Perceptions and acceptance of grapefruit-like model beverages that vary in taste, colour and aroma sensory properties: effects of sensitivity to bitter taste and *TAS2R38* and *TAS2R19* bitter receptor genes

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Declarations

General declaration

I declare that the thesis, which I hereby submit for the degree PhD Food Science at the University of Pretoria is my own work and has not been previously submitted by me for a degree at any other University or institution of higher education.

…………………………
Signature

…………………………
Date

Ethics declaration

I declare that this research has been subject to ethical review and received ethical approval from the Faculty of Natural and Agricultural Sciences Ethics Committee at the University of Pretoria (EC 130827-088).

I also declare that I have not deviated from the terms of the ethical approval issued by this Committee.

…………………………
Signature

…………………………
Date

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Summary

Perceptions and acceptance of grapefruit-like model beverages that vary in taste, colour and aroma sensory properties: effects of sensitivity to bitter taste and *TAS2R38* and *TAS2R19* bitter receptor genes

by

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Study leader: Prof. H.L. de Kock

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Department: Consumer and Food Sciences

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Grapefruit juice is an excellent source of many nutrients and phytochemicals that contribute to a healthy diet. Currently, there is an increasing interest in grapefruit products because consumption appears to be associated with a reduced risk of certain chronic diseases, such as obesity, diabetes, cancers and cardiovascular disease. The consumption of grapefruit (*Citrus paradisi* Macfadyen) however remains low in South Africa as some individuals like grapefruit and others do not and the reason/s for this variation is not clear. Taste, aroma and colour are important fruit product quality factors that influence consumer preferences. Perception of grapefruit flavour does not depend on only one individual sense, but is the result of multisensory integration of unimodal signals. Where there is a mixture of appearance, taste and aroma signals, cross-modal sensory interaction occurs which may potentially change the intensity and character of flavour perception. Sensory perception is interpreted differently across individuals. The main objective of the study was to determine the effect of varying the bitterness, sweetness, colour and aroma intensities of a grapefruit-like model beverage on the perception of sensory properties and consumer liking of the beverages with the aim of giving guidance to breeders on selection and improvement of grapefruit traits to optimize hedonic value. The second objective of this study was to determine the effects of sensitivity to bitter taste [as determined through 6-propylthiouracil (PROP) taster classification] and genetic

variation in *TAS2R38* and *TAS2R19* SNP genotypes on hedonic rating of the flavour of grapefruit-like beverages differing in bitter/sweet taste intensity.

A factorial design was used to formulate 36 grapefruit-like beverages with deflavoured clarified apple juice as base and modification of bitter taste (3 levels), sweet taste (3 levels), aroma intensity (2 levels) and colour (red or yellow). Descriptive analysis was used to describe the sensory profiles of the 36 beverages. Hedonic rating of colour, aroma and flavour of the 12 most diverse beverages from the design was measured with a consumer panel. Sensitivity to bitter taste of 96 young African females (18-24 years) was measure and the respondents classified into PROP taster groups. DNA was extracted from the saliva of the participants for genotyping of *TAS2R38* and *TAS2R19* bitter receptor genes. The subjects also rated the flavour of grapefruit-like beverages differing in bitter taste intensity for hedonic value.

The results showed that varying the bitterness, sweetness, colour and aroma intensity of the grapefruit-like model beverage have an effect on the sensory properties and consumer liking of the beverages. The concentration of naringin in the grapefruit-like beverage increased the bitter taste, aftertaste and grapefruit flavour intensity of the drink. Consumers preferred grapefruit-like beverages with a red colour and low bitterness. Sensitivity to the bitterness of grapefruit beverages and whether there is an association between genetics of bitter taste perception and liking of grapefruit were further explored. The results then showed that respondents' sensitivity to bitter taste, as well as genetic variation in *TAS2R38* and *TAS2R19* (single SNP genotypes) are partly responsible for the lower liking of grapefruit model beverages with higher naringin (more bitterness) concentration.

In this study, sensitivity of respondents to bitter taste (PROP status) has been linked to preference for red coloured grapefruit beverages, grapefruit beverages with low bitterness/high sweetness and grapefruit-like beverages with low intensity of grapefruit aroma. This is the first study to report on consumers' perception and acceptance of grapefruit-like model beverages that vary in taste, colour and aroma sensory properties. People differ genetically in bitter taste sensitivity and this research demonstrated the role of some genetic variables (notably rs10772420 of the *TAS2R19* SNP genotype and both rs713598 and rs1726866 of the *TAS2R38* SNP genotypes). It is the first study showing the effect of *TAS2R38* SNP genotypes on grapefruit liking. It is also the first study to determine the effect of PROP taster status, perception of grapefruit beverage characteristics (e.g. bitterness level, colour type and aroma level) and variation in *TAS2R38* and *TAS2R19* SNP genotypes on hedonic ratings for colour, aroma and flavour of grapefruit-like beverages in a group of South African females. So far populations from Africa have been under represented in similar studies. Most studies where a link between rs10772420 and lower bitterness perception and greater liking for unsweetened grapefruit juice was established, included only Caucasians. Studying the role of genetic differences in sensitivity to PROP bitterness (e.g. in taster status) in modulating multisensory grapefruit flavour perception is needed to determine why the liking for grapefruit varies between individuals.

The findings of this study can help researchers and breeders to change properties and traits in grapefruit varieties, can assist product formulators and quality assurance staff to optimize the flavour of grapefruit products for consumer acceptance and to make the generic product more acceptable to a larger portion of the South African population. However, the sample of respondents used in this research represents only a small portion of the South African population and therefore cannot be extrapolated to represent the population. The insights gained from this subgroup may be used to enhance the acceptance of grapefruit products for the larger population.

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CHAPTER 1:
Introduction

Grapefruit (*Citrus paradisi*; family: Rutaceae) is rich in citrus flavonoids and limonoids and has beneficial anti-oxidant and anti-inflammatory properties (Zargar, Al-Majet and Wani, 2018; Cristóbal-Luna, Álvarez-González, Madrigal-Bujaidar and Chamorro-Cevallos, 2018). South Africa's domestic consumption of fresh grapefruit remains low at 7000 MT per year on stable market demand versus 72,000 MT for fresh oranges (USDA Foreign Agricultural Service Gain Report, 2018) as local consumers have not acquired a strong preference for the taste of grapefruit. Some individuals like the flavour of grapefruit while others do not. The reasons for the dichotomy of opinions is not fully understood, but the bitter taste of grapefruit probably plays a role.

Bitterness in grapefruit juice is characteristic and expected. However, excessive bitterness is an undesirable attribute and adversely influence acceptance by consumers' which created an economic problem for the citrus industry in the USA (Manlan, Matthews, Rouseff, Littell, Marshall, Moye and Teixeira, 1990). Sami, Toma, Nelsen and Frank (1997) reported in a study that less than one-third of the households in the United States purchased grapefruit in 1983 on a regular basis. Naringin is by far the most dominant flavonoid in grapefruit and imparts a bitter and for some, objectionable taste to grapefruit and grapefruit juice (Nishad, Singh, Singh, Saha, Dubey, Varghese and Kaur, 2018; Cristóbal-Luna *et al*., 2018). Lee and Kim (2003) reported that grapefruit, as in all citrus, also contains the bitter limonoid, limonin. Fellers, Carter and De Jager (1987) reported the influence of limonin on consumers preference of processed grapefruit juice. Frozen concentrated grapefruit juice was reconstituted with limonin addition to produce five different level limonin juices. Preference by both users and non-users was lowest for the highest limonin content juice.

Taste perception plays a key role in determining individual food preferences (Bartoshuk, 2000). Individual differences in taste perception of consumers may influence preferences for grapefruit. Perception is the first step towards liking: what cannot be perceived cannot be liked or preferred. Flavour perception results from the integration of taste and smell perception sensory systems i.e. the combination of odours sensed ortho-nasally and retro-nasally with tastes sensed by receptors in the oral cavity (Lipchock, Reed and Mennella, 2011). Although the food flavours perceived are composed of distinct sensory properties, which are odours and tastes primarily, there is ample evidence that these properties are not perceived independently (Prescott, 2015). While eating or drinking, individuals experience a multifaceted combination of sensations, which will blend into a unitary perception. This process is known as multimodal sensory integration (Kremer, Bult, Mojet and Kroeze, 2007). Sensitivity to taste and other oral sensations shows considerable variation between individuals, and there is increasing evidence that these variations have a significant influence on food preferences and consumption behaviour (Drewnowski, 1997; Duffy and Barthoshuk, 2000; Reed, Tanaka and McDaniel, 2006; Tuorila, 2007), assumingly also with regards to grapefruit products.

Numerous intrinsic and extrinsic factors can influence consumers' food choices and purchase decisions (Enneking, Neumann and Henneberg, 2007). While there are certainly common precepts that all humans can relate to, the degree to which each person experience the effect of bitterness, sweetness, colour and aroma intensity on the cross-modal perception of sensory properties and consumer liking of grapefruit, can vary. Some of this variability, in relation to acceptance of grapefruit, comes from differences in experiences: e.g. how familiar individuals' are with grapefruit, what they have been told about it, or the personal value they assign to consuming citrus fruit or sensations of bitterness in foods. However, environmental factors and experience do not explain all of the differences in food preferences.

Common polymorphisms in genes involved in taste perception may account for some of the interindividual differences in food preferences within and between populations. Several taste receptors have been identified within taste cell membranes on the surface of the human tongue, and these include the *TASTE 2 receptor (TAS2R)* gene family of bitter taste receptors (Lee and Cohen, 2015). In humans, differences in bitter taste perception are controlled by the family of *TAS2R* genes (Drayna, 2005). The 25 human bitter receptors and their respective genes contain unusually high levels of allelic variation, which may influence responses to bitter compounds in the food supply (Hayes, Wallace, Knopik, Herbstman, Bartoshuk and Duffy, 2011).

It is possible that grapefruit consumption could be increased if the reason(s) for individuals' dislike of the sensory properties is better understood and can be overcome. The main objective of this study was to determine the effect of varying the bitterness, sweetness, colour and aroma intensity of a grapefruit-like model beverage on the cross-modal perception of sensory properties and liking by a specific group of South African females. The second objective was to relate genetic effects modulating sensitivity to bitter taste perception of the same group of respondents with hedonic ratings of the beverages.

The aim of the research was to contribute scientific insight on cross-modal sensory perception and on a practical applied level to give guidance: e.g. to grapefruit plant breeders on selection and improvement of fruit traits to optimise the hedonic value of grapefruit juice. Also for other role players in the industry in order to optimize product formulation and fruit and fruit juice blending quality to enhance grapefruit acceptance.

CHAPTER 2:
Literature review

2.1 Introduction

Grapefruit (*Citrus paradisi*; family: Rutaceae) can generally be found in three different varieties and the colour depends on the presence (or absence) of lycopene (Rao and Rao, 2007). The grapefruit varieties: Star Ruby, a red grapefruit type accounts for 79 % and Marsh, a white grapefruit type accounts for 16 % and Rose, a pink grapefruit type accounts for 3 % of the total grapefruit trees planted in South Africa (Citrus Growers Association, 2016). Grapefruit provides not only nutrients (carbohydrates, proteins, vitamins, minerals), but also phytochemicals such as flavonoids and limonoids, which are not essential for life, but may provide many health benefits (Zhang, 2007). Naringin and limonin are responsible for the bitter taste commonly associated with grapefruit (Ribeiro and Ribeiro, 2008). Naringin is by far the most dominant flavonoid bitter principle in grapefruit (Puri and Kalra, 2005). The bitterness of citrus juices (e.g. orange and grapefruit) can restrain its consumption and naringin is the main compound responsible for this undesirable attribute in grapefruit juice (Ribeiro, Rocha, Sepodes, Mota-Filipe and Ribeiro, 2008).

Taste, aroma, colour and mouthfeel all contribute to the flavour of foods and beverages (Drewnowski, 2001). The perception of flavour is perhaps the most multisensory aspect of our everyday experiences. Research by psychologists and cognitive neuroscientists increasingly reveals the complex multisensory interactions that give rise to the flavour experiences we all know and love, demonstrating how they rely on the integration of cues from all of the human senses (Spence, 2015). Flavour is defined 'as a perception that includes gustatory, oral-somatosensory, and retronasal olfactory signals that arise from the mouth as foods and beverages are consumed' (Small, 2012). The combination of these information signals, as well as additional sensory systems (e.g. touch/mouthfeel) (Lipchock *et al*., 2011) are combined at a higher level of processing in the brain and the overall cognitive perception of flavour can be modulated by cross-modal interactions (Abdi, 2002). Wang, Hayes, Ziegler, Roberts and Hopfer (2018) reported cross-modal aroma-taste interaction between vanilla and sucrose in skim milk, indicating vanilla aroma enhanced perceived sweetness. People show differences in their ability to detect many flavours (Hayes, Feeney and Allen, 2013). Bitter compounds are detected by a group of taste receptors (a type of G protein-coupled receptors) in the mouth. There are roughly 25 genes in the human genome that code for TASTE 2 receptor genes (*TAS2R*), the bitter taste receptors (Shi and Zhang, 2009). These 25 human bitter receptors genes (i.e. the TAS2Rs) are known to have high levels of allelic variation, which contribute to variations in taste (Hayes *et al*., 2011). Some might find grapefruit bitter, but not find beer, coffee or chocolate bitter. It appears that there may be genetic differences in bitter sensitivity between individuals. Understanding the numerous intermodal and cross-modal interactions that occur among sensory qualities (including taste, colour and aroma) when consuming grapefruit as fruit or juice is also essential to understanding the role of the human senses in perception and acceptance of the variable product quality.

2.2 Grapefruit

The first scientific description of grapefruit is attributed to the botanist James Macfadyen in 1837, who named it *Citrus paradisi* Macfadyen (Scora, Kumamoto, Soost and Nauer, 1982). However, this is only one of the various origins as stated in a few other research papers. It belongs to the Citrus genus, a taxon of flowering plants in the family Rutaceae. Other members of the genus include oranges, lemons, limes, citrons, pomelos (pummel, pommel) and mandarins (tangerines). The grapefruit is said to have developed by natural hybridization between pummel (*Citrus maxima*) and sweet orange (*Citrus sinensis*) (Nicolist, Deng, Gentile, Malfa, Continella and Tribulato, 2000). The fleshy part of the fruit is edible, and the seed extracts have been used as a food supplement and in the cosmetic industry (Ganzera, Aberham and Stuppner, 2006). Grapefruit has attracted much attention owing to its nutritional and antioxidant properties (Xu, Liu, Chen, Ye, Ma and Shi, 2008). *Citrus paradisi* juice can generally be found in South Africa in three different varieties, red, pink and white as seen in Figure 1. China leads the world in global grapefruit production, followed by the United States (Citrus Growers Association, 2016).

Red (Star Ruby) | White (Marsh) | Pink (Rose)

Figure 1 South African Grapefruit varieties. Variety in brackets

Grapefruit juice products (white, pink, or red) can be found commercially as concentrated juice or reconstituted single strength from concentrate or freshly squeezed juice from the fruit. It can be packed for the commercial market in glass, carton, plastic or metal (Hasbay, 2016). Typical grapefruit juice products in the retail market are 100 % juice, nectars (25-50 % juice content) and drinks (up to 25 % juice content) (Hasbay, 2016). Naringin imparts a bitter and for some consumers, objectionable taste to grapefruit juice. Excessive bitterness of the juice was considered as an important economic problem in commercial grapefruit juice production (Manlan *et al.*, 1990). To reduce the bitterness, a number of processes such as chemical treatments, physical separation processes, blending with nonbitter citrus juices and sugars, and enzymatic treatment has been reported and applied (Cavia-Saiz, Muniz, Ortega and Busto, 2011). Gous (2012) used two commercial enzymes (commercial enzymes from Amano Enzymes, Japan), aromase (β-primeverosidase and β-glucosidase) and laccase (polyphenol oxidase) in a study, for debittering grapefruit peel juice. The study recommended that aromase be used on its own or in combination with laccase, to debitter grapefruit peel juice. Current industrial technology for debittering is based on the adsorption of bitter compounds onto porous adsorbent resins (cellulose acetate or microporous resin beads orcross-linked styrene divinylbenzene resins (Shaw, Baines, Milnes and Agmon, 2000).

2.2.1 Vitamins and minerals of grapefruit

Grapefruit is an excellent source of the anti-oxidant vitamin C and contain moderate levels of the β-complex group of vitamins such as folate, riboflavin, pyridoxine, and thiamin in addition to some resourceful minerals such as iron, calcium, copper, and potassium (Sinclair, 1972).

2.2.2 Colour of grapefruit

Lycopene (red) and beta-carotene (yellow) have been identified as the major pigments in red or pink grapefruit (Lee, 2000). In the pink grapefruit cultivar, varying amounts of zeta-carotene (pale yellow) and phytofluene (pale orange) are also present. Zeta-carotene and phytofluene were reported as the predominant pigments in the white Marsh variety along with a trace of beta-carotene (Xu, Fraser, Wang and Bramley, 2006).

2.2.3 Bitterness of grapefruit

Bitterness in all citrus fruit is mainly caused by the accumulation of two different chemical compounds: limonin from the limonoid terpene group and naringin from the flavonoid phenolic group in the fruit tissues (Hasegawa, Berhow and Fong, 1995). In certain varieties of oranges, an increase in bitterness is observed in juices, after extraction, restraining their industrial use. This has been explained by the conversion of the nonbitter precursor, limonoate A-ring lactone, to a bitter compound, limonin, under acidic conditions (Ribeiro, Silveira, Elbert and Ferreira-Dias, 2003). Naringin is considered the foremost flavonoid bitter principle in grapefruit (Puri and Kalra, 2005). The distribution of the limonoid (limonin) and the flavonoid (naringin) in each fruit part of grapefruit can be seen in Figure 2 (Pichaiyongvongdee and Haruenkit, 2009).

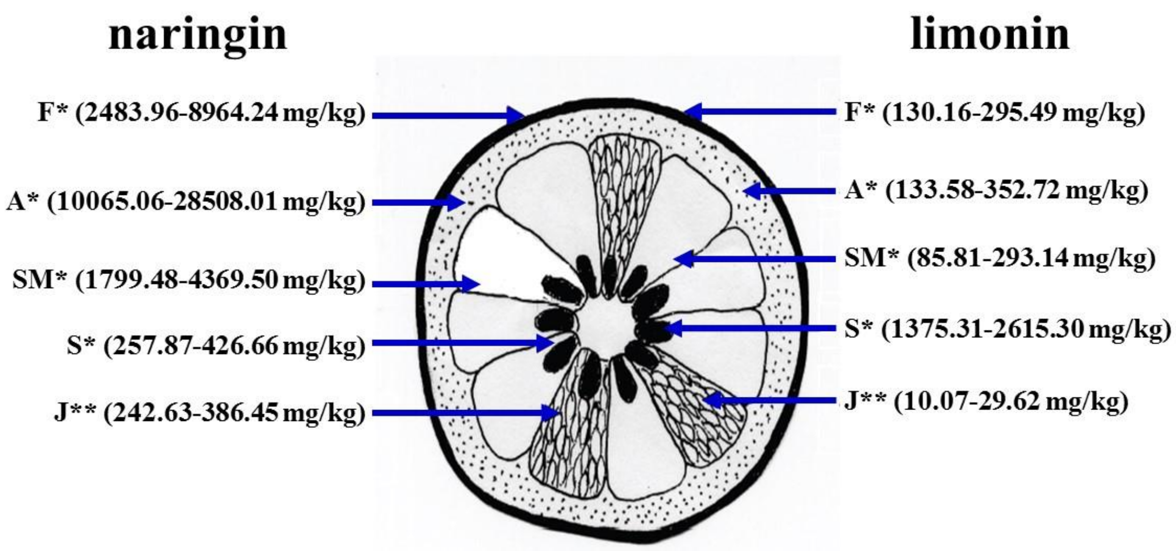

Figure 2 Cross-section of grapefruit showing typical variation in the distribution of limonin (mg/kg) and naringin (mg/kg) in each fruit part from seven pummelo cultivars grown in Thailand (Pichaiyongvongdee and Haruenkit, 2009)

F = Flavedo, A = Albedo, SM = Segment membranes, S = seeds, J = juice

*naringin and limonin concentration in flavedo, albedo, segment membranes and seeds

**naringin and limonin concentration in juice (Pichaiyongvongdee and Haruenkit, 2009)

The intense bitterness of naringin is said to exceed that of quinine and is detectable in water containing as little as 0.05 mg/kg (Prakash, Singhal and Kulkarni, 2002). Naringin occurs in high concentrations in the albedo layer or inner layer of the peel, in seeds, in the core and in membranes of grapefruit (Yusof, Ghazali and King, 1990). Naringin is abundant in immature grapefruit but its concentration decreases as the fruit ripen (Munish and Banerjee, 2000). Naringin in grapefruit flesh ranges from about 218-340 ppm with a general trend to diminish towards midseason and increase again towards the end of the season (Ortuno, Garcia-Puig, Fuster, Perez, Sabater, Porras, Garcia-Lidon and Del Rio, 1995). The flavonone glycoside naringin consists of an aglycone naringenin and a disaccharide consisting of rhamnose and glucose (Rouseff and Matthews, 1980). Enzymes containing α-rhamnosidase and β-glucosidase activities hydrolyse naringin to prunin and rhamnose by its α-rhamnosidase activity. Prunin is then broken down into naringenin and glucose by β-glucosidase activity (Prakash *et al.*, 2002). Narirutin is present at the second highest concentration among the flavonoids in grapefruit. Naringin and narirutin are closely related in their chemical structures (Tripoli, La Guardia, Giammanco, Di Majo and Giammanco, 2007). Both naringin and narirutin (glycosides) will be converted to naringenin (aglycone) in the human body (Erlund, Silaste and Alfthan, 2002).

2.2.4 Pectin, cellulose, fibre and carbohydrates of grapefruit

Grapefruit contains high concentrations of pectin, cellulose, hemicellulose and fibre (Sinclair, 1972).

The main portions of carbohydrates in citrus fruits are the free simple sugars: sucrose, glucose, and fructose as well as rhamnose. Sucrose is generally the main sugar observed in grapefruit juice (Lee, 2000).

2.2.5 Aroma of grapefruit

The peel oil has a strong and desirable aroma useful in the industrial flavouring of foods, beverages, pharmaceutical products, perfumes, and cosmetics (Njoroge, Koaze, Karanja and Sawamura, 2005). Limonene and β-caryophyllene were found to be most abundant components in grapefruit samples taken of Rio Red Grapefruit harvested at five different periods comprising from developmental to maturity stages (Chaudhary, Jayaprakasha and Patil, 2017). The peel oil of grapefruit has a high content of the monoterpene fraction, which is mainly d-limonene

(Kirbaslar, Tavman, Dulger and Turker, 2009) and the composition of grapefruit oil aroma can be seen in Table 1.

Table 1 Chemical Composition of Grapefruit oil aroma

	Fraction	Component	Composition (%)	Reference
Non-Aroma Components	Hydrocarbons	D-limonene	85-96 %	Kirbaslar *et al*., 2009
		Myrcene	< 2.5 %	Ahmad *et al*., 2006
		Sabinene		
		α-Pinene		
		γ-Terpinene		
Aroma Components	Nootkatone	Nootkatone	< 1.0 %	Ortuno *et al*., 1995
	Aldehydes	Octanal		
		Nonanal		
		Decanal		
		Dodecanal		
	Octyl acetate	Octyl acetate		
	Citronellyl acetate	Citronellyl acetate		
	Citral	Citral		
	Carvone	Carvone		
Other	Esters, Alcohols & Other	Esters, Alcohols & Other	± 6 %	Ortuno *et al*., 1995

2.2.6 Health benefits of grapefruit

Vitamin C (ascorbic acid) is one of the most important vitamins with antioxidant properties and take part in free radical scavenging, cancer prevention, collagen synthesis, ion absorption, wound healing, cholesterol metabolism and immunity improvements (Lee, Lee, Surh and Lee, 2003). Vitamin C supports important skin functions, stimulating collagen synthesis and assisting in antioxidant protection against UV-induced photo damage (Pullar, Carr and Vissers, 2017) and wound healing (Du, Cullen and Buettner, 2012).

Dietary carotenoids (β-carotene & lycopene) provide health benefits in decreasing the risk of disease, particularly certain cancers and eye disease with β-carotene's ability to be converted to vitamin A (Johnson, 2002). It was shown that lycopene could protect against various diseases such as cancer (Yang, Yang and Wang, 2013), atherosclerosis (Hu, Li and Jiang, 2008), and nonalcoholic steatohepatitis (Wang, Ausman and Greenberg, 2010). Studies have shown that lycopene had a positive influence on the cardiovascular system and reduced the total cholesterol level as well as reducing blood pressure in hypertensive patients (Kulczynski, Gramza-Michalowska, Kobus-Cisowska and Kmiecik, 2017).

Naringin and limonin have demonstrated antioxidant, hypolipidemic, and antihypertensive properties in cell culture, in an animal model, as well as in human clinical trials (Rizza, Muniyappa and Iantorno, 2011). Grapefruit promoting cardiovascular health is likely attributable to the flavonones, naringin and hesperidin found in the pith of grapefruit, which are converted to the aglycones naringenin and hesperidin in the gut (Erlund, Silaste and Alfthan, 2002). It is mainly the naringin aglycone (naringenin) that is bioavailable and is largely responsible for the beneficial properties of naringin. Research on naringin or naringenin has shown that naringin could act as a free radical scavenger and antioxidant (Jeon, Kim and Kim, 2007), reduce total cholesterol levels and enhance lipid metabolism (Da-Silva, De-Oliveira, Nagem, Pinto, Albino, De Almeida, De-Moraes and Pinto, 2001). Animal studies have shown that the active flavonoids in grapefruit, naringin and hesperidin, appear to improve glycemic control by potentiating insulin secretion, enhancing the transport of blood glucose to peripheral tissues, or by inhibiting endogenous glucose production (De la Garca, Etxeberria, Lostao, Roman, Barrenetxe, Martinez and Milagro, 2013).

Grapefruit is very low in calories, just 40 calories per 100 g juice (Cracknell and Nobis, 1989), and there has been an increase in research on its effects of grapefruit consumption on body metabolism.

Grapefruit has been reported to increase satiety due to its fibre content, as well as delay gastric emptying by increasing gastric acid (Chaw, Yazaki and Evans, 2001).

2.2.7 Grapefruit-drug interactions

Grapefruit juice and grapefruit product consumption have potential health benefits; however, their intake is also associated with interactions with certain drugs, including statins, calcium channel blockers, antibiotics, immunosuppressants and antihistamines (Hukkinen *et al*., 1995; Kupferschmidt *et al*., 1995; Benton *et al*., 1996; Kupferschmidt *et al*., 1998). Grapefruit can irreversibly inhibit cytochrome P450 metabolizing enzyme called CYP3A4, a metabolizing enzyme for almost 50 % drugs, and is found in the liver and small intestinal epithelial cells (Pirmohamed, 2013). If the drug is not metabolized, then the level of the drug in the blood can become too high and lead to fatal drug toxicity (Pirmohamed, 3013). The other effect is that grapefruit can block the absorption of drugs in the intestine. If the drugs is not absorbed, then not enough of it is in the blood, to have a therapeutic effect (Mitchell, 2016). Polyphenolics present the major group of citrus compounds interacting with drugs. Included are hydroxycinnamic acids; flavonoids, such as flavanones, flavones, and flavonols; anthocyanins; and coumarins (Berhow *et al*., 1998). In vitro studies with single compounds demonstrated that furanocoumarins and their dimmers may primarily be responsible for the interactions of grapefruit with drugs (De Castro *et al*., 2006). The most abundant furanocoumarins in grapefruit are bergamottin and 6’7’-dihydroxybergamottin (He *et al*., 1998; Schmiedlin-Ren *et al*., 1997; Guo *et al*., 2000). The furan ring in the furanocoumarins enhanced the inhibitory effect on CYP3A4 activity. Flavonoids with more phenolic hydroxyl (OH) groups produced stronger inhibition than those with less hydroxyl groups (Ho *et al*., 2001). Although naringin is the major flavonoid in grapefruit juice, it has a minor contribution to the well-known grapefruit juice-drug interactions. Naringin is hydrolyzed to its aglycone naringenin in the gut lumen (Ho *et al*., 2001). Naringenin was reported to be a potent competitive inhibitor of CYP3A4 (Kimura *et al*., 2010).

Initially, the liver was assumed to be the major site of grapefruit-drug interactions. However, it has been shown that the interactions only occur when drugs are administered orally, not intravenously, which indicates that the interaction may take place during the gastrointestinal absorption phase (Lundahl *et al*., 1997; Ducharme *et al*., 1995).

Some drugs such as cyclosporine, sirolimus, simvastatin, lovastatin and felodipine carry a warning label regarding the possibility of an interaction (FDA/CDER, 2004).

2.3 Food preferences and food choice

The development of food preferences begins at conception and continues across the life course (Scott, 1992). Any sensory input, for example, what a human tastes, smells, sees, hears and touches, can potentially influence food perception and preference (Spence and Piqueras-Fiszman, 2014). Understanding flavour integration is essential to understand consumers’ food choices (Köster, 2009). Food preferences appear to be partially genetically determined, with high coefficients of heritability for preferences for protein foods, fruit, vegetables and desserts (Falciglia and Norton, 1994). Yang and Hort (2018) reported a link between genetic variation and taste perception (sweet, bitter, salty, sour and fat) and food preferences. Some citrus fruits, cruciferous vegetables and green leafy vegetables are bitter (Drewnowski and Gomez-Carneros, 2000) and generally disliked due to the instinctive rejection of the bitter taste (Steiner, 1979). Conversely, other bitter foods such as alcohol or coffee (Lahti-Koski, Pietinen, Heliovaara and Vartiainen, 2002) are consumed and enjoyed by a large segment of the population.

2.3.1 Perception of grapefruit fruit and juice

Perception involves both the physical senses (sight, smell, hearing, taste and touch) as well as the cognitive processes involved in interpreting those senses (Krishna, 2012). Perception allows humans to interact with and learn from the environment. The sensory profiles of two types of fresh, white and pink, Malaysian pomelo [*Citrus grandis* (L.) Osbeck] juices were evaluated by experienced flavourists in a study by Cheong, Liu, Zhou, Curran and Yu (2012). Descriptive sensory evaluation indicated that white pomelo juice was milder in taste especially with regards to the acidity. Principal component analysis and partial least square regression in that study revealed a strong correlation between the chemical components and flavour attributes (i.e. acidic, fresh, peely and sweet) in pomelo juices. Buettner and Schieberle (2001) confirmed in a reconstitution experiment to simulate the aroma of fresh, hand-squeezed juice of the White Marsh seedless grapefruits, that the typical sulfurous, grapefruit-like odour was mainly due to the catty, blackcurrant-like 4-mercapto-4-methylpentan-2-one and the grapefruit-like smelling 1-*p*-methenene-8-thiol. The odourants quantified in the study as seen in Table 2, were dissolved in water in the exact amounts determined to be present in fresh white grapefruit juice. The overall aroma of the flavour model, evaluated by the sensory panel, in comparison to a hand-squeezed juice, was plotted as a spider web diagram. It revealed similarity with the natural aroma of the fresh juice, eliciting the same intensities of the grapefruit-like, fruity, terpene-like, and citrus-like odour qualities. Rosales and Suwonsichon (2015) reported a sensory lexicon of pomelo fruit as seen in Table 3, over various cultivars cultivated and consumed in Thailand. Results showed that the attributes were able to describe great variation in aroma, flavour and texture characteristics among pomelo cultivars.

2.3.2 Fruit as a food choice item

In the U.S., several campaigns and programs, such as Produce for Better Health Foundation (PBH) and the Nutrition assistant Program administered by USDA, have continuously and extensively promoted vegetable and fruit consumption among U.S. consumers to reduce the risk of diseases such as stroke, cancer and diabetes (Steward and Harris, 2004). Demand for fruits is expected to grow, from 164.5 pounds per capita in 2000 to 182.3 pounds by 2020 (Lin, Variyam, Allshouse, Cromartie, 2003). However, per capita consumption of fresh citrus (orange, tangerine, lemon, lime and grapefruit) experienced a decline from 26.6 pounds in 1998 to 20.6 in 2008 (USDA-ERS 2009).

Food choice decisions made by individuals in relation to fruit consumption can be a complex food choice process (Pollard, Kirk and Cade, 2002). Factors for example the food product itself, together with the sensory attributes (e.g. colour, flavour and taste) and non-sensory characteristics (e.g. product information and prices) (Roosen, Marette, Blanchemanche and Verger, 2007), can have an influence on food choice behaviour. It has been quite difficult to assess the impact of quality on consumer preferences and choice of fruit (Van der Pol and Ryan, 1996). Obenland, Campisi-Pinto and Lu Arpaia (2018) used more thorough methods of sensory evaluation and fruit sampling for analytical characteristics than what have been previously employed. Commercially packed grapefruit were obtained over a nine month period. The fruit were evaluated by panelists for overall like-ability, grapefruit flavour intensity, juiciness, tartness and bitterness. Panelists were also asked questions regarding purchase intent. The same grapefruit halves used in the sensory analysis were used to assay for soluble solids (SSC) and titratable acidity (TA). It was found that likeability was most strongly linked to sweetness, with bitterness having a lesser role. Purchase intent data gathered indicated that grapefruit should have a very high flavour quality (like moderately and above) to make more likely both an immediate and future purchase.

Table 2 Concentrations of potent odourants in Hand-Squeezed grapefruit juice (Buettner and Schieberle, 2001)

Odourant	Concentration[a] (μg/kg)
acetaldehyde*	6150
ethyl 2-methylpropanoate*	5.8
(R)-α-pinene*	42
ethyl butanoate*	70
(S)-ethyl 2-methylbutanoate*	3.9
hexanal	33
(Z)-hex-3-enal*	108
myrcene*	94
(R)-limonene*	2308
hept-1-en-3-one*	0.5
ethyl hexanoate*	4.3
octanal*	32
oct-1-en-3-one*	0.8
4-mercapto-4-methylpentan-2-one*	0.8
nonanal	9.3
methional	0.2
decanal*	89
(E)-non-2-enal*	0.5
linalool*	76
1-*p*-methene-8-thiol*	1.01
Ethyl 3-hydroxyhexanoate	117
(*E*, *E*)-deca-2,4-dienal*	1.0
Tr-4,5-epoxy-(*E*)-dec-2-enal*	3.1
3a, 4, 5, 7a-terahydro-3,6-dimethyl-2 (3*H*)-benzofuranone	1.1
vanillin	69

[a]Data are mean values of at least duplicates; Odourants used in the flavour reconstitution experiment are marked with an asterisk (*)

Table 3 Lexicon for describing appearance, aroma, flavour and texture/mouthfeel characteristics of five pomelo cultivars[1] at different storage periods. Attributes, definitions, references and Intensities (Rasales and Suwonsichon, 2015)

Attributes	Definition	Reference, intensity and preparation
Appearance		
Glossiness	Reflection of the light incident on the surface of the sample.	Halls mint candy = 3.5 20 g Mitr Phol Syrup in 2 oz cup = 12.0
Aroma/Flavour		
Citrus ID	Combination of aromatics associated with citrus fruits such as orange, lemon, lime, grapefruit and pomelo.	0.05 ml McCormick Pure Orange Extract in 200 ml water = 5.0 (aroma) = 4.5 (flavour)
		0.30 ml McCormic Pure Orange Extract in 200 ml water = 10.0 (aroma) = 9.0 (flavour)
Pomelo ID	An aroma blend which is bitter, sour, sweet and viney associated with pomelo.	100 % Chabaa Pomelo juice with pomelo flesh = 6.0 (aroma) = 7.5 (flavour)
Orange peel	Sour, slightly pungent, oily, bitter, citrus aromatic characteristic	Harvey Fresh Grapefruit Juice = 8.0 (aroma) = 7.0 (flavour)
Viney	Green, fresh aroma and flavour notes associated with newly cut vines and stems. Sometimes relate to cucumber.	Japanese cucumber (0.5 in cube) = 6.5 (aroma) = 5.0 (flavour)
Floral	A sweet, heavy aroma and flavour blend of a combination of flowers, which can be somewhat chemical and perfume-like.	0.02 g Winner Jasmine scent solution in 400 ml water = 2.5 (aroma) = 4.0 (flavour)
Overall sour	Aroma and flavour notes associated with the impression of all sour substances.	30 ml Heinz vinegar in 180 ml water = 5.0 (aroma) = 7.0 (flavour)
Overall sweet	Aroma and flavour notes associated with the impression of all sweet substances.	20 g Mitr Phol granulated sugar in 200 ml water = 3.0 (aroma) = 3.5 (flavour)
Sweet	The fundamental taste sensation of which sucrose is typical.	2 % sucrose solution = 2.0; 5 % sucrose solution = 5.0; 10 % sucrose solution = 10.0
Sour	The fundamental taste sensation of which citric acid is typical.	0.015 % citric solution = 1.5; 0.05 % citric acid solution = 3.0; 0.08 % citric solution = 5.5
Bitter	The fundamental taste sensation of which caffeine or quinine are typical.	0.02 % caffeine solution = 3.5
Texture/mouthfeel		
Hardness	Force to compress between molars required for sample deformation.	Sengheng Hard tofu (0.5 in cube) = 3.0; 3 % Agar (Telephone brand) (0.5 in cube) = 6.5
Firmness	The degree of denseness of sample on the first bite.	Kinu Soft tofu (0.5 in cube) = 1.0; Sengheng Hard tofu (0.5 in cube) = 3.5
Moisture	Amount of wetness/juiciness released from sample during chewing.	McGarett Pineapple in syrup (0.5 in cube) = 6.5
Chewiness	Difficulty in chewing sample with molar.	McGarett Pineapple in syrup (0.5 in cube) = 4.0
Fibrous	The degree to which fibers are present.	McGarett Pineapple in syrup (0.5 in cube) = 5.0
Numbing	Numbness felt on tongue after swallowing or spitting sample for 30 s.	Pepsi = 3.0
Astringent	The complex of drying, puckering, shrinking sensations in the oral cavity.	0.035 % McCormick Alum solution = 1.5

Table 3 Lexicon for describing appearance, aroma, flavour and texture/mouthfeel characteristics of five pomelo cultivars[1] at different storage periods. Attributes, definitions, references and Intensities (Rasales and Suwonsichon, 2015) (*continue*)

Attributes	Definition	Reference, intensity and preparation
Prickly	A sensation of stinging or tingling and slightly burning in the mouth.	20 ml Heinz White Vinegar in 160 ml water = 8.0
Particles	Amount of small hard particles remaining in the oral cavity (except on teeth) immediately after swallowing.	Lay's Stax original (0.5 in^2) = 2.5; Tong Garden salted peanut (2 pieces) = 5.0
Aftertaste		
Orange peel	Sour, slightly pungent, oily, bitter, citrus aromatic characteristic.	
Bitter	A bitter sensation remaining in the oral cavity after swallowing sample.	
Sweet	A sweet sensation remaining in the oral cavity after swallowing or splitting sample.	

[1]Kao Numpueng, Kao Tangkwa, Kao Yai, Thong Dee, Tubtim Siam

Food choice can further be influenced by personal factors of the consumer, such as demographics (Wadolowska, Babicz-Zielinska and Czarnocinska, 2008), motives (Steptoe and Wardle, 1999). Although demographics are not likely to explain all the variation in consumer preferences, some are often significantly related to preferences. For example, numerous studies have found location (city, region or country) to be an important factor in explaining heterogeneous consumer preferences (Fox, 1995; Jaeger, Andani, Wakeling and MacFie, 1998).

