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Jeeha Park

*University of Connecticut - Storrs*, jeeha92@gmail.com

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# Characterizing and Improving the Oral Sensations and Preference of Polyphenol-Rich Aronia Berry Juice

## Abstract:

**Background:** Aronia berries (chokeberries) have very high levels of health-promoting polyphenols yet cause "choking" sensations due to bitterness and astringency. We aimed to describe oral sensations and palatability of aronia juice by variations in harvest time and oral sensory phenotype. Sensory blocking combinations were tested to improve juice acceptability. **Methods:** Ripe aronia berries were harvested at 7 time points and juiced for oral sampling by 50 adults who underwent bitter taste phenotyping. The adults reported quality intensities of prototypical tastes, foods, and aronia juices. Ethyl butyrate (10ppm) and/or sucrose (0.15 and 0.3M) were added to prototypical oral stimuli and aronia juice for changes in liking and oral sensation. **Results:** The hedonic ratings of juice averaged weakly dislike, ranging from 'strongly dislike' to 'above moderately like.' Astringency was the strongest sensation, yet sweetness was the primary driver of liking in multiple regression analysis. Those who liked the juice reported a greater balance between astringency and either sourness or sweetness. The level of negative taste and astringency sensations and liking of berry juiced varied with bitter phenotype. In the sensory blocking procedures, adding ethyl butyrate alone increased sweetness but failed to improve juice acceptance. Ethyl butyrate acted synergistically with added sugar to enhance perceived juice sweetness. However a substantial increase in palatability was not achieved because the sweet and ethyl butyrate did not block astringency. **Conclusion:** The negative sour/bitter and astringency sensations impede aronia juice liking. Adding sugar and sweet flavors improved palatability by blocking sour/bitter sensations. Future research is needed to determine methods to block astringency and achieve an acceptable aronia juice for consumption as an enjoyable and health-promoting beverage.

## Introduction:

One of the trends in society today is the focus on healthy eating and nutrition. *Aronia melanocarpa*, or more commonly known as chokeberry, has recently been added to the list of nutrient dense foods. Chokeberries have high levels of health promoting anti-oxidants and polyphenols, including anthocyanins, laonoids, hydroxycinnamic acids, and proanthocyanidins (Taheri et al., 2013). However, its high level of nutrition is associated with negative taste for many people. Aronia "Chokeberries" cause a choking sensation due to bitterness, astringency, and sourness associated with the high levels of polyphenols. Astringency is the dryness of the mouth, a puckering sensation in the oral cavity (Bajec and Pickering, 2008). In food items, phenolic compounds at very low concentrations can be perceived with varying astringent and bitter intensities. (Brock and Hofmann, 2008). Sourness has been noted to evoke rejection of food items but appeared to be a positive factor in liking of food items in low concentration (Laaksonnen et al., 2013). Despite the health benefits, the combination of negative taste qualities, which is the result of high levels of micronutrients of aronia berries, may contribute to its low palatability.

### *Aronia Melanocarpa:*

Polyphenols have generated a great deal of interest for their antioxidant and anti-inflammatory capacities. *Aronia Melanocarpa* berries are one of the richest known sources of dietary polyphenols and anthocyanins. (Wu et al., 2006; Perez-Jimenez et al., 2010) Aronia berry's polyphenol-rich juice has shown a variety of bioactivities: regulation of endothelial function, blood cholesterol level, inflammation, oxidative stress, and blood pressure (Jurgon'ski et al., 2008; Naruszewicz et al., 2007; Valcheva-Kuzmanova et al., 2007; Li et al., 2012). Aronia berries are cultivated in Europe for use in wine, preservative spreads, juice, tea, salsa, and other food products. Although not widely used in the Americas, it has gained increased interest in the United States due to its high polyphenol levels.

### *Bitterness and Astringency as an Intake Inhibitor:*

Different motivational factors play a role in food choice and dietary preference. Healthy fruit drinks and dairy products are more pleasant for those people rating healthiness as a vital factor in food selection than for those that do not concern for the nutritional value when choosing food items (Pohjanheimo et al., 2010). Also, the interaction between genotype and sensitiveness to taste qualities and various behavioral factors play a role in liking and preference for food items. Both biological and behavioral factors must be accounted in evaluating and explaining liking.

Berries, in general, are considered to be a staple of a healthy diet because they contain high amounts of antioxidative polyphenols and fibers (Kallio et al., 2006; Laaksonen et al., 2011). However, they are consumed at less than the recommended amount. One of the reasons for the low consumption of Aronia chokeberries is due to its bitter and astringent taste qualities. The bitter perception of food items is biologically explained by interaction of bitter stimulus with G-protein-coupled TAS2R receptors (Kim et al., 2004; Kim and Drayna, 2004). One of the studied human bitter taste receptor gene is the *hTAS2R38* gene, which responds to the bitter compounds phenylthiocarbamide and propylthiouracil (Kim et al., 2003). Based on their ability to taste the bitterness of these thiourea compounds (-N-C=S), subjects can be phenotypically categorized as super-tasters, medium-tasters, and non-tasters (Bufe et al., 2005; Sandell and Breslin, 2010).

The perception of bitterness from food items varies, and the variability has a genetic basis. Propylthiouracil (PROP) is a well-studied thiourea compound characterizing non-tasters, medium-tasters, and super-tasters (Laaksonen et al., 2012). Super-tasters report greater bitterness than non-tasters from a broad range of compounds and foods or beverages including quinine hydrochloride (QHCl) (Hayes et al. 2008), naringin (Drewnowski et al. 1997), grapefruit juice (Drewnowski et al. 1997; Lanier et al. 2005), coffee (Lanier et al. 2005), green tea (Gayathri et al. 1997), alcoholic beverages (Intranuovo and Powers 1998; Lanier et al. 2005), and vegetables (Dinehart et al. 2006; Kaminski et al. 2000; Keller et al. 2002; Turnbull and Matisoo-Smith 2002). Those who taste PROP with greater bitterness intensity also report more sweetness from sucrose than those who taste PROP with less bitterness intensity (Hayes and Duffy 2007). PROP-sensitive tasters, super-tasters able to perceive greater bitterness of PROP, demonstrate greater sensitiveness to the astringency of red wine than those who are PROP-insensitive, non-tasters unable to perceive the bitterness of PROP (Pickering et al., 2004). Super-tasters also demonstrate lower acceptance for polyphenol-rich food items such as grapefruit, green tea, coffee, and soybean tofu (Dinehart et al., 2006; Drewnowski et al., 2001). The individual variation based on the *hTAS2R38* gene expression, which also correlates to personal preference for polyphenol-rich food items. Thus, the *hTAS2R38* genotype or the PROP phenotype may provide a marker for overall differences in oral sensations of juice from the aronia berry.

### *Food-related Sensory and Subject-related Non-sensory Factors Indicate Food Item Preference:*

Oral sensations play a vital role in determining dietary behaviors; therefore, PROP sensitiveness may serve as a marker for diet-related chronic illnesses (Duffy et al., 2007). PROP sensitivity phenotype has been shown to influence consumption of vegetables containing high amounts of N-C=S compounds. Super-tasters demonstrate more negative sensations from vegetables and a greater disliking for vegetables, leading to lower consumption of vegetables

(Dinehard et al., 2006). Similarly, PROP-sensitive preschool children consume less plain broccoli (Fisher et al., 2011). Bitterness and astringency are key taste qualities in Aronia berries, along with sourness and sweetness. The variation in TAS2R receptors or bitterness phenotype may have an impact on the liking of aronia berry juice.

