) as well as memory enhancement.

In this study the empirical flavor thresholds of ginseng, G-Rg_1, and G-Rb_1 were identified. These values show the amount of ginseng and the ginsenosides that can be added to a beverage without interfering with its current flavor profile. Threshold levels were calculated using the R-index by the rating method, and the percent contribution of G-Rb_1 and G-Rg_1 on the overall flavor and taste of ginseng was calculated using taste activity values (TAVs) and flavor activity values (FAVs).

The flavor threshold of ginseng was found to be ranging from $9.94x10^{-5}$ to $5.20x10^{-3}$ g/100 mL with an average value of $1.02x10^{-3} \pm 4.63x10^{-4}$ g/100 mL, while the thresholds of G-

Rg_1 and G-Rb_1 were found to be ranging from $1.05x10^{-4}$ to $1.52x10^{-3}$ g/100 mL with an average of $5.21x10^{-4} \pm 8.91x10^{-5}$ g/100 mL, from $1.64x10^{-4}$ to$7.66x10^{-4}$ g/100 mL with an average value of $7.67x10^{-4} \pm 1.78x10^{-4}$ g/100 mL, respectively. Group flavor thresholds for ginseng, G-Rg_1, and G-Rb_1 were identified to be $1.09x10^{-3}$ g/100 mL and $4.64x10^{-4}$ g/100 mL, $5.65x10^{-4}$ g/100 mL, respectively. All confidence intervals were calculated at α=0.05.

The percent contributions of G-Rb_1 and G-Rg_1 towards the TAV generated from the average taste threshold of ginseng were found to be 8.5% and 7.0%. The percent contributions of G-Rb_1 and G-Rg_1 towards the FAV generated from the average taste threshold of ginseng were found to be 9.0% and 13.3%, respectively. The percent contributions of G-Rb_1 and G-Rg_1 for the group taste threshold of ginseng were found to be 82.9% and 68.1%. The percent contributions of G-Rb_1 and G-Rg_1 for the average taste threshold value of ginseng were found to be 53.7% and 79.1%, respectively.

In this study the threshold of ginseng was found to be below the lowest recommended intake level for pharmacological benefits for a single serving beverage, but the total taste accounted for by G-Rb_1 and G-Rg_1 was below 100%. This means that companies could in theory substitute either G-Rb_1 or G-Rg_1 in place of ginseng in energy drinks at levels which could allow therapeutic benefits without having to deal with the full strength bitterness of ginseng.

Key Words: Ginseng, Ginsenosides, G-Rb1, G-Rg1, Threshold, Taste Activity Value, Flavor Activity Value

4.2 INTRODUCTION

Ginseng has been a common medicinal component of Asian folk medicine for thousands of years, and while there are over 200 compounds present in ginseng (Chu and Zhang 2009), it is generally assumed that ginseng saponins, also known as ginsenosides, are responsible for its

medicinal properties. Over 100 ginsenosides have been currently isolated and identified from various varieties of ginseng plants; however, the ginsenoside content of a specific plant varies widely depending on several factors including species, age at harvest, and location of cultivation (Jia and Zhao 2009).

Western cultures have recently become interested in investigating the pharmacological benefits of ginseng. As the conclusions of these studies have slowly spread into the knowledge of the general public, ginseng has become an increasingly more common food additive in functional foods and beverages (Heckman and others 2010) in the form of *Panax ginseng*, which is most often referred to as Asian ginseng. While there are 11 different types of ginseng grown throughout the world, Asian ginseng root is the most common form used in commercial products in Western cultures after it has been processed into white ginseng (Clauson and others 2008; Heckman and others 2010).

In 2009 the functional beverage market was estimated to be worth over $9 billion in sales with the energy drink category claiming a $1 billion share (Mintel 2010). While most energy drinks are marketed to ages 18-34 year olds (Heckman and others 2010), more energy drinks are expanding their claims from simply energy enhancement to also claims of improved cognitive function and memory retention, which has the potential to widen their consumer base. According to the 2010 Mintel Report an average 50% of consumers who purchase functional beverages are looking for a product that can provide memory enhancement benefits (Mintel 2010).

Companies producing energy drinks can use ginseng as an ingredient that has the ability to support both energy and memory enhancement claims. Ginseng is well promoted as having the capabilities to improve cognitive function, concentration, and memory as well as improve physical and athletic stamina (Clauson and others 2008). While ginseng has had many touted

benefits, most studies investigate ginsenosides as individual constituents as opposed to ginseng extract as a whole (Heckman and others 2010).

As ginseng is currently classified as a dietary supplement, the quantity of ginseng used in an energy drink does not need to be identified on the product label. Recommended therapeutic doses for ginseng start at 100-200 mg/day however, many energy drinks contain a subtherapeutic amount (Clauson and others 2008). This could be due to ginseng's well known bitter flavor which not well received by Western consumers.

A possible option for companies concerned about adding large amounts of ginseng to their product, may have the option of adding only specific ginsenosides to their product which tout the desired benefits. For companies to be able to consider this option the threshold of the desired ginsenoside must first be identified and it would need to be proven that the threshold of the ginsenoside in question is higher than that of the normally used ginseng sample.

To model this approach ginsenosides Rb_1 and Rg_1 (G-Rb_1 and G-Rg_1) were chosen to be a possible substitution for ginseng in an energy drink beverage. Both G-Rb_1 and G-Rg_1 have been extensively studied as possible drugs to be used to improve memory impairments in animal models. G-Rb_1 has been shown to improve induced memory disorder and synaptic loss (Tohda and others 2004) and facilitate spatial learning and memory (Liu and others 2011). G-Rg_1 has shown potential in enhancing learning and memory (Chu and Zhang 2009) and has been shown to improve all stages of learning (i.e. registration, consolidation and retrieval of memory) in rats (Zhang and others 1990). Both ginsenosides have also shown promise in enhancing aerobic performance (Wang and others 1998).

Previous research for the panelists participating in this study found the taste threshold of ginseng to be an average of 0.055 ± 0.019 g/100 mL (at α=0.05) with the group threshold of the

panel tested found to be 0.050 g/100 mL. These results showed that it could be possible to add enough ginseng in a single serving size (240 mL) energy drink to meet the lower limits recommended for therapeutic benefits (100 mg/day). However, consumers do not rely solely on taste when consuming a product; they also highly depend on factors such as aroma and aroma by mouth which panelists' ability to utilize were hindered in the previous study. There is one published report of the threshold of G-Rb_1 and G- Rg_1, which was found to be $5.0x10^{-7}$ and $5.0x10^{-6}$ M respectively; however, the medium in which these thresholds were identified in was not reported (Kim and others 2001).

As G-Rb_1 and G-Rg_1 are both major ginsenosides of Asian ginseng and are two of six ginsenoside standards available, it was also deemed a question of interest as to what percent contribution G-Rg_1 and G-Rb_1 have on the overall flavor and taste of ginseng. To determine this, the taste activity value (TAV) and flavor activity value (FAV) for each ginsenoside must be calculated. Then the percent contribution can be established by creating a ratio between the TAV or FAV and the found detection threshold of ginseng. Thresholds for all compounds were identified using R-index by the rating method which has been shown to be just as effective as the current ASTM recommended method of ascending limits (Robinson and others 2005; Kappes and others 2006).

4.3 MATERIALS AND METHODS

Sample Preparation

For this study all solutions contained 0.7% propylene glycol (PG, Fisher Scientific, Philadelphia, PA) as it was used to solubilize both G-Rb_1 and G-Rg_1, due to their insolubility in H_2O. Samples of ginseng (90% ginsenosides, AmaxNutra Source, Eugene, OH), G-Rb_1 and G-Rg_1 (Xi'an TonKing Biotech, Xi'an, China) were commercially purchased. Solutions for each

sample were prepared at seven concentrations, in threefold increments. Ginseng was presented in the following concentrations: $5.74x10^{-1}$, $1.91x10^{-1}$, $6.38x10^{-2}$, $2.13x10^{-2}$, $7.09x10^{-3}$, $2.36x10^{-3}$, and $7.87x10^{-4}$ g/ L. G-Rb_1 was presented in the following concentrations: $1.52x10^{-1}$, $5.15x10^{-2}$, $1.75x10^{-2}$, $5.92x10^{-3}$, $2.01x10^{-3}$, $6.80x10^{-4}$, $2.31x10^{-4}$ g/L. Sample G-Rg_1 was presented in the following concentrations: $8.16x10^{-2}$, $2.71x10^{-2}$, $9.04x10^{-3}$, $3.01x10^{-3}$, $1.00x10^{-3}$, $3.35x10^{-4}$, and $1.13x10^{-4}$ g/L.