Another factor that can influence food choice is prior expectations (Deliza and MacFie, 1996). Fernández-Vásquez, Hewson, Fisk, Vila, Mira, Vicario and Hort (2014) reported in a study how slight variations in orange juice hue (reddish to greenish hues) affect the perceived flavour intensity, sweetness, and sourness, and the expected and actual liking of orange juice. Another example of prior expectation on fruit choice is the fact that grapefruit juice and grapefruit product consumption have potential health benefits; however, their intake is also associated with interactions with certain drugs, including channel blockers, immunosuppressants and antihistamines (Seden, Dickinson, Khoo and Back, 2010). Patients or consumers' first response when choosing a fruit may choose to exclude grapefruit from their diets and consume other fruits, including other types of citrus, to avoid an interaction. An increasing number of adverse drug reactions might be avoided on the basis of knowledge about the interaction of grapefruit juice and relevant drugs. Therefore patients and consumers need to be educated about the hazards (and advantages) of grapefruit interaction with grapefruit and what medication can be used with grapefruit (Kiani and Iman, 2007).

Another factor that influence food choice is body image, physiological restrictions (Bisogni, Connors, Devine and Sobal, 2002) and weight status e.g. obesity (Berg, Lappas, Wolk, Strandhagen, Toren, Rosengren, Thelle and Lissner, 2009). Consumers tend to believe that grapefruit can help with weight loss. However, a study by Dow, Going, Chow, Patil, Thomson (2012) suggest that consumption of grapefruit daily for 6 weeks does not significantly decrease body weight, lipids, or blood pressure as compared with the control conditions.

Additional influences on the food choice can be the socio-economic environment and context where the food choice and consumption will take place (King, Weber and Meiselman, 2004).

2.4 Multisensory flavour perception

In order to really understand the experience of flavour, one needs to move beyond the traditional definitions of flavour [as captured by the International Standards Organization (ISO 5492, 2008)]. Flavour is defined as ‘a complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting’. From the first moments we encounter a food item during childhood humans develop associations, and even preferences, with particular sensory characteristics. This complex network of associations keeps expanding throughout the lifetime and marks how persons perceive and react towards foods. ‘It help to explain basic questions such as why red drinks tend to taste sweet, like red fruits, why some odours bring us back to childhood, and why we eat more of certain foods than others’ (Piqueras-Fiszman and Spence, 2015).

Taste, smell, and oral somatosensations (mixed sensory category and includes all sensations received from the skin and mucous membranes, as well as from the limbs and joints) combine to generate a largely unitary experience, namely flavour (Stevenson, 2016). One of the most fundamental phenomena of multisensory flavour perception is oral referral of the taste and olfactory sensations associated with substances in the mouth. In order to fully understand this truly multisensory perception, it is necessary to know the basics of the sensory modalities and systems that are directly related to flavour perception (i.e. gustation, olfaction, and somesthesis/chemesthesis) (Lim, 2016).

Colour is perhaps the single most important product-intrinsic sensory cue when it comes to setting expectations regarding the likely taste and flavour of a food or drink. To date a large body of research has demonstrated that changing the hue or intensity/saturation of the colour of a variety of different food and beverage items exerts a sometimes dramatic impact on the expectations, and often on the subsequent taste/flavour experience of participants in the laboratory, as well as consumers under the more naturalistic conditions of everyday life. It is important to note that food colours can have rather different meanings, and hence give rise to differing expectations in different age groups, not to mention in those from different cultures. By gaining a better understanding of the sensory and hedonic expectations that are elicited by food colour in different groups of individuals, researchers are now beginning to better understand the various ways in which, what the eyes see can modulate the multisensory perception of flavour, and alter food behaviours (Spence and Piqueras-Fiszman, 2016).

In food-related applications of flavour, both bottom-up (sensory), as well as top-down (expectations) processes, are at play. Most of the complex interactions that take place are outside the awareness of the perceiving subject (Dijksterhuis, 2016).

Flavour perception reflects the integration of distinct sensory signals, in particular odours and tastes, primarily through the action of associative learning. Associative learning involves pairing a flavour with attractive attributes such as the sensory affective features e.g. the pleasantness of the citrus aroma (Dickinson and Balleine, 2002). This process of integration has hedonic consequences largely derived from pairing odours with the innate hedonic properties of tastes and post-ingestive effects of nutrients. Odour becomes not only the defining characteristic of flavours but also a carrier of hedonic information that has adaptive significance through its effects on motivation to consume and on the identification of foods that are safe to ingest (Prescott, 2016).

Mojet and Köster (2016) reported that flavour memory is also strongly linked to the situational aspects of previous encounters with a flavour, but does not depend on the precise recollection of its sensory features as in vision and audition.

Over the last few decades, researchers have learned a great deal about the network of brain areas that are involved in the construction of both the sensory-discriminative and hedonic aspects of our multisensory flavour experiences. The scientific study of the brain on flavour is sometimes referred to as ‘neurogastronomy’ and understanding the neural networks that underlie multisensory flavour perception is important. Kringelbach and Berridge (2009) showed (Figure 3) the oral sensory pathways for taste (black circles), somatosensory (white circles), and olfactory (grey circles). The pathways involve the relevant primary sensory areas (situated in limbic and paralimbic cortex and in unisensory neocortex) as well as a number of associations, or integration sites, such as the insular cortex and orbitofrontal cortex.

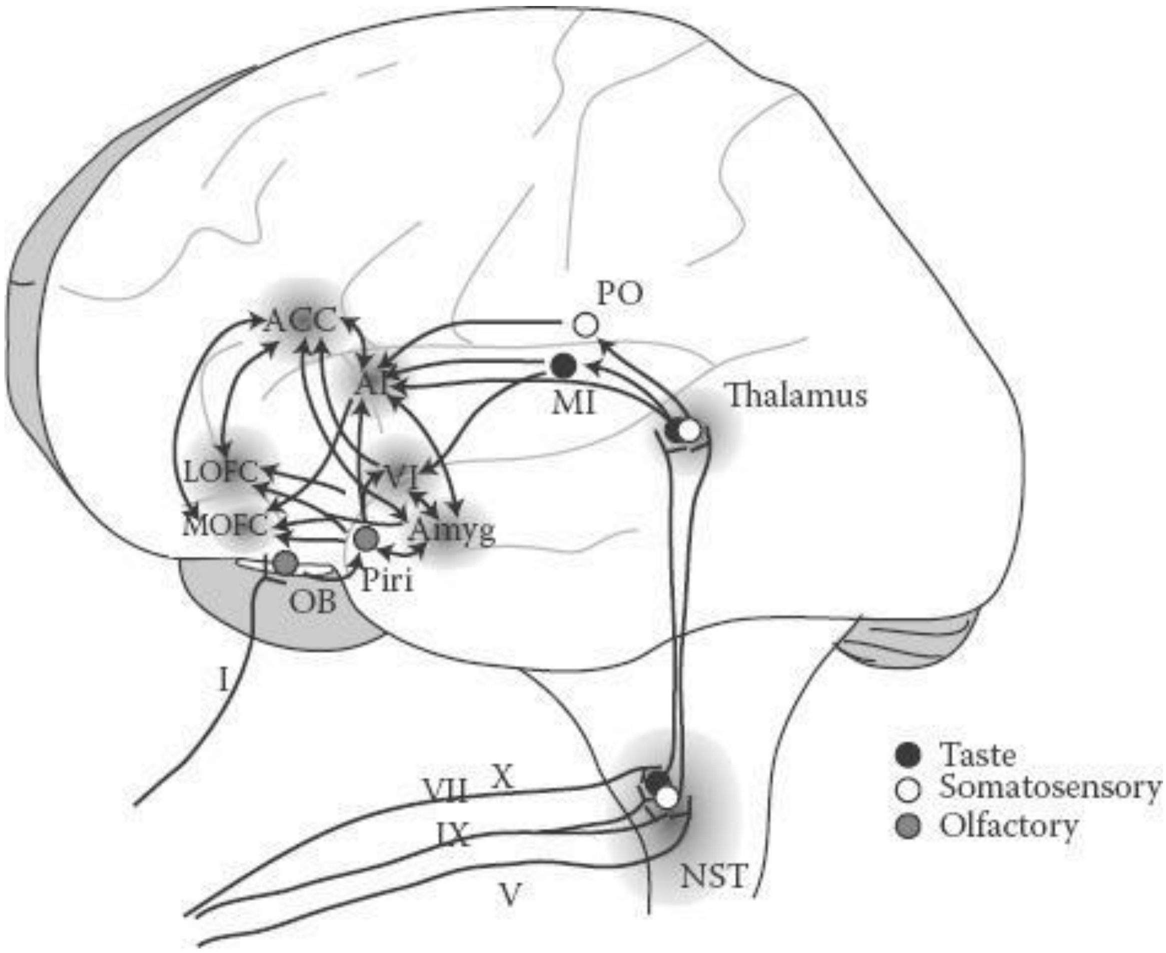

Figure 3 Neural networks that underlie multisensory flavour perception - taste (black circles), somatosensory (white circles), and olfactory (gray circles). Chorda tympani (VII), Glossophyarngeal (IX), Vagus nerve (X), rostral nucleas of the solitary tract (NST), mid insula (MI), anterior insula (AI), ventral insula (VI), medial orbitofrontal cortex (MOFC), lateral orbitofrontal cortex (LOFC), glossopharyngeal nerve (IX), trigeminal nerve (V), postcentral gyrus (PO), cranial nerve (I), anterior cingulated cortex (ACC), amygdale (Amyg), olfactory bulb (OB), piriform cortex (Piri) (Kringelbach and Berridge, 2009)

Anatomical locations in Figure 3 are only approximate and connectivity is not exhaustive. Information from taste receptors on the tongue is conveyed via the chorda tympani nerve (VII), glossopharyngeal nerve (IX), and vagus nerve (X) to the rostral nucleus of the solitary tract (NST), which then projects to the thalamus. From here, taste information projects to the mid-insula (MI) and anterior insula and overlying frontal operculum (AI). AI also projects to the ventral insula (VI), medial orbitofrontal cortex (MOFC), and lateral orbitofrontal cortex (LOFC). Somatosensory input reaches the NST via the glossopharyngeal nerve (IX) and trigeminal nerve (V), which then project to the thalamus. Oral somatosensory information is then relayed to the opercular region of the postcentral gyrus (PO). Olfactory information is conveyed via the cranial nerve I to the olfactory bulb, which projects to the primary olfactory cortex, including the piriform cortex (piri). The piriform cortex, in turn, projects to VI and the orbitofrontal cortex. The anterior cingulated cortex (ACC) and amygdale (Amyg) are also strongly interconnected with the insula and orbital regions representing taste, smell, and oral somato sensations. Gustatory stimuli project from the tongue to the primary taste cortex (more specifically, the anterior insula and the frontal or parietal operculum), whereas olfactory stimuli project directly to the primary olfactory (i.e. piriform) cortex. From there, the inputs from both senses project to the orbitofrontal cortex (OFC). Gustatory stimuli are thought to project to the caudolateral OFC, whereas olfactory stimuli project to the caudomedial OFC (as reported by Small in 2012). The orbitofrontal cortex, the anterior cingulated cortex, and the insula have been shown to be the important areas for integration of olfactory and gustatory information (Eldeghaidy, Marciani, Pheiffer, Hort, Head and Taylor, 2011). Small and Prescott (2005) proposed in a study that flavour perception depends upon neural processes occurring in chemosensory regions of the brain, including the anterior insula, frontal operculum, orbitofrontal cortex and anterior cingulated cortex, as well as upon the interaction of this chemosensory 'flavour network' with other heteromodal regions, including the posterior parietal cortex and possibly the ventral lateral prefrontal cortex.

Spence and Piqueras-Fiszman (2016) reported that one important distinction to be drawn is between the flavour expectations that normally precede consumption and the flavour experiences that follow, with the former typically influencing the latter more than is often realized. Branding, pricing, and labelling have a significant influence on the brain's response to flavour, as we rarely experience food and drink in the absence of such cues.

Schifferstein (2016) reported that food products are unique in that our interactions with them may involve all of the senses. Experiences with food products are inherently dynamic, implying that modalities may play different roles in various stages of user-product interactions. Starting with exploration from a distance (vision), followed by closer inspection and active engagement (touch), to intimate contact with the product that ultimately involves ingestion (smell and taste), followed by any post-ingestive effects.

Taste perception

The mechanisms proposed to explain the mutual influence of aroma and taste when perceived together can occur at physicochemical, physiological or psychological levels (Taylor, 2002). Taste perception occurs during gustation as taste-active compounds stimulating taste receptors on the tongue. Taste perception can be separated into five basic taste characters, including sweetness, saltiness, umami, sourness, and bitterness (Steward, DeSimone and Hill, 1997). Sour and salty tastes are detected via ion-channels, whereas bitter, sweet and umami tastes are sensed via G-protein coupled receptors (Bachmanov and Beauchamp, 2007). The information from all these receptors gets transmitted to the brain, where it is processed and integrated (Keast, Dalton and Breslin, 2004). Bitter is the most complex of human tastes, and is arguably the most important (Beckett, Martin, Yates, Veysey, Duesing and Lucock, 2014). Aversion to bitter taste is important for detecting toxic compounds in food; however, many beneficial nutrients also taste bitter and these may therefore also be avoided as a consequence of their bitter taste (Beckett *et al*., 2014). There are a variety of chemical compounds which are bitter, such as polyphenols, organic acids, peptides, salts, sulfimides, and acyl sugars (Drewnowski, 2001).

Aroma perception

Smell (olfaction) is the detection of volatile odour compounds by the olfactory sensory neurons in the olfactory epithelium which is located at the top of the nasal cavity (Rinaldi, 2007).

An odour is perceived when the concentration of volatiles in the headspace phase reaches the odour threshold level (Buettener and Schieberte, 2000). The odour threshold is defined as 'the level at which the human nose can detect a volatile compound'. The odour threshold for specific compounds may be different among individuals. Human beings are just able to discriminate between up to four odourants in chemically complex odourant mixtures (Livermore and Laing, 1998). If more components were presented, the task became more complex and discrimination became more difficult (Livermore and Laing, 1998). Each aroma compound has a perceivable character, however when perceived in combination with other compounds, they can interact with each other.

2.4.1 Cross-modal sensory interactions

The single most important factor in the acceptance of a product like grapefruit juice is probably its sensory character. Sensory character is the integrated response to the chemical and physical stimuli imparted by the food through its texture, taste, colour, aroma and irritant components or modalities (Forde and Delahunty, 2004). The combination of information from the olfactory system (the combination of odours sensed ortho-nasally, retro-nasally) and gustatory system (taste), as well as additional sensory systems (touch) (Lipchock *et al*., 2011), are combined at a higher level of processing in the brain and its overall cognitive perception of flavour can be modulated by cross-modal interactions (Abdi, 2002). Understanding the numerous intermodal and cross-modal interactions that occur among sensory qualities is essential to understanding the role of the human senses in food perception and acceptance (Meiselman, 1996). No literature was found on studies investigating intermodal or cross-modal sensory interaction in grapefruit or other citrus products. However, Wang *et al*. (2018) reported cross-modal aroma-taste interaction between vanilla and sucrose in skim milk, indicating vanilla aroma does enhance perceived sweetness. However, the enhancing effect of vanilla aroma was not as pronounced as that of sucrose on vanilla flavour.

It is important to understand how and where interactions occur as they often impact on the perception of flavour as well as other attributes affecting the key sensory profile of products. These interactions can occur at a number of levels, from physical interactions between components within the food or beverage matrix (e.g. pectin as a viscosifying agent in juice can have specific effects on odour and flavour attributes where it enhanced cereal odour or suppress honey odour and flavour, lemon odour and cooked apple flavour), leading to changes in taste, aroma and appearance (Walker and Prescott, 2000).

Two main types of mechanisms underpin these cross-modal interactions. First, physico-chemical mechanisms drive chemical stimuli release as a function of food consumption and structure, and food oral processing. Second, perceptual mechanisms result from sensory information integration from the receptors to the brain (Thomas-Danguin, Sinding, Tournier and Saint-Eve, 2016).

The interaction between different components in multicomponent mixtures might lead to enhancement, suppression or masking of certain flavours (Bitnes, Ueland and Moller, 2009). For example, different types of colours and sweeteners can have an effect on the bitterness of beverages. One of the classic studies to investigate colour’s influence on taste sensitivity was conducted by Maga (1974). He investigated the effects of colouring an aqueous solution red, green, or yellow on perceptual thresholds for four of the basic tastes (salty, sour, sweet, and bitter). Maga (1974) reported that red colour decreased caffeine bitterness while yellow and green had no such effect. Menella, Reed, Mathew, Roberts and Mansfield (2015) reported in a study using a group of children and adults and determining whether adding sucrose to urea, caffeine, denatonium benzoate, propylthiouracil (PROP), and quinine would reduce their bitterness using a forced-choice method of paired comparison. In both children and adults, sucrose suppressed the perceived bitterness of each agent. In adults, sucrose was effective in reducing the bitterness ratings from moderate to weak for all compounds tested, but those with the sensitive form of the sweet receptor, reported greater reduction for caffeine and quinine.

Cross-modal correspondences have been defined as ‘a tendency for a sensory feature, or attribute, in one modality, either physically present or merely imagined, to be matched (or associated) with a sensory feature in another sensory modality’ (Spence, 2012). Cross-modal correspondence can influence cross-modal integration during perceptual learning (Brunel, Carvalho and Goldstone, 2015). Experiencing a stimulus in one sensory modality is often associated with an experience in another sensory modality. Brunel *et al*. (2015) reported that seeing a lemon might produce a sensation of sourness which indicates some kind of cross-modal correspondence between vision and gustation. Another example may be when one sees a red coloured beverage it creates the impression that the beverage may be sweet. A specific correspondence that has received attention of late is the one between round forms and sweet taste. Machiels (2018) reported a study focusing on two different cup forms (round versus angular). The author tested this association for a buttermilk drink and a mate-based soft drink but were not able to corroborate the frequently suggested correspondence effect. However, a correspondence was found between the angular cup and a more bitter taste for the soft drink.

A schematic depiction of the two routes of olfactory perception: orthonasal and retronasal can be seen in Figure 4. Odours sensed orthonasally enter the body through the nose (nares) and travel directly to olfactory epithelium in nasal cavity. Kringelbach and Berridge (2009) reported that odours sensed retronasally enter the mouth during eating and drinking. Volatiles are released from food or drink and subsequently pass through the nasopharynx at the back of the oral cavity to enter the nasal cavity and reach the olfactory epithelium.

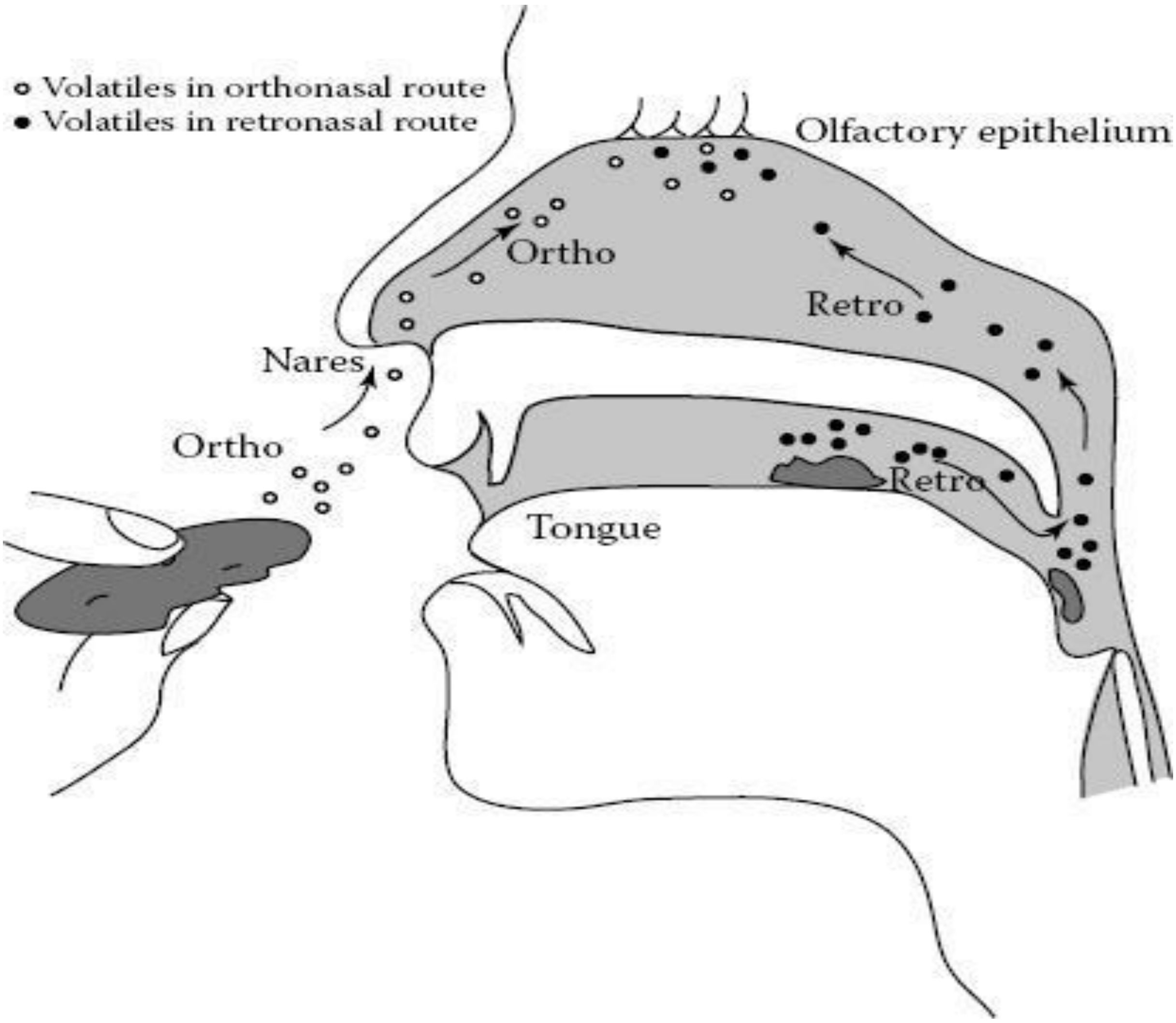

Figure 4 Orthonasal versus retronasal olfaction routes (Kringelbach and Berridge, 2009)

Cross-modal sensory interactions arise due to the simultaneous perception of stimuli, which influence each other across different modalities, which indicates that these mechanisms, therefore, operates at the cognitive level rather than the physical level (Noble, 1996). With the different neural networks that underlie multisensory flavour perception in mind, Verhagen and Engelen (2006) explored multisensory interactions and related them to oral-sensory (gustatory and somatosensory) and olfactory (orolfactory) interactions. Verhagen and Engelen (2006) illustrated the sequential processing steps involved in forming a Gestalt ('whole') perception of food. The cognitive model indicating the sequential processing can be seen in Figure 5, represented in separate neural structures, followed by obligatory pre-attentive multisensory integration (MSI) and conscious perception (both affective and 'objective'). MSI is divided into peripheral MSI (pMSI, occurring mainly between somatosensory and gustatory inputs) and central MSI (cMSI, occurring among all inputs). cMSI depends on the congruence of the information (e.g. quality congruence between taste and smell), and whether such integrated information reaches consciousness depends on attentional processes. Congruency is defined by Schifferstein and Verlegh (1996) as ‘the extent to which two stimuli are appropriate for combination in a food product’. They showed that strawberry odour enhanced sweet taste in whipped cream while the aromas peanut butter, bacon or wintergreen did not enhance sweet taste (Schifferstein and Verlegh, 1996). In visual-auditory MSI none of the stimuli makes physical contact with the transducing substrates (retina, cochlea), as these substrates are involved with distal perceptual processes. Hence, perceptual interactions are assumed to be related to central effects. However, as all physico-chemical aspects of oral stimuli directly interact with oral and olfactory surfaces, which transduce these properties, it is to be expected that many interactions among sensations derived from the oral cavity are at the peripheral level.

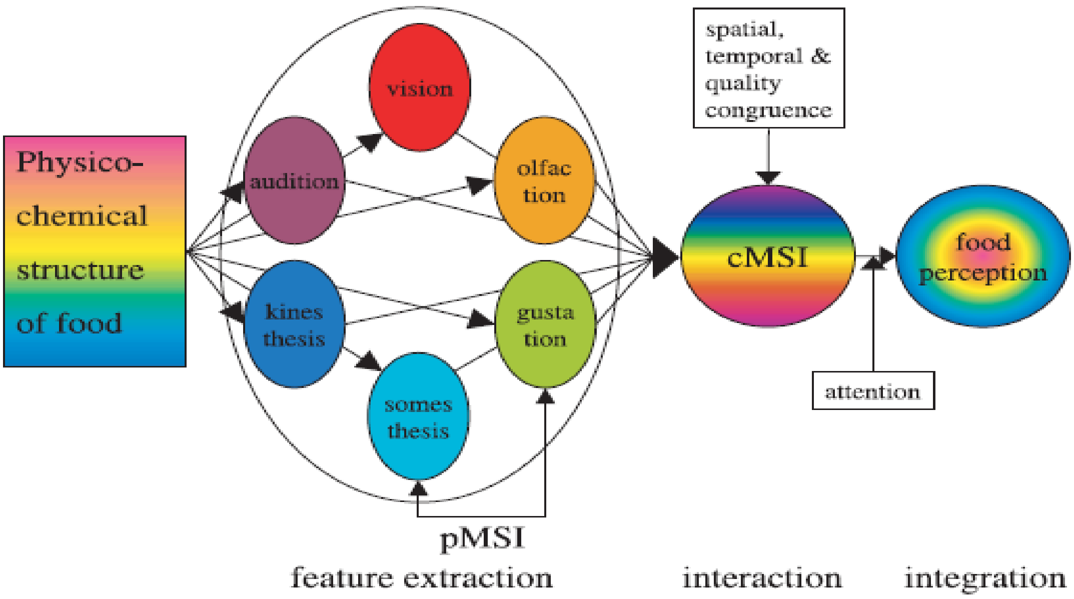

Figure 5 A cognitive model indicating the sequential processing (extraction, interaction and integration) of unimodal food properties (Verhagen and Engelen, 2006)

Aroma-aroma interactions reported at suprathreshold levels can shift the perceived aroma intensity by either enhancement or suppression, however, the latter occurs more frequently (Ferriera, 2012). This, also known as mutual suppression, has been observed in binary, ternary, and quaternary mixtures, and additional compounds to the mixture can further promote suppression (Laing, Eddy and Best, 1994). The general cause of aroma-aroma interactions is thought to occur through peripheral mechanisms and is considered as a combination of competitiveness between the volatile compounds at the site of the receptors (Bell, Laing and Panhuber, 1987) coupled with their configuration changes (Gillan, 1983). It is important to distinguish between orthonasal smell when sniffing and the retronasal smell when air is pulsed out from the back of the nose when swallowing (Rozin, 1982). Small, Gerber, Mak and Hummel (2005) have been able to provide empirical support for the claim that different neural substrates may actually be involved in processing these two kinds of olfactory information. It is the retronasal aromas that are combined with gustatory cues that give rise to flavours. Rozin (1982) reported that an odour that is presented orthonasally, is not as identifiable when presented retronasally.

Chartier (2012) suggested that as much as 80-90 % of the taste of food comes from the nose, explaining why food tastes of nothing much when a person has a head cold, thus providing support for the importance of olfactory input to the enjoyment of food and drink. Because gustation and olfaction are anatomically and physiologically distinct entities, taste and smell are considered as two modalities that may process the inputs independent of one another.

Cross-modal sensory interactions also occur between complex mixtures of tastes and aroma and can result in larger interaction effects compared to those that occur for mixtures of single tastes and aroma, as reported for enhancement in citrus flavour (Hewson, Hollowood, Chandra and Hort, 2008). Saenz-Navajas, Campo, Fernández, Valentin and Ferreira (2010) showed that the addition of volatile fruity extracts brought about a decrease in astringency and bitterness and an increase in sweet perception in white wines. Astringency and bitterness were also inversely related to the intensity of fruity aroma.

The addition of sucrose has been found to increase perceived aroma intensity in model solutions (Pfeiffer, Hollowood, Hort and Taylor, 2005). Hewson *et al.*, 2008 showed flavour perception of a citrus flavoured model beverage system was increased on addition of tastetants (sugars and acids), although, glucose showed a different profile (fructose appeared to sustain this enhancement effect over a wider concentration range than glucose) to fructose despite equi-sweet levels being used. Both addition of glucose and fructose enhanced perceived citrus flavour intensity (Hewson *et al*., 2008). This raises the intriguing possibility that glucose and fructose may differ not only in the make-up of the taste receptors they interact with, but at high concentrations, their binding may consequently trigger neuronal activation that ultimately results in the differing perceptual effects observed between the two monosaccharides. Both citric and lactic acids, in the absence of sugar, resulted in similar levels of perceived citrus flavour intensity. Citrus aroma is more congruent with sourness per se, and it could be postulated that acid of any taste quality could enhance flavour perception. It is therefore clear from this study that tastetants (sucrose, glucose, fructose and citric acid) can affect the enhancement of aroma intensity and taste-aroma interactions can be expected in the study using model grapefruit-like beverages as study material.

Veldhuizen, Siddique, Rosenthal and Marks (2018) reported in a study how lemon extract, sucrose, and citric acid, when presented separately and together, affected sweet, sour, and citrus flavours. Results showed that both lemon extract and citric acid increased the ratings of citrus and sour intensity. Lemon extract did not affect sweet, but citric acid did. The presence of citric acid significantly increased sweetness. Adding citric acid to water, sucrose, lemon, and sucrose plus lemon increased the mean ratings of sweetness, and each increase in sweetness was substantial except when adding citric acid to sucrose. None of the interactions of citric acid with the other flavorants was significant. The results further suggest that lemon flavour is complex, having citrus and sour qualities that may not be fully separable in perception.

In a study by Niimi, Eddy, Overington, Heenan, Silcock, Bremer and Delahunty (2014), it was found that cheese flavour intensity in a model solution containing aroma could be enhanced or suppressed depending on the tastetant added. Moreover, odours can enhance the perceived intensity of a taste when the mixture is composed of harmonious or congruent taste-odour pairs (Small, 2008). Such cross-modal enhancement has been described as odour-induced taste enhancement (Djordjevic, Zatorre and Jones-Gotman, 2004). Aromas cannot just enhance taste perception, but also decrease it, usually due to a lack of taste-smell congruency. Stevenson, Prescott and Boakes (1999) showed a decrease in sourness with increasing caramel notes. Sweetness perception has been reported to be enhanced by the addition of aromas related to sweet products, such as vanilla, caramel or fruity notes, even at subthreshold concentrations, due to the associations formed during previous exposures with complex stimuli (Boakes and Hemberger, 2012).

It is not known if the intensity of aroma compounds could suppress the detection of bitterness in grapefruit juice. It is necessary to investigate whether grapefruit aromas can suppress the bitterness in grapefruit.

Visual interactions with a food or beverage

Research demonstrated that changing the hue and/or intensity of the colour added to a food or beverage can influence the perceived identity and/or intensity of the flavour. While varying the colour intensity impacts the rated taste and flavour intensity in some studies, such a cross-modal effect was not always found (Spence, Levitan, Shankar and Zampini, 2010). The reason behind such mixed results may well be explained by different taste/flavour expectations that can sometimes be associated by different people with one and the same food colour. One of the most common observations has been that changing the hue of a drink changes the perceived flavour. Many people will, for example, say that a cherry-flavoured drink tastes of lime if coloured green while perceiving it to taste of orange if colour orange (Zampini, Wantling, Phillips and Spence, 2008). The influence of colour on food perception may be due to learned associations between specific colours and particular flavours. Maga (1974) reported that green significantly increased sweet sensitivity, while both yellow and green colours decreased sour sensitivity. Cross-modal interactions resulted from a colour cue setting up an expectation concerning the likely identity and intensity of the food or drink's taste or flavour by influencing olfactory qualities of food, oral-somatosensory attributes of food, and or the overall flavour perception (Spence *et al*., 2010). Fernández-Vázquez, Hewson, Fisk and Hort (2014) reported in a study how slight variations in orange juice hue (reddish to greenish hues) affect the perceived flavour intensity, sweetness, and sourness, and the expected and actual liking of orange juice. People are less accurate in identifying the odour of a food or beverage when the colour is inappropriate (or incongruent) than when it is appropriate (Zellner, 2013). On the other hand, people consistently match certain odours that they have come across before with certain colours, such as the odour of cucumber with the colour green, or the odour of strawberry with a pinkish-red colour, thus suggesting the existence of relatively stable crossmodal associations between odours and colours (Zellner, 2013). Even wine experts are heavily influenced by colour in their judgements of wine (Marrot, Brochet and Dubourdieu, 2001). Sugrue and Dando (2018) reported in a study how both colour of the cider and label significantly influences the perception and hedonic responses to the ciders. Zampini *et al*. (2008), reported in a study on multisensory flavour perception by assessing the influence of fruit acids and colour cues on the perception of fruit-flavoured beverages. The taster status of fourteen participants (9 females and 5 males; mean age of 36 years; range from 22 to 58 years) was evaluated using 6-n-propylthiouracil (PROP) filter paper strips. It resulted in 4 of the participants, classified as non-tasters, 5 as medium tasters, and 5 as supertasters. Thirty beverages were formulated using flavour (blackcurrant, orange, or flavourless), colour of the solution (yellow, grey, orange, red, or colourless), and fruit acids (present vs. absent). Participants had to identify the flavour of the beverages. Zampini *et al*., (2008), found that supertasters were significantly less influenced by the inappropriate colouring of a beverage than were medium tasters who, themselves, were less influenced than were the non-tasters. The results show that the modulatory effect of visual cues on peoples' flavour identification responses is more pronounced in non-tasters than in medium tasters who in turn showed more of an influence of visual cues on their flavour identification responses than did the supertasters.

Spence, Wan, Woods, Velasco, Deng, Youseff and Deroy (2015), showing a summary of published study results. The basic tastes (bitter, sweet, sour, salty and possibly also umami), are in some way associated with particular colours and the crossmodal correspondences can be seen in Table 4. The participants in these studies were either given the names of one of the basic tastes and had to pick a matching colour or else rate how well (or badly) the colour matched a given taste.

Table 4 Summary of results of published studies showing crossmodal correspondences between colours and basic tastes (Spence, Wan, Woods, Velasco, Deng, Youseff and Deroy, 2015)

Study	O'Mahony (35)	Heller (24)	Koch and Koch (28)	Tomasik-Krótki and Strojny (65)	Wan *et al.* (71)
Number of participants	51	2,000	45	519	452
Origin of participants	USA (CA)	Germany	USA (OR)	17 countries	4 countries
Type of participants	University students	Cross-section of the public	Students	High school and university students	Internet recruits
Black	(Bitter)	Bitter	(Bitter)	-	Bitter
Blue		Salty		Salty	
Green	(Bitter)	Sour	Sour	Sour (bitter)	Sour
Orange		Sweet	Sweet	Sweet	
Pink	-	Sweet	-	-	Sweet
Red	Sweet	Sweet	Sweet	Sweet	
Violet		Bitter		Bitter/umami	-
White	Salty	Salty	Salty	-	Salty
Yellow	Sour	Sour	Sour	Sour	
Brown		Bitter		-	
Grey		Salty	-	-	

The participants in these studies were either given the names of one of the four or five basic tastes and had to pick a matching colour or else rate how well (or badly) the colour matches a given taste. The strongest crossmodal correspondences are shown, while weaker correspondences appear in brackets.

Note: Dashes denote the fact that this colour was not tested in this study. Note also that not all of the colour options are shown for every study

Colour plays a definite role in flavour perception but no information was found on the effect of colour on grapefruit flavour perception. It is clear that there is a definite cross-modal interaction between colour and taste. The effect of colour on acceptability and liking of the grapefruit juices needs to be investigated. It may be that the red juice will be more acceptable as it gives the perception of sweetness.

2.5 Tasting and smelling disorders

Any loss in your sense of smell and taste can have a negative effect on your quality of life. It can also be a sign of more serious health problems (Naka, Riedl, Luger, Hummel and Mueller, 2010). Mattes, Cowart, Schiavo, Arnold, Garrison, Kare and Laowry (1990) reported that chemosensory disorders were frequently associated with decreases in food acceptability. It further report that although dietary responses to these dysfunctions varied greatly, patients with distorted or phantom smell and/or taste sensations tended to report weight loss whereas those with simple sensory loss were more likely to report weight gain.

2.5.1 Smell Disorders

People who have a smell disorder either have a decrease in their ability to smell or changes in the way they perceive odours (Razani, Davidson and Murphy, 1996). Hyposmia is a reduced ability to detect odours (Cowart, Flynn-Rodden, McGeady and Lowry, 1993). Anosmia is the complete inability to detect odours. In rare cases, someone may be born without a sense of smell, a condition called congenital anosmia (Gains, 2010). Parosmia is a change in the normal perception of odours, such as the smell of something familiar is distorted, or something that normally smells pleasant now smell foul (Landis, Frasnelli, Croy and Hummel, 2010). Phantosmia is the sensation of an odour that isn't there (Landis, Croy and Haehner, 2012).

2.5.2 Taste Disorders

The most common taste disorder is phantom taste perception, a lingering, often unpleasant taste even though there is nothing in your mouth (Naik, Shetty and Maben, 2010). People can also experience a reduced ability to taste sweet, sour, bitter, salty, and umami, a condition called hypogeusia (Kamel, 2004). Ageusia is when people can't detect any tastes. True taste loss, however, is rare. Mostoften, people are experiencing a loss of smell instead of a loss of taste (Welge-Lüssen, Dőrig, Wolfensberger, Krone and Hummel, 2011). Dysgeusia is a condition in which a foul, salty, rancid, or metallic taste sensation persist in the mouth. Dysgeusia is sometimes accompanied by burning sensation of the mouth (Naik, Shetty and Maben, 2010).

2.5.3 Genetics of flavour perception

People show differences in their ability to detect many flavours (Hayes *et al*., 2013). Each person lives in a unique flavour world, and part of these differences lies in their genetic composition, especially related to sensory receptor genes (Reed *et al*., 2006).

2.5.4 Taste genetics

Perception of taste may vary between individuals depending on genetic variations in certain taste receptor genes. Yang and Hort (2018) reported in a review the latest developments linking genetic variation with taste perception (sweet, bitter, salty, sour and fat) and food preferences. Genetically determined variation in taste sensitivity in human subjects was reported for four of the basic tastes: sweet (Mainland and Matsunami, 2009), bitter (Kim, Wooding, Ricci, Jorde and Drayna, 2005), sour (Wise, Hansen, Reed and Breslin, 2007) and umami, the savoury taste exemplified by the taste of monosodium glutamate (Shigemura, Shirosaki and Sanematsu, 2009). The five basic tastes are being sensed by taste-specific receptor cells (TRCs), which are clustered together in the taste buds on the tongue (Efeyan, Comb and Sabatini, 2015). Of the five basic tastes, sweet, umami and bitter taste are sensed through activation of G-protein-coupled receptors (GPCRs) (Lefkowitz, 2013). Sourness and saltiness are transduced via ion channel proteins (Wise *et al.*, 2007).

Sweet and umami tasting substances activate defined combinations (heterodimers) of the taste receptor type 1 (*TAS1R*). The combination *TAS1R2-TAS1R3* senses sweet, whereas the combination *TAS1R1-TAS1R3* detects umami (Kitagawa, Kusakabe, Miura, Ninomiya and Hino, 2001). Individual variation in the perception of bitter taste is a common human trait (Drewnowski and Gomez-Carneros, 2000) that reflects the rich allelic diversity in *TAS2R* receptors. Genetics play a significant role in the perception of different taste qualities, accounting for over 30 % of the variance in sweetness, sourness, and bitterness (Knaapila, Hwang, Lysenko, Duke, Fesi, Khoshnevisan, James, Wysocki, Rhyu and Tordoff, 2012). The perception of sweetness has been weakly-to-moderately correlated with perception of bitterness (Lim, Urban and Green, 2008), however, whether this association is due to shared genes has not been determined in humans.