#### *Ethyl Butyrate as Taste Quality Enhancer by Olfaction:*

Nutritional important foods and beverages that have bitterness and heightened negative oral sensations need flavor enhancement to increase the likeability and probably of regular consumption. For example, the bitterness of a product such as aronia berry juice can be blocked at the peripheral level, physical level, and cognitive level (Bennett et al., 2012). For peripheral blocking, salts interfere with bitter compound-receptor binding. Physical binding of bitterness can occur through adding fats or proteins to block this binding, such as adding whole milk with enhanced milk solids to the berry juice. Adding sweetness to target a mixture of perception sources can block the central cognitive bitter perception (Guadagni et al., 1974). On the cognitive level, retronasal olfactory sensation plays a part in overall flavor perception (Hort and Hollowood, 2004). For example, enhancing the fruity flavor of a bitter fruit juice can enhance the perceived sweetness of the juice, which in turn, can depress the negative taste qualities. In this case, the sweet odor is paired with a sweet taste to enhance the overall perceived sweetness (Schiferstein and Verlegh, 1996). Thus, ethyl butyrate, the sweet flavor of Juicy Fruit gum, enhances sweetness at both supra and subthreshold levels (Lavin and Lawless, 1998; Labbe et al., 2007). The question of interest is if adding fruity odor of ethyl butyrate to the aronia juice will increase the perceived sweetness of the juice, diminish the negative oral sensations (sour, bitter, astringency) enough to enhance the overall acceptance of the aronia juice. It is hypothesized that the congruency between sweetness of the juice and the sweet olfactory flavor will be enough to increase hedonic ratings of aronia juice by masking negative taste and astringency of aronia juice.

#### **Methods:**

##### *Purpose:*

The first objective of this present study was to phenotype chemosensory functions of 50 adults using the NHANES protocol, olfactometer, and PROP bitterness perception. Participants reported intensities of prototypical tastes (sweetness, sourness, saltiness, bitterness, and astringency), oral chemesthetic compound solutions (alum, quinine, sodium chloride, citric acid, and sucrose), food items, aronia berry juices, and non-oral stimuli. The ratings served as sensory standards to describe the participants' response to stereotypical tastes. The second objective was to determine the oral perceptions and the palatability of the juices as a function of harvest time. We characterized oral sensations and palatability of the aronia berry juice by including variation by harvest time and oral sensory phenotype. Lastly, different concentrations of sugar and/or ethyl butyrate were added to determine which combination of additives increased the palatability of aronia juice. We hypothesized that PROP super-tasters would demonstrate a greater dislike for aronia berry juice due to the increased perception of astringency, sourness, and bitterness of the juice. However, with the addition of ethyl butyrate, sweetness perception will be increased and sourness, bitterness, and astringency perception will be suppressed resulting in higher palatability of the aronia juice.

### *Aim:*

We conducted a sensory analysis of the taste, astringency, and hedonic value of the aronia juice as a function of the harvest time. We also searched for a relationship to determine if the sensory and hedonic ratings of the juice varied with oral phenotype, and how to improve the overall acceptance of aronia juice.

### *Participants:*

Individuals were recruited (via email responses) through posters distributed around the University of Connecticut community for this laboratory-based observational study (see appendix). Recruitment posters did not mention the aronia juice and asked for participation “in a study looking at why people eat what they do.” The University of Connecticut Institutional Review Board approved all methods. Participants provided informed and written consent and were paid for their time.

Fifty healthy adults, between the ages of 18 and 62 (mean age =  $26.9 \pm 11.7$ ), were recruited. There were 36 females (72%) and 14 males (28%). Eighty-six percent of the participants were Caucasian. Most participants did not report any dietary restrictions; however, for those that reported food allergies (peanuts, lactose intolerance, religion, and etc), certain food items were excluded. No participants showed dietary restrictions for aronia berry juice. More than 70% of the participants had normal BMI, but nine were overweight and five were obese under the BMI scale.

### *Procedure:*

The study was done in three parts. The first session consisted of tasting prototypical taste solutions, the second session included tasting the aronia juice samples and food items, and the third session involved tasting combinations of week 5 aronia juice and prototypical tastes with sucrose and/or ethyl butyrate. Following a written consent (see appendix), the participants were orientated to the general Labeled Magnitude Scale (gLMS), which they used to rate the intensity and hedonics of sensations. The gLMS scale is oriented vertically, ranging from ‘no sensation’ (0) to ‘strongest imaginable sensation of any kind’ at the top (100). ‘Barely detectable’ (1.4) ‘weak’ (6), ‘moderate’ (17), ‘strong’ (35), and ‘very strong’ (53) are between the two extremes. The participants were asked to formulate their own, personal strongest imaginable sensation and rate the intensity of the brightest light they have experienced, the intensity of light in a dimly lit restaurant, and the intensity of the light in the taste lab. Participants correctly ordered the light intensities, showing their understanding of the gLMS scale. Using the traditional Natick nine points scale for intensities of perceptions may give erroneous comparison among participants and sensations because a scale label may indicate different intensities within a group. The Natick nine points scale allows participants to rate intensities between 1 (extremely weak) to 9 (extremely strong). However, the gLMS unifies the group under individualized strongest sensation of all time, allowing for affective comparison within the group.

After introducing the subjects to the gLMS scale, taste function and phenotype were assessed. We utilized the National Health and Nutrition Examination Survey, which scales and identifies intensity ratings of 1mM Quinine and two concentrations of NaCl (1 M and 0.32 M) sampled regionally on the tongue tip and/or with the whole mouth. In addition, subjects reported

the bitterness from 3.2mM PROP, which associated with *TAS2R38* receptor genotype. Smell function was also assessed with a 40 item odor identification test administered with an OEI Olfactometer. Participants also reported intensities of the odors on gLMS. The variation test included eight randomly order solutions (PROP always at the last). Participants reported the intensities of sweetness, sourness, saltiness, bitterness, astringency, and like or dislike.

In the second session, the participants, once again, were orientated to the gLMS scale using the same procedure as the first day. Participants correctly ordered the light intensities as expected, still demonstrating understanding of the gLMS sale. The spatial test was re-administered using the same protocol as the one during the first session. For the last part of the procedure, participants were randomly served the Aronia juice of different harvest time (Week 1 to Week 7) with different food items between the Aronia juice samples. The participants swooshed the food items and the aronia juice in their mouths and spit it out. Then the participants ingested small sips of the either the juices or the food items. The participants reported the intensities of sweetness, sourness, saltiness, bitterness, astringency, and like or dislike. They also reported the overall intensity of the flavor with and without swallowing.

#### *Stimuli:*

As a part of taste phenotyping associated with the *TAS2R38* gene, participants were served three different concentrations of propylthiouracil (PROP): 0.32 mM, 1mM, and 3.2mM. The participants reported the bitter intensity from each of the PROP concentrations. The Spatial Test, used to test regional taste functions, incorporated 1mM Quinine, 1M NaCl, and 0.32M NaCl. The three solutions were applied regionally and to the whole mouth.

For the Variation Test, eleven different solutions were given randomly to the participants with PROP always given last. The solutions used were 0.32M sucrose, 0.32M NaCl, 1M sucrose, 1mM citric acid, 0.1M sucralose, 0.32mM quinine, 25% ethanol, 0.32mM PROP, 1.3M sodium acetate, 1g/L alum, and 32mM citric acid. The food samples used on the second visit were grapefruit juice, white icing, apple juice, heavy cream, instant coffee mix, and soy sauce. The food samples were presented between aronia juice samples. The orders of both the aronia juice samples (week 1-7) and the food samples were random. The aronia juice samples were removed from the freezer and stored in the refrigerator the day before the trials. All solution samples from the Variation test and the aronia juice test were presented in an amount enough for a small mouthful. Also, the required stimuli were prepared several hours before the trial to allow the food samples and solutions to reach room temperature.

#### *Blocking:*

Participants used the gLMS scale to rate the intensities and the degree of liking for sucrose (0.15 and 0.3M), citric acid (6 and 1M), tannic acid (1gm/L), and Week 5 aronia juice. Citric acid, tannic acid, and aronia juice were sampled with two concentraions of sucrose (strong and weak), 10ppm ethyl butyrate, and weak sucrose plus ethyl butyrate. Similar to the first two sessions, the subjects rated intensities of sweetness, sourness, saltiness, bitterness, astringency and hedonics of each solution with and without the additives.