A 2.12 g/L stock solution was created for G-Rb_1 by adding 0.032 g into a 50 mL vial to which 15 mL of PG was added. The vial was then sealed and placed in a 60ºC sonicating water bath for 45 minutes. After sonication subsequent amounts for each concentration were dispensed into a 1L graduated cylinder using a micropipetter. Supplemental amounts of PG were added to lower concentrations so that each solution contained equal amounts of PG (Table 4.1). Spring water (Absopure,Urbana, IL) was then used to bring the solution up to 1.2 liters.

A 1.14 g/L stock solution was created for G-Rg_1 by adding 0.017 g into a 50 mL vial to which 15 mL of PG was added. The vial was then sealed and placed in a 60ºC sonicating water bath for 45 minutes. After sonication subsequent amounts for each concentration were dispensed into a 1L graduated cylinder using a micropipetter. Supplemental amounts of PG were added to all concentrations so that each solution contained amounts of PG identical to that of G-Rb_1 solutions (Table 4.2). Spring water was then used to bring the solution up to 1.2 liters.

Each day a 0.115 g/L ginseng stock solution was prepared using 0.103 g of ginseng and 0.9 L of spring water, which was stirred for 5 minutes prior to further dilution. Solutions were then appropriately dispensed and diluted into concentrations using a graduated cylinder, spring water, and 8.6 mL of PG. Solutions were again stirred for 5 minutes, after which they were poured into individual dispensing pipettes (Cole Parmer, Veron Hills, IL). The pipettes were

used to dispense 10 mL of solution into 30 mL plastic cups with lids (Solo Cup Company, Urbana, IL) for the experimental samples. The noise reference (50 mL of a 0.7% PG and spring water solution) was available during the experiment in 163 mL plastic cups with lids. All samples were prepared the day before and were stored and served at room temperature (27ºC). The containers were coded with three-digit random numbers. The reference for noise was labeled as ‘‘noise.’’

Ginsenoside Analysis by High Performance Liquid Chromatography

Ginsenoside analysis was done following the procedure reported by Chung (2011), which is shown in its entirety as follows. Ginsenosides in the ginseng extract used in the study were profiled by high performance liquid chromatography (HPLC). The ginseng extract has been commercially sold as a food ingredient and labeled as *Panax ginseng* extract, containing 80% ginsenosides, from AmaxNutra Source Incorporated. Crude saponins were extracted according to Shibata and others (1966) and Do and others (1986). Two grams of the ginseng extract powder was extracted in a 250-mL Erlenmeyer flask for 1 hr by refluxing it with 50 mL of water-saturated *n*-butanol at 80°C. The upper layer of the mixture of water-saturated *n*-butanol and ginseng extract was decanted into another 250-mL Erlenmeyer flask, and another 50 mL of water-saturated *n*-butanol was added to the ginseng extract residue. The extraction of crude sapoinins from the ginseng extract with water-saturated n-butanol was replicated three times. The solvent containing the crude saponins was combined and filtered through a Whatman No. 4 filter paper (Whatman International, Maidstone, UK). The filtrate was cleansed with 20 mL of deionized water through vigorously shaking, and subsequently, evaporated in a vacuum. The dried residue was washed with 50 mL of diethyl ether to remove the fat and then weighed for crude saponin contents.

Ginsenosides of the crude saponin extracts were analyzed according to Hong and others (2009). The crude saponin extracts were dissolved by an appropriate volume of methanol, and filtered through a 0.45 μm polyvinylidene fluoride (PVDF) syringe filter prior to injection into an HPLC system. Ginsenoside analysis was performed on a Jasco 114 HPLC system (Jasco, Inc., Tokyo, Japan) with a PU-2089 Plus gradient pump equipped with a degasser, an AS-2075 Plus autosampler, and a UV-2075 Plus UV-vis detector. The HPLC system was controlled by a Jasco ChromPass software (Jasco, Inc., Tokyo, Japan). Comparative analyses were conducted using a μ-Bondapak (Waters, Milford, MA, USA) C18 column (3.9 × 300 mm i.d., 10 μm pore size) with the column temperature set to 35°C. Two mobile phases, water (A) and acetonitrile (B), were used with a linear gradient. The mobile phase A was maintained at 80% for the first 5 min, decreased from 80 to 67% over 33 min and from 67 to 20% in the next 25 min, maintained at 20% for 12 min, increased from 20% to 80% over 5 min, and equilibrated for 10 min before the next injection. The flow rate of mobile phases was 1 mL/min and the ginsenosides were detected at 203 nm. A stock solution of mixed ginsenoside standard containing the ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, and Rh_2 (Fleton Reference Substance Co., Ltd, Chengdu, China) was prepared and diluted to the appropriate concentration for calibration. All solvents were HPLC-grade and were obtained from SK Chemicals (Ulsan, Korea).

Rinse Preparation

A 0.55% solution of carboxymethlycellose (CMC) was prepared the day before testing to be utilized as a rinse in between samples. Eleven grams of CMC was weighed out and poured into a 2 L beaker. Using a 1000 mL graduated cylinder, 2 L of hot water (65ºC) was added to the beaker. The solution was then stirred until all CMC had dissolved. Solution was stored at room temperature until use.

Panel Selection

Panelists were recruited from the pool of panelists that participated in the earlier ginseng taste threshold panel. Panelists were screened for the ginseng taste threshold panel based off of allergies, smoker status, frequency of consumption of products containing ginseng, and schedule availability. Twenty-one panelists, 2 M and 19 F, ages 21–60 years old returned for this panel.

Sample Presentation

A randomized complete block design using ten replicates was used for seven different signal samples and one noise sample per compound. The randomization was done using the computer program Compusense *Five* 5.0 (Guelph, Canada). Five replicates of the noise and five replicates of the seven concentrations of one compound sample consisted of a complete set for the session. Testing was split across 6 days so that panelists saw only 1 ginseng sample a day and saw all 10 replications over the course of 2 consecutive days. Panelists were instructed to take the whole sample into their mouth, swirl it around for 2–3 seconds, expectorate, and complete the task given during the experiment. Samples were presented monadically and panelists were asked if the sample was a signal sure, signal unsure, noise sure, or noise unsure, where the 0.7% PG solution represented the noise and the ginseng and ginsenoside solutions represented signals. Panelists rinsed twice in-between samples, first using room temperature CMC, followed by warm water to prevent residual bitterness carryover. After every 8th sample panelists were required to wait 2 minutes before starting with the next group of samples. At any time during the experiment, panelists were allowed to retaste the noise reference in order to refamiliarize themselves with the noise sample. Panelists evaluated samples at 27 ºC in individual booths with incandescent lighting.

Data Analysis

R-index

For each ginseng sample thresholds were found using the R-index response matrix method (O'Mahony 1992). Then the ginseng concentration (g/100 mL) for each sample was plotted as a function of R-index percentage. The two points found directly above and below an R-index of 75% were identified and a linear trend line was created between these two points to identify the ginseng concentration at an R-index of 75%. Matrices were constructed for each individual panelists and all responses were combined together to create a single matrix for the group, allowing thresholds to be identified for each panelist and the group as a whole.

Taste Activity Value

Taste activity values (TAVs) were calculated using both the group threshold value and the average threshold value identified for all panelists in this study. Values were calculated by finding the ratio between the ginsenoside content found in ginseng (Table 4.3) and the identified threshold for that ginsenoside. Taste threshold values, both the average and group, were calculated using the panelist's data from an earlier ginseng taste threshold study (See Chapter 3). The TAV for ginseng was calculated from taking an assumed 100 g of ginseng divided by the identified taste threshold for ginseng, as the threshold of ginseng was presented in g/100 mL. Percent contribution was then identified by calculating the ratio between the TAV for each ginsenoside and the TAV for ginseng. For full calculations see Appendix C.