2.5.4.1 Bitter taste

Bitter taste detection evolved as a warning against toxin ingestion (Meyers and Brewer, 2008). Bitterness in food tends to trigger an innate negative response or aversion (Drewnowski, Henderson, Hann, Berg and Ruffin, 2000) and can be detected at low levels (Tepper, 2008) to protect against accidental ingestion of potential toxins, even in small amounts. Bitter-tasting substances activate receptors of the *TAS2R* family, which in humans consists of 25 members (Chandrashekar, Mueller, Hoon, Adler, Feng, Guo and Ryba, 2000), encoded by cluster of genes located on chromosomes 5, 7 and 12 (Shi, Zhang, Yang and Zhang, 2003), which appear to have evolved from gene duplication (Kaji, Karaki, Fukami, Terasaki and Kuwahara, 2009). Bitter receptors may also play a role in detection in other areas of the body, and have been found in the nasal passageways (Finger, Bottger, Hansen, Anderson, Alimohammadi and Silver, 2003; Tizzano, Gulbransen, Vandernbeuch, Clapp, Herman, Sibhatu, Churchill, Silver, Kinnamon and Finger, 2010) and in the gut (Wu, Rozengurt, Yang, Young, Sinnett-Smith and Rozengurt, 2002), although the consequences of these extra-oral receptors are still poorly understood. Variation has been observed in a number of bitter receptor genes, and in general, the variation observed in bitter receptors is higher than in most other genes (Kim, Wooding and Riaz, 2006), with a total of 151 non-synonymous single-nucleotide polymorphisms (SNPs) combinations or haplotypes identified within the members of the *TAS2R* gene family (Kim *et al*., 2006).

The area of bitterness sensitivity is the most extensively researched of all the taste qualities. According to Fast, Duffy and Bartoshuk (2002), it has been known since 1931 that some people are insensitive to the bitter compound phenylthiocarbamide (PTC), a chemical that was synthesized by Arthur Fox for making dyes. While he was working in his laboratory, Fox accidentally tasted the compound and found it bland, yet when his benchmate also accidentally tasted the compound, he found it very bitter. Individual differences in recognition threshold are partially determined by alleles at a putative bitter receptor gene on chromosome 7 (*TAS2R38*), but it is not known to what extent these alleles determine sensitivity to bitter compounds within the same chemical class or to other taste qualities (Bufe, Breslin, Kuhn, Reed, Tharp, Slack, Kim, Drayna and Meyerhof, 2005). *TAS2R38*, a receptor for the thiourea compounds, PTC and 6-n-propylthiouracil (PROP), explains most of the variance in human bitter taste. Both bitter compounds, PTC and PROP, contain a thiourea chemical group (N – C = S) and are recognized to a degree by the receptor encoded by the *TAS2R38* gene (Turnbull and Matisoo-Smith, 2002) and is considered modestly restrictive as this receptor also responds to compounds without the N – C = S motif (Meyerhof, Batram, Kuhn, Brockhoff, Chudoba, Bufe, Appendino and Beherens, 2010). PROP is now more commonly used in taste perception studies, as PTC has a slightly sulfurous odour (Reed *et al*., 2006) and it has been reported to be toxic in mice (St. John, Pour and Boughter, 2005).

Genetic variation in sensitivity to PTC and PROP is the most-studied bitter-taste phenotype in humans (Tepper, 2008). PROP-related differences in chemosensory perception have been shown to influence food preferences which are the primary determinants of food selection and dietary behaviour (Hayes *et al*., 2011). Through this mechanism, PROP status is thought to play an important role in defining body composition and nutritional status (Tepper, 2008).

Individuals can be defined as tasters or non-tasters based on their ability to discriminate threshold concentrations of PROP from plain water. In 1992, the phrase 'supertasters', in relation to bitter taste perception was coined (Bartoshuk, Fast, Karrer, Marino, Price and Reed, 1992) following the research in PROP perception, when it was discovered that the 'taster' group could be further divided into 'medium tasters' and supertasters of PROP, resulting in three groups of tasters: supertasters, medium tasters and non-tasters, depending on the perceived intensity of PROP, making up 20, 50 and 30 % of the population, respectively (Tepper, White, Koelliker, Lanzara and d'Adamo, 2009). On the basis of sensitivity to thiourea, the human population can be phenotypically classified into three categories: insensitive, sensitive, and hypersensitive to bitterness. The variance of this distribution is explained by the haplotypes generated by three polymorphisms in the *TAS2R38* gene accounting for 55-85 % of the variance in PTC sensitivity (Kim, Jorgenson, Coon, Leppert, Risch and Drayna, 2003). Supertasters perceive PROP as intensely bitter, and generally, also dislike the taste. When tested with suprathreshold (i.e. below threshold) concentrations of this compound, tasters can be further divided into those who are very sensitive, i.e. PROP supertasters, and those who are moderately sensitive, i.e. medium tasters (Barthoshuk, Duffy and Miller, 1994). It has been shown that PROP tasters are more sensitive to the bitterness of Brassica vegetables, as well as to other bitter-tasting foods, such as dark chocolate and beverages, as well as grapefruit juice and coffee, which do not contain the N – C = S moiety (Sandell and Breslin, 2006).

Variation in bitter taste perception has been linked to a preference for many different foods. Although PROP itself is not found in nature, PROP-related compounds occur in many fruit and vegetables and bitter tasting foods, including the brassica family of vegetables which contain glucosinates, and are hydrolysed to isothiocyanates (Vig, Rampala and Thinda, 2009). Isoflavones are bitter-tasting phenolic compounds found in soya and green tea, which might also taste bitter to PROP-sensitive individuals (Akella, Henderson and Drewnowski, 1997). Therefore, PROP-sensitive individuals may be more sensitive to the bitter taste of certain 'healthy' foods with known chemo-preventative effects (Vig *et al*., 2009), which may affect their preferences, and ultimately their health status (Keller, Steinmann, Nurse and Tepper, 2002).

The bitter taste phenotype is a complex trait not only expressed by *TAS2R38* but also by other genes (Roudnitzky, Behrens, Engel, Kohl, Thalmann and Hubner, 2015). Mutations in *TAS2R31* and *TAS2R43* (to a lesser extent) may be responsible for individual responses to the bitter aftertaste of saccharin and acesulfame-k (Roudnitzky, Bufe, Thalman, Gunn, Xing, Crider, Behrens, Meyerhof and Wooding, 2011). Genetic variation in the *TAS2R19* gene has also been associated with quinine bitterness (Reed, Zhu, Breslin, Duke, Henders, Campbell, Montgomery, Medland, Martin and Wright, 2010), grapefruit liking and bitterness differences among caucasians (Hayes *et al*., 2013; Duffy, Hayes, Sullivan and Faghri, 2009).

Perception of the bitter taste of grapefruit and other plant products

Structurally very different molecules can act as ligands for the bitter taste receptors, including both naturally occurring plant metabolites and synthetic compounds. The bitter-tasting substances contained in the diet include polyphenols, alkaloids, flavonoids, terpenoids, and thiol compounds (Behrens, Reichling, Batram, Brockhoff and Meyerhof, 2009). Grapefruit are a good source of polyphenols (Alam, Subhan, Rahman, Uddin, Reza and Sarker, 2014). Ligands have been described for most of the human *TAS2R*s (Thalman, Beherens and Meyerhof, 2013). More than 550 ligands for bitter receptors have been identified (Wiener, Shudler, Levit and Niv, 2011). However, this number represents only a tiny fraction of the thousands of plant-based bitter compounds that exist in nature. Since the number of compounds greatly exceeds the number of receptors, it seems likely that individual receptors respond to more than one bitter compound type (Behrens and Meyerhof, 2006). In fact, some receptors are narrowly-tuned, responding to a limited range of compounds. *TAS2R8* is an example of a highly-selective receptor that has only three known ligands which share common structural properties. On the opposite end of the spectrum are *TAS2R*10, -14 and -46 which are highly promiscuous, responding to 50 % of the bitter compounds applied in cell-based expression studies.

A number of studies as reviewed by Drewnowski, Henderson and Barratt-Fornell (1998) have shown no correlation between taster status and food preferences, while many others have reported links between taster status and preference for and/or consumption of various fruit and vegetables. Lower preference has been reported in PROP tasters (both medium tasters and super tasters) for citrus fruit (Drewnowski *et al*., 1998) and this group appears to consume less fruit in general than nontasters.

The extreme differences among individuals in the perception of the bitter compound (PROP) and structurally related compounds are due almost entirely to genetic variation of the bitter receptor *TAS2R38*, which accounts for 50-80 % of the PROP phenotype (Behrens, Gunn, Ramos, Meyerhof and Wooding, 2013; Bachmanov, Bosak, Lin, Matsumato, Ohmoto and

Reed, 2014). The remaining 20-50 % could be explained by other genetic or non-genetic factors (Lipchock, Reed and Menella, 2011).

There are three well-known single nucleotide polymorphisms (SNPs) in TAS2R38: rs713598 (C/G), rs1726866 (C/T), and rs10246939 (G/A). These SNPs encode for non-synonymous amino acid substitutions at position 49 (alanine/proline, A49P), 262 (valine/alanine, V262A), and 296 (isoleucine/valine, I296V) respectively (Drayna, 2005). While several haplotypes have been observed, the two most common are PAV (proline-alanine-valine at amino acid positions 49, 262 and 296 respectively), the 'taster' form, and AVI (alanine-valine-isoleucine at amino acid positions 49, 262 and 296 respectively), the non taster form (Drayna, 2005; Campbell, Ranciaro, Froment, Hirbo, Omar, Bodo and Breslin, 2012). By way of explanation, a 'haplotype' is the order of genetic variants along each chromosome; in the above example, 'AVI' is one haplotype and 'PAV' is another). An intriguing observation is that heterozygotes (people with one taster and one nontaster form of the receptor) can differ markedly in taste sensitivity. PAV homozygotes exhibit greater sensitivity to PTC/PROP bitterness, while AVI homozygotes are less sensitive (Bufe *et al.*, 2005). Heterozygotes (PAV/AVI) show intermediate bitter taste sensitivity (Caln, Padiglia and Zonza, 2011). Other haplotypes namely Alanine-Alanine-Valine (AAV), Alanine-Alanine-Isoleucine (AAI), Proline-Alanine-Isoleucine (PAI), Proline-Valine-Isoleucine (PVI) have been observed rarely (1-5 %). Mostly in combinations with PAV or AVI as heterozygotes and very infrequently as homozygotes in specific populations, such as from Africa (Hayes, Bartoshuk, Kidd and Duffy, 2008). Haplotypes (AAV, AAI, and PVI) that convey intermediate PROP/PTC response magnitudes have been rarely observed or limited to specific populations (Wooding, Kim, Bamshad, Larsen and Jorde, 2004).

Genetic variation in the *TAS2R19* gene on chromosome 12 (Hayes, Feeney, Nolden and McGeary, 2015) has also been associated with quinine bitterness, grapefruit liking and bitterness differences among caucasians (Hayes *et al.*, 2013; Duffy *et al.*, 2009). Bitter compounds (limonin and naringin) in grapefruit, do not activate TAS2R19 in vitro, however Hayes *et al*. (2015), observed strong linkage disequilibrium (LD) between TAS2R19 and TAS2R31 SNP's.

Drewnowski, Henderson and Shore (1997), reported in a study that increased taste acuity for both PROP and naringin was associated with a greater dislike for each bitter compound. PROP supertasters disliked bitter naringin solutions significantly more than did either tasters or non-tasters. PROP sensitivity was also associated with reduced acceptability of grapefruit juice. Acceptability of orange juice, which does not contain naringin, was unrelated to PROP taster-status (Drewnowski *et al*., 1997). No difference in sensitivity to denatonium, limonin, or quinine as a function of PROP status was found for either gender (Cubero-Castillo and Noble, 2004).

A variant in a cluster of bitter receptors on chromosome 12 was associated with quinine perception (Reed *et al*., 2010), and the bitterness of some high-intensity sweeteners was associated with alleles within a cluster of bitter receptors on chromosome 12 (Roudnitzky *et al*., 2011). Several reports showed that the perception of bitter taste is related not only to the specific taste of bitter compounds but also to the wide behaviour spectrum of the individual in relation to food choices (Tepper, 2008). PROP hypersensitive individuals had a more restricted diet, compared to sensitive or insensitive individuals (Duffy and Barthoshuk, 2000).

Fungiform papillae are one of the three types of papilla structures which house the tastebuds on the tongue. The bitter *TAS2R38* gene has been suggested to influence fungiform papillae density, as PROP sensitivity has been generally reported as positively correlated with fungiform papillae density (Essick, Chopra, Guest and McGlone, 2003) and thus tasters of PROP may exhibit higher densities of trigeminal (touch) fibres on the tongue than nontasters.

2.5.4.2 Sweet taste

The sweet taste receptor consists of two proteins, *TAS1R2* and *TAS1R3,* which form a heterodimer (Boughter and Bachmanov, 2008). Genetic studies suggest that people vary in their liking for sweetness (Reed *et al*., 2006). The variation is not well understood, but is likely to be due, at least in part, to allelic variation in the sweet receptor *TAS1R3* (Fushan, Simons, Slack, Manichaikul and Drayna, 2009). Perception of sweetness and sweet liking for highly concentrated solutions differ among people. For some individuals, as sweetness intensity increases, liking increases, with an eventual plateau for liking if the concentration is sufficiently high enough; for others, as intensity increases, liking may decrease (Kim, Prescott and Kim, 2017). The liking or dislike for high-intensity sweeteners (rather than sugar) may be due to their off-tastes; in fact, alleles in bitter receptors partially account for person-to-person differences in how these non-sugar sweeteners are perceived (Roudnitzky *et al*., 2011).

To get a better understanding of the variation seen in our response to sweet, we need to determine the relative contribution of our ability to detect and how intensely we perceive the taste of sweeteners versus our actual preferences for them. Additionally, we must consider that sweet taste perception and preferences can vary both between individuals and in the same individual over a period of time (Reed and McDaniel, 2006).

2.5.5 Aroma genetics

Odour perception is also influenced by genotypes of olfactory receptors (Pelchat, Bykowski, Duke and Reed, 2010). It might be expect from knowledge of bitter taste that differences in the human ability to smell certain compounds relate to variation in genes that encode odourant receptors. However, unlike the taste receptor families, the odourant receptor gene family is very large. Humans have nearly 400 potentially functional olfactory receptor genes, making this gene family one of the largest in the human genome (Niimura and Nei, 2003). The large number of olfactory receptors reflects the need to detect a wider variety of compounds than in the case of taste. The ability to smell some odourants is heritable, for other odourants, it is not. For instance, the ability to smell food odours like chocolate or lemon is associated with little or no heritability (Tuorila, 2007). However the pleasantness of cinnamon is heritable and has been mapped to chromosome 4 by linkage analysis (Knaapila, Keskitalo, Kallela, Wessman, Sammalisto and Hiekkalinna, 2007). Individual variation in perception of some odours has been attributed partly to specific olfactory receptors (OR) genes. The difference among people in their ability to smell androstenone is at least partially determined by genes (Knaapila *et al*., 2012) and an allele of an OR gene, OR7D4, contributes to this trait (Keller, Zhuang, Chi, Vosshall and Matsunami, 2007). However, unlike alleles of the taste receptor gene *TAS2R38*, which account for almost 70 % of person-to-person variation in perception of bitter taste from PTC (Kim *et al*., 2003). The OR7D4 alleles, account for only a small amount of variance in perception of androstenone (Keller *et al*., 2007). Two other OR genes have been associated with individual variation in the sense of smell: OR11H7 with isovaleric acid (sweaty odour) (Menashe, Abaffy, Hasin, Goshen, Yahalom, Luetje and Lancet, 2007) and OR2M7 with the smell of asparagus metabolites in urine (Eriksson, Macpherson, Tung, Hon, Naughton, Saxonov, Avey, Wojcicki, Peer and Mountain, 2010). Association between the gene OR2J3 and detection of cis-3-hexen-1-ol (green leaf odour) has also been suggested (Jaeger, McRae, Salzman, Williams and Newcomb, 2010). Many olfactory receptors combine to detect a particular odourant (Malnic, Hirono and Sato, 1999), and one odourant may stimulate many receptors, so if one is not working, others may compensate. Repeated exposures to individual odourants have been demonstrated to lower detection threshold (increased sensitivity) to these odourants, suggesting that genes do not entirely determine the perceptions (Dalton, Doolitle and Breslin, 2002).

It may be that humans, like other primates, began losing functional odourant receptors during the development of tricolour vision when the sense of sight began to dominate (Gilad, Przeworski and Lancet, 2004).

2.6 Concluding remarks

The bitterness of grapefruit fruit and juice can restrain its consumption. Naringin is the main compound responsible for this undesirable attribute. Some might find grapefruit bitter, but not find beer, coffee or chocolate bitter. It appears that there may be genetic differences in bitter sensitivity between individuals. Understanding the numerous intermodal and cross-modal interactions that occur among sensory qualities in the grapefruit fruit or juice is also essential to understanding the role of the human senses in perception and acceptance of grapefruit that vary in taste, colour and aroma sensory properties.

Red colour may be appears to decreased caffeine bitterness while yellow and green had no such effect. Sucrose suppressed the bitterness of urea, caffeine and propylthiouracil (PROP). Volatile fruity extracts brought about a decrease in astringency and bitterness and an increase in sweet perception in white wines. It is not known if colour (red or yellow), sucrose sweetness and the intensity of aroma compounds could suppress the perception of bitterness in grapefruit juice by effecting the cross-modal perception. No literature was found on studies investigating intermodal or cross-modal sensory interaction in grapefruit.

Perception of taste may vary between individuals depending on genetic variations in certain taste receptor genes. Individual differences in recognition threshold are partially determined by alleles at a putative bitter receptor gene on chromosome 7 (*TAS2R38*), but it is not known to what extent these alleles determine sensitivity to bitter compounds in grapefruit. The extreme differences among individuals in the perception of the bitter compound (PROP) and structurally related compounds are due almost entirely to an allele of the bitter receptor *TAS2R38*. PROP supertasters disliked bitter naringin solutions significantly more than did either tasters or non-tasters. PROP sensitivity was also associated with reduced acceptability of grapefruit juice. Genetic variation in the *TAS2R19* gene has been associated with quinine bitterness, grapefruit liking and bitterness differences among Caucasians. No study could be found reporting on the bitter taste sensitivity and bitter receptor genes of African females.

Insights provided by this research will be of benefit to the citrus processing industry. It will help to better understand the effect of grapefruit colour, flavour, aroma and the cross-modal interactions between them as well as the consumer's bitter sensitivity and bitter receptor genes on the perception and acceptance of grapefruit. This wil make it possible to improve its consumption.

CHAPTER 3:
Hypotheses and objectives

3.1 Hypotheses

- The sensory properties of grapefruit-based model solutions with varying grapefruit aroma, sucrose and/or naringin concentrations and colour type (red versus yellow) will vary according to multifactorial linear functions. Perceived bitterness of the grapefruit juice models will drive consumer's dislike but bitterness perception will be a function of cross-modal aroma-taste, colour-taste and sweet taste-bitter taste interactions.
    - Sweet-tasting compounds, odourants and textures have been employed by the pharmaceutical and food industries to mask bitterness and improve the taste properties, and thus improve the acceptance of these bitter beverages and food (Gaudette and Piclering, 2013).
    - Cross-modal aroma-taste interaction between vanilla and sucrose have been reported in skim milk, indicating vanilla aroma does enhance perceived sweetness (Wang, Hayes, Ziegler, Roberts and Hopfer, 2018).
    - Aroma could mask the perception of taste (Boakes and Hemberger, 2012).
    - Both colour of the cider and label significantly influenced the perception and hedonic responses to ciders (Sugrue and Dando, 2018).
    - The addition of sweeteners to vegetables reduces the bitterness that many consumers find unpleasant (Wilkie, Capaldi,, Phillips and Wadhera, 2013). Sucrose can reduce the bitterness of bitter substances (Nakamura, Tanigake, Miyanaga, Ogawa, Akiyoshi, Matsuyama and Uchida, 2002). Consumer perception for the bitterness of grapefruit juice decreased with increased sugar/acid ratio (Fellers, Carter and De Jager, 1988).
    - Lemon extract, sucrose, and citric acid, when presented separately and together, affected perception of sweet, sour, and citrus flavours (Veldhuizen *et al*., 2018).
    - The naringin content in red grapefruit is significantly lower than in white grapefruit (Wanwimolruk and Marquez, 2006). Red colour decreased bitter taste sensitivity of caffeine water solution with yellow and green colours having no effects (Maga, 1974).
- Genetic variation among individual respondents will have an effect on liking ratings for model grapefruit beverages that vary in flavour. Consumers with higher PROP sensitivity would be associated with a greater dislike for grapefruit-like beverages with higher bitterness and this will be related to their *TAS2R38* receptor genetic profile. Consumers' *TAS2R19*

receptor genetic profile would be associated with a greater dislike for grapefruit-like beverages.

- Genetic variation can be linked to taste perception (sweet, bitter, salty, sour and fat) and food preferences (Yang and Hort, 2018).
- Perceptual differences based on PROP taster status (PTS) extent to sensitivity to other bitterants and are also associated with individual food preferences (Pickering, Haverstock and DiBattista, 2006).
- Taste responses to naringin, a flavonoid, and the acceptance of grapefruit juice are related to genetic sensitivity to 6-n-propylthiouracil (Drewnowski *et al.*, 1997).
- The rs10772420 SNP was previously associated with the remembered liking of grapefruit juice (Duffy *et al*., 2009), with homozygotes reporting greater liking. The rs10772420 SNP was also associated with responses to sampled unsweetened grapefruit juice, with homozygotes reporting less bitterness and greater liking (Hayes *et al*., 2011).

3.2 Objectives

- To determine the effect of varying the bitterness, sweetness, colour and aroma intensity of a grapefruit-like model beverage on the cross-modal perception of sensory properties and consumer liking of the beverage.
- To determine the sensory drivers of liking of the grapefruit-like beverages.
- To determine the effect of sensitivity to bitter taste [as determined through propylthiouracil (PROP) taster classification] and genetic variation in *TAS2R38* and *TAS2R19* SNP genotypes in a group of young African females, on hedonic ratings for the flavour of grapefruit-like beverages differing in bitter/sweet taste intensity.

CHAPTER 4:
The Formulation and Physico-chemical Composition of the Grapefruit-like Beverages for the study

4.1 Abstract

Grapefruit juice can be yellow, pink or red in colour and is an excellent source of many nutrients and phytochemicals including vitamin C, folic acid, potassium, flavonoids, pectin, pigments and limonoids. Grapefruit juice and grapefruit consumption have potential health benefits, but it can be too bitter which can lead to some dislike. Naringin is by far the most dominant flavonoid bitter principle in grapefruit. In many populations, even among people who know the nutritional value, the consumption is often very low. The aim of this phase of the study was to develop grapefruit-like model beverages that could be used to determine the reasons for the liking or disliking of the sensory properties. To assess the effects of cross-modal interaction of sensory properties on consumer preferences for grapefruit juice and to determine the effects of the acid, flavourant, sweet, bitter and colour components of the grapefruit-like beverages on each other. The grapefruit-like model beverages were prepared with deionized apple juice concentrate as a stock base, using export requirements for fresh grapefruit to Japan and Europe as °Brix and acid guideline. The deionized apple juice base concentrate (acidified with citric acid) were modified using a factorial design with three naringin concentrations (158 mg/kg, 315 mg/kg and 473 mg/kg) x 3 sucrose concentrations (8, 10 and 12 °Brix) x 2 colours (yellow and red) x 2 mixed aroma compounds intensities (2.5 and 10.0 mg/kg of a cocktail consisting of caryophyllene, citral, nootkatone, octanal, nonanal and decanal). Different stock concentrates (containing the total required naringin, colours, aroma and sucrose) were mixed and diluted with water to get 36 different single strength grapefruit-like beverages. The °Brix, citric acid % and °Brix/acid ratio were determined. Reverse-phase HPLC was used to determine the % sugars (glucose, fructose, sucrose, lactose, maltose, galactose, trehalose) and the % sugar alcohols (mannitol and sorbitol) in the beverages. The colour was expressed as CIE L* a* b* values. The required composition of the 36 single strength grapefruit-like beverages showed that stock concentrates containing the concentration of each factor can be mixed to result in an accurately prepared set of single strength grapefruit-like beverages. Sucrose was inverted after exposure to citric acid. Mannitol and sorbitol were formed by the reduction of fructose. The bitter, sweet, aroma and colour factors had significant effects on the

°Brix, acidity, °Brix/acid ratio, sucrose, fructose, glucose and L* a* b* colour values of the grapefruit-like beverages. The physico-chemical composition of all the beverages was determined.

4.2 Introduction

Grapefruit (*Citrus paradisi* Macfadyen) is an important cultivar of the *Citrus* genus which contains a number of nutrients beneficial to human health (Castro-Vazquez, Alaňón, Rodrĭguez-Robledo, Pérez-Coello, Hermosĭn-Gutierrez, Dĭaz-Maroto, Jordăn, Galindo and Arroyo-Jiménez, 2016). Grapefruit juice can generally be found in three different varieties. The colour, red, pink and white (or yellow), depends on the presence (or absence) of lycopene (Rao and Rao, 2007; Rao, Ray and Rao, 2006). The Star Ruby, red grapefruit variety cultivated in South Africa accounted for 79 % of the total trees planted in South Africa (Citrus Growers Association, 2013). It was followed by 16 % Marsh, white grapefruit and 3 % Rose, pink grapefruit and 2 % other grapefruit cultivars. Grapefruit is a rich source of vitamin C, folic acid, potassium, flavonoids, pectin, pigments and limonoids (Manners, 2007; Ladaniya, 2008). Naringin was found to be the major flavonoid in grapefruit followed by narirutin and hesperidin (Ross, Ziska, Zhao and Elsohly, 2000). As in all citrus, the grapefruit also contains the bitter limonoid, limonin and together with naringin it imparts a bitter taste to grapefruit (Lee and Kim, 2003). By consuming grapefruit and grapefruit juice, individuals not only will obtain essential nutrients but also will consume health-beneficial flavonoids. In many populations, even among people who know the nutrition value, the consumption is however often very low (Nestle, Wing, Birch, DiSogra, Drewnowski, Middleton, Sigman-Grant, Sobal, Winston and Economos, 1998).

Naringin is by far the most dominant flavonoid bitter principle in grapefruit (Ho, Saville, Coville and Wanwimolruk, 2000). Grapefruit juice has a naringin content of 242.63 mg/kg to 386.45 mg/kg (Pichaiyongvongdee and Haruenkit, 2009). In South Africa, grapefruit packed for export to Japan and Europe needs to have a minimum °Brix of 8.0 and acidity of 1.33 % citric acid % (Dr. G.J. Begemann, Manager Process Quality, Golden Frontier Citrus, personal communication). Nootkatone, octanal, nonanal, decanal dodecanal, octyl acetate, citronellyl acetate, citral, and carvone have been reported in grapefruit oil at low levels (± 1.0 %) and these compounds were indicated as the major contributors to the aroma (Sawamura and Kuriyama, 1988; Ortuno *et al*., 1995; Wilson and Shaw, 1980). The aldehydes (primarily octanal and decanal), plus ß-caryophyllene, which is known for its 'woody or spicy' aroma, contribute significantly to the overall flavour profile (Cheong, Liu, Zhou, Curran and Yu, 2012). High-performance liquid chromatographic (HPLC) methods to analyse grapefruit showed three sugars (sucrose, glucose, fructose) and four organic acids (oxalic, citric, ascorbic and malic acid). The major organic acid in grapefruit is citric acid and sucrose is present in the largest amount of sugars (Kelebek, 2010). Hydrolysis of sucrose results in invert sugar, which is a mixture of equal amounts of glucose and fructose (Chinachoti, 1995). In the commercial production of invert sugars, using acids, enzymes and temperature, sucrose is hydrolyzed to invert into glucose and fructose monomers (L'Homme, Arbelot, Puigserver and Biagini, 2003). Lowering the pH of the solution by addition of an acid increases the concentration of protons in the solution and therefore increases the rate of the hydrolysis (Clark, Edye and Eggleston, 1997). Acid hydrolysis of sucrose, however, is not only dependent on pH but also on temperature and sucrose concentration as well (Wienen and Shallenberger, 1988).

The aim of this part of the research was to formulate the experimental material (a set of 36 single strength grapefruit-like beverages) for the next phase where the effects of factors contributing to the flavour of grapefruit juice on consumer preferences could be determined. The second objective was to determine if stock concentrates containing the concentration of each factor can be mixed to result in a set of single strength grapefruit-like beverages. The third objective was to determine the effect of naringin level, sucrose level, aroma mixture level, colour type and their interactions on the physico-chemical composition of a model grapefruit-like beverage.

4.3 Materials and methods

4.3.1 Experimental

4.3.1.1 Reagents

Deionized apple juice base concentrate was procured from Cape Fruit Processors (Pty) Ltd, Malelane, South Africa, citric acid anhydrous and naringin from Protea Chemicals (Pty) Ltd, Johannesburg South Africa, sunset yellow, ponceau red and quinoline yellow colours from Sensient Colours, Johannesburg, South Africa, sucrose from Selati Sugars, Malalane, South Africa. The grapefruit aroma compounds caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), aldehyde C10 (decanal) were procured from Clive Teubes (Pty) Ltd, Johannesburg, South Africa.

4.3.1.2 Preparation of the grapefruit-like beverages

The target specification range for the grapefruit-like beverages was set at 8.5 °Brix and acidity 1.33 % citric acid and a resulting °Brix/acid ratio of 6.59. A base of deionized apple juice concentrate with a 70.60 °Brix and acidity of 0.05 % was used to prepare 36 single strength grapefruit-like beverages. Since naringin is the most pronounced flavonoid bitter principle in grapefruit, it was decided to use naringin as a bitter component in the model grapefruit-like beverage in the study. Citric acid was used to adjust the acidity of the deionized juice base concentrate (Figure 6) to get an acidified deionized juice base with 68.00 °Brix (8.5 °Brix times 8) and acidity of 10.64 % (1.33 % times 8) which was modified in a 3 (naringin) x 2 (aroma) x 2 (colour) x 3 (sucrose) factorial design to get 36 grapefruit-like beverages (Table 5).

The acid adjustment of the deionized apple juice base was calculated to increase the acidity of 0.05 % to the target acidity 10.64 % and it was done by using the following formula:

$$x\ citric\ acid\ (g) = \frac{\%\ citric\ acid\ in\ target - \%\ citric\ acid\ in\ apple\ juice\ base}{100} \times mass\ apple\ juice\ concentrate$$

$$x\ citric\ acid\ (g) = \frac{10.64\ \% - 0.05\ \%\ x\ 17400.00\ g = 1842.66\ g\ citric\ acid}{100}$$

The °Brix of the deionized apple juice base increased with the addition of anhydrous citric acid (100 °Brix) and water addition was needed to reduce the °Brix to the target °Brix but it also reduced the acidity percentage below the target. A quantity of 1842.66 g citric acid was added to compensate for this resulting in a deionized apple juice base with 73.42 °Brix and acidity 10.88 %.

The amount of water needed was calculated using Pearson's Square (Mullan, 2008) in the following formula.

$$\text{mass of water to be added (g)} = \frac{\text{mass of apple juice concentrate (desired °Brix} - \text{measured °Brix)}}{\text{°Brix of water} - \text{desired °Brix}}$$

$$\text{mass of water to be added (g)} = \frac{19284.42\ (68.68 - 73.42) = 1330.93\ \text{g water}}{0 - 68.68}$$

Final water addition resulted in a deionized apple juice base with 68.68 °Brix and acidity of 10.87 %.

Deionized apple juice base concentrate (17400.00 g),°Brix (Refractometer) = 70.60 °Brix, Acidity % w/w = 0.05% w/w

← 1884.42 g Citric Acid

Deionized apple juice base concentrate with citric Acid (19284.42 g), °Brix (Refractometer) = 73.42 °Brix, Acidity % w/w = 10.88% w/w

← 1330.93g Water

*Deionized apple juice base concentrate (20615.35 g), °Brix (Refractometer) = 68.68 °Brix, Acidity % w/w = 10.87% w/w

naringin												aroma**											
158 mg/kg				315 mg/kg				473 mg/kg				2.5 mg/kg						10 mg/kg					
9[1]	18	27	36	8[2]	17	26	35	7[3]	16	25	34	25[4]	26	27	34	35	36	7[5]	8	9	16	17	18
6[9]	15	24	33	5[10]	14	23	32	4[11]	13	22	31	22[12]	23	24	31	32	33	4[13]	5	6	13	14	15
3[17]	12	21	30	2[18]	11	20	29	1[19]	10	19	28	19[20]	20	21	28	29	30	1[21]	2	3	10	11	12

colour												sucrose											
Yellow***						Red****						8 °Brix				10 °Brix				12 °Brix			
8[6]	16	18	26	34	36	7[7]	9	17	25	27	35	9[8]	18	27	36	8	17	26	35	7	16	25	34
4[14]	6	14	22	24	32	5[15]	13	15	23	31	33	6[16]	15	24	33	5	14	23	32	4	13	22	31
2[22]	10	12	20	28	30	1[23]	3	11	19	21	29	3[24]	12	21	30	2	11	20	29	1	10	19	28

8 °Brix	Formula for mixing 8 °Brix G-LB with the four factors (naringin, aroma, colour, sucrose) 538.48 g (4 x 134.62 g = 4 factors) DAJB + 3800 g water = 4338.48 g G-LB, °Brix (Refractometer) = 8.49 °Brix, Acidity % w/w = 1.32% w/w
10 °Brix	Formula for mixing 10 °Brix G-LB with the four factors (naringin, aroma, colour, sucrose) 538.48 g (4 x 134.62 g = 4 factors) DAJB + 96.41 g sucrose + 3800 g water = 4434.89 g G-LB
12 °Brix	Formula for mixing 12 °Brix G-LB with the four factors (naringin, aroma, colour, sucrose) 538.48 g (4 x 134.62g = 4 factors) DAJB + 197.20 g sucrose + 3800 g water = 4535.68 g G-LB

[1]134.62 g D A J B - 158 mg/kg nar in 4338.48 g G-L B	4	[9]134.62 g D A J B - 158 mg/kg nar in 4434.89 g G-L B	4	[17]134.62 g D A J B - 158 mg/kg nar in 4535.68 g G-L B	4
[2]134.62 g D A J B - 315 mg/kg nar in 4338.48 g G-L B	4	[10]134.62 g D A J B - 315 mg/kg nar in 4434.89 g G-L B	4	[18]134.62 g D A J B - 315 mg/kg nar in 4535.68 g G-L B	4
[3]134.62 g D A J B - 473 mg/kg nar in 4338.48 g G-L B	4	[11]134.62 g D A J B - 473 mg/kg nar in 4434.89 g G-L B	4	[19]134.62 g D A J B - 473 mg/kg nar in 4535.68 g G-L B	4
[4]134.62 g D A J B - 2.5 mg/kg aroma in 4338.48 g G-L B	6	[12]134.62 g D A J B - 2.5 mg/kg aroma in 4434.89 g G-L B	6	[20]134.62 g D A J B - 2.5 mg/kg aroma in 4535.68 g G-L B	6
[5]134.62 g D A J B - 10 mg/kg aroma in 4338.48 g G-L B	6	[13]134.62 g D A J B - 10 mg/kg aroma in 4434.89 g G-L B	6	[21]134.62 g D A J B - 10 mg/kg aroma in 4535.68 g G-L B	6
[6]134.62 g D A J B - Yellow colour in 4338.48 g G-L B	6	[14]134.62 g D A J B - Yellow colour in 4434.89 g G-L B	6	[22]134.62 g D A J B - Yellow colour in 4535.68 g G-L B	6
[7]134.62 g D A J B - Red colour in 4338.48 g G-L B	6	[15]134.62 g D A J B - Red colour in 4434.89 g G-L B	6	[23]134.62 g D A J B - Red colour in 4535.68 g G-L B	6
[8]134.62 g D A J B as is	12	[16]134.62 g D A J B as is plus sucrose	12	[24]134.62 g D A J B as is plus sucrose	12

*Deionized apple juice base concentrate needed for 144 x 134.62 g samples
**Carryophylene, Citral, Nootkatone, Aldehyde C8 (Octanal), Aldehyde C9 (Nonanal), Aldehyde C10 (Decanal)
***0.00125% Yellow solution (Quanolene Yellow)
****0.001% Red solution (30% Sunset Yellow and 70% Ponceua red)

DAJB = Deionized apple juice base concentrat   nar = naringin
G-LB = Grapefruit-like beverages

12 x 8 °Brix G-LB (Table 1) combinations | 12 x 10 °Brix G-LB (Table 1) combinations | 12 x 12 °Brix G-LB (Table 1) combinations

1 to 8 8 °Brix stock concentrates | 9 to 16 10 °Brix stock concentrates | 17 to 24 12 °Brix stock concentrates

134.62 g weight quantities used in the preparation of the complete set of grapefruit-like beverages

Figure 6 The preparation of 36 grapefruit-like beverages by using a 3 (bitterness) x 3 (sweetness) x 2 (aroma) x 2 (colour) factorial design

Table 5 Factorial design for the 36 grapefruit-like beverages for this study

Numbers*	Code**	Bitter***	Sweet****	Aroma*****	Colour
1	LMHR	158 mg/kg	10 °Brix	10 mg/kg	Red#
2	MMHR	315 mg/kg	10 °Brix	10 mg/kg	Red
3	HMHR	473 mg/kg	10 °Brix	10 mg/kg	Red
4	LHHR	158 mg/kg	12 °Brix	10 mg/kg	Red
5	MHHR	315 mg/kg	12 °Brix	10 mg/kg	Red
6	HHHR	473 mg/kg	12 °Brix	10 mg/kg	Red
7	LLHR	158 mg/kg	8 °Brix	10 mg/kg	Red
8	MLHR	315 mg/kg	8 °Brix	10 mg/kg	Red
9	HLHR	473 mg/kg	8 °Brix	10 mg/kg	Red
10	LMLR	158 mg/kg	10 °Brix	2.5 mg/kg	Red
11	MMLR	315 mg/kg	10 °Brix	2.5 mg/kg	Red
12	HMLR	473 mg/kg	10 °Brix	2.5 mg/kg	Red
13	LHLR	158 mg/kg	12 °Brix	2.5 mg/kg	Red
14	MHLR	315 mg/kg	12 °Brix	2.5 mg/kg	Red
15	HHLR	473 mg/kg	12 °Brix	2.5 mg/kg	Red
16	LLLR	158 mg/kg	8 °Brix	2.5 mg/kg	Red
17	MLLR	315 mg/kg	8 °Brix	2.5 mg/kg	Red
18	HLLR	473 mg/kg	8 °Brix	2.5 mg/kg	Red
19	LMHY	158 mg/kg	10 °Brix	10 mg/kg	Yellow##
20	MMHY	315 mg/kg	10 °Brix	10 mg/kg	Yellow
21	HMHY	473 mg/kg	10 °Brix	10 mg/kg	Yellow
22	LHHY	158 mg/kg	12 °Brix	10 mg/kg	Yellow
23	MHHY	315 mg/kg	12 °Brix	10 mg/kg	Yellow
24	HHHY	473 mg/kg	12 °Brix	10 mg/kg	Yellow
25	LLHY	158 mg/kg	8 °Brix	10 mg/kg	Yellow
26	MLHY	315 mg/kg	8 °Brix	10 mg/kg	Yellow
27	HLHY	473 mg/kg	8 °Brix	10 mg/kg	Yellow
28	LMLY	158 mg/kg	10 °Brix	2.5 mg/kg	Yellow
29	MMLY	315 mg/kg	10 °Brix	2.5 mg/kg	Yellow
30	HMLY	473 mg/kg	10 °Brix	2.5 mg/kg	Yellow
31	LHLY	158 mg/kg	12 °Brix	2.5 mg/kg	Yellow
32	MHLY	315 mg/kg	12 °Brix	2.5 mg/kg	Yellow
33	HHLY	473 mg/kg	12 °Brix	2.5 mg/kg	Yellow
34	LLLY	158 mg/kg	8 °Brix	2.5 mg/kg	Yellow
35	MLLY	315 mg/kg	8 °Brix	2.5 mg/kg	Yellow
36	HLLY	473 mg/kg	8 °Brix	2.5 mg/kg	Yellow

*36 Beverage numbers. **Code: 1st letter = naringin concentration (High, Medium, Low); 2nd letter = sucrose concentration (High, Medium, Low); 3rd letter = aroma concentration (High, Low); 4th letter = colour (Red or Yellow). ***Bitter naringin concentration. ****Sweet value in °Brix due to sucrose; *****Aroma blend = Caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), aldehyde C10 (decanal). #Red colour blend = 0.001 % Red solution (30 % Sunset Yellow and 70 % Ponceau Red); ##Yellow colour blend = 0.0125 % Yellow solution (Quinoline Yellow)

4.3.2 Methods

The following physico-chemical analyses were conducted on the deionized apple juice base concentrates as well as on the resulting grapefruit-like beverages:

4.3.2.1 Total soluble solids (°Brix), Titratable acidity (TA) and °Brix/acid ratio

Total soluble solids were determined with the Atago refractometer (Model RX-5000α, Atago Co. Ltd, Japan) and the results expressed in °Brix. Titratable acidity (TA) expressed as % was assessed by titrating samples with 0.1 N NaOH to the phenolphthalein endpoint of pH 8.1 measured with a pH meter (Model 702 SM Titrino, Metrohm Herisau, Switzerland) (fruitandnuteducation.ucdavis.edu/files/162035). The acid corrected °Brix was determined by adding the acid correction factor (obtained from an acid correction factor table) to temperature-compensated °Brix measured by a refractometer (Kimball, 1999). After determining the titratable acidity and calculating the acid corrected °Brix the °Brix/acid ratio was calculated by dividing the acid corrected °Brix value by titratable acidity.