#### *Aronia Berries:*

The cultivar, Aronia “Viking,” was first harvested on August 1, 2012 and every 7th day thereafter until September 9, 2012 at a test plot in Storrs, CT, USA. The berries were cleaned, dried, and frozen at -20°C in polyethylene bags to maintain proper quality for analysis. The juicing of the berries took place on October 24, 2012. On the day of the juicing, the frozen berries were thawed and mashed. Then they were placed on a cider press for juice extraction. The juices were frozen at -20°C until the day before the procedure, placed at 35°C until the procedure.

## **Results:**

### *Sensory and hedonic ratings of prototypical taste and oral sensations:*

We first determined individual taste perception ratings for prototypical tastes and several liquid food items. The participants rated the sweetness, sourness, saltiness, bitterness, astringency, and liking on the gLMS (1.4 = barely detectable, 6 = weak, 17 = moderate, 35 = strong, and 53 = very strong). The primary perception for each of the prototypical taste solutions is as follows: sucrose (sweet), citric acid (sour), NaCl (salty), quinine (bitter), sucralose (sweet), PROP (bitter), ethanol (bitter), sodium acetate (salty), and alum (astringency) (Table 1).

Prototypical taste solutions typically have one representative taste quality. However, several solutions showed more than one taste quality. Citric acid demonstrated multiple qualities including astringency, sourness, and bitterness. The astringency of citric acid drove the overall disliking of the solution. Participants also rated astringency for PROP along with bitterness. Overall, of sweet prototypical tastes, only sweet solutions had a mean positive hedonic rating. When participants perceive greater sweetness, they express greater liking ( $R=0.73$ ,  $P<0.01$ ). Even in basic tastes, the participants demonstrated taste quality variation. Sucrose-sweetness, citric acid-sourness, quinine-bitterness, and PROP-bitterness showed significant standard mean deviations. Genetic factors played a role in taste perception, especially PROP-bitterness perception. The variation in the perception of sweetness, sourness, and bitterness may influence the participants' taste perception of aronia berry juice. Overall, in unitary taste solutions, the representative taste quality drove liking.

Before analyzing the results of aronia juice, we determined how the participants responded to other food items. As seen in Table 2, grapefruit juice rated highest in sourness but it was accompanied by sweetness and bitterness. Overall, grapefruit juice was moderately liked. In a multiple regression analysis, grapefruit juice liking was predicted only by sweetness perception ( $P<0.01$ ), and not significantly by sour-bitterness. Apple juice had strong sweetness and hedonics ratings. Apple juice liking was driven also by sweetness perception ( $P<0.01$ ). Coffee rated the highest bitterness and the lowest hedonics rating. Coffee bitterness drove disliking ( $P<0.01$ ). Soy sauce had the highest saltiness. For soy sauce, both saltiness and bitterness contribute to the hedonics rating ( $P<0.01$ ). Saltiness of soy sauce drove liking; however, soy sauce dislike was driven by bitterness. Similarly to the prototypical taste solutions, all four food items showed hedonics ratings with significant standard deviations. In complex tastes, more than one quality can be perceived. The predominant taste quality drove the hedonics ratings, but a mixture of perceptions could also drive the palatability, as seen with soy sauce.

### *Sensory and hedonic ratings of aronia juice:*

After characterizing the participants based on their responses to prototypical tastes and food items, taste sensory profiles of aronia juice were obtained across harvest time (Figure 1). Week 1 harvest was the most disliked and Week 6 harvest was the most liked. Weeks 1, 2, and 3 had the most unfavorable sensory profiles (lower sweetness, greater sourness, and astringency). Weeks 5 and 6 harvests demonstrated the more favorable taste qualities (greater sweetness, lower sourness and astringency). Perceived sweetness increased with liking whereas perceived sourness, bitterness, and astringency decreased with each additional week after the first harvest. Week 5 aronia berry juice demonstrated the highest perceived sweetness but week 6 aronia berry juice had the greatest acceptance (Figure 2).

In a multivariate model predicting preference for each harvest from perceived taste qualities, for all weeks, sweetness positively contributed to aronia juice acceptance ( $R=0.482$ ,  $P<0.01$ ). In Week 6, sourness also positively contributed to liking (Figure 3,  $P<0.05$ ). In weeks 1, 2, 5, 6, and 7, astringency had a negative correlation to liking. Subjects reported significantly higher sweetness in favorable weeks (5 and 6) than unfavorable weeks (1, 2, and 3). Weeks 5 and 6 also demonstrated lower sourness, bitterness, and astringency (Figure 4). The sweet to sour ratio is greater in the favorable weeks than the unfavorable weeks. As shown in figure 3, sourness contributed positively to liking. This may indicate that if a balance is achieved between sweetness and sourness, sourness may also drive liking. Overall, the level of sweetness proved to be the most effective way to predict palatability of aronia juice.

Aronia juice liking for all harvest weeks showed the greatest variation in the hedonics rating. There was also notable variation in sourness, bitter, and astringency. There is often confusion differentiating sourness and bitterness. This confusion may have contributed to the variation of sourness and bitterness perception. When tasting a food item with both sour and bitter qualities, one may attribute more sourness to the food item due to “confusing” bitterness for sourness, and also vice versa.

When we compared sensory and hedonics ratings of aronia juice to sensory and hedonics ratings of prototypical tastes, we found that aronia juice astringency correlated with alum (1g/L) astringency. Also, those who rated higher citric acid sourness reported higher aronia juice sourness, indicating positive correlation. However, citric acid liking did not correlate with aronia juice liking. In comparison to complex food items, apple juice and grapefruit juice sweetness had a positive and negative, respectively, correlation to aronia juice sweetness.

#### *Individual variations:*

The participants represented a variety of PROP phenotypes. Figure 5 is a scatter plot of the relationship between quinine and PROP bitterness measured on the gLMS scale. There was variation in the perception of the quinine and PROP bitterness. All PROP and quinine bitterness categories were represented. However there were fewer subjects in the low PROP high quinine group. Subjects who perceived more PROP relative to quinine bitterness ( $n=10$ ) perceived the juice as less sour, more astringent, and disliked the juice more than those who perceived high bitterness from both PROP and quinine ( $n=15$ ) (Figure 6). There is significant correlation between quinine bitterness and aronia juice sourness ( $R=0.442$ ,  $P<0.01$ ). Although the high quinine bitterness group experienced greater sourness, the group also experienced less astringency and liked the juice more than low quinine bitterness group (Figure 6). A correlation test did not show significant correlation between aronia juice astringency and hedonics ratings;



therefore, dislike of aronia juice may be driven by a multi-variable combination of both sourness and astringency. Although both perceptions are negative qualities, astringency may have a greater influence in driving dislike of aronia juice.

Subjects demonstrated variability in taste qualities and liking of aronia juice samples. There was the greatest variance in liking of the juice. It ranged from 'very strong dislike' to almost 'strong like.' Sweetness showed the least variance with ratings ranging from 'no sensation' to almost 'strong sweetness.' Some subjects rated the astringency of the juice 'very strong.' The subjects also reported sourness as high as 'very strong sourness.' On average, the subjects reported high intensity ratings for sourness and astringency of aronia juice samples.

The participants could be split into a group who rated positive for hedonics ratings (likers) and a group who rated negative for hedonics ratings (dislikers). Controlling for age, sex, and intensity of tones as a cross-modal standard, Aronia juice likers (n=27) experienced greater sweetness than dislikers (Figure 7). They also demonstrated a greater balance between astringency-sourness and astringency-sweetness than dislikers. This may indicate that higher sweetness masks astringency and sourness, which may lead to greater liking of aronia juice.