Flavor Activity Value

Flavor activity values (FAVs) were calculated for both the group threshold value and the average threshold value identified for all panelists in this study. FAVs and percent contribution were calculated using the same calculations as those for TAVs except flavor threshold values of

ginseng (group and average) were substituted for the taste threshold values of ginseng. For full calculations see Appendix D.

4.4 RESULTS AND DISCUSSION

The flavor threshold values for ginseng, G-Rg_1, and G-Rb_1 were found to be $9.94x10^{-5}$ to $5.20x10^{-3}$ g/100 mL, $1.05x10^{-4}$ to $1.52x10^{-3}$ g/100 mL, $1.64x10^{-4}$ to $7.66x10^{-4}$ g/100 mL, respectively, with average values calculated as $1.02x10^{-3} \pm 4.63x10^{-4}$ g/100 mL for ginseng, $5.21x10^{-4} \pm 8.91x10^{-5}$ g/100 mL for G-Rb_1, and $7.67x10^{-4} \pm 1.78\ x10^{-4}$ g/100 mL for G-Rg_1 (Table 4.4). All confidence intervals were calculated at a significance level of 0.05.

As previously mentioned, Kim and others (2001) reported threshold values for G-Rb_1 and G-Rg_1 to be $5.0x10^{-7}$ and $5.0x10^{-6}$ M, respectively. Threshold values in this study were found to be above the previously reported threshold values for both compounds. The threshold of G-Rb_1 was found to be $6.91x10^{-5}$ M and the threshold of G-Rg_1 was found to be $6.50x10^{-5}$ M. It is difficult however, to discuss these differences as the sensory methodology was not reported by Kim and others (2001).

It is also interesting to note that this study did not find the flavor threshold of ginseng to be above 100 mg per serving, which would meet the lower limits of the daily recommended amount for optimal pharmacological benefits, for any of the panelists. This is different than what was seen in the previous taste threshold study, which found the threshold of ginseng to be high enough to allow for a full therapeutic dose to be added to a single serving size beverage without imparting off flavors.

As panelists were not specifically asked whether or not G-Rb_1 and G-Rg_1 were bitter, we cannot assume that the general population sees them as such. However, because they are saponins which are generally known to be bitter (Aldin and others 2004), comparing their

threshold levels to other known bitter compounds seems prudent. As seen here, the threshold values of a single compound can vary greatly depending on the group being tested. Thus for this comparison, a single literature reference has been chosen (Keast and Roper 2007) which found the detection threshold of three common bitter compounds, propylthiouracil (PROP), caffeine, and quinine-HCl (QHCl). In this study the group thresholds for caffeine, QHCl, and PROP were found to be 1.2 ± 0.12 mM, 0.0083 ± 0.001 mM, and 0.088 ± 0.07 mM. This places the group threshold values of G-Rb_1 (0.051 mM) and G-Rg_1 (0.058 mM) slightly below the known threshold of QHCL which has the lowest threshold of the three bitter compounds (Table 4.5).

The percent contribution for G-Rb_1 towards the TAV of ginseng was found to be 53.7% for the average and 82.9% for the group threshold. The percent contribution for G-Rg_1 towards the TAV of ginseng was found to be 79.1% for the average and 68.1% for the group threshold (Table 4.6). The percent contribution for G-Rb_1 towards the FAV of ginseng was found to be 9.0% for the average and 8.5% for the group threshold. The percent contribution for G-Rg_1 towards the FAV of ginseng was found to be 13.3% for the average and 7.0% for the group threshold (Table 4.7).

The percent contribution towards the FAV of ginseng for both G-Rb_1 and G-Rg_1 are significantly lower than their respective percent contributions towards the TAV of ginseng. This is most likely due to the aromatic compounds present in the ginseng extract. The TAV study done on ginseng, used nose-clips to restrict the use of panelists' nasal passages therefore blocking the perception of aromatic compounds. Panelists in the FAV studies did not use nose-clips and as a result had extra compounds to factor into the overall sensory experience.

By adding the two percent contribution values of G-Rb_1 and G-Rg_1, their total percent contribution on the overall flavor of ginseng can be identified. In this study, it was found that in

some instances this value equaled greater than 100%. We can conclude that the compounds in ginseng most likely work together to create an antagonistic effect in which bitter can be used to mask bitter. A similar effect was seen by Chung 2011, in which the bitterness of chocolate was reported to mask the bitterness of ginseng.

When viewed on an individual basis, it is to be noted that neither G-Rb_1 nor G-Rg_1 reached a percent contribution of 100%; therefore, it is possible to add either one of the two compounds in place of ginseng to reduce the amount of bitter flavor added to a product. This decision would have to be made on a product by product basis prior to making any conclusions as whether it would be practical in an industrial setting; further evaluations would have to be done to take in to account cost analysis, marketing implications, and the quantity of each ginsenoside required to make specific health claims.

G-Rb_1 and G-Rg_1 were found to be some of the top gensenosides present in our purchased sample, but they were not the most prevalent (Table 4.3). Therefore, further research should be done to test the other ginsenosides present in the samples, including G-Rd and G-Re, which were both more prevalent in our sample of ginseng than G-Rb_1 and G-Rg_1. They were not tested in this study due to their pharmacological properties not aligning with the objectives of this study. Further research studies can focus on not only these ginsenosides, but also the other ginsenosides that are used as standards for ginseng so attempt to create a better picture of the flavor interactions of ginsenosides.

The theory using TAVs and FAVs to quantify the overall taste contribution that individual ingredients or components has on part of a larger food system is a relatively new. TAVs were first used in 1996 by Warmke and others to identify the total contributions of mineral salts, amino acids, nucleotides, and peptides towards their overall contribution to the taste of

Swiss cheese. Since that study, TAVs have been successfully used to identify the key taste contributors in stewed beef juice (Schlichterle-Cerney 1998), the non-volatile taste active compounds in the meat of Chinese mitten crab (Chen 2007), and the key tastants that are generated during Maillard-type reactions in food processing (Hofmann and others).

4.5 CONCLUSIONS

The threshold of ginseng was found range from $9.94x10^{-5}$ to $5.20x10^{-3}$ g/100 mL (average value $1.02x10^{-3} \pm 4.63x10^{-4}$ g/100 mL). Threshold values for G-Rg_1 and G-Rb_1 were found to range from $1.05x10^{-4}$ to $1.52x10^{-3}$ g/100 mL (average value $5.21x10^{-4} \pm 8.91x10^{-5}$ g/100 mL) and $1.64x10^{-4}$ to $7.66x10^{-4}$ g/100 mL (average value $7.67x10^{-4} \pm 1.78x10^{-4}$ g/100 mL), respectively. Confidence intervals for all calculations were done at α=0.05. The flavor threshold values for ginseng were found to be greater than those identified in the previous taste threshold ginseng study. Threshold values for G-Rg_1 and G-Rb_1 were above those previously reported in literature and they fall slightly below the threshold of QHCl.

The calculated values for percent contribution towards the TAV of ginseng for G-Rb_1 and G-Rg_1 were found to be significantly greater than their respective percent contributions towards the FAV of ginseng. Total percent contribution accounted for was found in some instances to be greater than 100%. This is most likely due to the influence of aromatic compounds present in the ginseng extract which have a strong influence on the flavor of ginseng.

Future research should focus on the differences found between the taste threshold and the flavor threshold and the possible causes of the extreme differences between the threshold values. Other studies should focus on finding the threshold values for either the remaining 4 ginseng standards compounds or if using this particular ginseng sample in the future, studies should focus

on identifying the threshold values of the other ginsenosides that are present in a larger quantity

identify the full influence of the major ginsenosides on the taste and flavor of ginseng extract

4.6 References

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4.7 TABLES AND FIGURES

Concentration (g/100 mL)	Stock Solution Added (mL)	Supplemental PG Added (mL)
$1.52x10^{-3}$	8.61	0.00
$5.07x10^{-4}$	2.86	5.74
$1.69x10^{-4}$	0.95	7.65
$5.63x10^{-5}$	0.32	8.29
$1.88x10^{-5}$	0.11	8.50
$6.26x10^{-6}$	0.04	8.57
$2.09x10^{-6}$	0.01	8.60

Table 4.1: Amount of stock solution and supplemental PG added to make 1.2 L of each concentration of G-Rb_1.