4.3.2.2 Starch and pectin

Starch and pectin content of the acidified and unacidified Deionized apple juice base concentrates as well as the grapefruit-like beverages samples were tested qualitatively with the iodine test (Tajchakavit, Boye and Couture, 2001) and the alcohol test (Girard and Fukumoto, 1999), respectively. Each analysis was carried out in triplicate.

4.3.2.3 Sugars (sugars and sugar alcohols)

An internal method used by RCL Foods, Malalane, South Africa was used for the analysis of the sugars. Sucrose, glucose, fructose, lactose, maltose, galactose, trehalose, and sugar alcohols mannitol and sorbitol concentrations in the acidified and unacidified deionized apple juice base concentrates as well as the grapefruit-like beverages were determined using HPLC (High-performance liquid chromatography). The HPLC system consisted of a Waters 1525 binary HPLC pump and an ERC 7515A refractive index detector. All the solvents and chemicals used during this assay were of analytical grade.

The grapefruit-like beverage samples for the analysis of sucrose, glucose and fructose were filtered through a 0.2 µm PTFE (polytetrafluoroethylene) syringe filter prior to HPLC injection. The separation was accomplished by means of a high-performance carbohydrate (30 cm x 0.78 mm i.d.) column. The column temperature was set at 30°C. Breeze ™ software was used to monitor the separation process and after analysis, a chromatogram was obtained. The injection volume for all samples was 20 µl with the analysis conducted at a flow rate of 0.5 ml/min. The mobile phase consisted of 80 % acetonitrile and 20 % deionized water.

Pure standards of sucrose, glucose and fructose were purchased from Sigma Aldrich (St. Louis, USA). The sugar standards were prepared in 60 % acetonitrile and 40 % deionized water at concentrations of 2 %, 7 %, 9 % sucrose and 1 %, 4 %, 7 % glucose and 1 %, 4 %, 7 % fructose, respectively. Standards of 20 µl aliquots were chromatographed singularly and as mixtures by injection into the HPLC system. Standard calibration curves were obtained for each sugar by plotting peak areas versus concentrations. Regression equations that showed a high degree of linearity ($R^2 > 0.995$) were obtained for each sugar from the calibration curves. Sugars in the juice samples were identified by comparing the retention time of the unknown with those of the standard sugars. The analysis of lactose, maltose, galactose, trehalose, and sugar alcohols mannitol and sorbitol concentrations in the acidified and unacidified deionized apple juice base concentrates as well as the grapefruit-like beverages was determined by the analytical lab SGS (SGS, Johannesburg, South Africa) using the WI-AN-007 method (SGS, Johannesburg, South Africa).

4.3.2.4 Colour

Colour of the beverages was measured using a Minolta Colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The instrument was calibrated using a white reference tile. The 36 grapefruit-like beverages were individually poured into a glass cuvet. The cuvet was rinsed with distilled water in between samples. Colour values L, a, b were recorded as the mean of three triplicate recordings. Colour values were expressed as CIE L* a* and b*, where L* shows (whiteness or brightness/darkness), a* shows (redness/greenness) and b* shows (yellowness/blueness).

4.3.2.5 Absorbance and clarity

Both the deionized apple juice base concentrates with and without citric acid was diluted with distilled water and standardize to 11 °Brix. The absorbance and clarity were measured at 420 nm and the clarity was measured at 625 nm using a Metertek SP-839 spectrophotometer (Metertek Inc., Nangang, Taipei, Taiwan).

4.3.3 Statistical analysis

Data obtained in the study were expressed as means ± standard deviations for duplicate and triplicate measurements.

The attributes were analysed as for a completed random design with 36 treatment combinations (3 naringin x 3 sucrose x 2 aroma x 2 colour levels), testing for differences between all main effects and all their interactions using analysis of variance (ANOVA). The residuals from the analysis were acceptable for ANOVA. Means were compared using Fisher’s least significant difference test at the 5 % level. Data were analysed using the statistical program GenStat® [VSN International Ltd (VSNi), Hemel Hempstead, United Kingdom].

4.4 Results and discussion

4.4.1 Effect of the addition of citric acid on the physico-chemical composition of the deionised apple base concentrate

The sucrose concentration of the original deionised apple base concentrate at 1.25 g/100 g as seen in Table 6 reduced to 0.00 g/100 g after the addition of citric acid, possibly due to inversion. However, this is strange as one would expect an increase in both fructose and glucose after citric addition but fructose showed a reduction from 6.15 g/100 g (before citric acid addition) to 4.85 g/100 g after citric addition and glucose also showed a reduction after citric addition from 3.00 g/100 g to 2.55 g/100 g. In the study 8.94 % citric acid were add to 70.60°Brix deionized applejuice concentrate at ambient temperature. Shalaev, Qun, Shalaeva, Zografi (2000) showed in a study sucrose, colyophilized with an acid such as citric acid at a weight ratio of 1:10 citric acid:sucrose, undergoes significant acid-catalyzed inversion at 50°C despite the very low levels of residual water, i.e., > 0.1 % w/w. Hydrolysis of the disaccharide sucrose resulted in invert sugar, which is a mixture of equal amounts of glucose and fructose (Chinachoti, 1995). The splitting of sucrose is a hydrolysis reaction because one molecule of water is needed to break the reactant, in this case sucrose, into two products. The hydrolysis can be induced by using acids, enzymes and temperature (L'Homme *et al*., 2003). No cleavage occurs initially when sucrose is dissolved in water. The cleavage can be catalyzed by adding a catalyst. Lowering the pH of the solution by addition of an acid increases the concentration of protons in solution and therefore increases the rate of the hydrolysis (Clarke *et al*., 1997). Dwivedi, Singh, Sehra, Pandey, Sangwan and Mishra (2018) performed a study by processing wet Kinow mandarin (*Citrus reticulata)* fruit waste into novel Brønsted acidic ionic liquids and their application in hydrolysis of sucrose. Excellent yields of D-fructose and D-glucose were shown after sucrose hydrolysis by using the obtained Brønsted acidic ionic liquids at 85-95°C for 6 h. Both the acidified and unacidified deionized apple juice samples were however, stored below 20°C. The HPLC method use in the analysis of the apple juice samples used a column temperature of 30°C, which is a standard method used in analysis of sugars. There was also a slight increase in sorbitol from 0.60 g/100 g to 0.75 g/100 g and mannitol stayed the same at less than 0.50 g/100 g because the analysis method could not express exact values less than 0.50 which mean there could be a slight decrease in mannitol although it can only be express if less than 0.50. The presence of invertase enzymes residues in the apple juice were not tested.

The fate of the reduced fructose, glucose and reactions on sorbitol and mannitol concentrations is still unknown and needs further research.

Tests to determine the starch and pectin present in the deionized apple juice base concentrate without and with citric acid addition showed negative results. Both the tests were negative because it's not expected to found either starch or pectin present in the apple juice base concentrate after deionize and clarification process. The mechanism of enzymatic clarification of apple juice during the manufacturing of deionized apple includes the processes to hydrolyze pectin with pectinases and starch with amylases and cellulose with cellulases and hemicellulases (Höhn, Sun and Nolle, 2004).

Table 6 Effect of the addition of citric acid (8.94 %) on the physico-chemical composition of deionized apple juice base concentrate

	Without citric acid addition	**With citric acid addition**
°Brix (Refractometer)	70.60[1] (0.07)	70.70 (0.04)
Acidity as Citric Acid % w/w[2]	0.05 (0.01)	9.95 (0.01)
Acidity/°Brix ratio	1530.90 (204.91)	6.58 (0.04)
Sucrose g/100 g at 11.5 °Brix (RCL Foods Method[3])	1.25 (0.07)	0.00 (0.00)
Fructose g/100 g at 11.5 °Brix (RCL Foods Method)	6.15 (0.07)	4.85 (0.07)
Glucose g/100 g at 11.5 °Brix (RCL Foods Method)	3.00 (0.00)	2.55 (0.21)
Lactose g/100 g at 11.5 °Brix (WI-AN-007 Method[4])	< 0.5	< 0.5
Maltose g/100 g at 11.5 °Brix (WI-AN-007 Method)	< 0.5	< 0.5
Galacose g/100 g at 11.5 °Brix (WI-AN-007 Method)	< 0.5	< 0.5
Trehalose g/100 g at 11.5 °Brix (WI-AN-007 Method)	< 0.5	< 0.5
Manitol g/100 g at 11.5 °Brix (WI-AN-007 Method)	< 0.5	< 0.5
Sorbitol g/100 g at 11.5 °Brix (WI-AN-007 Method)	0.60 (0.14)	0.75 (0.09)
Absorbance at 11.5 °Brix A420 nm	0.03 (0.00)	0.02 (0.00)
Clarity (% Transmission) at 11.5 °Brix T625 nm	99.90 (0.00)	99.87 (0.06)
Starch	Negative	Negative
Pectin	Negative	Negative

[1]Values are means (± standard deviation)
[2]unit % weight/weight
[3]Internal Method used by RCL Foods, Malalane, South Africa
[4]Based on AOAC 982.14 (HPLC)

4.4.2 Physico-chemical characterisation of the 36 grapefruit-like beverages

The p-values of the effect of naringin level, sucrose level, aroma mixture level, colour type and their interactions on the °Brix, acidity, °Brix/acid ratio, sucrose, fructose and glucose content and L* a* b* colour values of model grapefruit-like beverages are shown in Table 7. Naringin level had a significant effect on the °Brix and L* a* b* colour values. The addition of naringin resulted in a white cloudiness in the beverages witch could have had an effect on the colour values. Since naringin is a natural source of polyphenol (Kaur, Singh, Singh, Schwarz and Puri, 2010), one can expect haziness to occur. Organic compounds such as proteins, polyphenols and carbohydrates (α-glucans, β-glucans) are known to form haze (Steiner, Becker and Gasti, 2012). Sucrose level resulted in significant differences in °Brix, acidity, °Brix/acid ratio and sucrose content L and a and the b colour value. It was expected for sucrose level to have a significant effect on sucrose content on the beverages. The increase in °Brix in Hamlin orange variety was attributed to a concurrent increase in sucrose content (Echeverria and Ismael, 1987). As a result of the increase in °Brix and a decrease in acidity, the °Brix/acid ratio increased during storage for Hamlin orange variety. Adding a aroma compound mixture resulted in significant differences in acidity, sucrose and in the L* and a* colour values of the beverages.

The type of colourant added resulted in significant differences in acidity and in the L* and a* and B* colour values of the beverages. It seems that there was a difference in acidity between the yellow and red colourants which were not quantified. The type of colourant (red versus yellow) added resulted in obvious significant differences in the L* a* b* colour values. The red beverages had higher a* values while the yellow beverages had higher L* and b* values.

The physico-chemical characterisation of the 36 grapefruit-like beverages are shown in Table 8. Significant differences in Brix ($F[35:5271.42] = 8.07$, $p < 0.001$) were shown between the 36 Grapefruit-like beverage samples which were expected due to the use of three levels sucrose and naringin. Sugars and acids, together with small amounts of dissolved vitamins, fructans, proteins, pigments, phenolics, and minerals, are commonly referred to as soluble solids (Chope, Terry and White, 2006). Total soluable solids can be measured using either a Brix scale hydrometer or a refractometer and reported as "degrees Brix" (°Brix) which is equivalent to percentage (%). In principle, the unit °Brix, which has been in common use in industry for many years, represent the dry substance content of solutions containing mainly sucrose (Dongare, Buchade, Awatade and Shaligram, 2014). For example,a juice sample that has 25 degree of Brix is assumed to contain 25 g of sugar/100 g of solution (Ball, 2006). This is however, not true in fruit and vegetables because sugars are not the only components contributing to total soluble solids and soluble solid content. Although the term 'Brix' is frequently used interchangeably with total soluble solids and soluble solid content, 'Brix' technically refers only to the sugar content of fruit juices. Considering that sugars (sucrose, glucose and fructose) and sugar alcohols (e.g. sorbitol and mannitol) constitute the majority (approximately 85 %) of total soluble solids in many fruits, it is therefore not surprising that both terms have become synonymous. However, this does not hold true for fruit such as limes, in which sugars constitute only 25 % of the total soluble solid content (Wardowski, Grierson and Westbrook, 1979). Titratable acidity show significant differences ($F[35:72.04] = 0.0017$, $p < 0.001$) between the samples. Different amounts of sucrose and naringin were added that changed the citric acid concentration responsible for the acidity value.

°Brix/acid Ratio showed significant differences ($F[35:2692.19] = 6.42$, $p < 0.001$) between the samples. This was expected as both the Brix and titratable acidity differed significally between the samples. The perception of taste in fruit and juices may be affected by other factors such as titratable acidity (Saftner, Polashock, Ehlenfeldt and Vinyard, 2008). The empirical Brix/acid ratio, found by dividing the acid-corrected and temperature-corrected Brix by the % titratable acidity w/w as citric acid (B/A ratio), is one of the most commonly used indicators of juice quality as well as fruit maturity (Kimball, 1999). Significant differences in sucrose were found between the samples which were expected as different amounts of sucrose were added to the different grapefruit-like samples. Both fructose ($F[35:1.06] = 0.0117$, $p = 0.436$) and glucose ($F[35:0.99] = 0.0032$, $p = 0.509$) did not show significant differences between the samples which were expected as nothing of both were added during the formulation/preparation of the grapefruit-like samples. Significant differences were shown by the L colour value ($F[35:103.73] = 1.3241$, $p < 0.001$), a colour value ($F[35:4103.01] = 0.0000$, $p < 0.001$) and b colour value ($F[35:498.76] = 0.2900$, $p < 0.001$)

Table 7 The p-values of the effect of naringin level, sucrose level, aroma mixture level, colour type and their interactions on the °Brix, acidity, °Brix/acid ratio, sucrose, fructose, glucose and L* a* b* colour values of model grapefruit-like beverages

Factor	°Brix	% Titratable Acidity w/w	°Brix/acid Ratio	Sucrose	Fructose	Glucose	L[1]	a[1]	b[1]
Naringin level	< 0.05	0.308	0.422	0.691	0.943	0.547	< 0.001	< 0.001	< 0.001
Sucrose level	< 0.001	< 0.001	< 0.001	< 0.001	0.039	0.270	0.003	0.016	< 0.001
Aroma mixture level	0.060	0.001	0.317	0.022	0.583	0.964	< 0.001	< 0.001	0.071
Colour type	0.285	< 0.001	0.052	0.078	0.583	0.452	< 0.001	< 0.001	< 0.001
Naringin x sucrose	0.001	0.011	0.672	0.046	0.383	0.140	< 0.001	0.265	< 0.001
Naringin x aroma	0.526	0.082	0.726	0.894	0.277	0.122	0.804	0.072	0.101
Sucrose x aroma	0.359	0.247	0.149	0.090	0.266	0.140	0.085	< 0.001	0.005
Naringin x colour	0.108	0.053	0.945	0.899	0.408	0.776	< 0.001	< 0.001	< 0.001
Sucrose x colour	0.108	0.197	0.559	0.418	0.054	0.205	0.104	< 0.001	0.738
Aroma x colour	0.017	0.329	0.246	0.018	0.831	0.700	< 0.001	< 0.001	0.235
Naringin x sucrose x aroma	0.844	0.961	0.924	0.872	0.408	0.610	0.085	0.584	< 0.001
Naringin x sucrose x colour	0.279	0.199	0.969	0.632	0.488	0.724	0.081	< 0.001	< 0.001
Naringin x aroma x colour	0.382	0.484	0.900	0.727	0.736	0.722	0.948	0.085	0.821
Sucrose x aroma x colour	0.160	0.608	0.187	0.129	0.390	0.144	0.014	0.342	< 0.001

[1]L* a* b* colour values

Table 8 Physico-chemical characterisation of the 36 grapefruit-like beverages

No[1]	Sample[2]	°Brix	% Titratable Acidity[3] w/w	°Brix/acid Ratio[4]	Sucrose[5]	Fructose[5]	Glucose[5]	L	a	b
1	LMHR	10.80b (0.01)	1.26cd (0.01)	8.75b (0.05)	1.54cd (0.03)	4.20b (0.11)	2.75a (0.13)	25.20ijkl (0.02)	2.28bc (0.01)	-0.26l (0.03)
2	MMHR	10.79b (0.02)	1.26cd (0.01)	8.77b (0.07)	1.52cd (0.00)	4.44ab (0.06)	2.82a (0.01)	24.87lmn (0.05)	2.17def (0.06)	-0.23ijkl (0.02)
3	HMHR	10.81b (0.02)	1.27bc (0.01)	8.74b (0.04)	1.46d (0.02)	4.43ab (0.08)	2.87a (0.01)	24.63n (0.07)	1.84jk (0.01)	-0.43o (0.01)
4	LHHR	12.74a (0.01)	1.23e (0.01)	10.58a (0.05)	3.52b (0.07)	4.31ab (0.01)	2.72a (0.04)	25.16ijklm (0.03)	2.43a (0.06)	-0.09fg (0.00)
5	MHHR	12.80a (0.04)	1.23e (0.07)	10.91a (0.64)	3.67ab (0.25)	4.36ab (0.28)	2.83a (0.13)	25.13jklm (0.03)	2.19cde (0.04)	-0.26l (0.02)
6	HHHR	12.80a (0.03)	1.23e (0.01)	10.57a (0.06)	3.62ab (0.03)	4.33ab (0.14)	2.79a (0.02)	24.81mn (0.20)	2.02gh (0.04)	-0.36mno (0.03)
7	LLHR	8.83c (0.01)	1.29a (0.01)	7.03c (0.04)	0.00e (0.00)	4.46ab (0.15)	2.84a (0.01)	24.95klmn (0.95)	2.59a (0.27)	-0.17hij (0.04)
8	MLHR	8.83c (0.00)	1.29a (0.01)	7.03c (0.03)	0.00e (0.00)	4.42ab (0.12)	2.79a (0.09)	25.15ijklm (0.31)	2.17def (0.03)	-0.24jkl (0.03)
9	HLHR	8.78c (0.05)	1.29a (0.01)	7.01c (0.09)	0.00e (0.00)	4.45ab (0.11)	2.82a (0.01)	25.35ghij (0.06)	1.97hi (0.03)	-0.38no (0.02)
10	LMLR	10.78b (0.06)	1.26cd (0.00)	8.76b (0.05)	1.63cd (0.06)	4.35ab (0.07)	2.78a (0.03)	25.15ijklm (0.30)	2.25bcd (0.07)	-0.14gh (0.07)
11	MMLR	10.76b (0.04)	1.25cd (0.08)	8.78b (0.81)	1.63cd (0.01)	4.41ab (0.04)	2.79a (0.04)	25.34ghij (0.02)	2.16def (0.02)	-0.25kl (0.01)
12	HMLR	10.77b (0.03)	1.26cd (0.00)	8.75b (0.03)	1.63cd (0.03)	4.35ab (0.07)	2.80a (0.12)	25.15ijklm (0.01)	1.81k (0.04)	-0.43no (0.02)
13	LHLR	12.73a (0.01)	1.23e (0.00)	10.54a (0.01)	3.71ab (0.01)	4.31ab (0.01)	2.79a (0.06)	25.51ghi (0.02)	2.31b (0.01)	-0.15ghi (0.01)
14	MHLR	12.92a (0.24)	1.23e (0.00)	10.54a (0.20)	3.74ab (0.49)	4.35ab (0.50)	2.81a (0.33)	25.42ghij (0.01)	2.12efg (0.05)	-0.27l (0.02)
15	HHLR	12.71a (0.03)	1.23e (0.00)	10.53a (0.02)	3.71ab (0.01)	4.27ab (0.02)	2.80a (0.03)	25.32ghij (0.05)	1.91ijk (0.02)	-0.39no (0.01)
16	LLLR	8.79c (0.03)	1.29a (0.01)	7.03c (0.04)	0.00e (0.00)	4.50ab (0.17)	2.83a (0.06)	25.57gh (0.04)	2.08fg (0.04)	-0.28lm (0.02)
17	MLLR	8.83c (0.02)	1.29a (0.01)	7.06c (0.04)	0.00e (0.00)	4.44ab (0.12)	2.81a (0.07)	25.23hijkl (0.07)	1.92ij (0.04)	-0.37no (0.02)
18	HLLR	8.78c (0.01)	1.29a (0.01)	7.03c (0.04)	0.00e (0.00)	4.35ab (0.02)	2.76a (0.04)	25.29hijk (0.02)	1.95hi (0.03)	-0.35mn (0.03)
19	LMHY	10.79b (0.00)	1.26cd (0.00)	8.77b (0.00)	1.63cd (0.05)	4.40ab (0.21)	2.76a (0.08)	26.60abc (0.06)	-0.20pqrs (0.03)	0.41ab (0.02)
20	MMHY	10.77b (0.01)	1.26cd (0.00)	8.75b (0.01)	1.64cd (0.31)	4.28ab (0.60)	2.76a (0.36)	26.59abc (0.01)	-0.23rs (0.02)	0.39abc (0.01)
21	HMHY	10.72b (0.05)	1.25cd (0.01)	8.76b (0.07)	1.64cd (0.00)	4.43ab (0.03)	2.81a (0.05)	26.19de (0.06)	-0.08m (0.01)	0.16e (0.01)
22	LHHY	12.77a (0.02)	1.23e (0.00)	10.58a (0.02)	3.67ab (0.11)	4.37ab (0.05)	2.75a (0.05)	26.63abc (0.06)	-0.25rs (0.01)	0.46a (0.02)
23	MHHY	12.76a (0.03)	1.23e (0.00)	10.57a (0.03)	3.79a (0.09)	4.36ab (0.07)	2.78a (0.01)	26.26cde (0.06)	-0.10mno (0.02)	0.20e (0.02)

Table 8 Physico-chemical characterisation of the 36 grapefruit-like beverages (*continue*)

No[1]	Sample[2]	°Brix	% Titratable Acidity[3] w/w	°Brix/acid Ratio[4]	Sucrose[5]	Fructose[5]	Glucose[5]	L	a	b
24	HHHY	12.75a (0.06)	1.23e (0.00)	10.56a (0.05)	3.70ab (0.06)	4.30ab (0.07)	2.77a (0.02)	26.29bcde (0.06)	-0.10mnop (0.01)	0.20e (0.02)
25	LLHY	8.81c (0.02)	1.29a (0.01)	7.05c (0.04)	0.00e (0.00)	4.44ab (0.10)	2.82a (0.02)	26.59abc (0.04)	-0.22rs (0.04)	0.38abc (0.03)
26	MLHY	8.82c (0.01)	1.29a (0.00)	7.04c (0.01)	0.00e (0.00)	4.33ab (0.15)	2.75a (0.06)	26.38abcd (0.05)	-0.16mnopqr (0.02)	0.29d (0.02)
27	HLHY	8.73c (0.10)	1.28ab (0.00)	7.02c (0.08)	0.00e (0.00)	4.52ab (0.03)	2.84a (0.04)	25.99ef (0.02)	0.02l (0.04)	-0.04f (0.01)
28	LMLY	10.81b (0.01)	1.26cd (0.00)	8.79b (0.00)	1.71c (0.08)	4.54a (0.04)	2.86a (0.05)	26.69a (0.08)	-0.23rs (0.03)	0.43a (0.04)
29	MMLY	10.78b (0.02)	1.25d (0.00)	8.83b (0.01)	1.62cd (0.06)	4.49ab (0.02)	2.88a (0.08)	26.42abcd (0.08)	-0.19nopqrs (0.02)	0.30d (0.00)
30	HMLY	10.77b (0.01)	1.25d (0.00)	8.82b (0.01)	1.60cd (0.02)	4.39ab (0.07)	2.81a (0.02)	25.67fg (0.01)	0.09l (0.02)	-0.17hijk (0.01)
31	LHLY	12.75a (0.03)	1.23e (0.01)	10.60a (0.03)	3.61ab (0.07)	4.30ab (0.09)	2.76a (0.02)	26.62abc (0.01)	-0.26s (0.03)	0.43a (0.01)
32	MHLY	12.72a (0.08)	1.23e (0.01)	10.57a (0.10)	3.73ab (0.17)	4.35ab (0.14)	2.83a (0.08)	26.45abcd (0.10)	-0.15mnopqr (0.02)	0.33bcd (0.04)
33	HHLY	12.81a (0.02)	1.23e (0.01)	10.58a (0.04)	3.67ab (0.01)	4.41ab (0.03)	2.79a (0.02)	26.29bcde (0.04)	-0.09mn (0.02)	0.20e (0.03)
34	LLLY	8.80c (0.01)	1.29a (0.00)	7.02c (0.01)	0.00e (0.00)	4.37ab (0.07)	2.77a (0.03)	26.63ab (0.04)	-0.21qrs (0.01)	0.38abc (0.01)
35	MLLY	8.80c (0.03)	1.28a (0.01)	7.06c (0.05)	0.00e (0.00)	4.26ab (0.02)	2.75a (0.00)	26.29bcde (0.05)	-0.12mnopq (0.02)	0.19e (0.01)
36	HLLY	8.71c (0.01)	1.28ab (0.00)	7.01c (0.01)	0.00e (0.00)	4.29ab (0.12)	2.70a (0.01)	26.52abcd (0.07)	-0.19opqrs (0.02)	0.32cd (0.03)

[1]Sample no [2]Sample code (refer to Table 1), Standard deviation in brackets, [3]Unit % weight/weight, [4]°Brix/Acidity, [5]Unit g/100 g, Mean values in a column with different letters are significantly different ($p < 0.05$)

The effect of naringin level, sucrose level, aroma mixture level, colour type and their interactions on °Brix, acidity and °Brix/acid ratio are shown in Table 9.

Sucrose resulted in significant differences ($p < 0.05$) in °Brix, acidity and the °Brix/acid ratio of beverages. The higher the sucrose concentration, the higher the resulting °Brix.

The variation of aroma levels resulted in significant differences ($p < 0.05$) between the acidity levels of the beverages. This is strange and unexpected. It seems that there was a difference in acidity between the aroma mixture levels which were not quantified. Colour type resulted in significant differences ($p < 0.05$) in acidity. There was a difference in acidity between the yellow and red colourants which were not quantified. Both the significant effects of aroma mixture content and colour type on the acidity might also be due to a mass balance effect.

The effect of varying naringin level, sucrose level, aroma mixture level and the addition of yellow and red colourants on fructose, glucose and sucrose content of grapefruit like beverages can be seen in Table 10. The difference in naringin level added did not result in significant differences in ($p > 0.05$) fructose, glucose or sucrose content of the grapefruit-like beverages. As expected sucrose level added resulted in significant differences ($p<0.05$) in sucrose content of the grapefruit-like beverages.

The level of aroma mixture added resulted in significant differences ($p < 0.05$) in sucrose content of the grapefruit-like beverages. This was not expected, although differences were statistically significant, it is in most cases negligible because the differences are so small and can therefore be largely ignored. As expected the type of colourant added did not have any significant effect on either the fructose, glucose or sucrose content of the beverages.

The effect of varying naringin level, sucrose level, aroma mixture level and the addition of yellow and red colourants and their interactions on L* a* b* colour values of grapefruit like beverages can be seen in Table 11. Almost all the main effects and their interactions caused significant differences ($p < 0.05$) in the colour values of the beverages. Polyphenolics provide a number of different functionalities in food including colour and astringency (Siebert, Troukhanova and Lynn, 1996). Agudelo, Barros, Santos-Buelga, Martinez-Navarrete and Ferreira (2017) reported naringin and narirutin to be the major phenolic compounds in grapefruit. Hydroxyl groups of phenolic compounds can bind with more than one polypeptide chain to link that leads to haze (Emmambux, 2004). Residues of polyphenol oxidase were not tested.

Polyphenol oxidase is important in the beneficial colouration of some of our foods such as prunes, dark raisins and teas. However in some cases, it is the damaging effect of enzymes in color deterioration (browning) of plant foods (Lozano, Drudis-Biscarri and Ibarz-Ribas, 1994). Enzymatic browning occurs in many fruit and vegetable tissues whenever they are injured. The injury can be the result of cutting, freezing or disease. The part of the injured fruit which is exposed to air undergoes a rapid darkening. This darkening reaction results from the polyphenol oxidase (PPO) catalyzed oxidation or phenolic compounds to O-quinones which subsequently polymerize to form dark-coloured pigments (El-Shimi, 1993).

Mailard reaction is often defined as nonenzymatic browning when foods are processed or cooked at high temperature, a chemical reaction occurs between amino acids and reducing sugars which generate different flavours and brown colour (Tamanna, Mahmood, 2015). Since the grapefruit-like beverages are stored at temperatures below 20°C, the possibility of colour changes due to the mailard reaction can be excluded.

The significant effects of sucrose level and aroma mixture level and their interactions on the colour values were unexpected. Although differences were significant these were small differences and can probably be largely ignored.

It was expected for the colour type to have a significant effect on the colour values of the beverages.

Table 9 Effects of varying concentrations of naringin, sucrose, aroma and addition of yellow and red colourants on mean °Brix, Acidity and °Brix/acid ratio values (± standard deviation) of grapefruit-like beverages

Main Effects		**°Brix**	**% Titratable Acidity w/w**	**°Brix/acid Ratio**
Naringin	158 mg/kg	10.783ab (1.633)	1.259a (0.026)	8.793a (1.467)
	315 mg/kg	10.782a (1.627)	1.258a (0.025)	8.798a (1.454)
	473 mg/kg	10.762b (1.664)	1.258a (0.023)	8.783a (1.468)
		*	NS	NS
Sucrose	8 °Brix	8.792c (0.048)	1.287a (0.006)	7.034c (0.042)
	10 °Brix	10.779b (0.035)	1.258b (0.007)	8.774b (0.045)
	12 °Brix	12.755a (0.051)	1.230c (0.004)	10.566a (0.050)
		**	**	**
Aroma	2.5 mg/kg	10.768a (1.630)	1.257b (0.024)	8.796a (1.452)
	10 mg/kg	10.783a (1.637)	1.260a (0.025)	8.787a (1.460)
		NS	**	NS
Colour	Yellow	10.771a (1.642)	1.257b (0.023)	8.800a (1.460)
	Red	10.779a (1.625)	1.260a (0.025)	8.782a (1.453)
		NS	**	NS
Naringin x sucrose	158 mg/kg x 12 °Brix	12.748a (0.024)	1.228c (0.004)	10.575a (0.032)
	315 mg/kg x 12 °Brix	12.749a (0.070)	1.230c (0.004)	10.560a (0.068)
	473 mg/kg x 12 °Brix	12.768a (0.052)	1.232c (0.004)	10.562a (0.048)
	158 mg/kg x 10 °Brix	10.795b (0.029)	1.261b (0.003)	8.769a (0.032)
	315 mg/kg x 10 °Brix	10.776b (0.023)	1.256b (0.008)	8.784a (0.055)
	473 mg/kg x 10 °Brix	10.767b (0.004)	1.258b (0.008)	8.770a (0.049)
	158 mg/kg x 8 °Brix	8.805c (0.021)	1.289a (0.005)	7.033a (0.031)
	315 mg/kg x 8 °Brix	8.821c (0.019)	1.258a (0.006)	7.050a (0.034)
	473 mg/kg x 8 °Brix	8.751d (0.059)	1.284a (0.007)	7.018a (0.054)
		**	*	NS
Aroma x Colour	2.5 mg/kg x yellow	10.773ab (1.661)	1.256a (0.024)	8.810a (1.477)
	10 mg/kg x yellow	10.769b (1.654)	1.258a (0.023)	8.790a (1.470)
	2.5 mg/kg x red	10.763b (1.631)	1.258a (0.024)	8.782a (1.454)
	10 mg/kg x red	10.796a (1.650)	1.262a (0.026)	8.783a (1.478)
		*	NS	NS

Mean values in a column for a specific section with different letters are significantly different
NS = not significant, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

Table 10 Effect of varying concentrations of naringin, sucrose, aroma and addition of yellow and red colourants on mean fructose, glucose and sucrose content (g/100 g) values (± standard deviation) of grapefruit-like beverages

Main Effects		Fructose	Glucose	Sucrose
Naringin	158 mg/kg	4.380a (0.121)	2.787a (0.059)	1.751a (1.517)
	315 mg/kg	4.387a (0.110)	2.805a (0.059)	1.766a (1.563)
	473 mg/kg	4.377a (0.091)	2.798a (0.052)	1.751a (1.538)
		NS	NS	NS
Sucrose	8 °Brix	4.402a (0.113)	2.791a (0.054)	0.000c (0.000)
	10 °Brix	4.406a (0.103)	2.812a (0.063)	1.593b (0.076)
	12 °Brix	4.334b (0.091)	2.787a (0.051)	3.676a (0.106)
		*	NS	**
Aroma	2.5 mg/kg	4.374a (0.100)	2.797a (0.056)	1.776a (1.532)
	10 mg/kg	4.388a (0.114)	2.796a (0.057)	1.737b (1.737)
		NS	NS	*
Colour	Yellow	4.388a (0.102)	2.792a (0.053)	1.771a (1.535)
	Red	4.374a (0.112)	2.802a (0.059)	1.741a (1.521)
		NS	NS	NS
Naringin x Sucrose	158 mg/kg x 12 °Brix	4.323a (0.049)	2.758a (0.043)	3.626a (0.094)
	315 mg/kg x 12 °Brix	4.352a (0.135)	2.814a (0.066)	3.727a (0.143)
	473 mg/kg x 12 °Brix	4.328a (0.080)	2.789a (0.021)	3.674a (0.045)
	158 mg/kg x 10 °Brix	4.374a (0.160)	2.788a (0.077)	1.627b (0.079)
	315 mg/kg x 10 °Brix	4.445a (0.048)	2.826a (0.049)	1.571b (0.065)
	473 mg/kg x 10 °Brix	4.400a (0.063)	2.823a (0.0058)	1.580b (0.080)
	158 mg/kg x 8 °Brix	4.442a (0.109)	2.816a (0.039)	0.000c (0.000)
	315 mg/kg x 8 °Brix	4.364a (0.115)	2.776a (0.057)	0.000c (0.000)
	473 mg/kg x 8 °Brix	4.402a (0.114)	2.781a (0.061)	0.000c (0.000)
		NS	NS	*
Aroma x Colour	2.5 mg/kg x yellow	4.378a (0.107)	2.795a (0.062)	1.770a (1.545)
	10 mg/kg x yellow	4.397a (0.099)	2.789a (0.044)	1.772a (1.570)
	2.5 mg/kg x red	4.370a (0.095)	2.800a (0.051)	1.781a (1.564)
	10 mg/kg x red	4.378a (0.129)	2.804a (0.068)	1.702b (1.702)
		NS	NS	*

Mean values in a column for a specific section with different letters are significantly different ($p < 0.05$)
NS = not significant, *$p \leq 0.05$, **$p \leq 0.01$

Table 11 Effect of varying concentrations of naringin, sucrose, aroma and addition of yellow and red colourants on mean L, a and b colour values (± standard deviation) of grapefruit-like beverages

	Main Effects or interaction Effects	L	a	b
Naringin	158 mg/kg	25.94a (0.72)	1.03a (1.28)	0.12a (0.31)
	315 mg/kg	25.79b (0.64)	0.98b (1.16)	0.01b (0.29)
	473 mg/kg	25.63c (0.61)	0.93c (1.01)	-0.14c (0.28)
		***	***	***
Sucrose	8 °Brix	25.83a (0.63)	0.97b (1.14)	-0.02c (0.31)
	10 °Brix	25.71b (0.73)	0.97b (1.14)	-0.09b (0.32)
	12 °Brix	25.82a (0.64)	1.00a (1.19)	0.02a (0.30)
		**	**	***
Aroma	2.5 mg/kg	25.86a (0.59)	0.95a (1.12)	-0.01a (0.32)
	10 mg/kg	25.71b (0.73)	1.01b (1.18)	0.00a (0.30)
		***	***	NS
Colour	Yellow	26.39a (0.26)	-0.15b (0.10)	0.27a (0.17)
	Red	25.18b (0.27)	2.11a (0.19)	-0.28b (0.10)
		***	***	***
Naringin x Sucrose	158 mg/kg Naringin x 12 °Brix Sucrose	25.98a (0.69)	1.06a (1.37)	0.16a (0.30)
	315 mg/kg Naringin x 12 °Brix Sucrose	25.82abc (0.58)	1.02a (1.19)	0.00d (0.28)
	473 mg/kg Naringin x 12 °Brix Sucrose	25.68c (0.67)	0.93a (1.08)	-0.09e (0.30)
	158 mg/kg Naringin x 10 °Brix Sucrose	25.91ab (0.78)	1.02a (1.30)	0.11b (0.33)
	315 mg/kg Naringin x 10 °Brix Sucrose	25.80abc (0.76)	0.98a (1.14)	0.05c (0.31)
	473 mg/kg Naringin x 10 °Brix Sucrose	25.41d (0.61)	0.92a (0.95)	-0.22f (0.26)
	158 mg/kg Naringin x 8 °Brix Sucrose	25.93ab (0.76)	1.02a (1.30)	0.08bc (0.32)
	315 mg/kg Naringin x 8 °Brix Sucrose	25.76bc (0.61)	0.95a (1.14)	-0.03d (0.29)
	473 mg/kg Naringin x 8 °Brix Sucrose	25.79abc (0.53)	0.94a (1.07)	-0.11e (0.30)
		***	NS	***
Naringin x Colour	158 mg/kg Naringin x Yellow	26.63a (0.05)	-0.23f (0.03)	0.41a (0.04)
	315 mg/kg Naringin x Yellow	26.40b (0.12)	-0.16e (0.05)	0.29b (0.07)
	473 mg/kg Naringin x Yellow	26.16c (0.28)	-0.06d (0.10)	0.11c (0.17)
	158 mg/kg Naringin x Red	25.26d (0.28)	2.30a (0.13)	-0.18d (0.08)
	315 mg/kg Naringin x Red	25.19de (0.21)	2.12b (0.10)	-0.27e (0.05)
	473 mg/kg Naringin x Red	25.09e (0.29)	1.92c (0.08)	-0.39f (0.04)
		***	***	***
Sucrose x Aroma	12 °Brix Sucrose x 2.5 mg/kg	25.94a (0.55)	0.97b (1.18)	0.02a (0.32)
	10 °Brix Sucrose x 2.5 mg/kg	25.74a (0.64)	0.98b (1.14)	-0.04c (0.32)
	8 °Brix Sucrose x 2.5 mg/kg	25.92a (0.60)	0.90c (1.11)	-0.02bc (0.33)
	12 °Brix Sucrose x 10 mg/kg	25.71a (0.72)	1.03a (1.23)	0.03a (0.29)
	10 °Brix Sucrose x 10 mg/kg	25.68a (0.83)	0.96a (1.18)	0.01ab (0.34)
	8 °Brix Sucrose x 10 mg/kg	25.73a (0.66)	1.04a (1.20)	-0.03bc (0.29)
		NS	***	**
Sucrose x Colour	12 °Brix Sucrose x Yellow	26.42a (0.17)	-0.16c (0.07)	0.30a (0.12)
	10 °Brix Sucrose x Yellow	26.36a (0.36)	-0.14c (0.12)	0.25a (0.22)
	8 °Brix Sucrose x Yellow	26.40a (0.23)	-0.15c (0.09)	0.26a (0.15)
	12 °Brix Sucrose x Red	25.23a (0.25)	2.16a (0.18)	-0.25a (0.11)
	10 °Brix Sucrose x Red	25.06a (0.27)	2.08b (0.20)	-0.29a (0.11)
	8 °Brix Sucrose x Red	25.26a (0.26)	2.09b (0.18)	-0.30a (0.08)
		NS	***	NS