#### *Enhancing prototypical samples:*

Once sensory profiling of aronia juice was attained, we investigated different ways to enhance the sweetness of aronia juice, including addition of 3-5% sucrose, and/or a sweet odorant (ethyl butyrate). Ethyl butyrate would mask unpleasant oral sensations from aronia juice and may increase their acceptability and palatability. As stated previously, a combination of sourness and astringency negatively influenced hedonics ratings. Therefore, we first focused on increasing liking of prototypical sour (12mM citric acid) and astringency (tannic acid) by suppressing negative oral sensations.

Sweetness needed to be improved to increase liking of a purely sour beverage (citric acid solution). Adding just ethyl butyrate did not demonstrate significant increase in liking. When only ethyl butyrate was added to the citric acid solution, there wasn't a significant increase in the hedonics rating. However, adding ethyl butyrate with a small amount of sugar resulted in a significant increase in the liking of citric acid solution. Adding ethyl butyrate and 0.15M sucrose to citric acid solution resulted in a significant increase in the hedonic rating compared to a 0.15M sucrose with citric acid solution. The increased hedonics rating was achieved by increasing sweetness. Sourness and astringency did not change between sucrose and sucrose plus ethyl butyrate. Noticeable reduction in bitterness and sourness are achieved only by adding more sugar. This indicates that sweetness primarily drives hedonics rating in purely sour citric acid solution. Unlike citric acid, tannic acid did not increase in hedonics rating with the addition of sucrose plus ethyl butyrate. Sucrose (0.3M) itself was enough to increase sweetness and decrease astringency. Sucrose plus ethyl butyrate did not change liking or oral sensory qualities (Figure 9).

#### *Enhancing aronia juice:*

Sucrose and ethyl butyrate combinations were added in week 5 aronia juice to increase overall liking. As seen in figure 10, the additives in the week 5 juices generated more variable responses. Sucrose (5%) alone increased liking by increasing sweetness and decreasing

sourness. Ethyl butyrate alone did not. Ethyl butyrate in combination with 3% sucrose achieved improved liking with less added sugar, but with much more variability in liking and oral sensory qualities. These additives did not influence perceived astringency. In several ways, the additives in aronia juice recorded similar oral sensory and hedonic responses to when they were added to citric acid.

## **Discussion:**

Aronia berries fit many of today's diets requiring high levels of antioxidants and micronutrients. Aronia berries were juiced across harvest time for this study. We first tested for intensity ratings of aronia juice taste qualities and likings across harvest time. The participants rated the hedonics and the taste qualities of aronia juice and other prototypical tasted on the gLMS scale. The responses to the prototypical tastes correlated to the response to aronia juice. A multivariable analysis was done to predict liking of aronia juice across harvest time. We also sought out a method to increase overall hedonics rating of aronia juice and prototypical sour by reducing negative taste qualities (sourness, astringency, and bitterness) with the inclusion of sugar and/or ethyl butyrate sweet odorant.

We found that the overall liking of aronia juice ranged from strongly dislike to above moderately liked across all of the harvest time. Liking correlated with perceived sweetness of the juice, indicating why juices harvested at later weeks, more ripe, were more liked. Sweetness was the most vital contributing factor to palatability of the aronia juice. In order to increase liking of aronia juice, we focused on increasing the perception of sweetness through sucrose and ethyl butyrate additives. Sugar improved juice liking by blocking sourness. Sucrose (0.3M) added moderate sweetness to citric acid while diminishing moderate/strong to weak sourness and shifting moderate/strong disliking to moderate liking. Ethyl butyrate increased sweetness in combination with sucrose but failed to diminish sourness or improve liking in citric acid or in aronia juice. Overall, the high antioxidant concentration of aronia berries caused participants to perceive high intensity of sourness and astringency. We were only able to reach a moderate liking ratings of the berry juice with sucrose and ethyl butyrate, which probably would not compel consumption for all consumers.

Our data are consistent with several previous studies. Comparing and contrasting the two widely used bitter markers (PROP and quinine) was the most useful taste phenotype in explaining differences in liking of the berries, as seen previously with other food by our lab (Duffy et al., 2006; Hayes et al., 2007) and other (Fischer, 2011). Our results suggest that high PROP/high Quinine group may experience a greater balance between sourness and astringency than high PROP/low Quinine group and hence may like the aronia juice more. Interestingly, high PROP/high Quinine group reported more sourness and yet liked the juice more. Similar patterns of sourness contributing positively to the liking of aronia juice were seen with week 4 harvests, and have been reported previously for other types of berries (Laaksonen et al., 2012). Based on these data and others from our lab, sour taste is liked if not coupled with unpleasant sensations of bitterness and astringency. As a negative taste quality, astringency negatively influences hedonics ratings. Sucrose improved perceived astringency in citric acid and tannic acid as shown previously (Lyman & Green, 1990) but not in aronia juice. The ethyl butyrate may have improved perceived sweetness, but the effect did not diminish astringency.

Based on the findings, healthy food items with substantial amounts of polyphenols contain some degree of bitter and sour oral sensory characteristics. As seen with previous studies, high concentrations of antioxidants and polyphenols are associated with significantly stronger sourness and bitterness, which discourage people from eating healthy food items. However, it is important to note that the relationship between sensory attributes in orosensory profiles may be more important than separate sensory properties of the food items. Therefore, increasing one of the taste qualities can change the dynamic of the sensory profile of the food item as a whole. Additives such as sucrose and ethyl butyrate help increase the overall acceptance of sour/astringent food items by increasing sweetness. Ethyl butyrate by itself does not suppress negative oral sensations and hence does not increase hedonics rating; ethyl butyrate is effective only when it is supplemented with a small amount of sugar. Although ethyl butyrate does not have any caloric content, it may raise concerns to those with calorie limitations if ethyl butyrate is added with sugar. Also, pediatricians often express concern regarding the possible adverse health effects of nonnutritive sweeteners. However, no negative side effects have been associated with ethyl butyrate, and it is already used in Juicy Fruit gum (the US Food and Drug Administration approved the additives used in this study). Ethyl butyrate only requires a small amount of sugar to improve sweetness and acceptance; therefore, if confidence can be attained with ethyl butyrate as an effective and safe enhancer aronia berry juice can be included in a healthy diet as a way to supplement daily antioxidant and polyphenol intake.

Berry juices are yet to be highly marketed in the United States due to the high sourness of most berry juices. If berry juices can be manufactured with sugar plus ethyl butyrate additives to increase sweetness with only a moderate increase in caloric content, people will have the option to consume micronutrient-rich drinks rather than resorting to soft drinks. The significance of this study can also be applied to the drinks that are available now. The concept of increasing hedonics ratings through sugar and ethyl butyrate can also be applied other food items. Many fruit juices and sports drinks contain very high sugar content. The high consumption of high sugar drinks is related to the obesity epidemic in the United States. If high preference of fruit juices and sports drinks can be maintained using smaller amount of sugar and adding ethyl butyrate instead, both children and adults will consume less calories throughout the day. Not just sugar + ethyl butyrate combination, but incorporating different additives combinations to healthy food items will increase overall consumption of nutrient rich foods by suppressing negative taste qualities and/or increasing positive taste qualities.

This study examined how aronia berry juice is perceived, while considering how individual variability in taste and oral sensations influenced aronia juice perception. Also, the study found ways to improve aronia juice through oral and olfactory additives. This paper extends research beyond suppression of negative oral sensations in model systems to a whole food item that may be a supplement to a healthy diet. Another strength of this study is that it looks at the sensory perception of aronia juice with additives, since most juices are marketed with additives. However, this study does have few limitations that need to be mentioned. First of all, the sample size of the study was relatively small and homogeneous, consisting mostly of healthy female adults, and testing occurred in a laboratory setting. Therefore, results may not be applicable to all ages, gender, health, and race. Although the implication of the study is that healthy berries are bitter, sour, and disliked, berries are diverse in oral sensory characteristics. Therefore, other healthy berries with or without additives may not produce similar results. Only two additives were used, sucrose and ethyl butyrate. However, many beverages use, beside

sucrose, artificial sweeteners such as aspartame. Finally, the present study did not test a 'combination-therapy' approach, which uses sodium ions and a sweetener simultaneously to exploit peripheral and central bitter suppression to improve aronia berry liking (Keast and Breslin 2005; Gaudette and Pickering 2012b).