Concentration (g/100 mL)	Stock Solution Added (mL)	Supplemental PG Added (mL)
$8.16x10^{-4}$	8.589	0.021
$2.71x10^{-4}$	2.855	5.755
$9.04x10^{-5}$	0.952	7.658
$3.01x10^{-5}$	0.317	8.293
$1.00x10^{-5}$	0.106	8.504
$3.35x10^{-6}$	0.035	8.575
$1.12x10^{-6}$	0.012	8.598

Table 4.2: Amount of stock solution and supplemental PG added to make 1.2 L of each concentration of G-Rg_1.

Ginsenoside	Amount present in sample (mg/g)
Rg_1	73.8
Re	216
Rf	2.7
Rb_1	77.2
Rc	36
Rb_2 + Rb_3	61.1
Rd	104
Rg_3	9.7
Rh_2	0.6
Rg_2 + Rh_1	44.4

Table 4.3: Ginsenosides identified present in the ginseng sample tested. Analysis was run by the Korean Food and Research Institute and confirmed by the USDA.

Panelist ID	G-Rg_1 Threshold (g/100ml)	G-Rb_1 Threshold (g/100ml)	Ginseng Threshold (g/100ml)
Panelist 1	1.64×10^{-4}	8.45×10^{-4}	1.49×10^{-3}
Panelist 2	1.84×10^{-4}	1.05×10^{-4}	1.17×10^{-4}
Panelist 3	2.51×10^{-4}	4.60×10^{-4}	9.94×10^{-4}
Panelist 4	2.71×10^{-4}	1.52×10^{-3}	1.34×10^{-3}
Panelist 5	2.98×10^{-4}	5.07×10^{-2}	1.16×10^{-3}
Panelist 6	4.30×10^{-4}	1.06×10^{-3}	1.76×10^{-4}
Panelist 7	4.47×10^{-4}	1.52×10^{-3}	1.28×10^{-3}
Panelist 8	4.84×10^{-4}	7.53×10^{-4}	2.85×10^{-4}
Panelist 9	5.18×10^{-4}	3.84×10^{-4}	1.34×10^{-3}
Panelist 10	5.37×10^{-4}	2.07×10^{-4}	1.14×10^{-3}
Panelist 11	5.68×10^{-4}	8.29×10^{-4}	1.66×10^{-4}
Panelist 12	5.82×10^{-4}	1.38×10^{-3}	2.13×10^{-4}
Panelist 13	6.02×10^{-4}	1.10×10^{-3}	1.35×10^{-3}
Panelist 14	6.30×10^{-4}	9.78×10^{-4}	1.22×10^{-3}
Panelist 15	6.45×10^{-4}	4.59×10^{-4}	8.35×10^{-4}
Panelist 16	6.86×10^{-4}	1.05×10^{-3}	1.92×10^{-3}
Panelist 17	6.86×10^{-4}	3.51×10^{-4}	1.03×10^{-3}
Panelist 18	6.95×10^{-4}	3.38×10^{-4}	1.21×10^{-3}
Panelist 19	7.38×10^{-4}	4.79×10^{-4}	1.09×10^{-3}
Panelist 20	7.66×10^{-4}	8.87×10^{-4}	1.58×10^{-4}
Panelist 21	7.66×10^{-4}	9.12×10^{-4}	1.24×10^{-3}
Average	$5.21 \times 10^{-4} \pm 8.19 \times 10^{-5}$	$7.67 \times 10^{-4} \pm 1.78 \times 10^{-4}$	$8.98 \times 10^{-4} \pm 2.41 \times 10^{-4}$

4.4: Detection thresholds in g/ 100 mL shown for G-Rg_1, G-Rb_1, and ginseng, for all panelists. Confidence intervals are calculated for an α=0.05 for all tables.

Compound	Detection Threshold (mM)
Caffeine	1.2
Propylthiouracil (PROP)	0.088
Quinine-HCl (QHCl)	0.0083
G-Rg_1	0.0058
G-Rb_1	0.0051

Table 4.5: Comparison of known group thresholds of common bitter compounds to those of G-Rb_1 and G-Rg_1. Threshold values of caffeine, PROP, and QHCl come from and Keast and Roper 2007.

	Taste Threshold Value (g/100 mL)	TAV	Ginseng Flavor Threshold (g/100 mL)	$TAV_{Ginseng}$	Percent Contribution
G-Rb_1 Average Threshold	$7.67x10^{-4}$	1006	$5.34x10^{-3}$	1872	53.7%
G-Rb_1 Group Threshold	$4.64\ x10^{-4}$	1663	$4.99x10^{-3}$	2005	82.9%
G-Rg_1 Average Threshold	$5.21x10^{-4}$	1481	$5.34x10^{-3}$	1872	79.1%
G-Rg_1 Group Threshold	$5.65x10^{-4}$	1366	$4.99x10^{-3}$	2005	68.1%

Table 4.6: Total taste activity values calculated for G-Rb_1 and G-Rg_1 using both the average taste threshold value and the group threshold value. Confidence intervals are calculated for an α=0.05 for all tables.

	Flavor Threshold Value (g/100 mL)	FAV	Ginseng Flavor Threshold (g/100 mL)	$FAV_{Ginseng}$	Percent Contribution
G-Rb_1 Average Threshold	$7.67x10^{-4}$	1006	$8.98x10^{-4}$	11142	9.0%
G-Rb_1 Group Threshold	$4.64x10^{-4}$	1663	$5.12x10^{-4}$	19512	8.5%
G-Rg_1 Average Threshold	$5.21x10^{-4}$	1481	$8.98x10^{-4}$	11142	13.3%
G-Rg_1 Group Threshold	$5.65x10^{-4}$	1366	$5.12x10^{-4}$	19512	7.0%

Table 4.7: Total flavor activity values calculated for G-Rb_1 and G-Rg_1 using both the average taste threshold values and the group threshold values. Confidence intervals are calculated for an α=0.05

Chapter 5: Impact of nose-clips on the empirical threshold of Asian ginseng

5.1 ABSTRACT

The use of nose-clips in sensory studies allows panelists to focus on taste and sensations in the mouth created by a product without the interference of aroma perceptions. However, it is unclear how the use of nose-clips affects a panelist's threshold level for individual compounds. Ginseng is a common ingredient found in functional beverages, specifically energy drinks, due to its wide variety of therapeutic uses. In this study, the influence of nose-clips is explored on the threshold of ginseng for 21 panelists. Threshold levels were calculated using the R-index by the rating method.

Threshold levels identified for ginseng while wearing nose-clips ranged from 0.014 to 0.213 g/100 mL (average value of $5.54x10^{-2}\pm1.85x10^{-2}$ g/100 mL, $\alpha=0.05$) and without nose-clips from $9.94x10^{-5}$ to $1.92x10^{-3}$ g/100 mL (average value of $1.04x10^{-3}\pm4.58x10^{-4}$ g/100 mL, $\alpha=0.05$). On average panelists saw a magnitude difference of 128 ±50 ($\alpha=0.05$) times lower threshold without nose-clips than the identified threshold while wearing nose-clips. Group threshold values for with nose-clips was found to be 0.05 g/100 mL and without nose-clips $1.09x10^{-3}$ g/100 mL, with a 46-fold difference. These values can be utilized to predict the changes in sensory characteristics when formulating energy drinks containing these popular functional ingredients. Further research should be done to identify the cause of the differences between the threshold levels focusing on two theories 1) the presence of aromatic compounds lowers the threshold value and 2) nose-clips used during testing increases a panelists potential to become mentally fatigued and distracted.

Key words: Ginseng, Threshold, R-index, Nose-clip, Distraction, Fatigue

5.2 INTRODUCTION

Tasting foods or beverages is a multi-sensory experience; however, at times during the product development stages, it is in the best interest of the developer to isolate sensory perceptions. In those instances, nose-clips can be utilized to isolate sensations in the mouth and the taste elicited by a product by blocking a panelist's ability to smell (Childs 2010). Recent applications reported have included partitioning taste from aromatic flavor notes of fresh tomato (Abegaz and others 2004), determining the sensory effects of incorporating cyclodextrins to minimize the bitterness of ginseng (Tamamoto and others 2010), identifying the consumer perception of astringency in clear acidic whey protein beverages (Childs 2010), and eliminating retronasal olfaction in determining the amount of ingested custard dessert within a first bite (de Wijk and others 2004).