Table 11 Effect of varying concentrations of naringin, sucrose, aroma and addition of yellow and red colourants on mean L, a and b colour values (± standard deviation) of grapefruit-like beverages (*continue*)

	Main Effects or interaction Effects	**L**	**a**	**b**
Aroma x Colour	2.5 mg/kg x Yellow	26.40a (0.30)	-0.15c (0.10)	0.27 (0.18)
	10 mg/kg x Yellow	26.39a (0.22)	-0.15c (0.09)	0.27 (0.15)
	2.5 mg/kg x Red	25.33b (0.17)	2.06b (0.17)	-0.29 (0.10)
	10 mg/kg x Red	25.03c (0.27)	2.17a (0.19)	-0.27a (0.10)
		***	***	NS
Naringin x Sucrose x Colour	158 mg/kg naringin x 12 °Brix Sucrose x Yellow	26.62a (0.04)	-0.25i (0.02)	0.44a (0.02)
	158 mg/kg naringin x 10 °Brix Sucrose x Yellow	26.65a (0.08)	-0.22hi (0.03)	0.42ab (0.3)
	158 mg/kg naringin x 8 °Brix Sucrose x Yellow	26.61a (0.04)	-0.22hi (0.02)	0.38ab (0.02)
	315 mg/kg naringin x 12 °Brix Sucrose x Yellow	26.36a (0.13)	-0.12fgh (0.03)	0.27cd (0.08)
	315 mg/kg naringin x 10 °Brix Sucrose x Yellow	26.50a (0.10)	-0.21ghi (0.03)	0.35bc (0.05)
	315 mg/kg naringin x 8 °Brix Sucrose x Yellow	26.33a (0.07)	-0.14fgh (0.03)	0.24d (0.06)
	473 mg/kg naringin x 12 °Brix Sucrose x Yellow	26.29a (0.04)	-0.10efg (0.02)	0.20de (0.02)
	473 mg/kg naringin x 10 °Brix Sucrose x Yellow	25.93a (0.29)	0.01e (0.10)	-0.01f (0.19)
	473 mg/kg naringin x 8 °Brix Sucrose x Yellow	26.25a (0.29)	-0.09ef (0.12)	0.14e (0.20)
	158 mg/kg naringin x12 °Brix Sucrose x Red	25.33a (0.20)	2.37a (0.08)	-0.12g (0.04)
	158 mg/kg naringin x 10 °Brix Sucrose x Red	25.18a (0.19)	2.27ab (0.05)	-0.20gh (0.08)
	158 mg/kg naringin x 8 °Brix Sucrose x Red	25.26a (0.43)	2.25b (0.19)	-0.23hi (0.07)
	315 mg/kg naringin x 12 °Brix Sucrose x Red	25.28a (0.16)	2.16b (0.06)	-0.27hi (0.02)
	315 mg/kg naringin x 10 °Brix Sucrose x Red	25.10a (0.26)	2.16b (0.04)	-0.24hi (0.02)
	315 mg/kg naringin x 8 °Brix Sucrose x Red	25.19a (0.20)	2.04c (0.14)	-0.31ij (0.07)
	473 mg/kg naringin x 12 °Brix Sucrose x Red	25.07a (0.31)	1.97c (0.07)	-0.38jk (0.03)
	473 mg/kg naringin x 10 °Brix Sucrose x Red	24.89a (0.29)	1.83d (0.03)	-0.43k (0.02)
	473 mg/kg naringin x 8 °Brix Sucrose x Red	25.32a (0.05)	1.96c (0.03)	-0.37jk (0.03)
		NS	***	***
Naringin x Sucrose x Aroma	158 mg/kg naringin x12 °Brix Sucrose x 2.5 mg/kg	26.07a (0.61)	1.03a (1.41)	0.14ab (0.32)
	158 mg/kg naringin x 10 °Brix Sucrose x 2.5 mg/kg	25.92a (0.86)	1.01a (1.36)	0.15ab (0.32)
	158 mg/kg naringin x 8 °Brix Sucrose x 2.5 mg/kg	26.10a (0.58)	0.93a (1.25)	0.05cd (0.36)
	315 mg/kg naringin x 12 °Brix Sucrose x 2.5 mg/kg	25.94a (0.57)	0.99a (1.24)	0.03cd (0.33)
	315 mg/kg naringin x 10 °Brix Sucrose x 2.5 mg/kg	25.88a (0.60)	0.99a (1.28)	0.03cd (0.30)
	315 mg/kg naringin x 8 °Brix Sucrose x 2.5 mg/kg	25.76a (0.58)	0.90a (1.11)	-0.09efg (0.31)
	473 mg/kg naringin x 12 °Brix Sucrose x 2.5 mg/kg	25.80a (0.53)	0.91a (1.10)	-0.10fg (0.32)
	473 mg/kg naringin x 10 °Brix Sucrose x 2.5 mg/kg	25.41a (1.29)	0.95a (0.94)	-0.30i (0.14)
	473 mg/kg naringin x 8 °Brix Sucrose x 2.5 mg/kg	25.91a (0.67)	0.88a (1.18)	-0.02de (0.37)
	158 mg/kg naringin x12 °Brix Sucrose x 10 mg/kg	25.89a (0.81)	1.09a (1.47)	0.18a (0.30)
	158 mg/kg naringin x 10 °Brix Sucrose x 10mg/kg	25.90a (0.77)	1.04a (1.36)	0.07bc (0.37)
	158 mg/kg naringin x 8 °Brix Sucrose x 10 mg/kg	25.77a (0.93)	1.10a (1.45)	0.11abc (0.30)
	315 mg/kg naringin x 12 °Brix Sucrose x 10 mg/kg	25.70a (0.62)	1.05a (1.26)	-0.03def (0.25)
	315 mg/kg naringin x 10 °Brix Sucrose x 10 mg/kg	25.738a (0.94)	0.97a (1.31)	0.08bc (0.34)
	315 mg/kg naringin x 8 °Brix Sucrose x 10 mg/kg	25.77a (0.70)	1.01a (1.27)	0.03cd (0.30)
	473 mg/kg naringin x 12 °Brix Sucrose x 10 mg/kg	25.55a (0.82)	0.96a (1.17)	-0.08efg (0.31)
	473 mg/kg naringin x 10 °Brix Sucrose x 10 mg/kg	25.41a (0.86)	0.88a (1.05)	-0.14gh (0.33)
	473 mg/kg naringin x 8 °Brix Sucrose x 10 mg/kg	25.67a (0.35)	1.00a (1.07)	-0.21h (0.19)
		NS	NS	***

Table 11 Effect of varying concentrations of naringin, sucrose, aroma and addition of yellow and red colourants on mean L, a and b colour values (± standard deviation) of grapefruit-like beverages (*continue*)

Main Effects or interaction Effects		L	A	b
Sucrose x Aroma x Colour	12 °Brix Sucrose x 2.5 mg/kg x Yellow	26.45a (0.15)	-0.17a (0.08)	0.32a (0.11)
	10 °Brix Sucrose x 2.5 mg/kg x Yellow	26.26a (0.46)	-0.11a (0.15)	0.19b (0.08)
	8 °Brix Sucrose x 2.5 mg/kg x Yellow	26.48a (0.16)	-0.17a (0.05)	0.30a (0.09)
	12 °Brix Sucrose x 10 mg/kg x Red	25.03de (0.20)	-0.15a (0.07)	-0.24c (0.12)
	10 °Brix Sucrose x 10 mg/kg x Red	24.90e (0.25)	-0.17a (0.07)	-0.31de (0.10)
	8 °Brix Sucrose x 10 mg/kg x Red	25.15cde (0.31)	-0.12a (0.11)	-0.26cd (0.10)
	12 °Brix Sucrose x 2.5 mg/kg x Red	25.42b (0.09)	2.11a (0.18)	-0.27cde (0.10)
	10 °Brix Sucrose x 2.5 mg/kg x Red	25.21bcd (0.18)	2.07a (0.21)	-0.27cde (0.13)
	8 °Brix Sucrose x 2.5 mg/kg x Red	25.36bc (0.16)	1.98a (0.08)	-0.33e (0.04)
	12 °Brix Sucrose x 10 mg/kg x Yellow	26.39a (0.18)	2.22a (0.07)	0.29a (0.13)
	10 °Brix Sucrose x 10 mg/kg x Yellow	26.46a (0.21)	2.10a (0.07)	0.32a (0.12)
	8 °Brix Sucrose x 10 mg/kg x Yellow	26.32a (0.27)	2.19a (0.11)	0.21b (0.19)
		*	NS	**

Mean values per comparison in a column with different letters are significantly different
NS = not significant, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

4.5 Conclusions

In this chapter, the preparation of a range of 36 grapefruit-like beverages that vary in added naringin level, sucrose level, aroma mixture level and colour type, was described. It showed that stock concentrates of deionised apple juice concentrate with citric acid, containing the concentration of each experimental factor can be mixed and diluted to result in a constant set of single juice grapefruit-like beverages. Characterisation of the physico-chemical properties of the deionized apple juice base concentrates and beverages shows that sucrose gets inverted with the addition of citric acid. From this study, the fate of the mannitol is still unclear due to the limitation of the detection accuracy of the analysis and will need further research. Naringin level, sucrose level, aroma mixture level and type of colourants added to the deionized apple juice acidified base concentrate have an effect on °Brix, acidity, °Brix/acid ratio, sucrose content, fructose content, glucose content and L* a* b* colour values of grapefruit-like beverages.

The next phase of the study will describe the sensory properties of the 36 grapefruit like beverages as evaluated by a trained sensory panel.

CHAPTER 5:
Effect of varying the bitterness, sweetness and aroma intensity as well as colour of grapefruit-like model beverages on sensory properties and consumer liking

5.1 Abstract

There are multiple reasons for individual differences in food preferences and one important contributor is sensory perception. Food perception and food liking are the result of multiple sensory modalities. Colour, aroma, sweet and bitter taste contribute to the sensory perception of grapefruit juice. The objective of the study was to determine the effect of varying the bitterness, sweetness, colour and aroma intensity of grapefruit-like model beverages using a full factorial design on the cross-modal perception of sensory properties and consumer liking of the beverages. A factorial design was used to create 36 grapefruit-like beverages with deflavoured clarified apple juice as base and modification of bitter taste (3 levels), sweet taste (3 levels), aroma intensity (2 levels) and colour (red or yellow). Descriptive analysis was used to describe the sensory profiles of the 36 beverages. Hedonic rating of colour, aroma and flavour of the 12 most diverse beverages from the design was conducted by a consumer panel. Colour, flavour and aroma liking were not strongly correlated requiring three Partial Least Square Regression (PLSR) models to relate liking to descriptive profiles. Descriptive analysis results showed that both bitterness and sweetness of the beverages had a significant effect on the flavour and after taste attributes. Aroma concentration variations had a significant effect on the majority of the sensory attributes. Colour had a significant effect on some of the aroma attributes as well as grapefruit flavour. Very few 2-factor interactions had significant effects on the sensory attributes. Consumers preferred red beverages over those with a yellow colour and low aroma over the high aroma samples. Consumers preferred low naringin/-high sucrose beverages over those with high naringin/-low sucrose levels. There were no significant attribute drivers for colour liking. Pungent and grapefruit aroma were negative drivers of aroma liking. The intensity of sweet and citrus flavours was positive and sour and bitter negative drivers of flavour liking.

This study showed that fruit juice processors should aim to develop a sensory profile for grapefruit juice and nectars with low bitterness, high sweetness, low grapefruit aroma levels and red colour to increase consumer liking of the beverages.

Keywords: Cross-modal interactions, Grapefruit-like beverages, Naringin, Consumer acceptance and preferences, Partial Least Square Regression (PLSR) models

Highlights

- Red grapefruit colour is preferred above yellow grapefruit colour
- Low bitterness/-high sweetness grapefruit is preferred above high bitterness/-low sweetness grapefruit
- Low grapefruit aroma is preferred above high grapefruit aroma

Abbreviations used: LMS labelled magnitude scale; SLAM, simplified labelled affective magnitude scale; DA, Descriptive analysis

5.2 Introduction

Grapefruit are the fifth most consumed fruit in the world (Grapefruit Market Reports - Trends, Analysis and Statistics, 2018). The annual world's consumption of grapefruit stands at 83.97 million metric tons. The consumption of grapefruit (*Citrus paradisi* Macfad) remains low in South Africa (USDA Foreign Agricultural Service Gain Report, 2017) as some individuals like grapefruit and others do not and the reasons for the variation is quite unknown. Food preferences have a significant impact on eating behaviours (Mok, 2010) and may be influenced by sensory perception (Hayes, Sullivan and Duffy, 2010). Food perception does not just depend on one individual sense, but appears to be the result of multisensory integration of unimodal signals (Pereira and Van der Bilt, 2016). The chemical sense of gustation (taste) is generally believed to involve the detection of five basic taste categories; sweet, sour, bitter, salty and umami (Chauhari and Roper, 2010). Tastetant molecules bind (either directly or indirectly) to ion channels in the membranes of taste receptor cells which are organized into taste buds (Chandrashekar, Hoon, Ryba and Zuker, 2006). From there, the signal is converted and sent to the brain in a process known as transduction (Frank and Hettinger, 2005). However, substances are very rarely delivered into the mouth in the form of pure tastants. The central nervous system then interprets information from different modalities taste, aroma, appearance, sound and texture and interaction of these signals results in the appropriate response to accept or to reject the stimulus (Frank and Hettinger, 2005; Rolls, 2006).

Flavour perception is complex and occurs during the simultaneous stimulation of a number of the senses. When different senses are stimulated concurrently and perceptually interact with each other, the perceived flavour that results can be regarded as cross-modal sensory interaction (Delahunty and Drake, 2004). Cross-modal interactions can change the intensity and character of individual tastes and aromas, and flavour overall (Delwiche, 2004). The perception and evaluation of food and drink is an inherently multisensory experience.

Gustatory, olfactory, visual, oral-somatosensory, auditory, and even nociceptive cues can all play a role in determining our perception of what we eat and drink (Delwich, 2004; Stillman, 2002). Aroma was shown to influence the basic tastes and vice versa (Forde and Delahunty, 2004; Fujimaru and Lim, 2013; Green, Nachtigal, Hammond and Lim, 2012; Lim, Fujimaru and Linscott, 2014; Niimi *et al*., 2014).

Odour-taste interactions can result in complicated changes in perceived flavour when complex stimuli are used e.g. the addition of an aroma can elevate the bitterness threshold (Gaudette and Pickering, 2013; Haraguchi, Yoshida, Hazekawa and Uchida, 2011). The bitterness inhibitory effect of five different taste-less aromas, green-tea, coffee, vanilla, apple and strawberry were examined on bitter branched-chain amino acid solutions. The green-tea and coffee aromas predominantly evoked bitterness, while the vanilla aroma predominantly evoked sweetness. Apple and strawberry aromas evoked both sweetness and sourness, with the apple aroma having stronger sourness and the strawberry aroma stronger sweetness (Mukai, Tokuyama, Ishizaka, Okada and Uchida, 2007). When sucrose is added to fruit juices, not only are the perceived level of bitterness and sourness reduced but the sweet odour intensity rating also change (Von Sydow, Moskowitz, Jacobs and Meiselman, 1974). The colour of food and drinks has impacts on people's subsequent experience of taste, flavour and overall sensory perception. It is reported in several studies that colour greatly impacts the ability to identify the type of food and beverage. Uncoloured flavoured orange beverages were perceived as being less tasty when compared to beverages which were orange coloured (Stillman, 1993). Flavour expectations showed that certain colours were strongly associated with particular flavours: red with cherry, orange with orange and green with lime (Du Bois, Cardello and Maller, 1980); yellow with lemon, blue with spearmint and red with strawberry, raspberry and cherry (Zampini, Sanabia, Philips and Spence, 2007). Roth, Radle, Gifford and Glydesdale (1988) altered the relationship of green and yellow colours in lemon and lime flavoured sucrose solutions and found that these colour changes had an impact on sweetness ratings. Zellner and Kautz (1990) found with a colour-odour pairing that solutions were rated as having more intense odours with colour cues than without, regardless of colour-odour appropriateness. This cross-modal effect presumably results from the colour cue setting up an expectation concerning the likely identity and intensity of a food or drink's taste or flavour (Spence *et al*., 2010).

The objective of the study was to determine the effect of varying the bitterness, sweetness, colour and aroma intensity of grapefruit-like model beverages on the cross-modal perception of sensory properties and consumer liking of the beverages. The second objective was to determine the sensory drivers of liking of the grapefruit-like beverages.

5.3 Materials and methods

5.3.1 Ethics statement

Ethical approval for this study was obtained from the Faculty of Natural and Agricultural Sciences Ethics Committee at the University of Pretoria (EC 130827-088).

5.3.2 Experimental design

A factorial design was used to create 36 grapefruit-like beverages with deflavoured clarified apple juice as base and modification of bitter taste (3 levels), sweet taste (3 levels), aroma intensity (2 levels) and colour (red or yellow) as shown in Table 12.

The sensory profiles of the 36 beverages were described by a trained sensory panel. The hedonic rating of colour, aroma and flavour of the 12 most diverse beverages from the design were measured using a untrained consumer panel (n = 96 females).

5.3.3 Samples

Standard preparation and mixing procedures were used for all added stimuli to ensure uniformity. The grapefruit-like beverages were filled in 250 ml plastic bottles with lids for easy handling and uniformity and kept frozen at -18°C until use. The beverages were defrosted overnight at ambient temperature and kept at 14°C until served.

5.3.4 Descriptive analysis

5.3.4.1 Participants

Sixteen judges (8 female and 8 male), 20-50 years, with one to two years of experience on descriptive sensory panels, participated in the descriptive study. They were advised not to eat, drink (except for water) or smoke for at least 1 h prior to a session.

5.3.4.2 Descriptive analysis procedure

Descriptive analysis consisted of two (1.5-2 h each) training sessions for descriptive attribute and methodology development. The generic descriptive analysis method (Einstein, 1991) was used in the training of the panel and performance monitoring was done to test reproducibility and consistency of the sensory panel ratings using PanelCheck software version 1.3.2 (www.panelcheck.com; Nofima Mat, Ås, Norway) and hence improve calibration. A total of 21 attributes were generated to characterize the appearance, aroma, flavour and after taste of the grapefruit-like beverages (Table 13). Reference standards used to define these sensory descriptors were present during training and evaluation sessions. The attributes were evaluated on a structured nine-point horizontal line scale (10 cm) with descriptors at the scale ends ranging from 'not intense' (at the left end of the scale, 0 cm) to 'very intense' (at the right end of the scale, 10 cm).

Data was captured using Compusense® *five* (Compusense® *five*, release 4.6; Compusense Inc., Guelph, ON, Canada).

Table 12 Factorial design for the 36 grapefruit-like beverages for this study

Numbers*	Code**	Bitter***	Sweet****	Aroma*****	Colour
1	LMHR	158 mg/kg	10 °Brix	10 mg/kg	Red#
2	MMHR	315 mg/kg	10 °Brix	10 mg/kg	Red
3	HMHR	473 mg/kg	10 °Brix	10 mg/kg	Red
4	LHHR	158 mg/kg	12 °Brix	10 mg/kg	Red
5	MHHR	315 mg/kg	12 °Brix	10 mg/kg	Red
6	HHHR	473 mg/kg	12 °Brix	10 mg/kg	Red
7	LLHR	158 mg/kg	8 °Brix	10 mg/kg	Red
8	MLHR	315 mg/kg	8 °Brix	10 mg/kg	Red
9	HLHR	473 mg/kg	8 °Brix	10 mg/kg	Red
10	LMLR	158 mg/kg	10 °Brix	2.5 mg/kg	Red
11	MMLR	315 mg/kg	10 °Brix	2.5 mg/kg	Red
12	HMLR	473 mg/kg	10 °Brix	2.5 mg/kg	Red
13	LHLR	158 mg/kg	12 °Brix	2.5 mg/kg	Red
14	MHLR	315 mg/kg	12 °Brix	2.5 mg/kg	Red
15	HHLR	473 mg/kg	12 °Brix	2.5 mg/kg	Red
16	LLLR	158 mg/kg	8 °Brix	2.5 mg/kg	Red
17	MLLR	315 mg/kg	8 °Brix	2.5 mg/kg	Red
18	HLLR	473 mg/kg	8 °Brix	2.5 mg/kg	Red
19	LMHY	158 mg/kg	10 °Brix	10 mg/kg	Yellow##
20	MMHY	315 mg/kg	10 °Brix	10 mg/kg	Yellow
21	HMHY	473 mg/kg	10 °Brix	10 mg/kg	Yellow
22	LHHY	158 mg/kg	12 °Brix	10 mg/kg	Yellow
23	MHHY	315 mg/kg	12 °Brix	10 mg/kg	Yellow
24	HHHY	473 mg/kg	12 °Brix	10 mg/kg	Yellow
25	LLHY	158 mg/kg	8 °Brix	10 mg/kg	Yellow
26	MLHY	315 mg/kg	8 °Brix	10 mg/kg	Yellow
27	HLHY	473 mg/kg	8 °Brix	10 mg/kg	Yellow
28	LMLY	158 mg/kg	10 °Brix	2.5 mg/kg	Yellow
29	MMLY	315 mg/kg	10 °Brix	2.5 mg/kg	Yellow
30	HMLY	473 mg/kg	10 °Brix	2.5 mg/kg	Yellow
31	LHLY	158 mg/kg	12 °Brix	2.5 mg/kg	Yellow
32	MHLY	315 mg/kg	12 °Brix	2.5 mg/kg	Yellow
33	HHLY	473 mg/kg	12 °Brix	2.5 mg/kg	Yellow
34	LLLY	158 mg/kg	8 °Brix	2.5 mg/kg	Yellow
35	MLLY	315 mg/kg	8 °Brix	2.5 mg/kg	Yellow
36	HLLY	473 mg/kg	8 °Brix	2.5 mg/kg	Yellow

*36 Beverage numbers. **Code: 1st letter = naringin concentration (High, Medium, Low); 2nd letter = sucrose concentration (High, Medium, Low); 3rd letter = aroma concentration (High, Low); 4th letter = colour (Red or Yellow). Samples which are yellow highlighted were used in hedonic ratings. ***Biter naringin concentration. ****Sweet value in °Brix due to sucrose; *****Aroma blend = Caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), aldehyde C10 (decanal). #Red colour blend = 0.001 % Red solution (30 % Sunset Yellow and 70 % Ponceau Red); ##Yellow colour blend = 0.0125 % Yellow solution (Quinoline Yellow)

The grapefruit-like beverages were poured as ± 30 ml aliquots in 125 ml polystyrene cups with plastic lids and marked with random three-digit numbers and kept at 14°C until serving. The panellists were provided with bland crackers (Cream crackers, Snackworks, South Africa) to eat in between sample tasting and filtered water for neutralizing and cleansing the palate before and between sample tasting.

The 36 grapefruit-like beverage samples were evaluated by the trained panellists in duplicate, 12 beverages per session, with a total of six sessions being required. The presentation order of samples was randomized for each session using a William's latin square design. The evaluation (See Appendix 1 for a copy of the questionnaire) was performed by panellists in a standard sensory laboratory seated in individual evaluation booths with daylight illumination.

Table 13 Attributes used for the descriptive analysis to describe the sensory characteristics of the grapefruit-like beverage samples

Attribute	Definition References indicated where applicable
Aroma	
Overall aroma intensity	The aroma of the beverage upon taking the first few sniffs
Citrus aroma	The aromatic associated with the general impression of citrus fruits
Grapefruit aroma	The aroma of fresh grapefruit
Chemical aroma	A very general term associated with many different types of compounds, such as solvents and cleaning compounds
Deteriorated/rotten aroma	Aroma associated with rotten, deteriorated, decayed fruit/material
Muddy/mouldy aroma	Aromatic characteristic of damp soil, wet foliage or slightly undercooked boiled potato
Fruity aroma	Aromatic associated with a mixture of non-specific fruits (apples, pears, melons and guava)
Green/grassy aroma	Aromatic characteristic of freshly cut leaves, grass or green vegetables (green beans)
Peely/peel oil aroma	Aroma associated with grapefruit peel or skin flavour. Ref: Grapefruit oil extracted from grapefruit
Soapy aroma	Aroma associated with unscented soap
Pungent aroma	Describe a product causing a sharp sensation of the nasal mucous membranes. Ref: vinegar
Woody/spicy aroma	Aromatic associated with dry, fresh-cut wood; balsamic or bark-like. Ref: 10 ppm alpha-humulene in water
Sweet aroma	Aromatic associated with high sugar content vegetables. Ref: Freshly boiled sweet corn
Flavour	
Overall flavour intensity	The intensity of the flavour that is released from the beverage upon taking the first sip
Sour taste	Basic taste on tongue stimulated by acids. Ref: citric acid
Sweet taste	Taste on the tongue stimulated by sugars. Ref: 5 % sugar (sucrose) in water
Bitter taste	Taste on tongue stimulated by bitter solutions. Ref: 473 mg/kg naringin in water
Astringent flavour	The chemical feeling factor on the tongue or skin surface of the oral cavity described as puckering/dry and associated with tannins. Ref: Strong black tea
Citrus flavour	Flavour associated with the general impression of citrus fruits. Ref: Cut lemon fruit and lime cordial
Grapefruit flavour	The flavour of fresh grapefruit. Ref: Cut red and white grapefruit
After taste	
Bitter after taste	Bitter taste remaining after swallowing

5.3.5 Consumer testing

5.3.5.1 Participants

Ninety-six young South African female consumers aged 18-24 years took part in the hedonic study. They were recruited by fieldworkers. Each panellist had to complete an online screening survey (Appendix 2) prior to the tasting session. Panellists were screened for a self-reported good state of health, by completing two questions; by rating their health by clicking on one of the options (poor, fair, good, very good, excellent), followed by a question to indicate how satisfied they are with their health (very dissatisfied, somewhat dissatisfied, somewhat satisfied, very satisfied). Persons that suffered from any food intolerance(s) and/or allergies were excluded from the study. All panellists included were informed about the nature of the task (that they will be asked to taste and rate how much they like or dislike small portions of different fruit beverages), had a chance to ask questions and gave consent before testing. They were advised not to eat, drink (except for water) or smoke for at least 1 h prior to a session.

5.3.5.2 Consumer testing procedure

The twelve most diverse grapefruit-like beverages (indicated in yellow on Table 12) were evaluated using the Simplified Labeled Affective Magnitude (SLAM) scale (Lawless, Cardello, Chepman, Lesher, Given and Schutz, 2010). The horizontal scale (10 cm) consisted of descriptors ranging from 'greatest imaginable dislike' (at the left end of the scale, 0 cm), 'neither like nor dislike' (at the middle of the scale, 5 cm) and 'greatest imaginable like' (at the right end of the scale, 10 cm). All the questions were displayed on a computer screen (Appendix 4). They were asked three questions during the tasting of each sample and had to use the computer mouse to click on the horizontal line scale to show their response. Firstly they had to look at the beverage (sample) and indicate how much they like or dislike the colour of the beverage. Secondly, they had to smell the sample before they taste it and indicate how much they like or dislike the smell/-aroma of the beverage. Lastly, they were asked to drink the beverage and indicate how much they like or dislike the taste/flavour of the beverage.

5.3.6 Statistical analysis

5.3.6.1 Descriptive analysis

One-way analysis of variance (ANOVA) was performed to determine the effect of the beverages on the sensory attributes. Tukey's HSD test was used to identify the nature of significant differences ($p \leq 0.05$). Another ANOVA model included the main effects of judge, bitter, sweet, aroma and colour together with the respective 2-way interactions. The ANOVA model was fitted using PROC GLM in SAS v9.4 (SAS Institute Inc., Cary, North Carolina, USA). Principal component analysis (PCA) was performed with XLSTAT 2014 (Addinsoft, Paris, France) and was applied to the correlation matrix formed from the sensory panel means for all attributes of grapefruit-like beverages. Two PCAs, one with all 36 samples included and a second PCA with only the 12 samples that were tested by the consumers were performed.

5.3.6.2 Consumer testing

ANOVA was used to determine the effect of the 12 beverages (3 bitter-sweet x 2 aroma x 2 colour) on the consumer liking of appearance, aroma and flavour of the beverages. Means were compared using Fisher's least significant difference test at the 5 % level. Data were analysed using GenStat® (VSN International Ltd., Hertfordshire, United Kingdom). Correlation analysis was done between liking of colour, aroma and flavour of the beverages. Ward’s hierarchical cluster analysis, PROC CLUSTER in SAS v9.4 (SAS Institute Inc., Cary, North Carolina, USA) was used to identify clusters of consumers based on similar preference rating scores.

5.3.6.3 Regression of descriptive analysis and consumer internal and external preference mapping

Consumer liking (y) of colour, aroma and flavour of beverages was modelled as a function of the sensory attributes (x) using partial least square (PLS) regression. Preliminary models were run with all sensory attributes and their squared terms included. VIP (or variable importance), which measures how valuable a variable is in terms of modelling the liking attributes, was used to select a smaller number of linear and squared terms for the final model. Only those linear terms with a VIP value greater than 0.8 as well as the five squared terms with the highest contribution were retained. The PLS models were used to determine the positive and negative drivers of colour, aroma and flavour liking, and also to predict consumer liking of the 24 samples that were profiled by the descriptive sensory panel, but not evaluated by the consumers. The SIMCA package (Umetrics, Umea, Sweden) was used for the PLS modelling.

5.4 Results

5.4.1 Descriptive analysis

A summary of the main and interaction ANOVA effects on sensory attributes of grapefruit-like beverages as evaluated by the trained sensory panel are shown in Table 14 (p 1 of 4). The bitterness of the beverages did not have any significant effect on any of the aroma attributes. It did however have a significant effect on overall flavour intensity as well as astringent flavour with the highest values for beverages with medium and high naringin concentrations. Bitterness had a significant effect on sweet, sour, bitter and grapefruit flavour and bitter after taste with the highest sweetness, and lowest sourness, bitter and grapefruit flavour and bitter after taste perceived in the beverages with low and medium naringin concentrations Table 14 (p 1 of 4). Sweetness had a significant effect on soapy aroma with the highest values perceived in the beverages with low and medium sucrose concentration. The sweetness of the beverages had a significant effect on sour, sweet, bitter, astringent and grapefruit flavour and bitter after taste with the highest sour, bitter, astringent flavour and bitter after taste and lowest sweet flavour perceived in the low sucrose concentration and the lowest grapefruit flavour perceived in the high sucrose concentration Table 14 (p 1 of 4). It was also clear that aroma concentration had a significant effect on the majority of the sensory attributes, namely overall aroma intensity and citrus, grapefruit, chemical, muddy/mouldy, fruity, green/grassy, peely/peel oil, soapy, pungent, woody/spicy and sweet aroma with the lowest aroma perceived in the beverages with the low aroma concentration. Aroma concentration had a significant effect on bitter, astringent, and citrus flavour and bitter after taste with the highest bitter and astringent flavour and bitter after taste perceived in the beverages with the low aroma concentration and the highest citrus flavour perceived in the beverages with the high aroma concentration Table 14 (p 1 of 4). Colour of the beverages had a significant effect on overall aroma intensity and grapefruit, deteriorated/rotten, muddy/mouldy, fruity and sweet aroma and grapefruit flavour with the highest values in all of them perceived in the red coloured beverages. This effect of aroma and colour on the attributes can be seen as evidence of cross-modal interaction. The citrus industry can benefit from this information during juice formulations by trying to use a higher level of aroma concentration. It will increase the citrus flavour and reduce the negative attributes, bitter and astringent flavour as well as the bitter after taste. This information of the effect that colour has on the attributes will also help the industry to use rather a red colour instead of a yellow colour. This will help to get the lowest values in grapefruit, for deteriorated/rotten and muddy/mouldy aroma. Very few 2-factor interactions of the beverage had significant effects on the sensory attributes. Bitter x aroma interaction had a significant effect on chemical aroma and overall flavour intensity, bitter flavour and bitter after taste with the lowest chemical aroma perceived in the beverages with low naringin and low aroma concentration as well as the beverages with the high naringin and low aroma concentration. The highest overall flavour intensity was perceived in the beverages with the medium naringin and low aroma concentration, the beverages with the high naringin and low aroma concentration and beverages with high naringin and high aroma concentration. The highest bitter flavour and bitter after taste were perceived in the beverages with the high naringin and low aroma concentration Table 14 (p 2 of 4). Bitter x colour interaction had a significant effect on bitter after taste with the highest value perceived in the red and yellow beverages with the high naringin concentration Table 14 (p 2 of 4). Bitter x sweet interaction had a significant effect on pungent aroma with the lowest value perceive in the beverages with medium naringin (bitter) and medium sucrose (sweet) concentration followed by beverages with medium naringin (bitter) and high sucrose (sweet) concentration and then beverages with low naringin (bitter) and low sucrose (sweet) concentration Table 14 (p 3 of 4). Aroma x colour interaction had a significant effect on bitter flavour with highest values perceived in the red and yellow beverages with low aroma concentration Table 14 (p 3 of 4). The sweet x aroma interaction did not have any significant effect on any of the sensory aroma attributes Table 14 (p 4 of 4). Sweet x colour interaction of the beverages had a significant effect on astringent and citrus flavour with the highest astringent flavour perceived in the beverages with the low sweet concentration and red colour and the lowest citrus flavour noted in the yellow beverages with the low sweetness concentration Table 14 (p 4 of 4).

PCA (Figure 7) shows a spread of the samples over the two-dimensional space. The distribution of samples was related to the intensities of descriptive attributes as seen in Table 14.