In conclusion, aronia berry juice was not widely accepted because of its low sweet and high sour/astringent qualities. However, addition of a small amount of sugar and ethyl butyrate odorant depressed sourness/astringency and improved sweetness leading to higher hedonics ratings. Future studies could look at whether variance in perception of the bitter/sour qualities from aronia berries maps onto differences in biological responses to the berries (e.g., in their oxidative and inflammatory stress reducing activities after a high calorie meal). PROP supertasters are likely to have greater levels of bitter taste receptors in the enteroendocrine cells (Jansse & Depoortere, 2013), and hence may also show a greater bioavailability of Aronia polyphenols after consumption relative to nontasters. Also, an interdisciplinary collaborations between sensory, nutrition, and plant scientists are key to producing palatable foods that appeal to health conscious consumers in growing methods that are sustainable as shown with tomatoes (Bartoshuk & Klee, 2013) and strawberries (Schwieterman et al, 2013).

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	Perception	Range	Mean	Std. Deviation		Perception	Range	Mean	Std. Deviation
Sucrose 1M	Sweetness	0 to 98	42.38	21.359	PROP 0.32mM	Sweetness	0 to 14	0.43	2.16
	Sourness	0 to 17	0.44	2.442		Sourness	0 to 69	5.84	16.209
	Bitterness	0 to 31	0.9	4.528		Saltiness	0 to 52	1.96	7.89
	Saltiness	0 to 32	0.98	4.627		Bitterness	0 to 99	43.1	31.526
	Astringency	0 to 14	0.52	2.252		Astringency	0 to 85	13.67	22.159
	Hedonics	-20 to 84	29.68	21.955		Hedonics	-99 to -1	-46.94	32.092
Citric Acid 0.032mM	Sweetness	0 to 42	1.8	6.63	Ethanol 25%	Sweetness	0 to 23	3.22	5.509
	Sourness	0 to 90	33.62	22.354		Sourness	0 to 31	3.12	6.83
	Saltiness	0 to 69	6.56	13.709		Saltiness	0 to 21	1.43	4.163
	Bitterness	0 to 83	9.06	17.326		Bitterness	0 to 91	12.31	17.049
	Astringency	0 to 83	14.7	17.558		Astringency	0 to 41	5.8	9.859
	Hedonics	-95 to 52	-13.14	30.814		Hedonics	-89 to 18	-16.24	22.761
Quinine 0.32mM	Sweetness	0 to 12	0.26	1.7	Sodium Acetate 1.3M	Sweetness	0 to 33	1.62	5.038
	Sourness	0 to 83	4.8	13.655		Sourness	0 to 47	11.74	13.575
	Saltiness	0 to 42	1.56	6.516		Saltiness	0 to 89	31.8	20.675
	Bitterness	4 to 99	47.54	24.533		Bitterness	0 to 94	12.42	17.725
	Astringency	0 to 71	9.96	15.16		Astringency	0 to 65	6	11.278
	Hedonics	-99 to 3	-51.72	27.792		Hedonics	-99 to 43	-26.12	30.309
NaCl 0.32M	Sweetness	0 to 14	0.34	2.016	Alum 1g/mL	Sweetness	0 to 20	4.14	5.932
	Sourness	0 to 28	2.36	6.343		Sourness	0 to 47	6.42	10.527
	Saltiness	7 to 95	37.08	20.115		Saltiness	0 to 31	2.56	6.961
	Bitterness	0 to 71	3.64	11.565		Bitterness	0 to 53	7.66	13.514
	Astringency	0 to 68	3.68	12.099		Astringency	0 to 100	24.94	24.702
	Hedonics	-99 to 50	-11.56	27.904		Hedonics	-99 to 50	-11.94	24.809
Sucralose 0.1mM	Sweetness	0 to 41	12.5	8.999					
	Sourness	0 to 13	0.5	2.053					
	Saltiness	0 to 17	0.48	2.46					
	Bitterness	0 to 21	0.62	3.05					
	Astringency	0 to 20	1.74	4.444					
	Hedonics	-38 to 80	11.30	20.503					

Table 1: Intensity ratings of prototypical taste solutions (sucrose 1M, citric acid 0.032M, quinine 0.32mM, 0.32 NaCl, sucralose 0.1mM, PROP 0.32mM, ethanol 25%, sodium acetate 1.3M, and alum 1g/mL). The participants reported intensity ratings of sweetness, sourness, saltiness, bitterness, astringency, and hedonics.



	Perception	Range	Mean	Std. Deviation
Apple Juice	Sweetness	3 to 84	32.71	19.826
	Sourness	0 to 23	2.73	5.678
	Saltiness	0 to 8	0.29	1.429
	Bitterness	0 to 22	0.65	3.278
	Astringency	0 to 47	1.48	6.888
	Hedonics	-55 to 92	37.42	27.761
Grapefruit Juice	Sweetness	0 to 67	9.94	13.094
	Sourness	0 to 92	19.83	15.692
	Saltiness	0 to 26	1.15	4.463
	Bitterness	0 to 67	11.94	15.254
	Astringency	0 to 53	6.08	11.683
	Hedonics	-21 to 90	21.85	25.518
Coffee	Sweetness	0 to 13	0.47	2.135
	Sourness	0 to 34	1.09	5.124
	Saltiness	0 to 8	0.3	1.443
	Bitterness	0 to 89	32.23	18.767
	Astringency	0 to 51	6.49	12.609
	Hedonics	-91 to 69	-10.02	33.725
Soy Sauce	Sweetness	0 to 13	0.75	2.32
	Sourness	0 to 37	4.94	10.079
	Saltiness	0 to 95	35.88	23.933
	Bitterness	0 to 72	5.38	11.88
	Astringency	0 to 33	2.1	5.915
	Hedonics	-87 to 77	2.58	35.962

Table 2: Intensity ratings of food items (apple juice, grapefruit juice, coffee, and soy sauce). The food items, unlike prototypical taste solutions, had multiple taste qualities. The participants reported intensity ratings of sweetness, sourness, saltiness, bitterness, astringency, and hedonics.