When consumers ingest a product, they use a wide variety of sensory attributes such as appearance, flavor, aroma, and aroma-by-mouth to determine overall liking (Meilgaard 2007). It is important to note that these perceptions do not work independently but work cohesively to create an overall experience for the consumer, unless the specific sensory attribute is purposely isolated. The interactions of multiple senses can create different biases such as the halo and horns effects when more than a single attribute of a sample is evaluated at the same time. The attributes of a product can either work together in a positive manner to increase each other's ratings (halo effect) or they can work against each other and decrease each other's ratings (horns effect, Lawless and Heymann 2010).

The halo and horns effects have been well documented in the literature**.** Wansink and others (2006) observed both a halo and horns effects in a study where the consumers'

perceptions of the physical appearance of their bottle of wine influenced not only their overall perception of the wine, but also of their overall experience and liking scores of the meal. Kappes and others (2006) documented a halo effect in carbonated beverages that showed the perceived bite, burn, carbonated, and mouthcoating attributes increased when the beverages were rated for mouthfeel attributes alone compared to when beverages were rated for all attributes. Tamamoto and others (2010) observed a horns effect when increased ginseng levels in a model energy drink system lowered intensity ratings of sweet, artificial lemon-lime, pear, mango, and pineapple attributes.

While the use of nose-clips is often common practice in descriptive analysis, it is rarely used in threshold studies. The few threshold studies that have reported using nose-clips have used the ASTM recommended method of ascending limits (Omur-Ozbek and Dietrich 2011). To date, it has not been used in a threshold test using the R-index by the rating method which has been shown to be as effective as the current ASTM recommended method of ascending limits (Robinson and others 2005; Kappes and others 2006). The goal of this study is to identify the difference in the threshold of ginseng when it is consumed under two different conditions, with and without nose-clips.

5.3 MATERIALS AND METHODS

Solutions of ginseng (90% ginsenosides, AmaxNutra Source, Eugene, OH) were prepared at seven different concentrations, in threefold increments. Ginseng was presented in the following concentrations for the without nose-clips tests: $5.74x10^{-2}$, $1.91x10^{-2}$, $6.38x10^{-3}$, $2.13x10^{-3}$, $7.09x10^{-4}$, $2.36x10^{-4}$, and $7.87x10^{-5}$ g/ 100 mL. For with nose clips, the concentrations tested were $5.74x10^{-1}$, $1.91x10^{-1}$, $6.38x10^{-2}$, $2.13x10^{-2}$, $7.09x10^{-3}$, $2.36x10^{-3}$, and $7.87x10^{-4}$ g/ 100 mL.

With Nose-Clips

Each day a 1.148 g/L ginseng stock solution was prepared using 1.03 g of ginseng and 0.90 L of spring water (Absopure,Urbana, IL), which was stirred for 5 minutes prior to further dilution. Solutions were, then, appropriately dispensed and diluted into concentrations using a graduated cylinder, spring water, and 8.6 mL of propylene glycol (Fisher Scientific, Philadelphia, PA). Propylene glycol (PG) was added to each solution so that the results from this test could be cross compared to previous studies, in which pure ginseng saponins (ginsenosides) were used, as they are not water soluble. Solutions were again stirred for 5 minutes, after which they were poured into individual dispensing pipettes (Cole Parmer, Veron Hills, IL). The pipettes were used to dispense 10 mL solution into 30 mL plastic cups with lids (Solo Cup Company, Urbana, IL) for the experimental samples. The noise reference (50 mL of a 0.7% PG and spring water solution) was available during the experiment in 163 mL plastic cups with lids. All samples were prepared the day before and were stored and served at room temperature (27ºC). The containers were coded with three-digit random numbers. The reference for noise was labeled as ‘‘noise”.

Without Nose-Clips

Each day a 0.114 g/L ginseng stock solution was prepared using 0.103 grams of ginseng and 0.90 L of spring water (Absopure,Urbana, IL), which was stirred for 5 minutes prior to further dilution. Solutions were, then, appropriately dispensed and diluted into concentrations using a graduated cylinder, spring water, and 8.6 mL of propylene glycol (Fisher Scientific, Philadelphia, PA). Propylene glycol (PG) was added to each solution so that the results from this test could be cross compared to previous

studies, in which pure ginseng saponins (ginsenosides) were used, as they are not water soluble. Solutions were again stirred for 5 minutes, after which they were poured into individual dispensing pipettes (Cole Parmer, Veron Hills, IL). The pipettes were used to dispense 10 mL solution into 30 mL plastic cups with lids (Solo Cup Company, Urbana, IL) for the experimental samples. The noise reference (50 mL of a 0.7% PG and spring water solution) was available during the experiment in 163 mL plastic cups with lids. All samples were prepared the day before and were stored and served at room temperature (27ºC). The containers were coded with three-digit random numbers. The reference for noise was labeled as ‘‘noise.’’

Rinse Preparation

A 0.55% solution of carboxymethlycellose (CMC) was prepared the day before testing to be utilized as a rinse in-between samples. Eleven grams of CMC was weighed out and poured into a 2.5 L beaker, to which 2 L of hot spring (65ºC) water was added. The solution was then stirred until all CMC had dissolved. Solution was stored at room temperature.

Panel Selection

Panelists were recruited from the pool of panelists that participated in the earlier ginseng flavor and taste threshold panels. Panelists were screened for the ginseng taste threshold panel based on allergies, smoker status, frequency of consumption of products containing ginseng, and availability. Twenty-one panelists, 2M and 19F, ages 21–60 years old participated in this panel.

Sample Presentation

A randomized complete block design using ten replicates was used for seven different signal samples and one noise sample. The randomization was done using the Compusense *Five* 5.0 program (Guelph, Canada). Five replicates of the noise and five replicates of each of the seven concentrations consisted of a complete set for the session. Panelists saw all 10 replications over the course of 2 consecutive days. Panelists were instructed to take the whole sample into their mouth, swirl it around for 2–3 seconds, expectorate, and complete the task given during the experiment. Samples were presented monadically and panelists were asked if the sample was a signal sure, signal unsure, noise sure, or noise unsure, where the 0.7% PG solution represented the noise and the ginseng solutions represented the signal. Panelists rinsed with room temperature CMC, followed by warm water (65ºC) to prevent residual bitterness carryover. After every 8th sample, panelists were required to wait 2 minutes before starting with the next group of samples to reduce physiological fatigue. At any time during the experiment, panelists were allowed to retaste the noise reference in order to refamiliarize themselves with the noise sample. Panelists evaluated samples at 27ºC in individual booths with incandescent lighting. During the taste threshold test, panelists were required to wear nose-clips.

Data Analysis

R-index

For both conditions (with and without nose-clips), thresholds were found using the R-index response matrix method (O'Mahony 1992). The ginseng concentration (g/100 mL) for each condition was plotted as a function of calculated percent R-index. The two points found directly above and below an R-index of 75% were identified and a linear trend line was created between these two points to identify the ginseng concentration at

an R-index of 75%. Matrices were constructed for each individual panelist and all responses were combined to create a single matrix for the group, allowing thresholds to be identified for each panelist and the group as a whole.

5.4 RESULTS AND DISUCSSION

Threshold levels identified for ginseng while wearing nose-clips ranged from 0.014 to 0.213 g/100 mL with an average value of $5.54x10^{-2}\pm1.85x10^{-2}$ g/100 mL (α=0.05, Figure 5.1) and without nose-clips from $9.94x10^{-5}$ to $1.92x10^{-3}$ g/100 mL with an average value of $1.04x10^{-3} \pm 4.58x10^{-4}$ g/100 mL, (α=0.05, Figure 5.2). Panelists saw on average of 128-fold difference between threshold levels for with and without nose-clips conditions (Figure 5.3). Group threshold values for with nose-clips was found to be 0.050 g/100 mL and without $1.09x10^{-3}$ g/100 mL with a 46-fold difference observed.