Table 14 Summary of the main and interaction ANOVA effects (colour hue, aroma, bitter and sweet levels) on mean values[1] (± SEM) for sensory attributes of grapefruit-like beverages as evaluated by a trained sensory panel (n = 16)

Attributes	Colour[2]			Aroma[3] mg/kg			Bitter (Naringin mg/kg)				Sweet (Sucrose mg/kg)			
	Red	Yellow		2.5	10		158 Low	315 Medium	473 High		8 °Brix	10 °Brix	12 °Brix	
Overall aroma intensity	**6.06[1]a (0.05)**	**5.83b (0.05)**	**	**5.52b (0.05)**	**6.37a (0.05)**	***	5.87a (0.06)	6.04a (0.06)	5.93a (0.06)	NS	5.96a (0.06)	5.91a (0.06)	5.96a (0.06)	NS
Citrus aroma	4.54a (0.05)	4.55a (0.05)	NS	**4.23b (0.05)**	**4.86a (0.05)**	***	4.51a (0.06)	4.58a (0.06)	4.55a (0.06)	NS	4.51a (0.06)	4.58a (0.06)	4.55a (0.06)	NS
Grapefruit aroma	**4.40a (0.05)**	**4.15b (0.05)**	**	**4.06b (0.05)**	**4.49a (0.05)**	***	4.28a (0.06)	4.23a (0.06)	4.32a (0.06)	NS	4.23a (0.06)	4.35a (0.06)	4.25a (0.06)	NS
Chemical aroma	4.09a (0.06)	4.03a (0.06)	NS	**3.86b (0.06)**	**4.25a (0.06)**	***	3.99a (0.07)	4.13a (0.07)	4.06a (0.07)	NS	4.06a (0.07)	4.03a (0.07)	4.09a (0.07)	NS
Deteriorated/rotten aroma	**2.14a (0.04)**	**2.00b (0.04)**	**	2.09a (0.04)	2.05a (0.04)	NS	2.08a (0.05)	2.04a (0.05)	2.09a (0.05)	NS	2.05a (0.05)	2.04a (0.05)	2.12a (0.05)	NS
Muddy/moldy aroma	**2.20a (0.03)**	**2.09b (0.03)**	**	**2.09b (0.03)**	**2.20a (0.03)**	**	2.12a (0.04)	2.13a (0.04)	2.18a (0.04)	NS	2.18a (0.04)	2.13a (0.04)	2.13a (0.04)	NS
Fruity aroma	**3.93a (0.05)**	**3.78b (0.05)**	*	**3.71b (0.05)**	**4.00a (0.05)**	***	3.89a (0.06)	3.83a (0.06)	3.85a (0.06)	NS	3.83a (0.06)	3.90a (0.06)	3.83a (0.06)	NS
Green/grassy aroma	3.13a (0.04)	3.14a (0.04)	NS	**2.91b (0.04)**	**3.36a (0.04)**	***	3.12a (0.05)	3.09a (0.05)	3.19a (0.05)	NS	3.12a (0.05)	3.18a (0.05)	3.10a (0.05)	NS
Peely/peel oil aroma	3.53a (0.05)	3.47a (0.05)	NS	**3.20b (0.05)**	**3.79a (0.05)**	***	3.53a (0.06)	3.47a (0.06)	3.49a (0.06)	NS	3.49a (0.06)	3.55a (0.06)	3.46a (0.06)	NS
Soapy aroma	3.29a (0.05)	3.23a (0.05)	NS	**3.16b (0.05)**	**3.36a (0.05)**	**	3.27a (0.06)	3.20a (0.06)	3.31a (0.06)	NS	**3.41a (0.06)**	**4.33a (0.06)**	**3.04b (0.06)**	**
Pungent aroma	3.16a (0.05)	3.11a (0.05)	NS	**2.84b (0.05)**	**3.43a (0.05)**	***	3.10a (0.06)	3.14a (0.06)	3.18a (0.06)	NS	3.17a (0.06)	3.13a (0.06)	3.11a (0.06)	NS
Woody/spicy aroma	2.49a (0.04)	2.40a (0.04)	NS	**2.33b (0.04)**	**2.57a (0.04)**	***	2.43a (0.04)	2.44a (0.04)	2.47a (0.04)	NS	2.48a (0.04)	2.48a (0.04)	2.37a (0.04)	NS
Sweet aroma	**3.79a (0.05)**	**3.62b (0.05)**	*	**3.56b (0.05)**	**3.86a (0.05)**	***	3.73a (0.06)	3.69a (0.06)	3.70a (0.06)	NS	3.69a (0.06)	3.72a (0.06)	3.72a (0.06)	NS
Overall flavor intensity	6.39a (0.05)	6.34a (0.05)	NS	6.37a (0.05)	6.36a (0.05)	NS	**6.14b (0.06)**	**6.51a (0.06)**	**6.46a (0.06)**	***	6.41a (0.06)	6.29a (0.06)	6.40a (0.06)	NS
Sour flavor	5.09a (0.06)	5.17a (0.06)	NS	5.14a (0.06)	5.11a (0.06)	NS	**4.90b (0.08)**	**5.08b (0.08)**	**5.40a (0.08)**	***	**5.93a (0.08)**	**5.07b (0.08)**	**4.38c (0.08)**	***
Sweet flavor	4.48a (0.05)	4.43a (0.05)	NS	4.38a (0.05)	4.53a (0.05)	NS	**4.64a (0.06)**	**4.51a (0.06)**	**4.21b (0.06)**	***	**3.04c (0.06)**	**4.50b (0.06)**	**5.82a (0.06)**	***
Bitter flavor	4.56a (0.07)	4.48a (0.07)	NS	**4.77a (0.07)**	**4.27b (0.07)**	***	**3.94c (0.08)**	**4.46b (0.08)**	**5.17a (0.08)**	***	**5.24a (0.08)**	**4.43b (0.08)**	**3.89c (0.08)**	***
Astringent flavor	4.95a (0.06)	4.81a (0.06)	NS	**4.97a (0.06)**	**4.79b (0.06)**	*	**4.62b (0.07)**	**4.91a (0.07)**	**5.12a (0.07)**	***	**5.35a (0.07)**	**4.88b (0.07)**	**4.41c (0.07)**	***
Citrus flavor	4.47a (0.05)	4.43a (0.05)	NS	**4.30b (0.05)**	**4.60a (0.05)**	***	4.52a (0.06)	4.44a (0.06)	4.39a (0.06)	NS	4.43a (0.06)	4.45a (0.06)	4.48a (0.06)	NS
Grapefruit flavor	**4.53a (0.05)**	**4.36b (0.05)**	*	4.49a (0.05)	4.40a (0.05)	NS	**4.19b (0.07)**	**4.41b (0.07)**	**4.73a (0.07)**	***	**4.53a (0.07)**	**4.54a (0.07)**	**4.26b (0.07)**	**
Bitter aftertaste	4.32a (0.07)	4.26a (0.07)	NS	**4.49a (0.07)**	**4.08b (0.07)**	***	**3.69c (0.08)**	**4.26b (0.08)**	**4.91a (0.08)**	***	**4.90a (0.08)**	**4.25b (0.08)**	**3.71c (0.08)**	***

Table 14 Summary of the main and interaction ANOVA effects (colour hue, aroma, bitter and sweet levels) on mean values[1] (± SEM) for sensory attributes of grapefruit-like beverages as evaluated by a trained sensory panel (n = 16) (*continue*)

Attributes	**Bitter (Naringin mg/kg) x Aroma[3] (mg/kg)**							**Bitter (Naringin mg/kg) x Colour[2]**						
Bitter (Naringin mg/kg)	**158 (low)**		**315 (medium)**		**473 (high)**			**158 (low)**		**315 (medium)**		**473 (high)**		
Aroma (mg/kg)	**2.5 Low**	**10 High**	**2.5 Low**	**10 High**	**2.5 Low**	**10 High**	**Colour**	**Red[5]**	**Yellow[6]**	**Red**	**Yellow**	**Red**	**Yellow**	
Overall aroma intensity	5.40[1]a (0.08)	6.33a (0.08)	5.71a (0.08)	6.37a (0.08)	5.46a (0.08)	6.39a (0.08)	NS	5.94a (0.08)	5.79a (0.08)	6.18a (0.08)	5.89a (0.08)	6.05a (0.08)	5.81a (0.08)	NS
Citrus aroma	4.19a (0.08)	4.83a (0.08)	4.33a (0.08)	4.82a (0.08)	4.17a (0.08)	4.93a (0.08)	NS	4.45a (0.08)	4.57a (0.08)	4.67a (0.08)	4.49a (0.08)	4.50a (0.08)	4.60a (0.08)	NS
Grapefruit aroma	4.01a (0.08)	4.54a (0.08)	4.07a (0.08)	4.39a (0.08)	4.10a (0.08)	4.53a (0.08)	NS	4.43a (0.08)	4.13a (0.08)	4.36a (0.08)	4.10a (0.08)	4.40a (0.08)	4.23a (0.08)	NS
Chemical aroma	**3.65c (0.10)**	**4.34a (0.10)**	**4.04a (0.10)**	**4.22a (0.10)**	**3.91b (0.10)**	**4.20a (0.10)**	*	4.04a (0.10)	3.94a (0.10)	4.18a (0.10)	4.08a (0.10)	4.04a (0.10)	4.08a (0.10)	NS
Deteriorated/rotten aroma	2.07a (0.06)	2.09a (0.06)	2.08a (0.06)	1.99a (0.06)	2.12a (0.06)	2.06a (0.06)	NS	2.18a (0.06)	1.98a (0.06)	2.05a (0.06)	2.03a (0.06)	2.19a (0.06)	1.99a (0.06)	NS
Muddy/mouldy aroma	2.02a (0.05)	2.23a (0.05)	2.12a (0.05)	2.15a (0.05)	2.13a (0.05)	2.23a (0.05)	NS	2.19a (0.05)	2.06a (0.05)	2.20a (0.05)	2.07a (0.05)	2.22a (0.05)	2.13a (0.05)	NS
Fruity aroma	3.70a (0.08)	4.08a (0.08)	3.69a (0.08)	3.98a (0.08)	3.74a (0.08)	3.95a (0.08)	NS	3.93a (0.08)	3.85a (0.08)	3.89a (0.08)	3.78a (0.08)	3.97a (0.08)	3.72a (0.08)	NS
Green/grassy aroma	2.85a (0.07)	3.40a (0.07)	2.92a (0.07)	3.26a (0.07)	2.96a (0.07)	3.43a (0.07)	NS	3.15a (0.07)	3.09a (0.07)	3.10a (0.07)	3.08a (0.07)	3.15a (0.07)	3.24a (0.07)	NS
Peely/peel oil aroma	3.17a (0.08)	3.90a (0.08)	3.22a (0.08)	3.72a (0.08)	3.22a (0.08)	3.77a (0.08)	NS	3.56a (0.08)	3.50a (0.08)	3.49a (0.08)	3.45a (0.08)	3.54a (0.08)	3.45a (0.08)	NS
Soapy aroma	3.08a (0.09)	3.45a (0.09)	3.12a (0.09)	3.28a (0.09)	3.28a (0.09)	3.34a (0.09)	NS	3.25a (0.09)	3.28a (0.09)	3.30a (0.09)	3.10a (0.09)	3.31a (0.09)	3.32a (0.09)	NS
Pungent aroma	2.73a 0.08)	3.47a (0.08)	2.95a (0.08)	3.32a (0.08)	2.85a (0.08)	3.50a (0.08)	NS	3.12a (0.08)	3.08a (0.08)	3.19a (0.08)	3.09a (0.08)	3.18a (0.08)	3.18a (0.08)	NS
Woody/spicy aroma	2.27a (0.06)	2.59a (0.06)	2.35a (0.06)	2.53a (0.06)	2.36a (0.06)	2.58 (0.06)	NS	2.43a (0.06)	2.43a (0.06)	2.53a (0.06)	2.35a (0.06)	2.52a (0.06)	2.42a (0.06)	NS
Sweet aroma	3.53a (0.09)	3.93a (0.09)	3.56a (0.09)	3.82a (0.09)	3.58a (0.09)	3.83 (0.09)	NS	3.85a (0.09)	3.61a (0.09)	3.72a (0.09)	3.66a (0.09)	3.81a (0.09)	3.60a (0.09)	NS
Overall flavour intensity	**6.06c (0.08)**	**6.22bc (0.08)**	**6.62a (0.08)**	**6.39b (0.08)**	**6.43a (0.08)**	**6.48a (0.08)**	*	6.19a (0.08)	6.09a (0.08)	6.62a (0.08)	6.40a (0.08)	6.37a (0.08)	6.54a (0.08)	NS
Sour flavour	4.83a (0.11)	4.98a (0.11)	5.20a (0.11)	4.95a (0.11)	5.40a (0.11)	5.41a (0.11)	NS	4.86a (0.11)	4.94a (0.11)	5.08a (0.11)	5.07a (0.11)	5.32a (0.11)	5.48a (0.11)	NS
Sweet flavour	4.53a (0.09)	4.75a (0.09)	4.45a (0.09)	4.58a (0.09)	4.18a (0.09)	4.24a (0.09)	NS	4.65a (0.09)	4.63a (0.09)	4.59a (0.09)	4.43a (0.09)	4.19a (0.09)	4.23a (0.09)	NS
Bitter flavour	**4.00d (0.12)**	**3.88d (0.12)**	**4.87bc (0.12)**	**4.05d (0.12)**	**5.44a (0.12)**	**4.89b (0.12)**	*	4.01a (0.12)	3.86a (0.12)	4.58a (0.12)	4.34a (0.12)	5.08a (0.12)	5.25a (0.12)	NS
Astringent flavour	4.60a (0.10)	4.63a (0.10)	5.04a (0.10)	4.76a (0.10)	5.25a (0.10)	4.98a (0.10)	NS	4.71a (0.10)	4.52a (0.10)	5.01a (0.10)	4.80a (0.10)	5.12a (0.10)	5.11a (0.10)	NS
Citrus flavour	4.42a (0.08)	4.62a (0.08)	4.29a (0.08)	4.59a (0.08)	4.20a (0.08)	4.57a (0.08)	NS	4.54a (0.08)	4.50a (0.08)	4.41a (0.08)	4.48a (0.08)	4.45a (0.08)	4.32a (0.08)	NS
Grapefruit flavour	4.17a (0.09)	4.21a (0.09)	4.57a (0.09)	4.25a (0.09)	4.73a (0.09)	4.73a (0.09)	NS	4.37a (0.09)	4.01a (0.09)	4.49a (0.09)	4.33a (0.09)	4.73a (0.09)	4.72a (0.09)	NS
Bitter aftertaste	**3.70c (0.12)**	**3.68c (0.12)**	**4.54b (0.12)**	**3.97c (0.12)**	**5.23a (0.12)**	**4.60b (0.12)**	*	**3.79bc (0.12)**	**3.59c (0.12)**	**4.40b (0.12)**	**4.11b (0.12)**	**4.77a (0.12)**	**5.06a (0.12)**	*

Table 14 Summary of the main and interaction ANOVA effects (colour hue, aroma, bitter and sweet levels) on mean values[1] (± SEM) for sensory attributes of grapefruit-like beverages as evaluated by a trained sensory panel (n = 16) (*continue*)

Attributes	Bitter (Naringin mg/kg) x Sweet (°Brix)										Aroma[3] mg/kg x Colour[2]				
	158 (low)			315 (medium)			473 (high)				2.5 (low)		10 (high)		
	8 Low	10 Medium	12 High	8 Low	10 Medium	12 High	8 Low	10 Medium	12 High		Red[6]	Yellow[7]	Red	Yellow	
Overall aroma intensity	5.90[1]a (0.10)	5.90a (0.10)	5.80a (0.10)	6.20a (0.10)	5.89a (0.10)	6.03a (0.10)	5.80a (0.10)	5.94a (0.10)	6.05a (0.10)	NS	5.59a (0.06)	5.45a (0.06)	6.52a (0.06)	6.21a (0.06)	NS
Citrus aroma	4.31a (0.10)	4.68a (0.10)	4.54a (0.10)	4.64a (0.10)	4.49a (0.10)	4.60a (0.10)	4.58a (0.10)	4.56a (0.10)	4.51a (0.10)	NS	4.22a (0.07)	4.24a (0.07)	4.86a (0.07)	4.86a (0.07)	NS
Grapefruit aroma	4.19a (0.10)	4.39a (0.10)	4.25a (0.10)	4.20a (0.10)	4.27a (0.10)	4.22a (0.10)	4.29a (0.10)	4.38a (0.10)	4.38a (0.10)	NS	4.12a (0.06)	4.00a (0.06)	4.67a (0.06)	4.30a (0.06)	NS
Chemical aroma	3.91a (0.12)	4.02a (0.12)	4.04a (0.12)	4.33a (0.12)	3.98a (0.12)	4.08a (0.12)	3.94a (0.12)	4.08a (0.12)	4.16a (0.12)	NS	3.91a (0.08)	3.82a (0.08)	4.26a (0.08)	4.25a (0.08)	NS
Deteriorated/rotten aroma	2.03a (0.08)	2.02a (0.08)	2.19a (0.08)	2.06a (0.08)	2.05a (0.08)	2.01a (0.08)	2.06a (0.08)	2.04a (0.08)	2.15a (0.08)	NS	2.18a (0.05)	2.01a (0.05)	2.10a (0.05)	1.99a (0.05)	NS
Muddy/mouldy aroma	2.12a (0.06)	2.13a (0.06)	2.12a (0.06)	2.23a (0.06)	2.11a (0.06)	2.06a (0.06)	2.17a (0.06)	2.16a (0.06)	2.20a (0.06)	NS	2.15a (0.04)	2.02a (0.04)	2.26a (0.04)	2.15a (0.04)	NS
Fruity aroma	3.89a (0.10)	4.02a (0.10)	3.75a (0.10)	3.78a (0.10)	3.87a (0.10)	3.85a (0.10)	3.82a (0.10)	3.82a (0.10)	3.90a (0.10)	NS	3.79a (0.07)	3.63a (0.07)	4.07a (0.07)	3.94a (0.07)	NS
Green/grassy aroma	3.10a (0.08)	3.22a (0.08)	3.05a (0.08)	3.15a (0.08)	3.09a (0.08)	3.02a (0.08)	3.11a (0.08)	3.24a (0.08)	3.24a (0.08)	NS	2.92a (0.06)	2.90a (0.06)	3.35a (0.06)	3.37a (0.06)	NS
Peely/peel oil aroma	3.58a (0.10)	3.57a (0.10)	3.45a (0.10)	3.50a (0.10)	3.52a (0.10)	3.39a (0.10)	3.39a (0.10)	3.66a (0.10)	3.54a (0.10)	NS	3.25a (0.06)	3.16a (0.06)	3.81a (0.06)	3.78a (0.06)	NS
Soapy aroma	3.29a (0.11)	3.46a (0.11)	3.05a (0.11)	3.39a (0.11)	3.23a (0.11)	2.99a (0.11)	3.54a (0.11)	3.31a (0.11)	3.09a (0.11)	NS	3.18a (0.07)	3.14a (0.07)	3.39a (0.07)	3.32a (0.07)	NS
Pungent aroma	**2.96ab (0.10)**	**3.20a (0.10)**	**3.13a (0.10)**	**3.40a (0.10)**	**2.96b (0.10)**	**3.05ab (0.10)**	**3.15a (0.10)**	**3.23a (0.10)**	**3.14a (0.10)**	*****	2.87a (0.07)	2.81a (0.07)	3.45a (0.07)	3.41a (0.07)	NS
Woody/spicy aroma	2.44a (0.07)	2.51a (0.07)	2.34a (0.07)	2.51a (0.07)	2.43a (0.07)	2.38a (0.07)	2.49a (0.07)	2.52a (0.07)	2.40a (0.07)	NS	2.39a (0.05)	2.26a (0.05)	2.60a (0.05)	2.54a (0.05)	NS
Sweet aroma	3.77a (0.10)	3.78a (0.10)	3.64a (0.10)	3.72a (0.10)	3.70a (0.10)	3.66a (0.10)	3.57a (0.10)	3.68a (0.10)	3.86a (0.10)	NS	3.65a (0.07)	3.46a (0.07)	3.93a (0.07)	3.79a (0.07)	NS
Overall flavour intensity	6.02a (0.10)	6.15a (0.10)	6.24a (0.10)	6.57a (0.10)	6.37a (0.10)	6.58a (0.10)	6.63a (0.10)	6.35a (0.10)	6.35a (0.10)	NS	6.38a (0.07)	6.36a (0.07)	6.41a (0.07)	6.32a (0.07)	NS
Sour flavour	5.77a (0.14)	4.71a (0.14)	4.23a (0.14)	5.94a (0.14)	5.14a (0.14)	4.15a (0.14)	6.09a (0.14)	5.37a (0.14)	4.76a (0.14)	NS	5.20a (0.09)	5.10a (0.09)	4.99a (0.09)	5.24a (0.09)	NS
Sweet flavour	3.15a (0.11)	4.78a (0.11)	5.99a (0.11)	3.02a (0.11)	4.57a (0.11)	5.95a (0.11)	2.95a (0.11)	4.16a (0.11)	5.51a (0.11)	NS	4.34a (0.07)	4.43a (0.07)	4.61a (0.07)	4.44a (0.07)	NS
Bitter flavour	4.64a (0.15)	3.91a (0.15)	3.27a (0.15)	5.28a (0.15)	4.20a (0.15)	3.89a (0.15)	5.81a (0.15)	5.19a (0.15)	4.50a (0.15)	NS	**4.91a (0.10)**	**4.64a (0.11)**	**4.21c (0.11)**	**4.33b (0.11)**	*****
Astringent flavour	5.10a (0.13)	4.71a (0.13)	4.05a (0.13)	5.42a (0.13)	4.85a (0.13)	4.45a (0.13)	5.53a (0.13)	5.07a (0.13)	4.75a (0.13)	NS	5.04a (0.08)	4.89a (0.08)	4.85a (0.08)	4.73a (0.08)	NS
Citrus flavour	4.61a (0.10)	4.45a (0.10)	4.50a (0.10)	4.29a (0.10)	4.56a (0.10)	4.47a (0.10)	4.38a (0.10)	4.33a (0.10)	4.46a (0.10)	NS	4.34a (0.07)	4.27a (0.07)	4.59a (0.07)	4.60a (0.07)	NS
Grapefruit flavour	4.24a (0.12)	4.28a (0.12)	4.04a (0.12)	4.46a (0.12)	4.51a (0.12)	4.26a (0.12)	4.87a (0.12)	4.83a (0.12)	4.48a (0.12)	NS	4.55a (0.08)	4.42a (0.08)	4.50a (0.08)	4.29a (0.08)	NS
Bitter aftertaste	4.21a (0.15)	3.67a (0.15)	3.19a (0.15)	5.10a (0.15)	4.14a (0.15)	3.54a (0.15)	5.40a (0.15)	4.93a (0.15)	4.41a (0.15)	NS	4.59a (0.10)	4.40a (0.10)	4.05a (0.10)	4.12a (0.10)	NS

Table 14 Summary of the main and interaction ANOVA effects (colour hue, aroma, bitter and sweet levels) on mean values[1] (± SEM) for sensory attributes of grapefruit-like beverages as evaluated by a trained sensory panel (n = 16) (*continue*)

Attributes	**Sweet °Brix x Aroma[3]**							**Sweet °Brix x Colour[2]**						
	8 Low		**10 Medium**		**12 High**			**8 Low**		**10 Medium**		**12 High**		
	2.5 Low	**10 High**	**2.5 Low**	**10 High**	**2.5 Low**	**10 High**		**Red**	**Yellow**	**Red**	**Yellow**	**Red**	**Yellow**	
Overall aroma intensity	5.57[1]a (0.08)	6.36a (0.08)	5.44a (0.08)	6.38a (0.08)	5.57a (0.08)	6.35a (0.08)	NS	6.11a (0.08)	5.82a (0.08)	5.94a (0.08)	5.88a (0.08)	6.13a (0.08)	5.79a (0.08)	NS
Citrus aroma	4.17a (0.08)	4.84a (0.08)	4.27a (0.08)	4.88a (0.08)	4.24a (0.08)	4.85a (0.08)	NS	4.60a (0.08)	4.42a (0.08)	4.52a (0.08)	4.64a (0.08)	4.50a (0.08)	4.60a (0.08)	NS
Grapefruit aroma	4.01a (0.08)	4.44a (0.08)	4.16a (0.08)	4.53a (0.08)	4.00a (0.08)	4.50a (0.08)	NS	4.43a (0.08)	4.03a (0.08)	4.46a (0.08)	4.23a (0.08)	4.30a (0.08)	4.20a (0.08)	NS
Chemical aroma	3.93a (0.10)	4.20a (0.10)	3.79a (0.10)	4.26a (0.10)	3.88a (0.10)	4.30a (0.10)	NS	4.15a (0.10)	3.98a (0.10)	3.99a (0.10)	4.06a (0.10)	4.12a (0.10)	4.06a (0.10)	NS
Deteriorated/rotten aroma	2.06a (0.06)	2.04a (0.06)	2.02a (0.06)	2.06a (0.06)	2.20a (0.06)	2.04a (0.06)	NS	2.16a (0.06)	1.93a (0.06)	2.10a (0.06)	1.98a (0.06)	2.16a (0.06)	2.08a (0.06)	NS
Muddy/mouldy aroma	2.14a (0.05)	2.21a (0.05)	2.05a (0.05)	2.22a (0.05)	2.08a (0.05)	2.18a (0.05)	NS	2.25a (0.05)	2.10a (0.05)	2.16a (0.05)	2.10a (0.05)	2.20a (0.05)	2.06a (0.05)	NS
Fruity aroma	3.66a (0.08)	4.01a (0.08)	3.72a (0.08)	4.09a (0.08)	3.75a (0.08)	3.92a (0.08)	NS	3.95a (0.08)	3.71a (0.08)	3.90a (0.08)	3.91a (0.08)	3.94a (0.08)	3.73a (0.08)	NS
Green/grassy aroma	2.87a (0.07)	3.36a (0.07)	2.94a (0.07)	3.42a (0.07)	2.91a (0,07)	3.30a (0.07)	NS	3.12a (0.07)	3.11a (0.07)	3.17a (0.07)	3.19a (0.07)	3.10a (0.07)	3.10a (0.07)	NS
Peely/peel oil aroma	3.13a (0.08)	3.84a (0.08)	3.30a (0.08)	3.80a (0.08)	3.18a (0.08)	3.74a (0.08)	NS	3.54a (0.08)	3.43a (0.08)	3.55a (0.08)	3.55a (0.08)	3.49a (0.08)	3.42a (0.08)	NS
Soapy aroma	3.35a (0.09)	3.46a (0.09)	3.18a (0.09)	3.48a (0.09)	2.96a (0.09)	3.12a (0.09)	NS	3.42a (0.09)	3.39a (0.09)	3.29a (0.09)	3.37a (0.09)	3.14a (0.09)	2.94a (0.09)	NS
Pungent aroma	2.92a (0.08)	3.42a (0.08)	2.78a (0.08)	3.48a (0.08)	2.83a (0.08)	3.39a (0.08)	NS	3.20a (0.08)	3.14a (0.08)	3.13a (0.08)	3.13a (0.08)	3.16a (0.08)	3.06a (0.08)	NS
Woody/spicy aroma	2.32a (0.06)	2.64a (0.06)	2.38a (0.06)	2.59a (0.06)	2.27a (0.06)	2.47a (0.06)	NS	2.53a (0.06)	2.44a (0.06)	2.52a (0.06)	2.45a (0.06)	2.43a (0.06)	2.31a (0.06)	NS
Sweet aroma	3.44a (0.09)	3.93a (0.09)	3.58a (0.09)	3.86a (0.09)	3.64a (0.09)	3.79a (0.09)	NS	3.74a (0.09)	3.63a (0.09)	3.76a (0.09)	3.68a 0.09)	3.88a (0.09)	3.56a (0.09)	NS
Overall flavour intensity	6.46a (0.08)	6.36a (0.08)	6.31a (0.08)	6.28a (0.08)	6.35a (0.08)	6.46a (0.08)	NS	6.51a (0.08)	6.31a (0.08)	6.29a (0.08)	6.30a (0.08)	6.39a (0.08)	6.42a (0.08)	NS
Sour flavour	5.96a (0.11)	5.91a (0.11)	5.07a (0.11)	5.08a (0.11)	4.40a (0.11)	4.35a (0.11)	NS	6.01a (0.11)	5.86a (0.11)	4.96a (0.11)	5.19a (0.11)	4.31a (0.11)	4.44a (0.11)	NS
Sweet flavour	2.92a (0.09)	3.16a (0.09)	4.38a (0.09)	4.63a (0.09)	5.85a (0.09)	5.78a (0.09)	NS	3.09a (0.09)	2.99a (0.09)	4.49a (0.09)	4.52a (0.09)	5.85a (0.09)	5.78a (0.09)	NS
Bitter flavour	5.51a (0.12)	4.98a (0.12)	4.80a (0.12)	4.07a (0.12)	4.02a (0.12)	3.76a (0.12)	NS	5.30a (0.12)	5.18a (0.12)	4.55a (0.12)	4.32a (0.12)	3.82a (0.12)	3.96a (0.12)	NS
Astringent flavour	5.42a (0.10)	5.28a (0.10)	4.96a (0.10)	4.79a (0.10)	4.52a (0.10)	4.31a (0.12)	NS	**5.62a (0.10)**	**5.08b (0.10)**	**4.92b (0.10)**	**4.83b (0.10)**	**4.30c (0.10)**	**4.52c (0.10)**	*****
Citrus flavour	4.29a (0.08)	4.56a (0.08)	4.33a (0.08)	4.56a (0.08)	4.29a (0.08)	4.67a 0.08)	NS	**4.59a (0.08)**	**4.26b (0.08)**	**4.40a (0.08)**	**4.50a (0.08)**	**4.42a (0.08)**	**4.54a (0.08)**	*****
Grapefruit flavour	4.45a (0.09)	4.60a (0.09)	4.69a (0.09)	4.38a (0.09)	4.32a (0.09)	4.20a (0.09)	NS	4.60a (0.09)	4.45a (0.09)	4.60a (0.09)	4.48a (0.09)	4.39a (0.09)	4.14a (0.09)	NS
Bitter aftertaste	5.03a (0.12)	4.77a (0.12)	4.54a (0.12)	3.96a (0.12)	3.91a (0.12)	3.52a (0.12)	NS	4.94a (0.12)	4.87a (0.12)	4.36a (0.12)	4.13a (0.12)	3.65a (0.12)	3.77a (0.12)	NS

[1]Attribute intensity scale from 'not intense' (0) to 'very intense' (10); [2]Red = 0.001 % solution (30 % Sunset yellow and 70 % Ponceau red); Yellow = 0.0125 % Quinoline yellow. [3]Aroma blend [caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), aldehyde C10 (decanal)]. abc: Different letters (abc) indicate significantly different mean values across design variable levels; Means represent the average of duplicate ratings by 16 panelists; *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.0001; NS = not significantly different

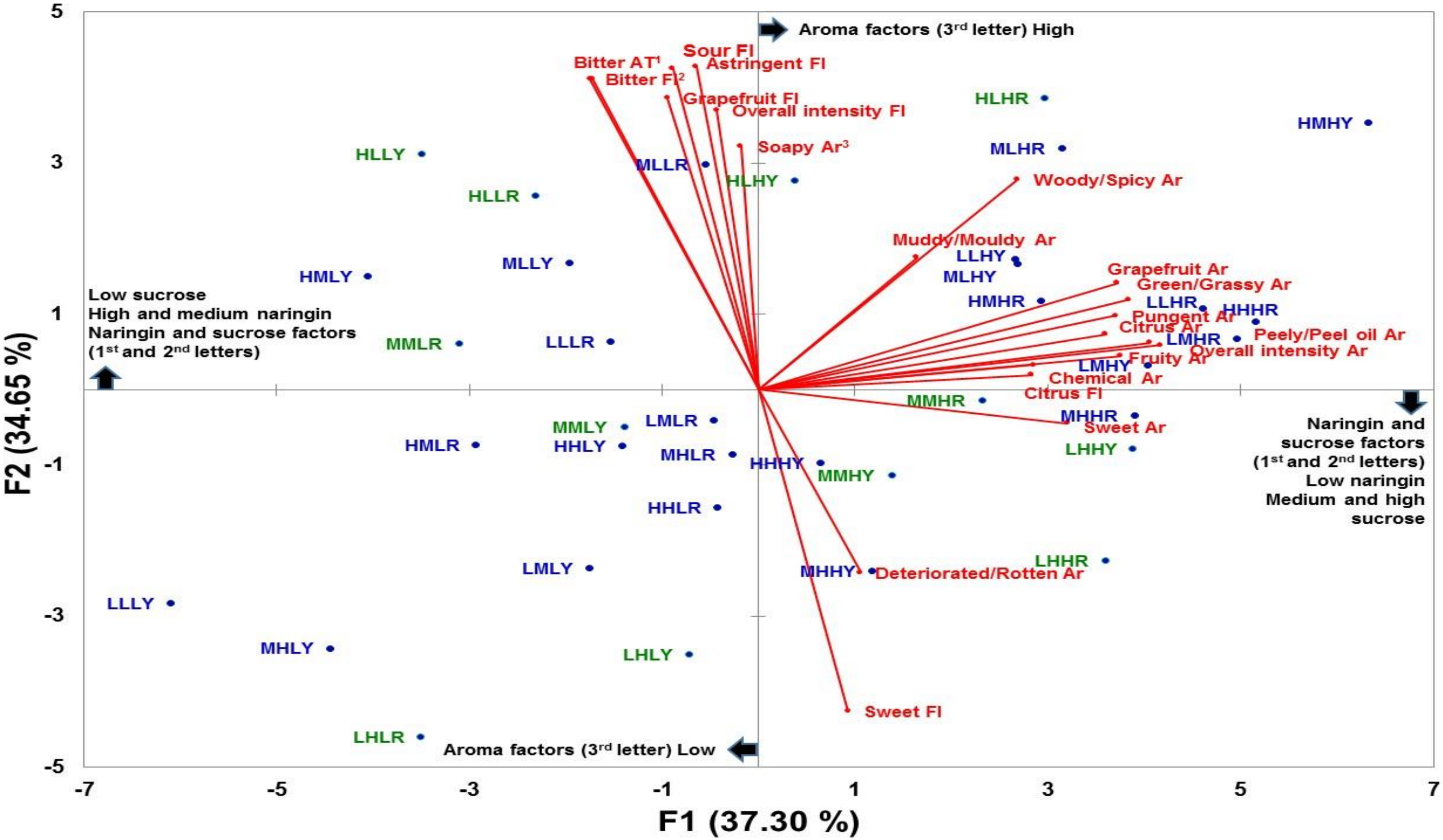

Figure 7 Principal Component Analysis (PCA) of the 36 grapefruit-like beverages. The vectors indicate the loadings for sensory attributes while the position of the sample codes indicate the score values. 4-Letter codes for the beverages indicate different levels of naringin (1st letter L = Low, M = Medium, H = High), sucrose (2nd letter L = Low, M = Medium, H = High), aroma (3rd letter L = Low, H = High) as well as type of colour (4th letter R = red or Y = yellow). Sensory attributes [1]AT = After Taste, [2]Fl = Flavour, [3]Ar = Aroma. Beverages in green font were selected for consumer test.

The first and second principal components (F1 and F2) explained 37 % and 35 % of the variance across the samples, respectively and accounted for a total of 72 % of the explained variance. F1 clearly separates beverages based on overall aroma intensity, peely/peel oil aroma, citrus aroma, sweet aroma and pungent aroma. Beverages that were more intense in terms of the mentioned attributes are located on the right of the plot with an H as 3rd letter and those with lower intensity located on the left of the plot has an L as 3rd letter. F2 clearly separated beverages based on ‘taste’ (naringin-sucrose) level with beverages with high and medium naringin concentration and low sucrose at the top, with a low concentration of naringin but medium and high sucrose at the bottom. Beverages HLLY, HLLR with high and MLHY with medium levels of naringin, at the top, were characterised by more intense ‘astringency, sour and bitter tastes, and grapefruit and overall flavour intensities’. Beverages LHHR with low naringin levels at the bottom, was characterized by the sweet taste. Citrus flavour intensity in the middle of the plot does not discriminate beverages on the first two PCs.

5.4.2 Consumer liking

5.4.2.1 General consumer preferences

The effect of varying concentrations of naringin/sucrose combinations, low and high aroma concentrations and yellow and red colourants applied to declarified apple juice on consumers' acceptance of colour, aroma and flavour of model grapefruit-like beverages are presented in Table 15.

Grapefruit-like beverages with the red colour were preferred over those with a yellow colour ($p < 0.05$). The low aroma samples were preferred over the high aroma samples. The low naringin/-high sucrose samples were preferred over the high naringin/-low sucrose samples. The aroma of the beverages with 473 mg/kg naringin at 8 °Brix sucrose was significantly more preferred than those with low naringin/-high sucrose and medium naringin/-medium sucrose concentrations. The flavour of the beverage with 158 mg/kg naringin at 12 °Brix sucrose was significantly more preferred than those with medium naringin/-medium sucrose followed by high naringin/-low sucrose concentrations. There was no significant interaction effect of the naringin/sucrose combinations, low and high aroma concentrations and yellow and red colours on consumer acceptance (liking) of colour, aroma and flavour of grapefruit-like beverages.

Table 15 The effect of varying colour, aroma and bitter/sweet gustatory flavourants on mean liking ratings[1] (± standard deviation) for colour, aroma and flavour of grapefruit-like beverages by (n = 90) consumers

	Colour[2]			**Aroma[3] mg/kg[3]**			**Bitter-Sweet Naringin mg/kg /Sucrose °Brix**			
	Red	**Yellow**		**2.5**	**10 mg/kg**		**158 / 12 Low/High**	**315 / 10 Medium/Medium**	**473 / 8 High/Low**	
Liking of **colour**	64[1]b (30)	60a (30)	**	63a (30)	61a (31)	NS	62a (30)	62a (31)	61a (30)	NS
Liking of aroma	51a (30)	51a (30)	NS	53a (29)	49b (31)	***	50ab (30)	53a (30)	49b (29)	*
Liking of **flavour**	45a (33)	45a (34)	NS	45a (34)	45a (34)	NS	55a (33)	46b (33)	34c (32)	***

[1]Simplified Labelled Affective Magnitude Scale (SLAM) 0 = greatest imaginable dislike, 100 = greatest imaginable liking. Different letters (abc) indicate significantly different mean values across design variable levels. NS = not significant, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. [2]Red = 0.001 % solution (30 % Sunset yellow and 70 % Ponceau red); Yellow = 0.0125 % Quinoline yellow. [3]Aroma blend [caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), aldehyde C10 (decanal)].

5.4.2.2 Consumer preference clusters

Three distinct subgroups of consumers were distinguished for colour liking and two for aroma and three for flavour liking of the beverages (Table 16).

5.4.2.2.1 Colour liking

Consumers in cluster 1, as well as those in cluster 2, preferred the red samples and there was a smaller group of consumers (cluster 3) that preferred the yellow beverages. Cluster 1 consumers showed the highest preference for the high aroma and high bitter low sweet sample HLHR and the lowest preference for the high aroma and medium bitter and sweet sample MMHY. Cluster 2 consumers showed the highest preference for the low aroma and high bitter and low sweet sample HLLR and the lowest for high aroma and low bitter and high sweet sample LHHY. Cluster 3 consumers showed the highest preference for MMHY and the lowest for HLHR.

5.4.2.2.2 Aroma liking

There are two clusters which are driven mainly by a very different response to sample LHHY, and to a lesser extent LHHR, which are the red and yellow samples with low naringin, high sucrose and high aroma content (Table 16). Cluster 1 consumers showed the highest preference for the low aroma sample LHLY and the lowest preference for the high aroma sample LHHY.

Table 16 Mean liking[1] scores for 12 grapefruit-like beverages for clusters of consumers

	Sample key	Cluster 1 (n = 44)	Cluster 2 (n = 29)	Cluster 3 (n = 22)
Mean colour liking scores	LHHR	77.0ab (2.6)	56.7abc (3.2)	48.0bcd (4.8)
	HLHR	78.3a (3.1)	63.7ab (2.8)	37.1d (5.4)
	MMHR	74.0ab (2.4)	54.3cb (3.0)	50.5bcd (5.6)
	LHLR	74.0ab (2.9)	59.2abc (2.5)	62.7abc (3.8)
	HLLR	65.1abc (3.0)	72.9a (3.4)	42.0cd (5.5)
	MMLR	75.1ab (2.8)	54.2cb (4.7)	63.0abc (5.8)
	MMHY	45.9d (3.5)	61.8ab (2.2)	85.5a (4.2)
	LHHY	53.2cd (3.2)	44.5c (4.0)	75.0a (4.4)
	HLHY	56.0cd (3.7)	58.4abc (3.7)	67.0ab (4.0)
	MMLY	52.0cd (3.1)	64.1ab (4.1)	65.5abc (6.3)
	LHLY	63.6bc (2.5)	52.4bc (3.9)	67.4ab (3.8)
	HLLY	57.5cd (2.7)	58.9abc (2.3)	66.5ab (4.7)
	Sample	**Cluster 1 (n = 45)**	**Cluster 2 (n = 50)**	
Mean aroma liking scores	LHLR	56.0abc (3.8)	54.2ab (2.4)	
	LHLY	57.6ab (3.0)	45.1b (3.2)	
	MMLR	57.4ab (3.0)	54.0ab (4.5)	
	MMLY	59.8a (3.7)	47.0ab (2.9)	
	HLLR	54.4abc (3.3)	51.4ab (3.0)	
	HLLY	44.0bcd (3.2)	56.2ab (3.0)	
	LHHR	34.4d (2.7)	52.3ab (2.4)	
	LHHY	33.8d (2.9)	61.6a (3.4)	
	MMHR	41.9cd (2.7)	59.1ab (3.1)	
	MMHY	47.4abcd (3.6)	54.9ab (2.8)	
	HLHR	51.3abc (3.7)	47.2ab (2.8)	
	HLHY	41.6cd (2.5)	54.9ab (3.3)	
	Sample	**Cluster 1 (n = 40)**	**Cluster 2 (n = 18)**	**Cluster 3 (n = 37)**
Mean flavour liking scores	LHHR	72.5a (2.7)	43.9abc (5.9)	39.8cdef (4.2)
	LHLR	64.0ab (2.9)	25.4c (5.0)	58.5abc (5.0)
	LHHY	59.9ab (4.1)	39.2abc (5.9)	57.8abc (4.1)
	LHLY	47.8bcd (3.9)	34.7abc (5.8)	75.0a (3.8)
	MMHR	29.1e (3.8)	57.7ab (7.3)	49.2bcd (3.5)
	MMLR	41.4cde (3.3)	38.5abc (5.1)	60.4ab (4.6)
	MMHY	47.8bcd (4.3)	48.0abc (5.3)	44.8bcde (3.8)
	MMLY	51.8bc (3.9)	46.5abc (4.1)	36.4def (4.1)
	HLHR	31.8de (2.5)	62.2a (7.2)	24.9f (3.1)
	HLLR	39.1cde (4.5)	26.9c (5.4)	29.9def (3.5)
	HLHY	34.6cde (3.2)	47.9abc (6.7)	28.1ef (3.9)
	HLLY	37.6cde (3.3)	31.9cb (5.1)	31.4def (4.1)

[1]Simplified Labelled Affective Magnitude Scale (SLAM) 0 = greatest imaginable dislike, 50 = neither like nor dislike, 100 = greatest imaginable liking. Standard deviations in brackets. Mean values in a column with different letters was significantly different. Sample key: 1st letter = naringin factor, 2nd letter = sucrose factor, 3rd letter = aroma, 4th letter = colour factor

Cluster 2 consumers most prefered sample LHHY and the least prefered sample LHLY.

5.4.2.2.3 Flavour liking

There are three clusters where cluster 2 is small, while clusters 1 and 3 larger and more similar in number. Cluster 1 consumers showed the highest preference for sample LHHR and the lowest for MMHR. Cluster 2 consumers showed the highest preference for sample HLHR and the lowest for LHLR. Cluster 3 consumers showed the highest preference for sample LHLY and the lowest for sample HLHR.