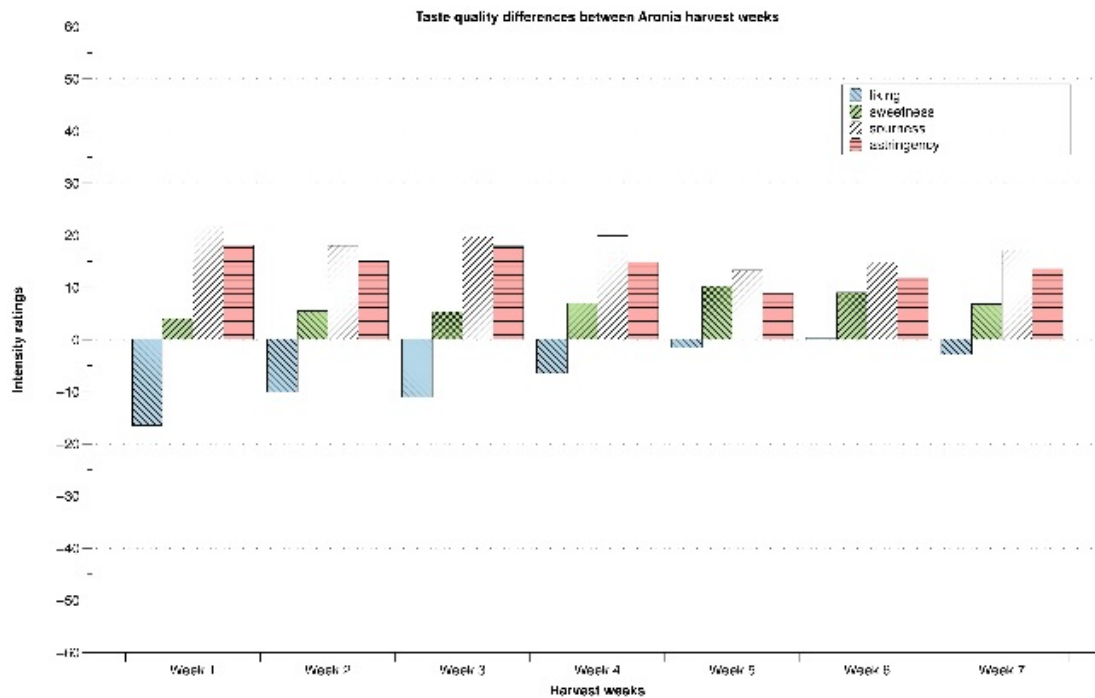


Figure 1: Week 1 harvests were the most disliked and Week 6 harvests were the most liked. Weeks 1, 2 and 3 had the most unfavorable sensory profiles (lower sweetness, greater sourness and astringency) whereas week 5 and 6 had the most favorable sensory profiles (greater sweetness, lower sourness and astringency).

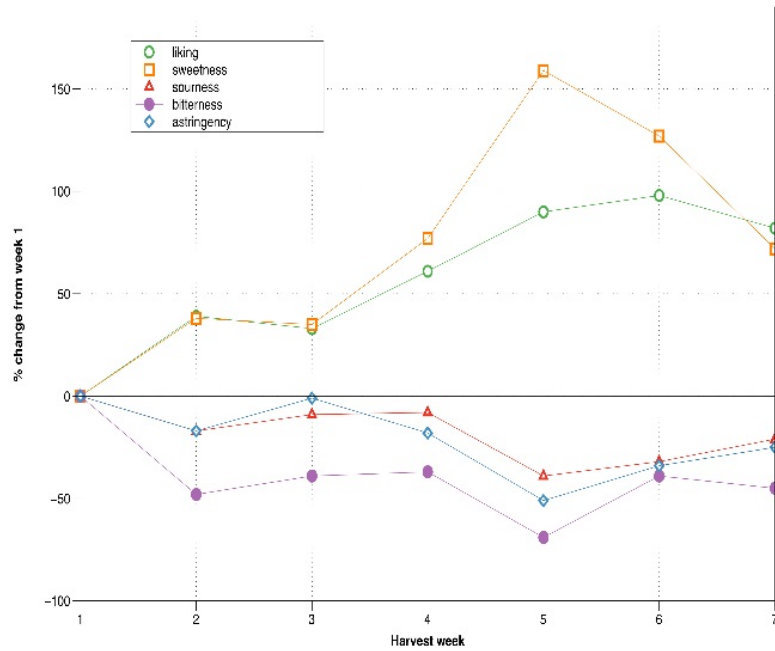


Figure 2: Perceived sweetness and liking increased whereas perceived sourness, bitterness and astringency decreased with each additional week after the first harvest, with peaks and troughs at week 1 through 7.

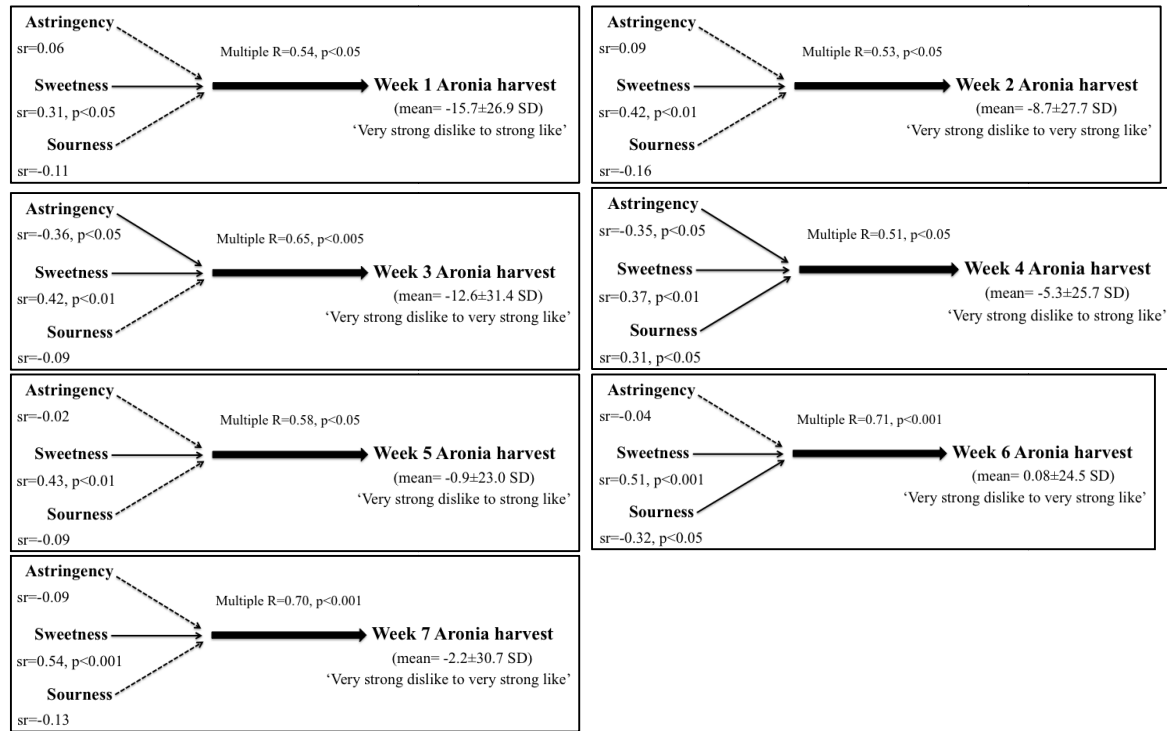


Figure 3: Multivariate models predicting preference for each harvest from perceived taste qualities, controlling for age, sex and intensity of tones. Semipartial correlation coefficients (sr) and the multiple regression coefficients (r) are reported. The dotted line represents a non-significant association.

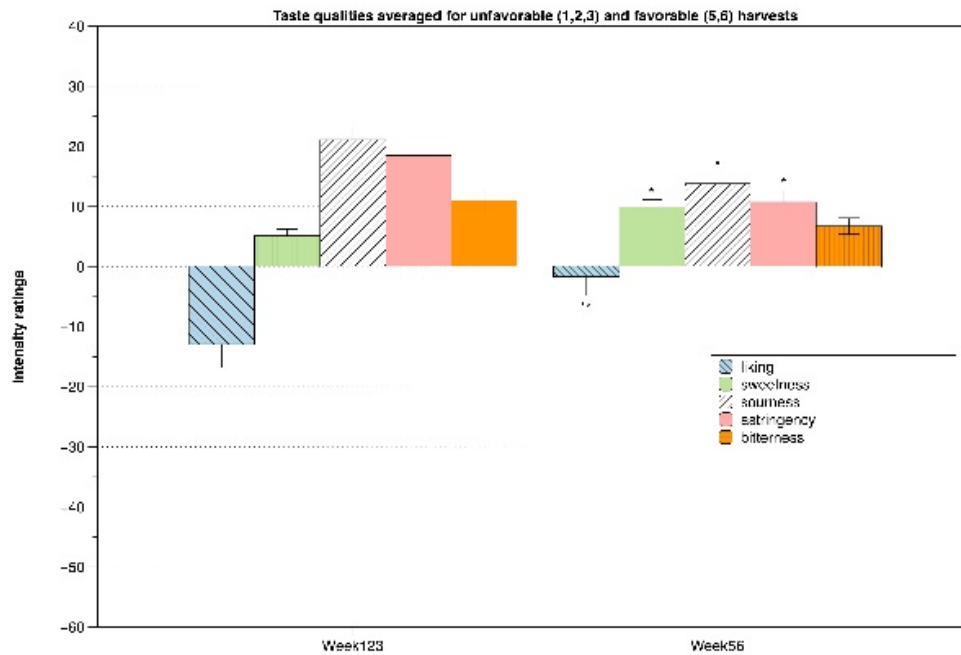


Figure 4: In paired t-tests, subjects reported, on average, significantly more sweetness and lower disliking, sourness, and astringency from favorable harvests (weeks 5 &6) than unfavorable harvests (weeks 1, 2 &3); (\* $p < 0.05$ ; \*\* $p < 0.01$ ).