Panelists were found to be more sensitive to ginseng when not wearing nose-clips. There are two different theories that could account for this difference, the first being that the difference is due to the aromatic compounds present in the ginseng extract. Previous descriptive panels done on red ginseng reported a strong presence of an earthy aroma (Chung 2010) and other studies have cited earthy and woody aroma present in ginseng as well (Kim and Sung 1985; Park and others 1999). However in this study, while panelists are wearing nose-clips they are unable to detect these aromas, which may be a key cue for detection threshold, thus, increasing the threshold. While this study was done on white ginseng and not red ginseng, there is a possibility that white ginseng contains similar aromatic compounds.

Another possible theory to account for the discrepancy is that using the nose-clips for an extended amount of time was a factor in increasing the amount of mental fatigue or

distraction experienced by the panelists. Panelists wore nose-clips on average for 30 minutes per session and were not allowed to remove the nose-clips until the testing session was completed. Having the constant pressure from the nose-clips and removing the panelists' ability to use their nasal cavity could have made it harder for panelists to focus, and therefore, made it more difficult to correctly distinguish the signal from the noise.

While panelist mental fatigue has not been previously reported in threshold testing using nose-clips, concerns about mental fatigue and distractions are a common concern in research studies. These concerns are carefully taken into consideration when designing an experiment focusing on the type of presentation used for certain protocols, the number of samples presented in a single setting, the environmental setting, and the rest time enforced in-between sample testing.

Fatigue can be broken down into two different types, mental and physical. Mental fatigue causes panelists to be less sensitive, and each panelist has a different threshold for mental fatigue (Amerine 1983). Studies that focus on mental fatigue typically come from a medical point of view. These studies focus on the mental fatigue caused by dehydration (Shirreffs and others 2004), sleep deprivation (Barker 2011), and substances that can take to alleviate it (Howard 2010).

Physical fatigue is well documented in sensory analysis. Colyar and others (2009) showed that fatigue effects were more prevalent when assessing products with strong lingering attributes when using a side-by-side protocol. Ömür-Özbek and Dietrich (2008) reported fatigue as the main reason that they limited the number of odor samples presented in a single setting to six samples, while using flavor profile analysis.

Desrochers and others (2002) cite the benefits for using analytical equipment as part of a quality control regimen due to equipment's inability to become fatigued. Oraguzie and others (2009) expressed concern as to the ability of panelists to become easily fatigued in contrast to analytical equipment in the post-harvest assessment of fruit quality parameters in apples. Brett and Johnsen (1996) found that panelists experienced fatigue while evaluating farm-raised catfish, due to 2-methylisobrneol, when samples were presented at intervals less than 7 minutes.

Distractions that occur during sensory testing are also taken into account when designing the experiment, but are not as well documented in the literature and have not been researched on the effect on thresholds. Most laboratory setting experiments are conducted in a quiet environment to minimize auditory distractions, individual booths to minimize visual distractions, and in a positive airflow and temperature controlled environment to minimize olfactory and temporal distractions (Meilgaard and others 2007). In a review by Spence and Shankar (2009), it was determined that current literature supports the theory that auditory cues influence many different aspects of our eating/dining experiences, however, auditory cues appear to be the most document in sensory. The influence of distractions on panelists' decisions has been investigated in other areas of science including psychology. Wright (1974) concluded that auditory distractions have a negative influence in the decision making process of male panelists who were making purchase intent statements for new vehicles. Olfactory distractions have been found to decrease the ability of a panelist to visually focus on a target (Michael and others 2005). Brunstorm and Mitchell (2006) found that the combination visual and

auditory distractions (such as a video game or television show) affected the development of satiety in panelists.

5.5 CONCLUSIONS

Panelists were found to be more sensitive to ginseng when not wearing nose-clips. Panelists averaged a 128-fold difference between identified flavor and taste thresholds, and a group difference of 46-fold. Without nose-clips threshold levels ranged from $9.94x10^{-5}$ to $1.92x10^{-3}$ g/100 mL (average value of $1.04x10^{-3}\pm4.58x10^{-4}$ g/100 mL, $\alpha=0.05$) and with nose-clips from 0.014 to 0.213 g/100 mL (average value of $5.54x10^{-2}\pm1.85x10^{-2}$ g/100 mL, $\alpha=0.05$). The group threshold value found with nose-clips was found to be 0.050 g/100 mL, compared to $1.09x10^{-3}$ g/100 mL for without nose-clips. Current theories to account for the difference between threshold values are that the difference is caused by the aromatic compounds present in ginseng, and that panelists could have become either more distracted or fatigued from the use of the nose-clips. If the difference is due to these compounds found in ginseng, then, there should be no discrepancies between a test run with and without nose-clips on a substance that has no volatile chemicals. If the difference is due to panelist mental fatigue or distraction, then, the difference will still remain when a similar test is conducted with substances with or without volatile compounds.

To further clarify this issue, future research should be done on both compounds known to be aromatic and those that are not. This can help determine whether the difference seen here is simply a function of the panelist not being able to use aromatic cues for identification or whether mental fatigue sets in earlier when using nose-clips for an extended period of time.

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5.7 TABLES AND FIGURES

Panelist ID Number	Threshold With Nose-clips g/100 mL	Threshold Without Nose-clips g/100 mL	Factor Difference Between Conditions
Panelist 1	0.029	5.201×10^{-3}	6
Panelist 2	0.014	1.917×10^{-3}	7
Panelist 3	0.043	1.917×10^{-3}	22
Panelist 4	0.034	1.207×10^{-3}	28
Panelist 5	0.038	1.087×10^{-3}	35
Panelist 6	0.037	1.029×10^{-3}	36
Panelist 7	0.046	1.237×10^{-3}	37
Panelist 8	0.054	1.280×10^{-3}	42
Panelist 9	0.051	1.138×10^{-3}	45
Panelist 10	0.058	1.164×10^{-3}	50
Panelist 11	0.094	1.223×10^{-3}	76
Panelist 12	0.138	1.339×10^{-3}	103
Panelist 13	0.018	1.657×10^{-4}	106
Panelist 14	0.034	2.848×10^{-4}	118
Panelist 15	0.017	1.168×10^{-4}	142
Panelist 16	0.213	8.355×10^{-4}	255
Panelist 17	0.055	2.131×10^{-4}	257
Panelist 18	0.028	9.943×10^{-5}	283
Panelist 19	0.056	1.756×10^{-4}	319
Panelist 20	0.045	1.340×10^{-4}	339
Panelist 21	0.061	1.580×10^{-4}	388
Average	0.055 ± 0.019	$1.04 \times 10^{-3} \pm 4.58 \times 10^{-4}$	128

Table 5.1: Panelists and their corresponding thresholds identified under both conditions for ginseng. The factor difference between the ginseng thresholds is also shown. Confidence intervals are calculated for α=0.05.

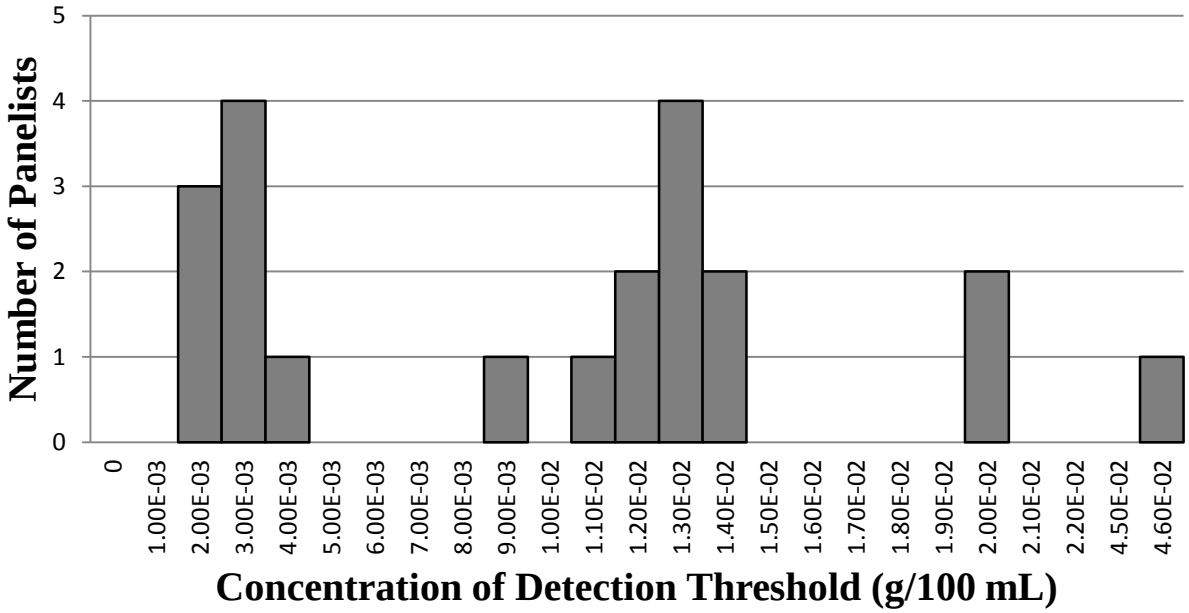

Figure 5.1: Detection thresholds (g/100 mL) for panelists with nose-clips.