5.4.2.3 Internal Preference Mapping of colour, aroma and flavour liking

5.4.2.3.1 Internal Preference Mapping of colour liking

The internal preference map of consumer clusters and descriptive properties of the 36 grapefruit-like beverages are shown in Figure 8. It indicates that the preference of cluster 2 consumers was best represented by the red sample MMLR which showed a stronger astringent, grapefruit flavour with bitter after taste and moderate deteriorated/rotten aroma, muddy/mouldy aroma and grapefruit aroma-intensities. Consumers in cluster 2 also liked samples HLHR, MMHR and LHHR with strong grapefruit flavour and aroma and bitter after taste but disliked yellow samples LHHY and LHLY which were positioned in the opposite direction of this preference vector. These samples were described as having a sweet flavour, citrus and chemical aroma by descriptive analysis. These sensory attributes were considered as the main drivers of dislike for this cluster of consumers. Cluster 1 consumers' preference was best represented by red samples HLHR, MMHR and LHLR with stronger grapefruit and woody/spicy aroma but they disliked yellow samples HLLY, HLHY which were positioned in the opposite direction of this preference vector. These samples were described as having a citrus flavour, soapy aroma and peely/peel oil aroma by descriptive analysis. These sensory attributes were considered as the main drivers of dislike for consumers in this cluster. Cluster 3 consumers preferred the yellow samples MMLY, MMHY with more intense citrus flavour, peely/peel oil aroma, soapy aroma and moderately citrus aroma. The consumers in cluster 3 disliked red samples LHLR and LHHR which were positioned in the oposite direction of this preference vector. These samples were described as having grapefruit, woody/spicy, pungent and muddy/mouldy aroma by descriptive analysis, these attributes were considered as the main drivers of dislike for this cluster of consumers.

5.4.2.3.2 *Internal Preference Mapping of aroma liking*

Consumers in the two clusters showed different preferences as presented by the vector directions. Figure 9 indicated that the preference of cluster 1 consumers was best represented by low-aroma, yellow samples LHLY, MMLY with a bitter, astringent flavour and bitter after taste. The consumers in cluster 1 disliked the high-aroma samples LHHY, LHHR and MMHR which were positioned in the opposite direction of the vector. These high-aroma, red samples were described as having strong fruity, grapefruit, citrus, pungent, chemical and muddy/mouldy aroma by descriptive analysis, these sensory attributes were considered as the main drivers of dislike for this cluster of consumers. Consumers in cluster 2 liked the high-aroma samples HLHY, MMHR and LHHY with more intense sweet, green/grassy, woody/spicy, peely/peel oil aromas and dislike the low-aroma yellow samples MMLY and LHLY which were positioned in the opposite direction of this preference vector. These samples were described as having a bitter flavour and bitter after taste by descriptive analysis, these sensory attributes were considered as the main drivers of dislike for this cluster of consumers.

5.4.2.3.3 *Internal Preference Mapping of flavour liking*

Three consumer clusters showed different preferences as presented by the vector directions. Figure 10 indicated that the preference of cluster 1 consumers was best represented by high aroma and low bitterness-high sweetness samples LHHR, LHHY with a deteriorated/rotten, chemical and sweet aroma. The consumers in cluster 1 disliked the red, high aroma samples HLHR and MMHR which are positioned in the opposite direction of the vector. These samples were described as having fruity, citrus and grapefruit aroma by descriptive analysis, these sensory attributes were considered as the main drivers of dislike for this cluster of consumers. The preferences of cluster 2 consumers are the opposite of cluster 1. Preferences of cluster 3 consumers were best represented by low aroma samples LHLY, MMLR and LHLR with a sweet and citrus flavour. The consumers in cluster 3 disliked the low aroma samples with high bitterness-low sweetness HLLR and HLLY which are positioned in the opposite direction of the vector.

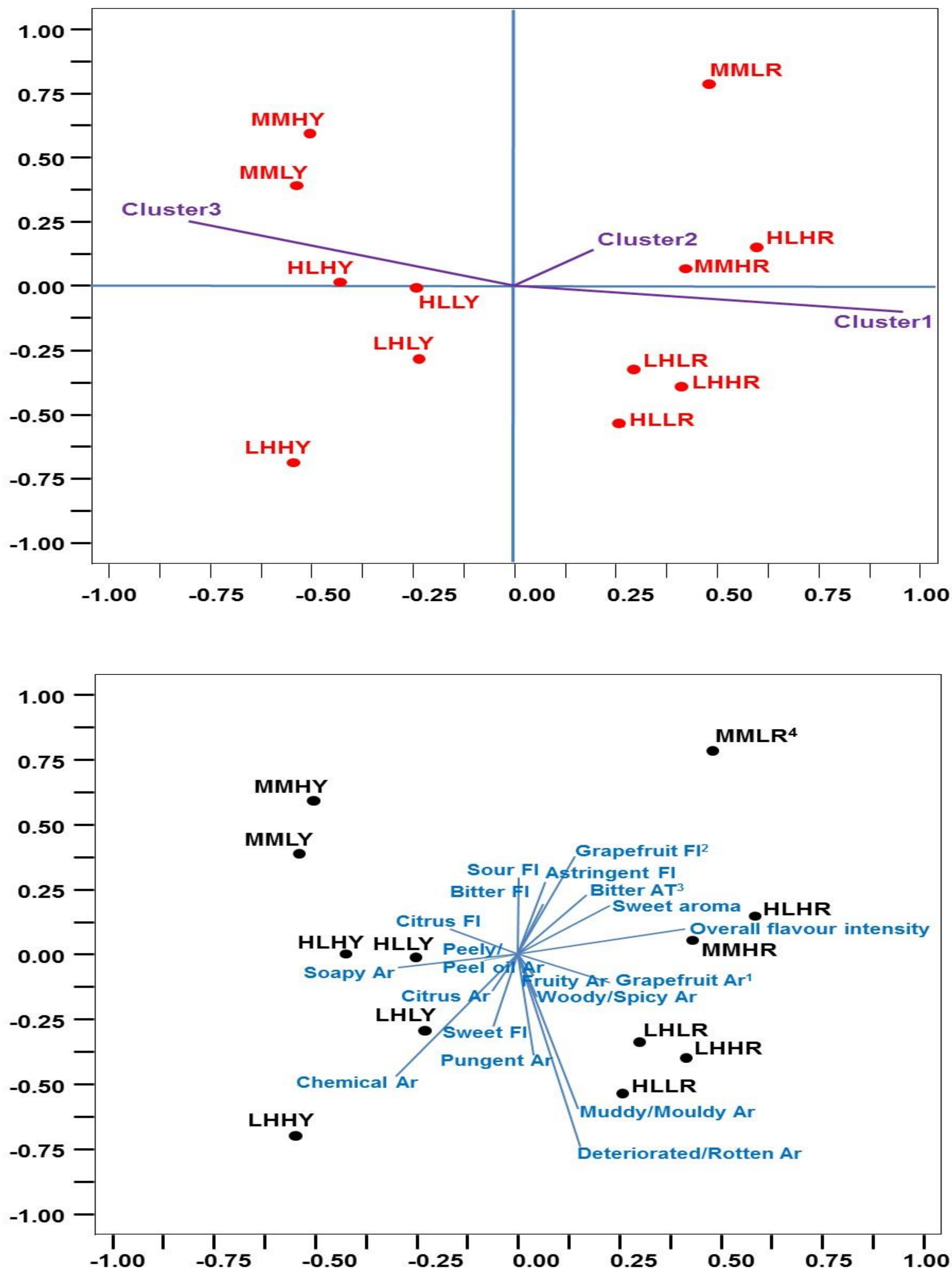

Figure 8 Internal Preference Mapping of consumer clusters and descriptive properties of the 36 grapefruit-like beverages (n = 12) with vectors representing the direction of colour liking for the consumer clusters. Sensory attributes [1]AT = After taste, [2]Fl = Flavour, [3]Ar = Aroma, [4]Sample key: 1st letter = naringin factor, 2nd letter = sucrose factor, 3rd letter = aroma, 4th letter = colour factor

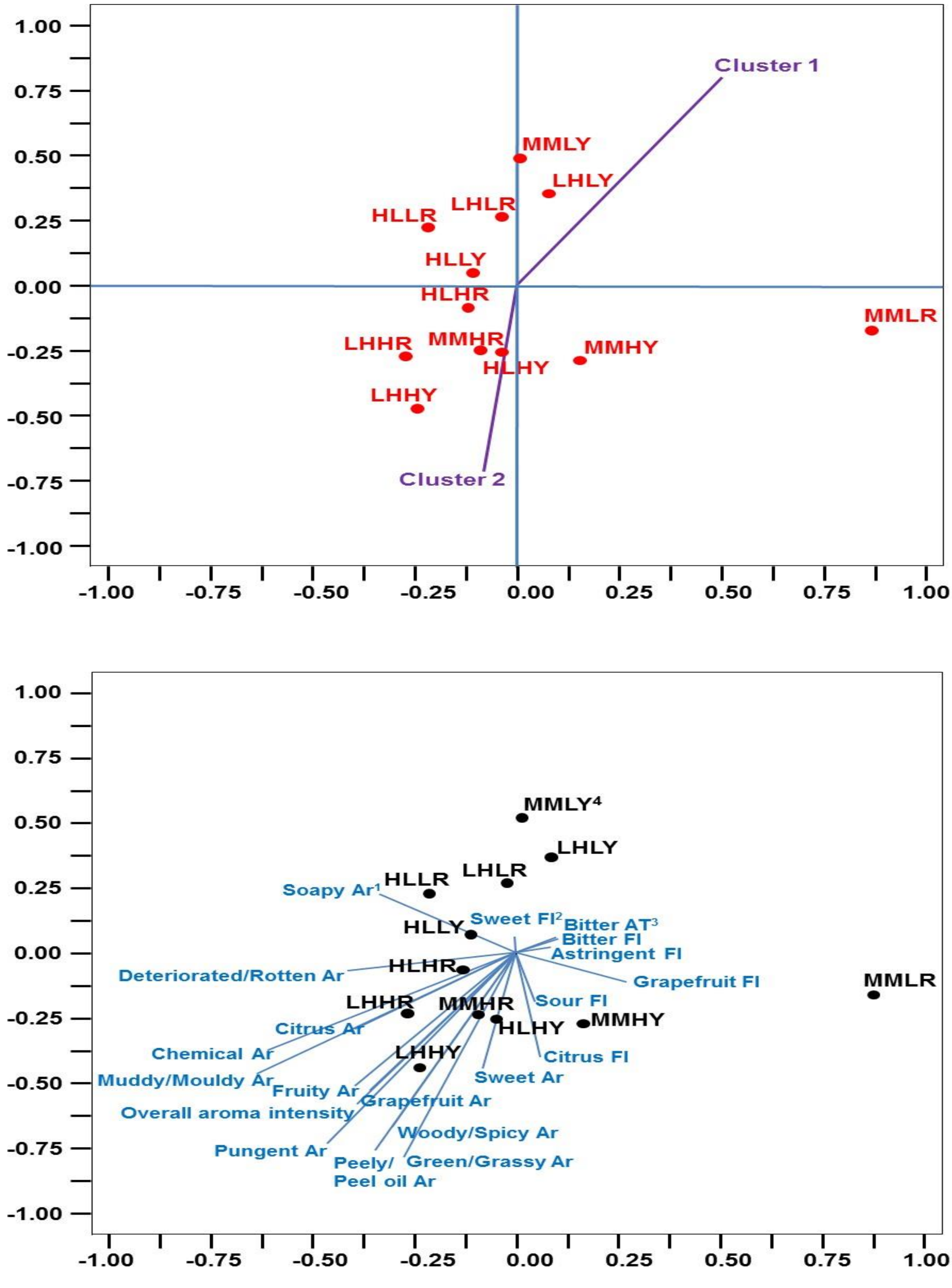

Figure 9 Internal Preference Mapping of consumer clusters and descriptive properties of the 36 grapefruit-like beverages (n = 12) with vectors representing the direction of aroma liking for the consumer clusters. Sensory attributes [1]AT = After taste, [2]Fl = Flavour, [3]Ar = Aroma, [4]Sample key: 1st letter = naringin factor, 2nd letter = sucrose factor, 3rd letter = aroma, 4th letter = colour factor

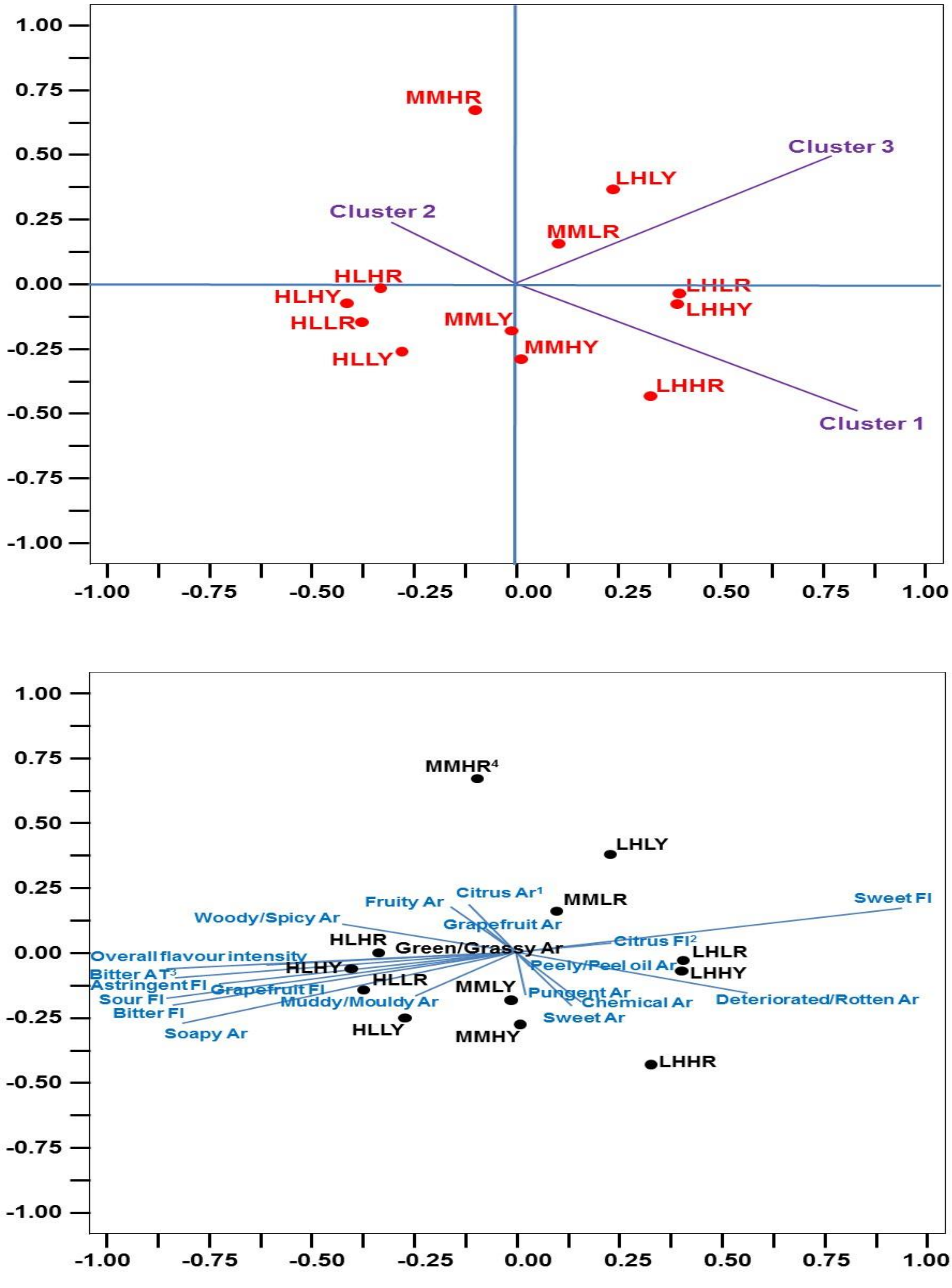

Figure 10 Internal Preference Mapping of consumer clusters and descriptive properties of the 36 grapefruit-like beverages (n = 12) with vectors representing the direction of flavour liking for the consumer clusters. Sensory attributes [1]AT = After taste, [2]Fl = Flavour, [3]Ar = Aroma, [4]Sample key: 1st letter = naringin factor, 2nd letter = sucrose factor, 3rd letter = aroma, 4th letter = colour factor

These samples were described as having muddy/mouldy, soapy aroma and sour, grapefruit flavour and bitter after taste by descriptive analysis, these sensory attributes were considered as the main drivers of dislike for this cluster of consumers.

5.4.2.4 Partial least squares regression of descriptive and liking data

The model with 2 PLS components (Figure 11) is poorest for colour liking with $R^2 = 0.871$. The models with 2 PLS components (Figure 12) for aroma liking with $R^2 = 0.970$ and flavour liking (Figure 13) with $R^2 = 0.982$ are better.

For better predictive ability, the PLS models included both linear and squared terms (indicated in Table 17 with superscript 2's). Preliminary models were run with all sensory attributes and their squared terms. Note that we have only fitted squared terms for some of the attributes, i.e. those that are contributing significantly to the model.

Positive drivers of aroma liking were fruity aroma^2, citrus flavour and sweet flavour against negative drivers, sweet aroma^2, sweet flavour^2, and pungent aroma. Positive drivers of flavour liking were sweet flavour, chemical aroma^2, and citrus flavour against negative drivers, soapy aroma, bitter after taste and sour flavour.

Standardised PLS regression coefficients for factors (Table 17) to summarise the relationship between predictors (X, customer liking variables) and Y, sensory response variables.

Expected errors of prediction for the 3 PLS models were low, lying between +/- 1.288 for the aroma model to +/- 2.458 for the colour model and +/- 2.678 for the flavour model with a 95 % confidence interval, indicating reliable prediction estimations of the liking variables.

The PLSR models predicted the hedonic ratings for all 36 beverages. The predicted hedonic scores for colour, aroma and flavour liking of the untested 24 grapefruit-like samples can be seen in Table 18.

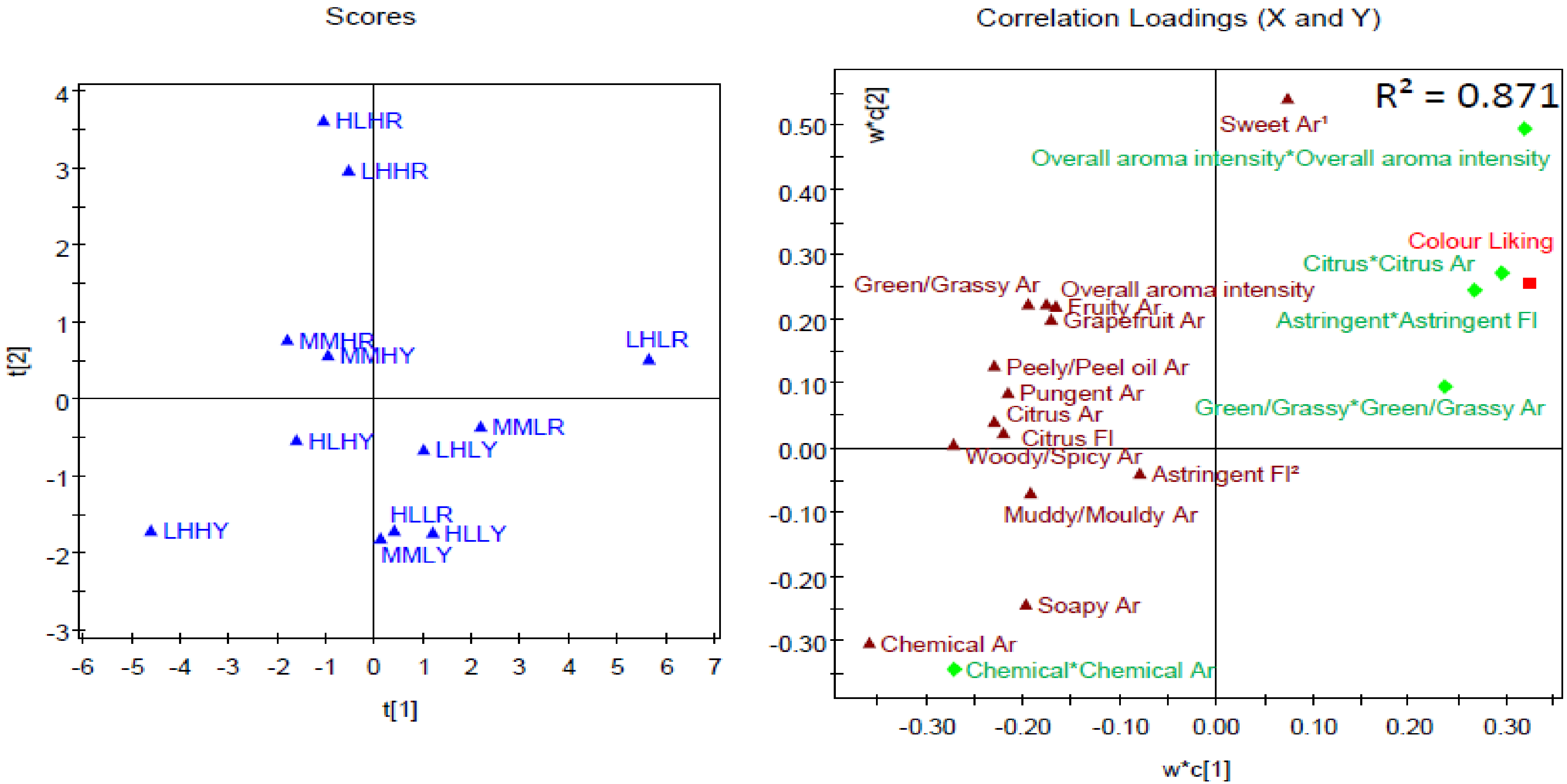

Figure 11 PLSR biplots for description (by trained panel) and liking (by consumers) of colour of grapefruit-like beverages. Square (sensory attributes) terms in green and sensory attributes in purple. Sensory attributes [1]AT = After taste, [2]Fl = Flavour, [3]Ar = Aroma. Sample key (Blue): 1st letter = naringin factor, 2nd letter = sucrose factor, 3rd letter = aroma, 4th letter = colour factor

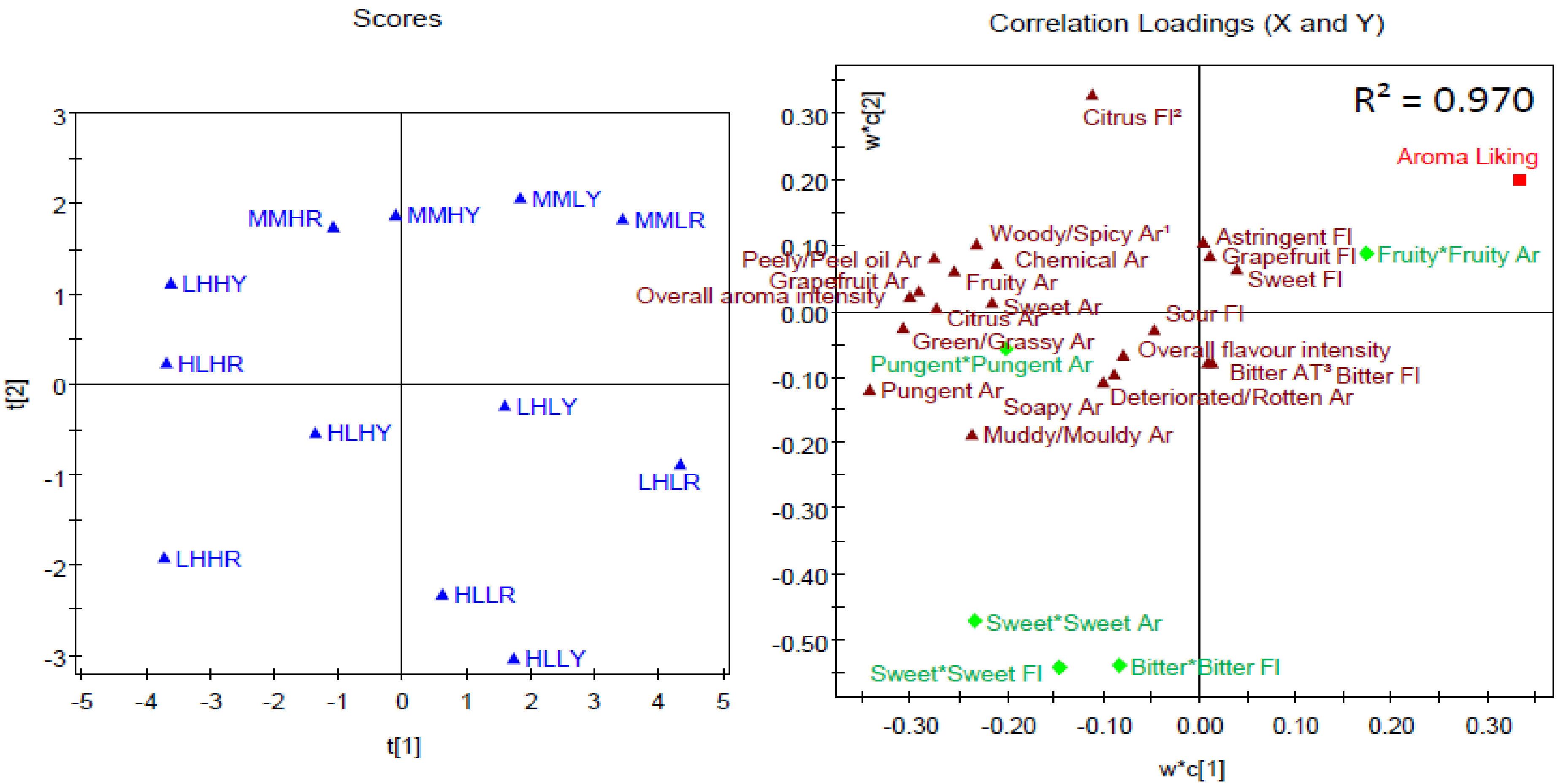

Figure 12 PLSR biplots for description (by trained panel) and liking (by consumers) of aroma of grapefruit-like beverages. Square (sensory attributes) terms in green and sensory attributes in purple. Sensory attributes [1]AT = After taste, [2]Fl = Flavour, [3]Ar = Aroma, Sample key (Blue): 1st letter = naringin factor, 2nd letter = sucrose factor, 3rd letter = aroma, 4th letter = colour factor

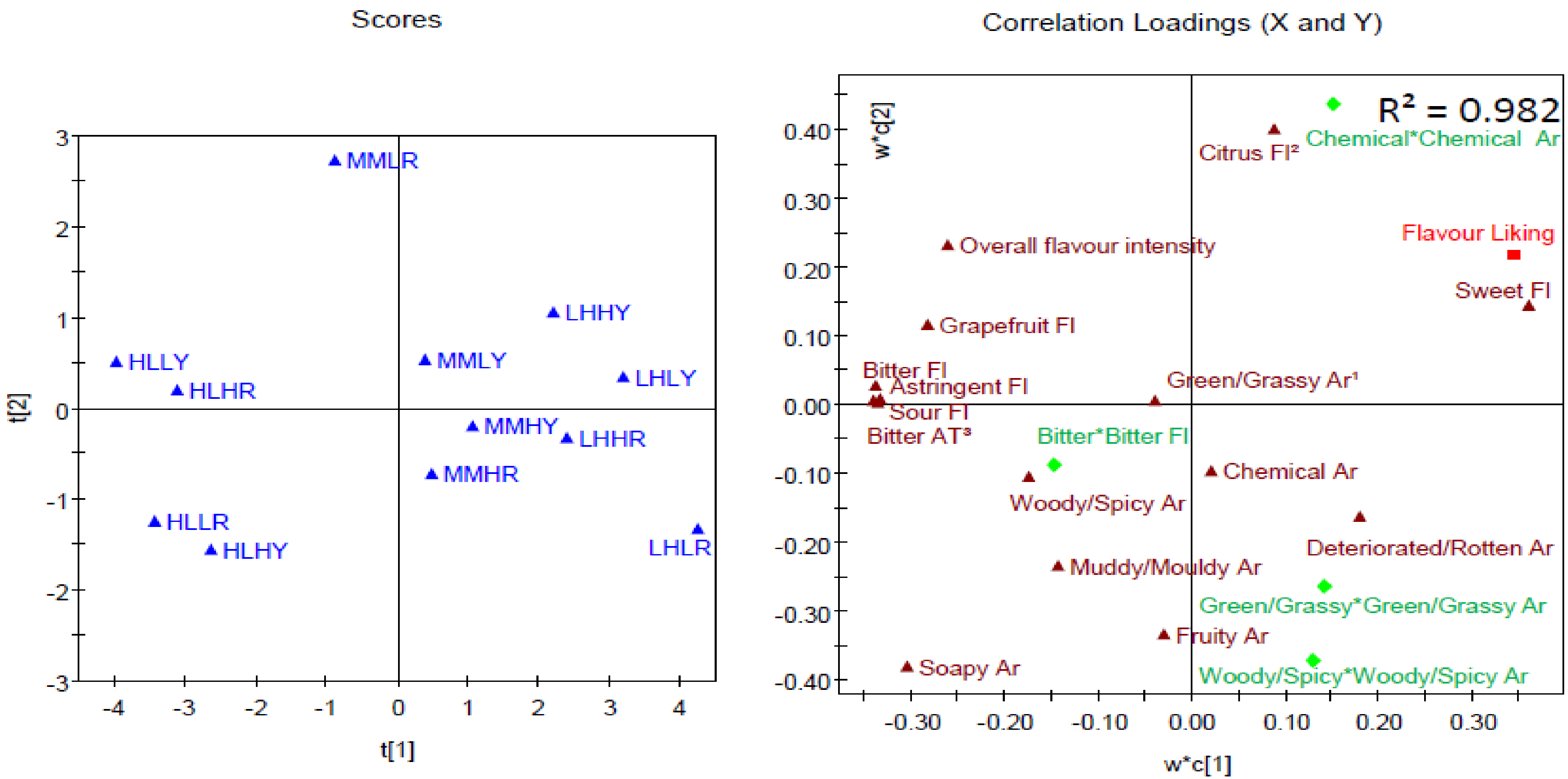

Figure 13 PLSR biplots for description (by trained panel) and liking (by consumers) of flavour of grapefruit-like beverages. Square (sensory attributes) terms in green and sensory attributes in purple. Sensory attributes [1]AT = After taste, [2]Fl = Flavour, [3]Ar = Aroma, Sample key (Blue): 1st letter = naringin factor, 2nd letter = sucrose factor, 3rd letter = aroma, 4th letter = colour factor

Table 17 Standardised partial least squares (PLS) regression coefficients for factors to summarise the relationship between predictors (X, customer liking variables) and Y, sensory response variables. Only selected important variables (main effects and squared effects, noted²) from the refined models are shown.

Liking of the colour $R^2 = 0.871$		**Liking of the aroma** $R^2 = 0.970$		**Liking of the flavour** $R^2 = 0.982$	
Overall aroma intensity ²	0.23	Fruity aroma ²	0.08	Sweet aroma	0.16
Citrus aroma ²	0.16	Citrus flavour	0.03	Chemical aroma ²	0.15
Sweet aroma	0.16	Sweet flavour	0.03	Citrus flavour	0.12
Astringent flavour ²	0.15	Astringent flavour	0.02	Deteriorated/rotten aroma	0.03
Green/grassy aroma ²	0.10	Grapefruit flavour	0.02	Green/grassy aroma ²	-0.01
Fruity aroma	0.00	Bitter after taste	-0.01	Greed/grassy aroma	-0.01
Overall aroma intensity	0.00	Bitter flavour	-0.01	Chemical aroma	-0.01
Grapefruit aroma	0.00	Sour flavour	-0.02	Woody/spicy aroma ²	-0.04
Green/grassy aroma	-0.01	Overall flavour intensity	-0.04	Overall flavour intensity	-0.04
Astringent flavour	-0.04	Deteriorated/rotten aroma	-0.05	Bitter flavour	-0.07
Peely/peel oil aroma	-0.04	Soapy aroma	-0.05	Grapefruit flavour	-0.07
Pungent aroma	-0.05	Chemical aroma	-0.06	Woody/spicy aroma	-0.08
Citrus aroma	-0.06	Woody/spicy aroma	-0.06	Fruity aroma	-0.08
Citrus flavour	-0.07	Sweet aroma	-0.07	Muddy/mouldy aroma	-0.10
Muddy/mouldy aroma	-0.08	Fruity aroma	-0.07	Bitter flavour	-0.11
Woody/spicy aroma	-0.09	Peely/peel oil aroma	-0.08	Astringent flavour	-0.11
Soapy aroma	-0.13	Pungent aroma ²	-0.08	Sour flavour	-0.12
Chemical aroma ²	-0.18	Citrus aroma	-0.09	Bitter after taste	-0.12
Chemical aroma	-0.19	Grapefruit aroma	-0.09	Soapy aroma	-0.19
		Overall aroma intensity	-0.10		
		Green/grassy aroma	-0.11		
		Muddy/mouldy aroma	-0.12		
		Bitter flavour	-0.14		
		Pungent aroma	-0.14		
		Sweet flavour ²	-0.16		
		Sweet aroma ²	-0.17		

The most important squared terms are the ones with a negative coefficient, as this indicates that there might be an optimum in the attribute range where liking is maximal

Table 18 PLS regression (PLSR) model predicted liking ratings for colour, aroma and flavour of grapefruit-like beverages

		Colour Liking		Aroma Liking		Flavour Liking	
Number[1]	Code[2]	Observed[3]	Predicted	Observed	Predicted	Observed	Predicted
2	***MMHR***	***61.1ab (30.6)***	***60.5***	***50.7ab (30.3)***	***50.8***	***43.5abc (31.0)***	***45.0***
4	***LHHR***	***64.1ab (29.4)***	***63.5***	***45.0b (30.4)***	***45.4***	***54.4a (32.3)***	***51.7***
9	***HLHR***	***62.8ab (32.5)***	***63.5***	***47.2ab (30.4)***	***46.9***	***34.5bc (32.7)***	***35.7***
11	***MMLR***	***66.1a (29.4)***	***63.6***	***56.7a (28.7)***	***55.9***	***48.3a (32.9)***	***47.6***
13	***LHLR***	***66.5a (29.5)***	***67.8***	***54.7ab (28.6)***	***55.1***	***55.4a (34.2)***	***55.4***
18	***HLLR***	***62.0ab (30.0)***	***60.8***	***51.3ab (28.6)***	***50.0***	***31.6c (29.3)***	***31.8***
20	***MMHY***	***61.5ab (31.5)***	***61.2***	***51.6ab (29.3)***	***52.0***	***47.8a (34.9)***	***47.8***
22	***LHHY***	***54.9b (32.1)***	***55.7***	***47.9ab (32.5)***	***47.6***	***53.8a (34.3)***	***53.8***
27	***HLHY***	***59.3ab (29.5)***	***59.7***	***48.6ab (30.7)***	***49.0***	***34.7bc (33.6)***	***33.7***
29	***MMLY***	***60.4ab (32.3)***	***60.4***	***53.9ab (30.9)***	***54.3***	***45.6ab (34.2)***	***47.1***
31	***LHLY***	***61.3ab (28.5)***	***62.2***	***52.2ab (29.2)***	***52.5***	***54.6a (32.8)***	***55.5***
36	***HLLY***	***60.5ab (27.8)***	***61.5***	***50.4ab (28.1)***	***50.8***	***34.6bc (32.0)***	***33.7***
1	LMHR		58.9		44.2		41.1
3	HMHR		61.5		48.7		40.8
5	MHHR		62.8		47.2		47.4
6	HHHR		59.6		46.0		45.4
7	LLHR		61.8		46.1		41.3
8	MLHR		59.9		46.6		37.6
10	LMLR		60.6		53.2		46.5
12	HMLR		63.6		54.2		44.5
14	MHLR		60.8		52.0		49.6
15	HHLR		63.0		52.1		48.0
16	LLLR		62.2		52.6		42.7
17	MLLR		59.0		48.2		32.3
19	LMHY		59.1		48.6		44.2
21	HMHY		62.1		40.8		38.3
23	MHHY		61.6		51.8		56.9
24	HHHY		62.4		52.1		51.3
25	LLHY		59.9		48.2		35.6
26	MLHY		60.5		45.3		39.8
28	LMLY		62.8		54.7		56.0
30	HMLY		62.2		53.7		40.3
32	MHLY		65.6		55.3		56.1
33	HHLY		62.5		52.1		46.3
34	LLLY		67.9		57.3		53.0
35	MLLY		59.6		52.1		39.0

[1]Refer to Table 10 for number. [2]Code: 1st letter = bitter level (High, Medium, Low); 2nd letter = sweet level (High, Medium, Low); 3rd letter = aroma level (High, Low); 4th letter = colour (Red or Yellow). Samples in bold italic were used for consumer evaluation. [3]Mean observed values with standard deviation in brackets; Different letters indicate significantly different mean values across design variable levels

5.5 Discussion of results

The overall flavour experience we perceive whenever we eat or drink is a result of the sensory input from our senses. Information from different modalities, taste, aroma, appearance, sound and texture, is relayed to the brain and integration of these signals results in our perception of flavour (Hewson *et al*., 2008).

The research studied the effect of varying the bitterness, sweetness, colour and aroma intensity of grapefruit-like beverages on the cross-modal perception of sensory properties and consumer liking. A total of 21 attributes were generated to characterize the appearance, aroma, flavour and after taste of the grapefruit-like beverages.

The bitterness of the grapefruit-like beverages did not have a significant effect on any of the aroma attributes. Fellers *et al*. (1987) reported that consumers did not find any difference in the aroma with increased levels of naringin (bitter) in processed grapefruit juice. The bitterness of the grapefruit-like beverages had a significant effect on the flavour attributes (astringent, sweet, sour, bitter and grapefruit flavour and bitter after taste). Fellers *et al*. (1987) similarly reported with an increase of limonin (bitter) in processed grapefruit juice the perceived amounts of bitterness and tartness increased while that of sweetness decreased.

The sweetness of the beverages did not have a significant effect on fruity or sweet aroma. Increasing the sugar concentration of blueberry and cranberry fruit juices increased their fruitiness (evaluated by sipping) even though no difference in the aroma was perceived by sniffing alone (Von Sydow *et al*., 1974). The opposite observations were made by Hort and Hollowood (2004); Lethuaut, Weel, Boelrijk and Brossard (2004); and Tournier, Sulmont-Rosse, Semon, Vignon, Issanchou and Guichard (2009). An increase in the intensity of different 'fruity' aromas was perceived in a multichannel flavour delivery system, model dairy desserts and custard desserts when increasing the sweetness with sucrose. The sweetness of the beverages had a significant effect only on the soapy aroma of the grapefruit-like beverages. The reason for this finding is not clear. Sucrose intensity had a significant effect on sour, sweet, bitter, astringent and grapefruit flavour and bitter after taste of the grapefruit-like beverages. Beck, Jensen, Bjoern and Kidmose (2014) reported that sucrose had a masking effect on the bitter taste of sinigrin, goitrin and quinine. Bonnans and Noble (1993) reported a greater suppression of sweetness by increasing acid levels than of sourness by increasing sweetener levels in orange flavoured solutions. When sucrose is added to fruit juices, not only are the perceived level of bitterness and sourness reduced (as was found in this research) but the sweet odour intensity rating also changes (although this was not found here) (Von Sydow *et al.*, 1974).

Aroma concentration had a significant effect on the majority of the sensory attributes. The sensation of flavour is elicited by a combination of nasal and oral stimulation. The consumption of foods and beverages results in the simultaneous perception of aroma and taste coupled with tactile sensations, all of which contribute to an overall impression of flavour. Tastes can increase the apparent intensity of aromas, conversely, the perceived intensity of tastes is increased when we taste flavoured solutions, especially if there is a logical association between them, such as between sweetness and fruitiness (Noble, 1996). The aroma compound (containing a citral component) of the grapefruit-like beverages had a significant effect on the citrus aroma attribute. Hewson *et al*. (2008) highlighted an additive effect of sweet components with citral and/or limonene volatiles having a 'citrus'-like aroma.