<i>Spearman's Rho Correlations</i>					
	<i>Apple Juice_sweet</i>	<i>Aronia_sweet</i>	<i>Grapefruit Juice_sweet</i>	<i>Aronia_astringency</i>	<i>Aronia_sour</i>
<i>1M Sucrose_Sweet</i>	0.423**	0.027	-0.350*		
<i>Alum_astringency (1g/L)</i>				0.716**	
<i>32mM Citric Acid_sour</i>					0.338*

Table 3: The juice qualities correlated with alum astringency, and citric acid sourness but, unlike apple or grapefruit juices, did not correlate with sucrose sweetness; (\*p<0.05; \*\*p<0.01).

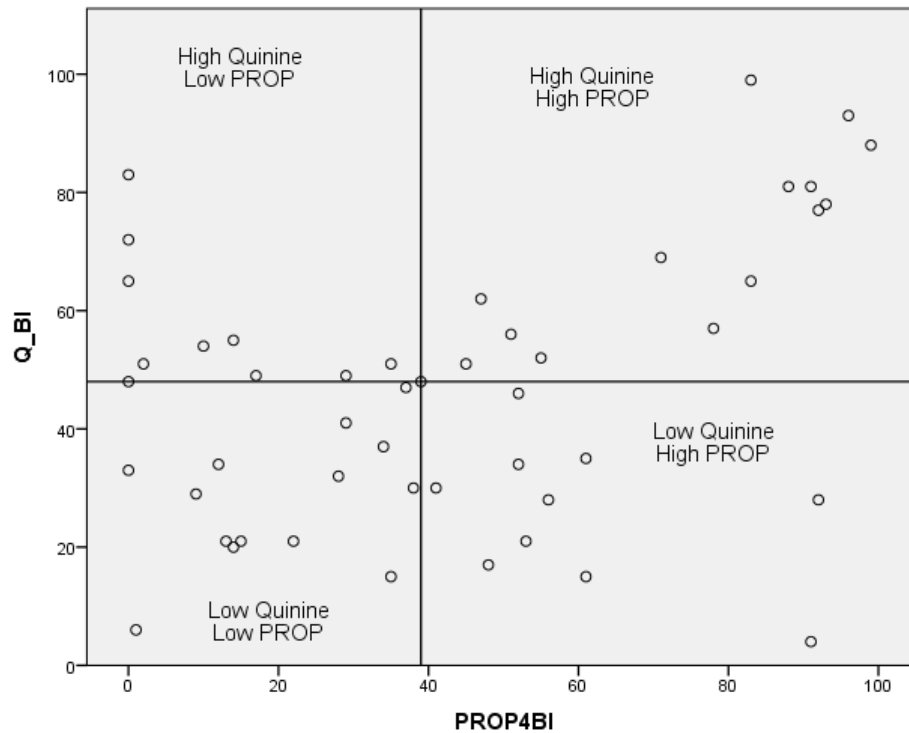


Figure 5: Scatter plot distribution of the perception of PROP bitterness and quinine bitterness. Intensity rating above 48 is considered a 'high quinine group' and a rating below 48 is considered 'low quinine group.' A rating above 39 is considered 'high PROP group' and a rating below 39 is considered 'low PROP group.'

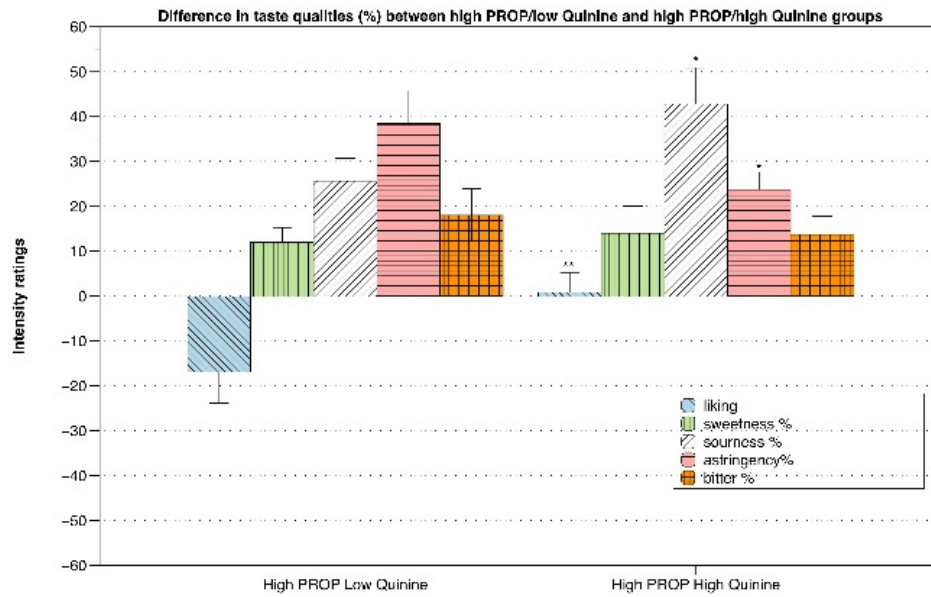


Figure 6: Subjects who perceived more PROP relative to quinine bitterness (n=10) reported the juice as less sour, more astringent, and more disliked than those who perceived high bitterness from both PROP and quinine (n=15); (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).



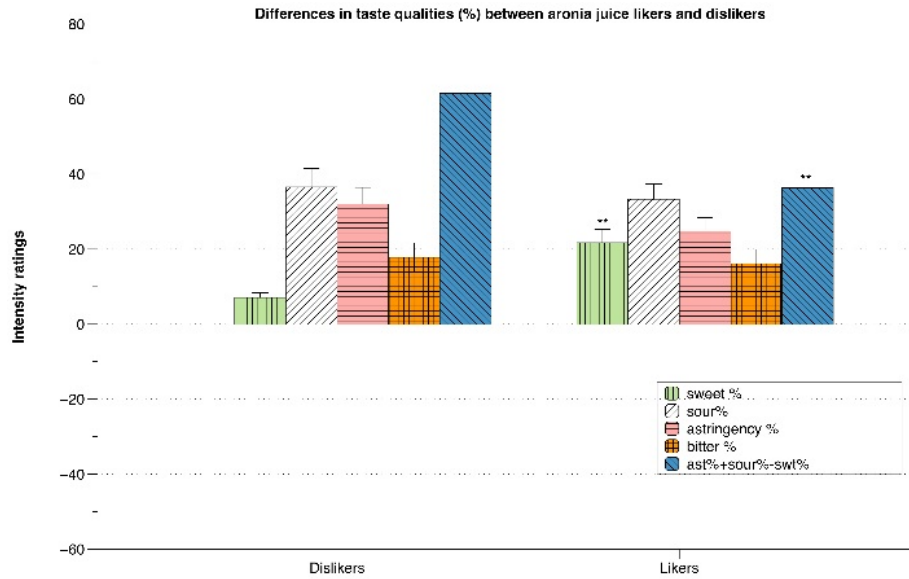


Figure 7: In analysis of covariance controlling for age, sex and intensity of tones as a cross-modal standard (for Fig 6 as well), aronia juice likers (n=27) experienced greater sweetness, and a balance between astringency, sourness and sweetness than dislikers of Aronia juice (n=20); (\* $p < 0.05$ ; \*\* $p < 0.01$ ).