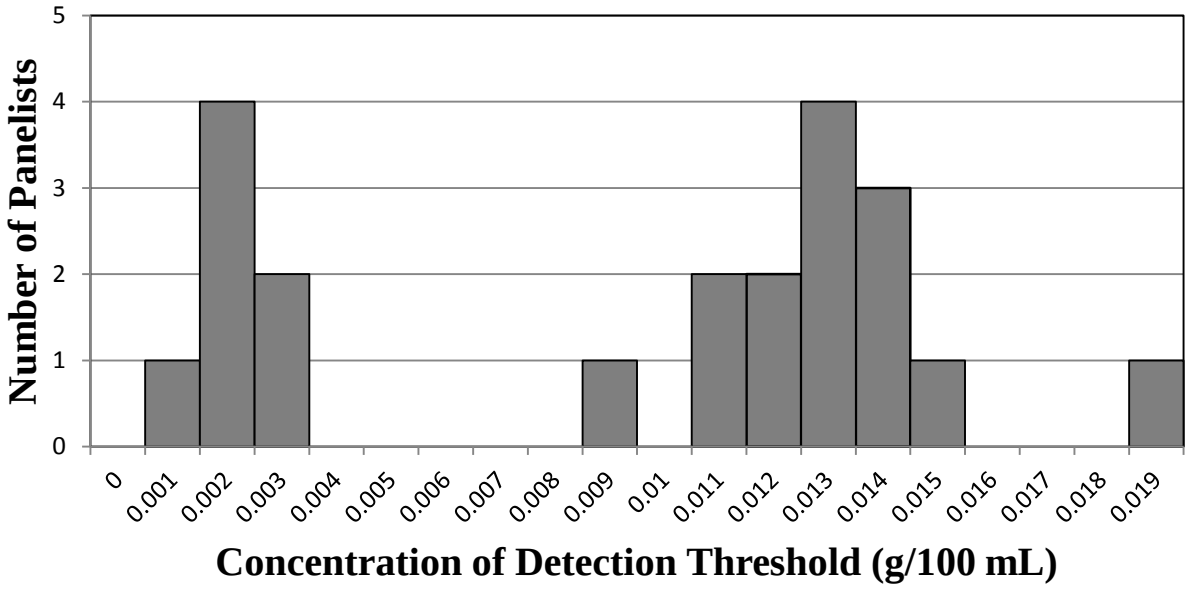

Figure 5.2: Detection thresholds (g/100 mL) for panelists without nose-clips.

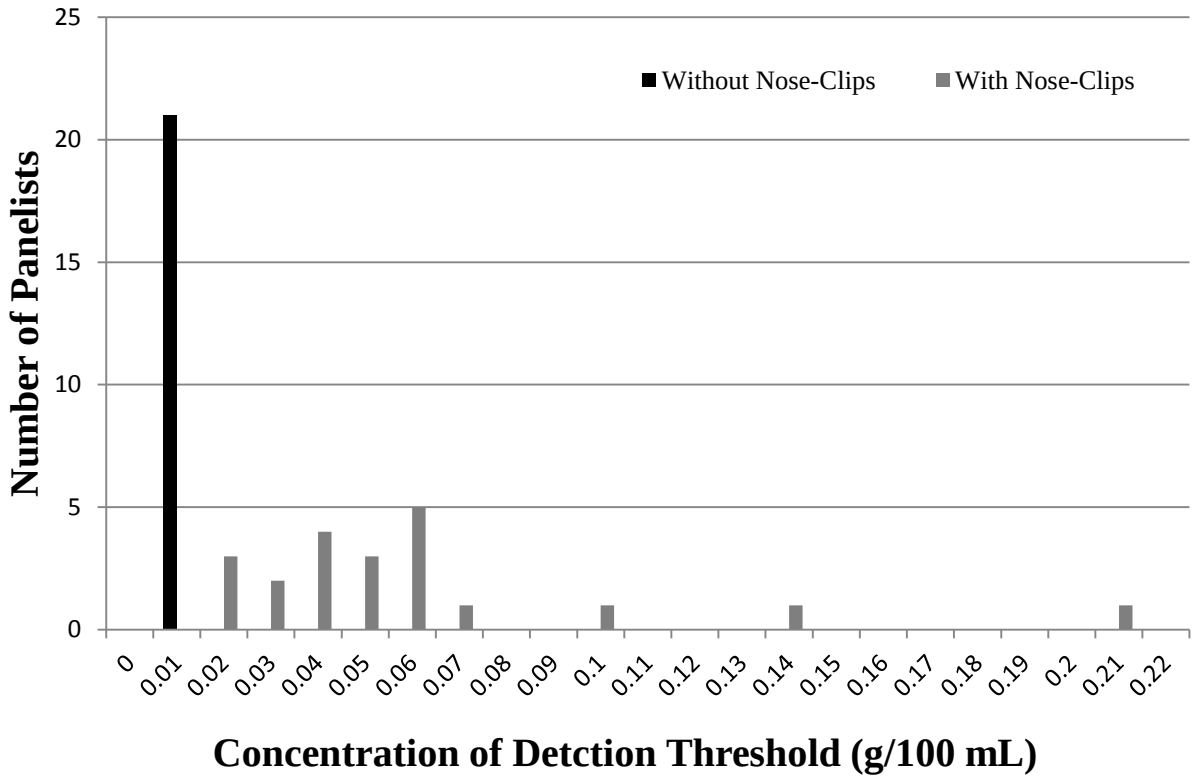

Figure 5.3: Comparison of distributions of detection thresholds (g/100 mL) for panelists while wearing nose-clips versus without nose-clips.

CHAPTER 6 - SUMMARY

As the knowledge of the therapeutic benefits of ginseng have become well known by the general public, ginseng's popularity as an ingredient in functional beverages, specifically energy drinks, has increased. However due to its bitter taste, most companies add sub-therapeutic levels of ginseng to their products, which allows them to place the ingredient on their ingredient list but does not allow them to make any health claims.

To identify the amount of ginseng that a company could add without compromising the current flavor profile of their beverage, the taste threshold was identified for three commercially available extracts of *Panax Ginseng*. Two of the samples were white ginseng (Sample A and B), which is the most common variety used in product formulations in the United States. The third was red ginseng (Sample C), which is typically used to create a tonic. Each ginseng sample had a different ginsenoside content, Sample A contained 90% ginsenosides, Sample B contained 70%, and Sample C contained 1.7%.

Through a baseline solution containing 0.7% propeleyene glycol and water, taste thresholds were identified for all three samples utilizing the R-index by the rating method. Thresholds for individual panelists for Samples A, B, and C were found to range from 0.014 to 0.213 g/100 mL, 0.023 to 0.450 g/100 mL, and 0.009 to 2.176 g/100 mL respectively. Average threshold values were found to be 0.053 ± 0.005 g/100 mL for Sample A, 0.090 ± 0.010 for Sample B, and 0.787 ± 0.085 for Sample C at α=0.05. Group threshold values were identified by combining data for all panelists to identify a single threshold for each sample. These values were found to be 0.050 g/100 mL, 0.092 g/100 mL, and 1.07 g/100 mL, for Samples A, B, and C, respectively. The average values

and group thresholds of all three samples were found to be inferring the possibility that companies could add the lower limits for therapeutic benefits (100 mg/day) into a single serving of a beverage (240 mL) without affecting the beverages current flavor profile. However, this experiment identified the taste threshold and not the flavor threshold of ginseng, which would be the defining factor as to whether or not companies could increase the ginseng content in their products without interfering with its current flavor profile.

Therefore a second experiment was conducted to identify the flavor threshold of ginseng. During this study the flavor thresholds of two of the main ginsenosides found in ginseng, G-Rb_1 and G-Rg_1. G-Rb_1 and G-Rg_1 were chosen as they have been shown to elicit therapeutic effects that one might wish to receive from an energy drink. They are also two of the six ginsenosides that are used as standards for distinguishing between ginseng varieties.

From a theoretical stand point if the flavor threshold of ginseng is above the recommended level for therapeutic benefits it may be possible to add one or both of the ginsenosides in place of ginseng. This could help avoid many of the off-flavors produced by ginseng, as long as the ginsenosides themselves account for only a small amount of the overall flavor of ginseng. To determine this, flavor thresholds were identified for a single ginseng sample (Sample A from the taste threshold study) as well as for G-Rb_1 and G-Rg_1. The flavor threshold of ginseng was found to be ranging from $9.94x10^{-5}$ to $5.20x10^{-3}$ g/100 mL with an average value of $1.02x10^{-3} \pm 4.63x10^{-4}$ g/100 mL, while the thresholds of G-Rg_1 and G-Rb_1 were found to be ranging from $1.05x10^{-4}$ to $1.52x10^{-3}$ g/100 mL with an average of $5.21x10^{-4} \pm 8.91x10^{-5}$ g/100 mL, from $1.64x10^{-4}$ to$7.66x10^{-4}$

g/100 mL with an average value of $7.67x10^{-4} \pm 1.78x10^{-4}$ g/100 mL, respectively. Group flavor thresholds for ginseng, G-Rg_1, and G-Rb_1 were identified to be $1.09x10^{-3}$ g/100 mL and $4.64x10^{-4}$ g/100 mL, $5.65x10^{-4}$ g/100 mL, respectively. All confidence intervals were calculated at α=0.05.

Taste activity values (TAVs) and flavor activity values (FAVs) for G-Rb_1, G-Rg_1, and ginseng were calculated using their respective average and the group thresholds and were used to identify their respective percent contribution towards the TAV and FAV of ginseng. The percent contributions of G-Rb_1 and G-Rg_1 towards the TAV generated from the average taste threshold of ginseng were found to be 8.5% and 7.0%. The percent contributions of G-Rb_1 and G-Rg_1 towards the FAV generated from the average taste threshold of ginseng were found to be 9.0% and 13.3%, respectively. The percent contributions of G-Rb_1 and G-Rg_1 for the group taste threshold of ginseng were found to be 82.9% and 68.1%. The percent contributions of G-Rb_1 and G-Rg_1 for the average taste threshold value of ginseng were found to be 53.7% and 79.1%, respectively. Results showed that alone, G-Rb1 and G-Rg1 only accounted for a small amount of the flavor threshold but a large part of the taste threshold.

To investigate the difference between the taste threshold and the flavor threshold, the results from first and second study were compared. The main difference in their methodologies is that identifying the taste threshold required to wear nose-clips while evaluating samples. A comparison of the two identified thresholds showed that there was a 128-fold difference between the average thresholds and a 46-fold difference between the group thresholds.

There are two possible theories to explain these differences. The first theory is that ginseng contains strong aromatic compounds that panelists may use as cues to identify a signal from the noise. As nose-clips were used in the first study panelist would have been unable sense these aromatic compounds. The second theory is that the continuous use of nose-clips for a long period of time (30 minutes) increased the panelists' sensitivity to fatigue. Not being able to breathe or smell for an extended period could have been taxing on the mental state of the panelists in the study thus making them more susceptible to fatigue and distractions than when in the flavor threshold study.

In conclusion, while the average and group taste thresholds of ginseng show potential for adding ginseng in therapeutic levels in a single serving size beverage, the flavor thresholds do not. However, the percent contribution of G-Rb_1 and G-RG_1 to the TAV and FAV of ginseng show potential for adding a single ginsenoside in place of ginseng, as neither alone accounts for 100% of the TAV or FAV of ginseng. Also, these studies have shown a large difference in-between the taste threshold and the flavor threshold of ginseng. These discrepancies could take place due to two different causes, the aromatics present in the ginseng sample or an increased susceptibility to fatigue and distractions due to the use of nose-clips.

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Appendix A- CONSUMER TEST RECRUITMENT SCREENER A
Name: _____

Email Address: _____

1. **Are you interested in participating in a ginseng threshold study?**
   ☐ YES ☐ NO

2. **Are you over 18 years old?** ☐ YES ☐ NO

3. **Are you allergic to any foods?** ☐ YES ☐ NO

   If yes, please list the foods you're allergic to:

4. **Do you smoke?** ☐ YES ☐ NO

5. **You identify yourself as: (check all that apply)**

   ☐ American Indian or Alaska Native
   ☐ South Asian
   ☐ Other Asian
   ☐ Black or African American
   ☐ Caucasian
   ☐ Hispanic or Latino
   ☐ Native Hawaiian or Other Pacific Islander
   ☐ Other:

6. **Desired time to participate: (Check times when you are available to participate. You MUST be able to attend at least 1 hour each day)**

Time of Day	Test Days				
	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
9-10am	☐	☐	☐	☐	☐
10-11am	☐	☐	☐	☐	☐
11-12pm	☐	☐	☐	☐	☐
12-1pm	☐	☐	☐	☐	☐
1-2pm	☐	☐	☐	☐	☐
2-3pm	☐	☐	☐	☐	☐
3-4pm	☐	☐	☐	☐	☐
4-5pm	☐	☐	☐	☐	☐

Appendix B - CONSUMER TEST RECRUITMENT SCREENER B

Question 1:
On your Sensory Questionnaire you marked your familiarity with ginseng as either "Very Familiar" or "Somewhat Familiar". Looking at the options below, could you please pick the description of which source of ginseng you feel you are familiar with. You may be familiar with both options.

Option A: Traditional
- Defined as products or beverages purchased for the sole reason that it contains ginseng,
Ex: Asian teas, Red ginseng tea, Snacks such as ginseng infused chocolate or candied ginseng root, Supplements, Bottled ginseng drinks, or ginseng extract

Option B: Western Sources
- Defined as products or beverages that have ginseng added as an additive, but is not the main marketing point
Ex: Energy drinks or Commercially bottled teas (such as those made by Lipton)

Question 2:
Please choose the statement that best describes how long you have been exposed to ginseng products.

I have been consuming ginseng products for
a. 1 year or less
b. 1-3 years
c. 3-5 years
d. 5-10 years
e. Ginseng has always been an integrated part of my food/beverage consumption

Appendix C -- TASTE ACTIVITY VALUE CALCULATIONS

Calculation followed is for the is taste activity value of G-Rb_1 using the group taste threshold values

Group Threshold of G-Rb_1	$4.64x10^{-3}$ g/100 mL
Group Threshold of Ginseng	$4.99x10^{-2}$ g/100 mL
Content of G-Rb1 in Ginseng Extract	77.2 mg/g

For the TAV of G-Rb_1

$$\frac{7.72\ g/100\ mL}{4.64x10^{-3}g/100\ mL} = 1663$$

For the TAV of ginseng

$$\frac{100\ g/100\ mL}{4.99x10^{-2}g/100\ mL} = 2005$$

For the TAV_{Total}

$$\frac{1663}{2005}x100 = 82.9\%$$

Appendix D – Flavor Activity Value Calculations

Calculation followed is for the is Flavor activity value of G-Rb_1 using the group taste threshold values

Group Threshold of G-Rb_1	$4.64x10^{-3}$ g/100 mL
Group Threshold of Ginseng	$5.12x10^{-3}$ g/100 mL
Content of G-Rb1 in Ginseng Extract	77.2 mg/g

For the FAV of G-Rb_1

$$\frac{7.72\ g/100\ mL}{4.64x10^{-3}g/100\ mL} = 1663$$

For the FAV of ginseng

$$\frac{100\ g/100\ mL}{5.12x10^{-3}g/100\ mL} = 19512$$

For the TAV_{Total}

$$\frac{1663}{19512}x100 = 8.5\%$$