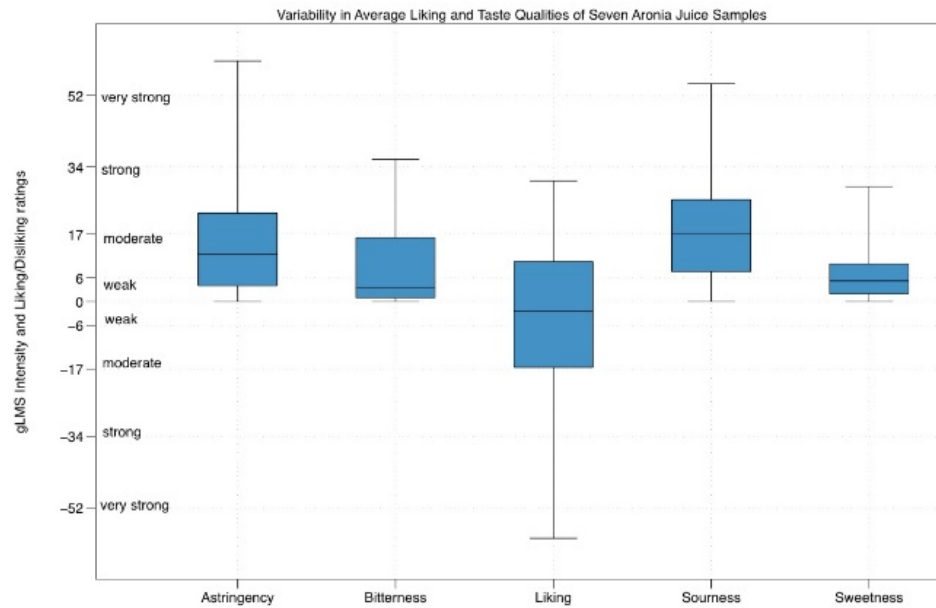


Figure 8: Subjects showed variability in average liking and taste qualities from Aronia juice samples. Liking showed the most variance, ranging from more than 'very strong dislike' to almost 'strong like.' Sweetness showed the least variance, ranging from 'no sensation' to almost 'strong like.'

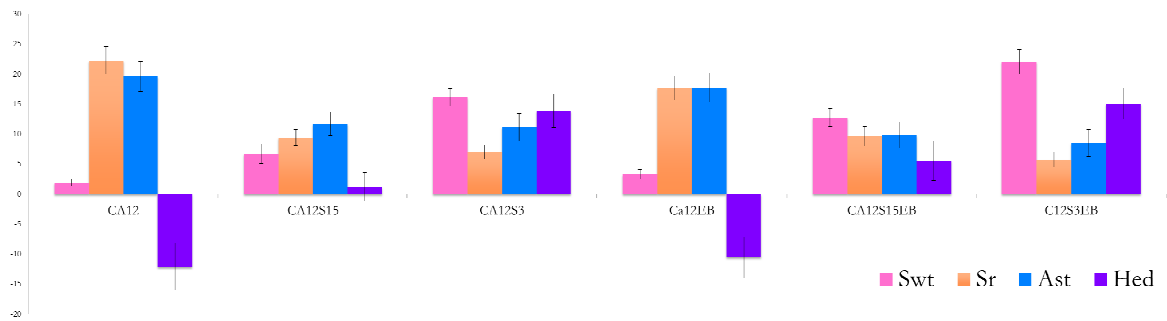


Figure 9: Addition of .15 to .3 M sucrose alone increased liking by increasing sweetness, decreasing sourness/astringency. Ethyl butyrate alone did not increase liking. Ethyl butyrate plus sucrose was sweeter than sucrose alone but not less sour/astringent.

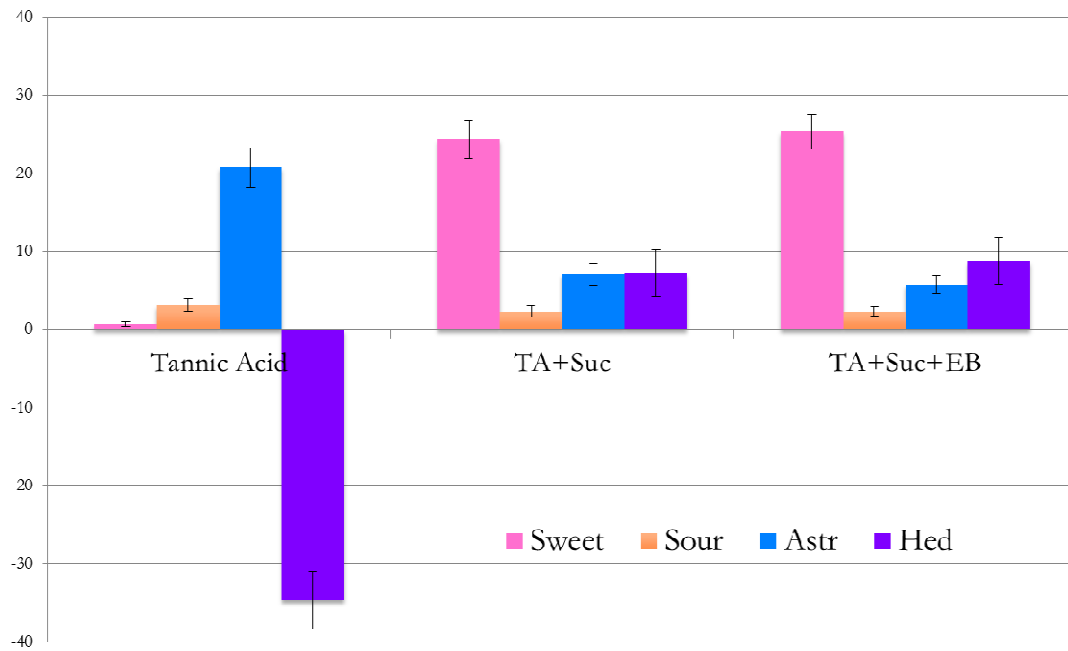


Figure 10: Addition of .3 M sucrose alone increased liking by increasing sweetness and decreasing astringency. Ethyl butyrate plus sucrose did not change liking or the oral sensory qualities.

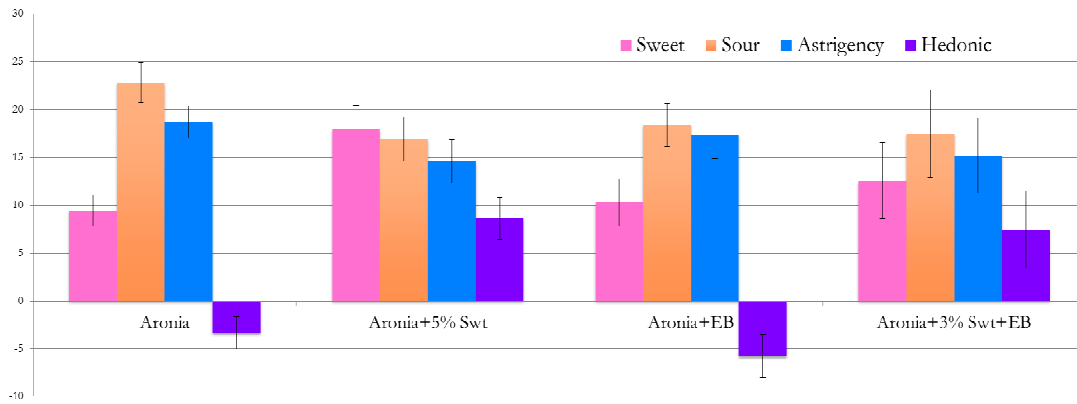


Figure 10: Addition of 5% Sucrose alone increased liking by increasing sweetness and decreasing sourness. Ethyl butyrate alone did not. Ethyl butyrate plus 3% sucrose achieve improved liking with less added sugar, but with much more variability in liking and oral sensory qualities. These additives did not influence perceived astringency.