Colour of the beverages had a significant effect on the perception of overall aroma intensity and grapefruit, deteriorated/rotten, muddy/mouldy, fruity and sweet aroma intensity. The red colour Star Ruby grapefruit variety is the benchmark standard of grapefruits regarding colour, flavour and fragrance (Sunday River Citrus Company, 2017). Zellner and Kautz (1990) suggest that the colour-induced olfactory enhancement seen with solutions smelled orthonasally might be the result of a conditioned olfactory percept caused by the colour. The orthonasal colour-induced odour enhancement might thus be due to a combination of the actual odour the subject experienced from smelling the solution and the colour-induced conditioned percept caused by the previous pairing of particular colours with different odours (most red-coloured beverages are fruit odours). Colour also had a significant effect on grapefruit flavour with the highest value perceived in the red coloured beverages. The Star Ruby variety with red colour is the most planted grapefruit variety in South Africa due to its global demand (USDA Foreign Agricultural Service GAIN Report 2016).

Grapefruit-like beverages with a red colour were preferred by consumers over those with yellow colour. Spence *et al*. (2010) reported that red colour decreased the perception of bitter taste sensitivity. Colouring a clear bitter solution red decreased bitter taste sensitivity, while the addition of yellow and green colouring had no such effect (Maga, 1974).

The low aroma samples were preferred over the high aroma samples. The low naringin/high sucrose samples were preferred over the high naringin/low sucrose samples. Fellers *et al.*, 1988 reported that with an increase in the ratio of °Brix/acidity of reconstituted grapefruit juice the consumer perception of sweetness increased and bitterness and aroma decreased. Some bitterness in processed grapefruit products is acceptable to consumers but excessive bitterness is one of the major consumer objections to such products as pointed out by Bell (1955) and Birdsall (1955) and confirmed here.

5.6 Conclusions

This study indicated that aroma, bitterness, and sweetness levels, and also product colour (hue) influences the perception of grapefruit-like beverages, as well as their hedonic value. A grapefruit-like beverage model was created and a lexicon to describe the sensory properties of the cross-model interaction of stimulus components of the model beverage was developed. From the descriptive sensory profiles, prediction models for liking of the colour, aroma, and flavour of grapefruit-like beverages were developed. In the next phase, the models should be applied to a wide range of grapefruit juice samples to determine validty and reliability in real juices. The models can then be optimized for application in grapefruit quality control and productdevelopment programs.

CHAPTER 6:
The effect of PROP taster status and genetic variation in *TAS2R* genes on hedonic ratings for flavour of bitter/sweet grapefruit-like beverages by young South African women

6.1 Abstract

Previously it was found that the concentration of naringin in a grapefruit-like beverage affected the bitter taste, aftertaste and grapefruit flavour intensity of the drink. On average, a group of young South African women prefer grapefruit-like beverages with low bitterness but large variation in preferences was noted. The objective of this follow up study was to determine if sensitivity to bitter taste [as determined through propylthiouracil (PROP) taster classification] and genetic variation in *TAS2R38* and *TAS2R19* SNP genotypes in the same group of South African women could explain the variation in hedonic (or pleasantness) ratings for the flavour of the bitter/sweet grapefruit-like beverages.

Ninety-six young South African women (18-24 years) were classified into PROP taster groups and rated the flavour of grapefruit-like beverages differing in bitter taste intensity for hedonic value. DNA was extracted from the saliva of the participants for genotyping of *TAS2R38* and *TAS2R19* bitter receptor genes. Non-tasters (9 % of the participants) and medium tasters (65 %) liked the flavour of the grapefruit-like beverages significantly more than supertasters (26 %). Of the two SNP variants, rs1868769 and rs10772420 of the *TAS2R19* gene, only rs10772420 did have an effect on the liking of the model grapefruit beverages. The findings support previous research with Caucasians where a link between rs10772420 and lower bitterness perception and greater liking for unsweetened grapefruit juice was established. Three SNPs, rs713598, rs1726866 and rs10246939, were associated with *TAS2R38*. Both rs713598 and rs1726866 SNPs were associated with greater liking of the flavour of grapefruit-like beverages. This has not been reported previously.

This research shows that the genetic variation in *TAS2R38* and *TAS2R19* SNP genotypes are partly responsible for the dislike of bitter grapefruit beverages.

Key words: Genetic variation, 6-n-propylthiouracil (PROP), Single nucleotide polymorphisms (SNPs), *TAS2R38*, *TAS2R19*, naringin, grapefruit

Highlights

- PROP taster status affect the liking of the flavour of grapefruit model beverages
- Polymorphisms of *TAS2R38* affect liking of the flavour of grapefruit model beverages
- *TAS2R19* rs10772420 affect liking of the flavour of grapefruit model beverages

Abbreviations used: LMS, labelled magnitude scale; SLAM, simplified labelled affective magnitude scale

6.2 Introduction

Grapefruit consumption in South Africa is relatively low at 7000 metric tones with a total annual production of 400,000 metric tones (Global Agricultural Information Network, 2017) compared to a total annual world production of 6.6 million metric tons (Citrus: World Markets and Trade, 2018). Although food preferences are subject to multiple influences, bitterness can be the key reason for food dislikes or food rejection (Drewnowski, Henderson and Shore, 1997b). Naringin imparts a bitter and objectionable taste to grapefruit juice, and excessive bitterness of the juice was considered as an important economic problem in commercial grapefruit juice production (Lee and Kim, 2003). Bitter taste has been cited as the main reason for disliking coffee, alcohol, some cheeses, bitter cruciferous vegetables and some citrus fruit (Reed, Li, Li, Huang, Tordoff, Starling-Roney, Taniguchi, West, Ohmen, Beauchamp and Bachmanov, 2004; Drewnowski and Rock, 1995). Perception of taste may vary between individuals depending on genetic variations in certain taste receptor genes (Kim *et al*., 2005). Although humans are born with an innate dislike for bitter and preference for sweet (Steiner, Glaser, Hawilo and Berridge, 2001), the ability to taste bitter and sweet varies widely. The genetic trait of taste sensitivity to the bitter compound 6-n-propylthiouracil (PROP) has been proposed as a marker for individual differences in taste perception (Duffy and Barthoshuk, 2000). Individuals can be defined as bitter tasters or non-tasters based on their ability to discriminate threshold concentrations of PROP from plain water. When tested, below threshold, concentrations of PROP, tasters can be further divided into those who are very sensitive, i.e. PROP supertasters, and those who are moderately sensitive, i.e. medium tasters (Deshaware and Singhal, 2017). Non-tasters were reported as more likely to be ‘bitter likers’ than PROP-sensitive individuals (Looy and Weingarten, 1992) while ‘supertasters’ have shown lower acceptance of whole-grain bread presumably due to the more bitter taste (Bakke and Vickers, 2007). The frequency of non-tasters varies greatly among populations around the globe, from as low as 7 % to more than 60 % (Guo and Reed, 2001).

Bitter-tasting substances activate receptors of the taste receptor type 2 (*TAS2R*) family (Chandrashekar *et al*., 2000), which is the largest family of taste receptors, encoding over 25 different taste receptors. *TAS2R* genes contain unusually high levels of allelic variation, which may indicate local adaptation for the avoidance of plant toxins (Kim and Drayna, 2005). Most of the bitter receptor genes are located on chromosomes 7 and 12, likely as a result of gene duplications (Kim *et al*., 2005). The gene most closely associated with PROP phenotype variance is *TAS2R38* that express receptors that bind the N – C = S group responsible for the bitter taste of thiourea compounds (Tepper, Koelliker, Zhao, Ullrich, Lanzara, d'Adamo, Ferrara, Ulivi, Esposito and Gasparini, 2008; Bufe *et al*., 2005; Kim *et al*., 2003). Polymorphisms of the *TAS2R38* gene explain the majority of variability in PROP thresholds (Kim *et al*., 2003). Bering, Pickering and Liang (2014) reported in a study that no association exists between genetic variation in *TAS2R19* single nucleotide polymorphisms (rs10772420, rs1868769, rs12578654, rs4763235) and PROP sensitivity. Single-nucleotide polymorphisms (SNPs) are the most abundant type of human genetic variation, meaning that one nucleotide has been substituted for another (Cai, White, Torney, Deshpande, Wang, Marrone and Nolan, 2000). SNPs can be further divided into synonymous and non-synonymous SNPs. Synonymous SNPs do not change the amino acid sequence of the resulting protein, due to degeneracy of genetic code, while non-synonymous SNPs do (Bromberg and Rost, 2007). The allelic diversity in *TAS2R38* is mainly due to three common SNPs, namely rs713598, rs1726866 and rs10246939 (Kim and Drayna, 2005; Deshaware and Singhal, 2017). These three SNPs with amino acid substitutions (Pro49Ala, Ala262Val, and Val296Ile) give rise to the two main haplotypes observed in over 90 % of the Caucasian population (Kim *et al*., 2005) PAV, the dominant taster variant and AVI, the non-taser recessive one. Also, rare haplotypes (AAV, AAI, and PVI) have been observed to contribute to intermediate PROP sensitivity (Bufe *et al*., 2005; Kim *et al*., 2003).

Genetic variation in the *TAS2R19* gene has also been associated with quinine bitterness, grapefruit liking and bitterness differences among caucasians (Hayes *et al*., 2013; Duffy *et al*., 2009). A genome-wide study of taste associations in over 700 twin pairs, using a range of tastants, found an association between the rs10772420 SNP (Arg299Cys) in *TAS2R19* on chromosome 12 and quinine bitterness (Reed *et al*., 2010). Hayes, Feeney, Nolden and

McGeary (2015) reported individuals who vary in the Arg299Cys SNP (rs10772420) in *TAS2R19* differ in the remembered liking of grapefruit juice. The rs10772420 SNP was also previously associated with responses to sampled unsweetened grapefruit juice, with Arg299 homozygotes reporting less bitterness and greater liking (Hayes *et al*., 2011). Dias (2014) reported that polymorphisms of *TAS2R19*, rs10772420 (A > G) and rs4763235 (C > G) were associated with naringin sensitivity and grapefruit and grapefruit preference. No association in previous studies could be found reporting on naringin sensitivity and grapefruit preference linked to the other two known *TAS2R19* polymorphisms, rs1868769 and rs12578654.

The objective of this study was to determine the effect of PROP taster status, and variation in *TAS2R38* and *TAS2R19* genotypes on hedonic ratings for flavour of grapefruit-like beverages that differ in bitterness level, colour type and aroma intensity. This study specifically focused on a group of young black South African women, due to the under-representation of non-Caucasians and specifically Africans in similar studies.

6.3 Materials and methods

6.3.1 Ethics statement

Ethical approval for this study was obtained from the Faculty of Natural and Agricultural Sciences Ethics Committee at the University of Pretoria (EC 130827-088).

6.3.2 Participants

Ninety-six young black South African women (18-24 years) were recruited from Pretoria, South Africa. All participants reported being healthy and not suffering from any food intolerances and/or allergies, were naïve to sensory testing and were asked to not eat or drink for at least 1 hour prior to the scheduled session. Participants gave informed consent prior to taking part in the study.

6.3.3 PROP classification

In order to classify participants based on PROP sensitivity, a paper disk method (Zhao, Kirkmeyer and Tepper, 2003) was used. The amount of PROP on the filter paper discs was verified with a T80+ UV/VIS Spectrometer (PG Instruments Ltd., Leicester, UK) following Zhao *et al.* (2003). PROP and NaCl paper discs were evaluated by the participants. NaCl is used as a control because taste intensity to NaCl is not related to PROP taster status (Tepper, Christensen and Cao, 2001). The procedure of Zhao *et al.* (2003) was followed and instructions for using the scale were provided (Appendix 3). The NaCl disc was tasted first followed by the PROP disc. Participants rated the intensity of the sensation from tasting each disc using a 100 mm labelled magnitude scale (LMS), with label descriptors placed at quasi-logarithmic intervals along the length of the scale from 'barely detectable' (at the bottom of scale, 0 mm) to 'strongest imaginable' (at the top of the scale, 100 mm) (Zhao *et al*., 2003). Bottled water and cream crackers were used as pallet cleansers before and after tastings. Data capturing was done using Compusense® five release 5.4 (Compusense Inc., Guelph, ON, Canada). Evaluation sessions were held in a sensory laboratory with individual cubicles. The sensory evaluation was conducted during six 30 min sessions at a time by 16 of the total 96 panelist,on one day.

6.3.4 DNA extraction and SNP genotyping

Saliva was collected from the participants (Appendix 4) on the same day before the PROP tasting using the Oragene DNA sample collection kit (OG-500, DNA Self-Collection kit, Genotek, Ottawa, Ontario, Canada) following the manufacturer's instructions. DNA was extracted from 91 participants' saliva with the Orangene OG-500 prepIT.L2P-5 DNA extraction kit (DNA Genotype) according to the manufacturer's instructions. DNA impurity was determined using a Nano Drop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA) and DNA quality further determined on a 1 % agarose electrophoresis gel, using loading dye and a 1 kb DNA ladder from KAPA Biosystems (Cape Town, South Africa).

Gene segments of *TAS2R38* and *TAS2R19* were PCR amplified in two separate reactions. The locations of the primers (Table 19) were chosen so that all well-known SNPs rs713598, rs1726866 and rs10246939 in *TAS2R38* (Duffy, Hayes, Davidson, Kidd, Kidd and Bartoshuk, 2010) and rs10772420 in *TAS2R19* (Duffy *et al*., 2009) fell within the amplified regions (970 bp and 702 bp respectively). Each 25 µl PCR amplification reaction contained 0.3 µM of each primer, 50 ng of DNA and 1 x KAPA HiFi HotStart Ready mix (Kappa Biosystems, Cape Town, South Africa). *TAS2R38* samples were amplified in an Applied biosystems 2720 Thermal cycler under the following PCR conditions: initial denaturation, 95°C for 3 min; denaturation, 98°C for 20 sec, annealing, 60.0°C for 15 sec and extension, 72°C for 1 min for 35 cycles; final extension, 72°C for 2 min. PCR conditions for *TAS2R19* samples were identical, except that annealing was 60.5°C for 15 sec. PCR products were analyzed by agarose gel electrophoresis on a 1 or 2 % agarose gel containing a one kb DNA ladder from KAPA Biosystems (Cape Town, South Africa). PCR amplification was successful for 82 *TAS2R38* samples and 91 *TAS2R19* samples.

Table 19 Primers used to amplify sections of the *TAS2R38* and *TAS2R19* genes

TAS2R38	
Forward primer	5’ TCGCATCCGCACTGTGTCCTAT 3’
Reverse primer	5’ ATCTGCCTTGTGGTCGGCTCTT 3’
TAS2R19	
Forward primer	5’ CTGGGCTGTAACGAACCATT 3’
Reverse primer	5’ GCTAGAAGACCCACGATGCT 3’

The obtained *TAS2R38* and *TAS2R19* PCR products were purified using QIAquick PCR purification kits (Qiagen, Southern Cross Biotechnology, Johannesburg, South Africa) or High pure PCR product purification kits (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. All PCR products were sequenced in an Applied biosystems 2720 Thermal cycler. The forward and reverse sequencing reaction volume was 10 µl in total, consisting of 2 µl of PCR template (about 50 ng), 3.2 µM of either forward or reverse primer (Table 19), 5 µl of sabex water, 1 µl of Big dye sequencing buffer and 1 µl of Big dye (version 3.1). Sequencing conditions for 25 cycles were: denaturation, 96°C for 10 sec; annealing of either forward or reverse primer, 50°C for 5 sec; extension of primers, 60°C for 4 min.

6.3.5 Hedonic ratings of the flavour of grapefruit-like beverages

The day after PROP classification, 6 x 30 min sessions were conducted for the evaluation of the flavour liking of the grapefruit-like beverages. A deflavoured clarified apple juice base concentrate was modified using a factorial design: 3 levels of naringin/sucrose (flavour) x 2 aroma intensity levels x 2 colours (red or white) to create 12 grapefruit-like beverages as shown in Table 20.

Table 20 Composition of 12 grapefruit-like beverages used for the hedonic rating of flavour by the participants (n = 96)

Sample Code[1]	Bitterness level	Naringin concentrations mg/kg	Sucrose concentrations °Brix	Aroma[2] concentrations mg/kg	Colours
MMHR[1]	Medium	315	10	10	Red[3]
LHHR	Low	158	12	10	Red
HLHR	High	473	8	10	Red
MMLR	Medium	315	10	2.5	Red
LHLR	Low	158	12	2.5	Red
HLLR	High	473	8	2.5	Red
MMHY	Medium	315	10	10	Yellow[4]
LHHY	Low	158	12	10	Yellow
HLHY	High	473	8	10	Yellow
MMLY	Medium	315	10	2.5	Yellow
LHLY	Low	158	12	2.5	Yellow
HLLY	High	473	8	2.5	Yellow

[1]Sample key: M = medium concentration; L = low concentration; H = high concentration; R = red; Y = Yellow
[2]Caryophylene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), aldehyde C10 (decanal)
[3]0.001 % Red solution (30 % Sunset Yellow and 70 % Ponceau Red); [4]0.0125 % Yellow solution (Quinoline Yellow)

Designing of the consumer test and data capturing were done using Compusense® *five* release 5.4 (Compusense Inc., Guelph, ON, Canada). Sessions were held in a sensory laboratory with daylight lighting conditions. The grapefruit-like beverages were kept at 14°C and 30 ml was poured into 125 ml white polystyrene cups and blind coded with 3-digit random codes prior to serving. The 12 beverages were served simultaneously on a white tray. Participants had to taste each beverage individually and rate their liking of the flavour by placing a mark on the screen on the Simplified Labeled Affective Magnitude Scale (SLAM) (Lawless and Heymann, 2010). The horizontal scale (100 mm) included descriptors ranging from ‘greatest imaginable dislike’ (at the left end of the scale, 0 mm), neither like nor dislike’ (at the middle of the scale, 50 mm) and ‘greatest imaginable like’ (at the right end of the scale, 100 mm). Sample presentation order was randomly balanced over the group of participants according to a William’s latin square design. Bottled still water and cream crackers were used as neutralizing agents before and after tasting the beverages.

6.3.6 Statistical analysis

Participants were initially classified into non-tasters (PROP disc bitterness rating ≤ 16.5 mm), medium tasters (16.6-50 mm) and supertasters (≥ 51 mm), following Zhao *et al*. (2003). The group means were calculated and cut off scores were determined at 95 % confidence intervals (Zhao *et al*., 2003). Participants were then further classified according to the cut off scores. Taster group differences in intensity ratings for PROP and NaCl were investigated by two-way ANOVA (taster status x taste stimuli) and the least significant difference (LSD) test. Another ANOVA was used to test the effect of taster group and beverage characteristics (e.g. bitterness, colour and aroma) on the hedonic ratings of the flavour of grapefruit-like beverages. Finally, five separate ANOVA's were conducted to test the effect of SNP genotypes (*TAS2R38*: rs713598, rs1726866 and rs10246939; *TAS2R19*: rs10772420 and rs1868769) and beverage characteristics on the hedonic ratings of the flavour of grapefruit-like beverages. Means were compared using Fisher's LSD test at 5 % level. All statistical analyses were performed with Genstat® (VSN International Ltd., Hemel Hempstead, United Kingdom).

6.4 Results

6.4.1 PROP classification and salt and bitter intensity ratings

Thirty-three percent of participants were classified as supertasters, 53 % as medium tasters and 14 % as non-tasters in the initial classification, while 26 % of participants were classified as supertasters, 65 % as medium tasters and 9 % as non-tasters in the final classification.

Significant differences in intensity ratings for PROP ($F [2:93] = 223.65$, $P < 0.001$) and NaCl ($F [2:93] = 87$, $p < 0.001$) were found among the three taster groups. As expected, the highest average rating > 84.1 mm for PROP was given by supertasters and the lowest rating < 10.3 mm, given by non-tasters, with medium tasters in between (Figure 14). Contrary to expectation, the rating for the NaCl disc by the supertasters was slightly but significantly higher ($p < 0.05$) compared to medium tasters and non-tasters (Figure 14), but there was no significant difference between the medium tasters and non-tasters' ratings.

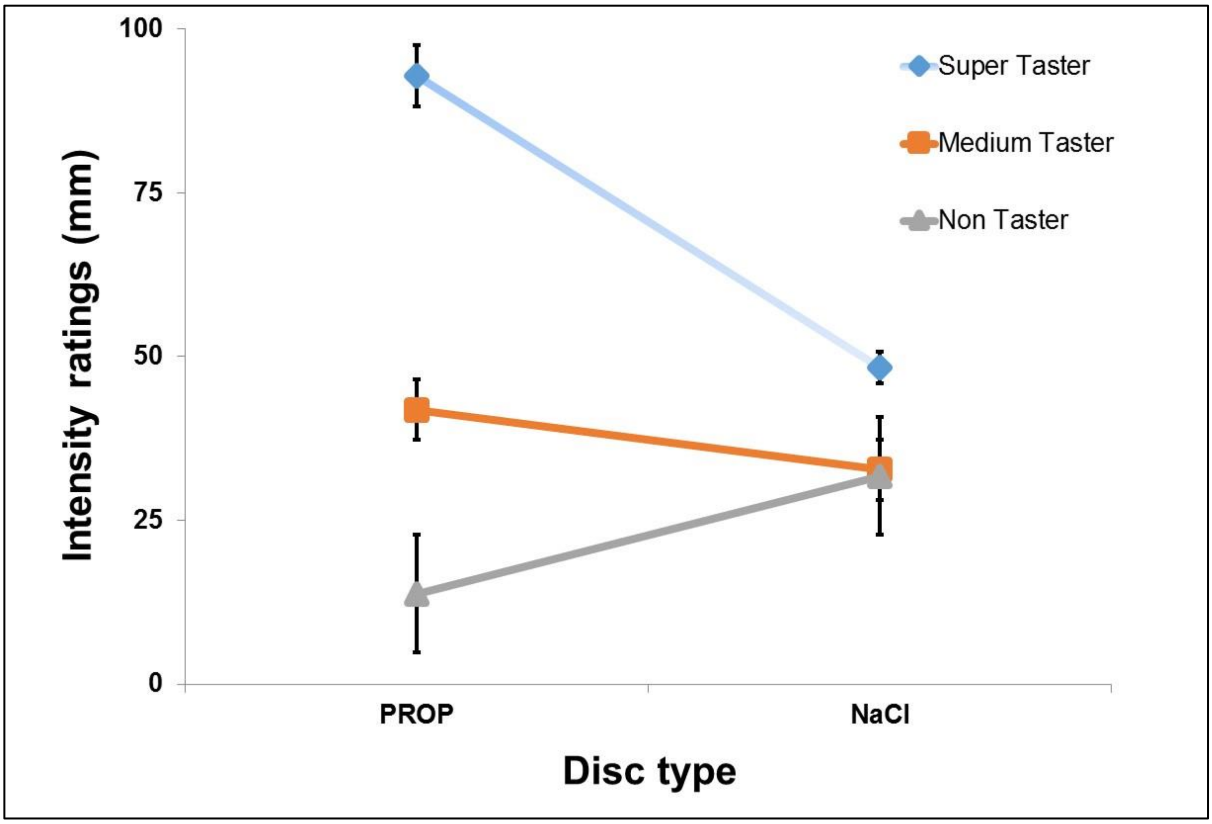

Figure 14 Intensity ratings of PROP taster groups for PROP and NaCl impregnated discs. Values are means with LSD (least significant difference) bars representing the 95 % confidence intervals. Means with LSD bars which do not overlap indicate a significant difference.

6.4.2 Hedonic rating of the flavour of grapefruit-like beverages

6.4.2.1 The effect of PROP taster group and beverage characteristics on the hedonic rating of the flavour of grapefruit-like beverages

The ANOVA showed that only PROP taster status and the bitterness of the beverages significantly influenced the liking of grapefruit-like beverages. The three PROP taster groups gave significantly different liking ratings for the flavour of the grapefruit-like beverages ($F [2:1108] = 10.56$, $p < 0.001$). Non-tasters [48 (± 37)] and medium tasters [48 (± 34)] liked the flavour of the beverages significantly more ($p < 0.05$) than supertasters [37 (± 32)]. Bitterness had a significant effect on the hedonic ratings of the flavour of grapefruit-like beverages ($F [2:1108] = 38.88$, $p < 0.001$), in that liking for both the low bitter [52 (± 33)] and medium bitter [47 (± 33)] beverages were significantly ($p < 0.05$) higher than the high bitter beverages [34 (± 32)]. Beverage aroma level and colour had no significant effect on the hedonic ratings of the flavour of grapefruit-like beverages and there were no significant interactions (all $p > 0.1$).

6.4.2.2 The effects of *TAS2R38* SNP genotypes and beverage characteristics on the hedonic rating of the flavour of grapefruit-like beverages

The three previously studied SNPs (rs713598, rs1726866 and rs10246939) were the only common SNPs identified in the amplified region of *TAS2R38*. The results showed that rs1726866 ($F [2,852] = 29.753$, $p < 0.001$) and rs713598 ($F [2,852] = 3.336$, $p = 0.05$) significantly affected liking of the flavour of grapefruit-like beverages, while rs10246939 did not affected liking of flavour ($F [2,852] = 2.918$, $p = 0.07$). Participants with the CC genotype for rs1726866 rated liking of the flavour of the grapefruit-like beverages (52 ± 34) significant higher ($p < 0.05$) than those with the CT (36 ± 33) and TT genotypes (33 ± 33). Participants with the CC genotype for rs713598 genotype rated the liking of the flavour of the grapefruit-like beverages (49 ± 34) significant higher ($p < 0.05$) than those with CG genotype (42 ± 34) but not significantly different to those with GG genotype (46 ± 35). There was no significant difference in the liking of flavour of grapefruit-like beverages by participants between the three *TAS2R38* rs10246939 genotypes.

As seen before, bitter level of the beverages significantly affected flavour liking ($F [2,852] = 34.915$, $p < 0.001$) by *TAS2R38* rs713598 groups. They rated the flavour liking of the low bitter grapefruit-like beverages (55 ± 33) significantly higher ($p < 0.05$) than the medium bitter beverages (46 ± 34), followed by the high bitter beverages (34 ± 33) and ($F [2,852] = 34.915$, $p < 0.001$) by TAS2R38 rs1726866 groups. They rated the flavour liking of the low bitter beverages (55 ± 33) significant higher ($p < 0.05$) than the medium bitter beverages (46 ± 34), followed by the high bitter (34 ± 33) and ($F [2,852] = 34.215$, $p < 0.001$) by *TAS2R38* rs10246939 groups. They rated the flavour liking of the low bitter beverages (55 ± 33) significant higher ($p < 0.05$) than the medium bitter beverages (46 ± 33), followed by the high bitter grapefruit-like beverages (34 ± 33). Beverage aroma level and colour type had no significant effect on the hedonic ratings of flavour of grapefruit-like beverages and there were no significant interactions (all $p > 0.05$).

6.4.2.3 The effects of *TAS2R19* SNP genotypes and beverage characteristics on the hedonic rating of the flavour of grapefruit-like beverages

We identified two common SNP's in the *TAS2R19* amplified region: rs1868769 and rs10772420. The results showed that rs10772420 ($F [2,1044] = 7.000$, $p = 0.002$), but not rs1868769 ($p > 0.1$) significantly affected liking of the flavour of grapefruit-like beverages. Participants with the CT genotype for rs10772420 rated the liking of the flavour of the grapefruit-like beverages (40 ± 33) significantly lower ($p < 0.05$) than both those with SNP CC (47 ± 35) and SNP TT (50 ± 34). As before, bitter level of the beverages significantly affected liking ($F [2,1044] = 39.363$, $p < 0.001$) by *TAS2R19* rs10772420 groups. These two SNP groups rated the flavour liking of the low bitter grapefruit-like beverages (54 ± 33) significant higher ($p < 0.05$) than the medium bitter grapefruit-like beverages (46 ± 34), followed by the high bitter beverages (34 ± 33) and ($F [2,852] = 34.915$, $p < 0.001$) by *TAS2R19* rs1868769 groups. They rated the flavour liking of the low bitter beverages (55 ± 33) significant higher ($p < 0.05$) than the medium bitter beverages (46 ± 34), followed by the high bitter beverages (34 ± 33). Beverage aroma level and colour type had no significant effect on the hedonic ratings of the flavour of grapefruit-like beverages and there were no significant interactions observed (all $p > 0.05$).

6.5 Discussion

Individuals were classified according to their PROP taster status. The final PROP taster status distribution of the group was 26 % supertasters, 65 % tasters and 9 % non-tasters. The percentage non-tasters fell within the range observed in other African populations (2.3 to 36.5 % non-tasters; Guo and Reed, 2001). Future research can be done in a South African population between taster groups to determine the density of the fungiform taste papillae on the anterior surface of the tongue to determine if supertasters in this population also have a greater density.

Contrary to expectation, a higher intensity rating was recorded for the disc with NaCl by supertasters compared to the other two taster groups. Tepper *et al*. (2001) reported that taste intensity to NaCl is not influenced by PROP taster status. A possible explanation for this unexpected finding could be due to the fact that NaCl can produce perceptible sensations of irritation by the trigeminal nerve at high concentrations (Zhao *et al*., 2003). Possibly, the NaCl filter paper disc with 1.0 mol/l NaCl could have caused some extent of irritation which was more prominent to supertasters. Other studies found that supertasters were more sensitive to a wide range of oral stimuli and oral irritation to compounds such as capsaicin, ethanol, caffeine, quinine, isohumulones, naringin, benzyl alcohol, sodium benzoate and potassium chloride, as well as sucrose, saccharin and neohesperidin dihydrochalcone etc. (Zhao *et al*., 2003; Tepper *et al*., 2001).

Non-tasters and medium tasters (who are less sensitive to the bitterness of PROP) liked the flavour of the grapefruit-like beverages significantly more than the supertasters. This finding is consistent with Bartoshuk *et al*. (1994), which reported an association between higher taste responsiveness to PROP and greater perception of bitterness from caffeine and naringin (from grapefruit). The finding is also consistent with previous work that found an association between increased sensitivity to PROP and reduced acceptability of grapefruit juice (Drewnowski *et al*., 1997). The bitterness of the beverages, but not the colour or aroma of the beverages, significantly influenced participants' hedonic ratings. All groups preferred high bitter beverages less than the low and medium beverages. Bitterness can be seen as a reason for dislike of grapefruit-like beverages. Lanier *et al.* (2005) also found in a study where participants tasted grapefruit juice in the laboratory, the sweetness was positively associated with liking, whereas bitterness was negatively associated with liking.

We found significant associations between genetic variation in *TAS2R38* (rs1726866 and rs713598) and the liking of grapefruit-like beverages, which is a finding not reported before. Participants with the CC genotype for rs1726866 preferred the grapefruit-like beverages significantly more than participants with the CT and TT genotypes. In addition, participants with the CC genotype for rs713598 preferred grapefruit-like beverages significantly more than those with the CG genotype, but preference was not significantly different to participants with the GG genotype.

In previous work, children with the bitter-sensitive genotypes (CG, GG; rs713598, ala49pro) preferred higher levels of sucrose than those with the bitter-insensitive genotype both in laboratory-based measures and in reported preferences of real-world foods like cereal and beverages (Mennella, Finkbeiner and Reed, 2012). Adults with the bitter-sensitive alleles of *TAS2R38* also rated foods such as brassica vegetables as more bitter compared to adults with the bitter-insensitive alleles (Sandell and Breslin, 2006).

Similarly we also found significant associations between genetic variation in *TAS2R19* (rs10772420) and the liking of grapefruit-like beverages. Participants with the CC and TT genotypes for rs10772420 (homozygotes) preferred grapefruit-like beverages significantly more than those with the CT genotype (heterozygotes).

Genetic variation in rs10772420 has also previously been associated with other dietary preferences e.g. guanine bitterness, grapefruit liking and bitterness sensitivity differences among individuals (Duffy *et al.*, 2009; Hayes *et al*., 2013). The results here are in agreement with those found in a study by Hayes *et al*. (2011). In that study, the rs10772420 SNP was also associated with responses to unsweetened grapefruit juice, with homozygotes reporting less bitterness and greater liking (Hayes *et al.*, 2011). The findings of the study are also novel because it is the first time that the effect of taster status and *TAS2R38* and *TAS2R19* genotypes on the liking of the flavour of grapefruit model beverages were described for a non-caucasian population.

6.6 Conclusions

Sensitivity to PROP limits the liking of the flavour of grapefruit model beverages. Polymorphisms of *TAS2R38* and *TAS2R19* genotypes are partly responsible for the dislike of the bitter taste of grapefruit beverages in young South African non-caucasian women. The group studied represents only a small portion of one gender of the population. This knowledge allows marketers to use additional marketing techniques to make grapefruit more attractive by e.g. focusing specifically on the health benefits of bitter compounds in grapefruit as well as researchers at nurseries to grow grapefruit trees that can yield fruit that exhibits less bitter taste.

CHAPTER 7:
General Discussion

Citrus paradisi Macfadyen is one of the most important world fruit crops, and consumption of citrus fruit or juice is found to be inversely associated with several diseases (Joshipura, Hu, Manson, Stampfer, Rimm and Speizer, 2001). Consumption of fruit and vegetables has been strongly associated with reduced risk of cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts, and age-related functional decline (Liu, 2003). The health benefits of citrus fruit have mainly been attributed to the presence of bioactive compounds, such as phenolics (e.g. flavanone glycosides, hydroxycinnamic acids) (Knekt, Ritz, Pereira, O'Reilly, Augustsson and Fraser, 2004), and carotenoids (Craig, 1997). The nutritional value of grapefruit makes it among the top most consumed fruit (Zhang, 2007). Over the past few decades, per person consumption of grapefruit has however been declining. Per capita consumption world wide of grapefruit (fresh and juiced) peaked at nearly 11 kg in 1978; however, by 2014, fresh grapefruit consumption was 1 kg per person and grapefruit juice consumption was 2 kg per person (Citrus, Agricultural marketing resource center, November 2015). The consumption of grapefruit remains low in South Africa (USDA Foreign Agricultural Service Gain Report, 2017) as some individuals like grapefruit and others do not. However, the reason for this individual liking of grapefruit is unclear. The main purpose of the study was to examine the effect of changing the sweetness, bitterness and aroma intensity and the colour type of grapefruit juice on the cross-modal perception of sensory properties and consumer liking by a group of young South African female consumers. It further investigated the effects of sensitivity to bitter taste [as determined through propylthiouracil (PROP) and genetic variation in TAS2R38 and TAS2R19 SNP genotypes, on hedonic rating of the flavour of grapefruit-like beverages differing in bitter/sweet taste intensity.

This general discussion will discuss if the consumption of grapefruit-like beverages can be related to multisensory perception and genotypes. It will cover the methodology that was used, its application and where it could have been improved. It is followed by a discussion of the results obtained and suggestions and recommendations for future research.

It was decided to use grapefruit-like model beverages rather than real grapefruit to control the complexity and allow for an analyzable experimental design. This choice of model has advantages but also various limitations. Many food ingredients are multifunctional, performing numerous roles within the food matrix (Gillette, 1985; Breslin and Beauchamp, 1997). This multiplicity of ingredient functions greatly complicates efforts to systematically study how taste perception potentially influences ingestive behavior and food choices. That is, changing a single ingredient typically alters multiple sensory properties of a food product (Breslin and Beauchamp, 1997). The grapefruit-like model beverages were prepared using deflavoured/ decoloured/deionized apple juice concentrate. It is produced by clarification, filtration, deionization and evaporation (concentration) of apple juice. Naturally occurring substances which give the acidity, colour and the taste (aroma) of apple juice are removed by the deionization process where the natural sugar content of the apple juice is preserved in the final product. A sweet taste was present only because of the natural sugars while it was colourless and odourless which made it ideal as a neutral fruit juice base concentrate. The neutral deionized apple juice base concentrate (acidified with citric acid to give a constant acid of 1.33 % citric acid found in single strenght grapefruit juice) was modified using a factorial design with 3 naringin concentrations (158 mg/kg, 315 mg/kg and 473 mg/kg) x 3 sucrose concentrations (8, 10 and 12 °Brix) x 2 colours (yellow and red) x 2 mixed aroma compounds intensities (2.5 and 10.0 mg/kg of a mixture of caryophyllene, citral, nootkatone, octanal, nonanal and decanal). Naringin content varies in grapefruit juice from 218-340 mg/kg (Pichaiyongvongdee and Haruenkit, 2009). There was decided to use levels lower, in-between and higher than 280 (average of 218-340 mg/kg naringin) as 158, 315 and 473 mg/kg. In South Africa, grapefruit packed for export to Japan and Europe needs to have a minimum of 8.0 °Brix and acidity 1.33 % citric acid (Dr. G.J. Begemann, Manager Process Quality, Golden Frontier Citrus, personal communication). It was decided to use two additional levels, 10 and 12 °Brix (general °Brix values found in packhouse fruit) which are higher than 8 °Brix (Dr. G.J. Begemann, Manager Process Quality, Golden Frontier Citrus, personal communication). It was decided to use 2.5 and 10 mg/kg aroma compounds intensities, based on the minimum and maximum amount found in grapefruit juice in practice (Clive Teubes, Owner, Clive Teubes (Pty) Ltd, personal communication). The use of pre-prepared concentrated stock solutions ensured equal distribution of grapefruit components during the preparation of the 36 single strength grapefruit-like beverages. Naringin was used for varying the bitterness of the model beverages. Naringin is a very distinctive bitter compound in grapefruit but there are also other bitter phytochemicals responsible for bitterness. Limonin is the major bitter limonoid in orange but can also cause bitterness in grapefruit. Future research could look at the effect of limonin only and in combination with naringin on the sensory properties and consumer liking of grapefruit juice. The aroma compounds, caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), aldehyde C10 (decanal) was obtained from Clive Teubes (Pty) Ltd and are natural aromas recovered from grapefruit essential oil. Although the major aroma components present in grapefruit were used to vary the aroma intensity of the beverages, it was impossible with this research to synthetically include all the possible aroma compounds present in grapefruit. Human beings are just able to discriminate between up to four odourants in chemical complex odourant mixtures (Livermore and Laing, 1998a). Up to date, however, there is little literature available informing which specific citrus odourants may be effective in bitterness amelioration. Future research can focus on other combinations of citrus aroma, as well as different aroma thresholds and the precise role that odour plays in bitterness perception. Synthetic colours (sunset yellow, ponceau red and quinolone yellow) were used to ensure a stable colour during the testing procedure. There are more colour losses related to natural pigments during processing and storage due to lower stability (oxidation) as compared to synthetic colourants (Cortez, Luna-Vital, Margulis and Mejia, 2017). Water and sucrose were used to standardize the °Brix or percentage of soluble solids (expressed in percentage sucrose) according to the grapefruit export specification guideline used by Golden Frontier Citrus, Malelane. The total acidity was expressed in % w/w of citric acid. An increase in the °Brix/acid ratio (Jha, Chopra and Kingsly, 2007) or total soluble solids/total acids led to an increase in the sweetness of fruits (Venkatachalam and Meenune, 2012).

Physico-chemical characterization of the grapefruit-like beverages showed sucrose was inverted at the acidic conditions (Figure 15) with the addition of citric acid. The addition of citric acid may have an effect on sucrose by possibly inverting it (Echeverria and Burns, 1989).

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} \xrightarrow[\text{Water}]{H+} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

Figure 15 Sucrose inversion using acid (Wienen and Shallenberger, 1988)

Sclafani and Mann (1987) reported a difference in perceived sweetness between sucrose, glucose and fructose. Each one on its own or in different combinations might have a different effect on the cross-modal perception of sensory properties and liking of grapefruit juice. Future research can also look at the effect of different combinations of other sugars and the cross-modal interactions applied to grapefruit juice perception and liking.

A descriptive analysis of the grapefruit-like beverages was used to obtain detailed information about the beverages including subtle differences in important sensory attributes. Descriptive analysis uses panellist that are trained to detect and describe differences among products. A generic descriptive analysis method (Einstein, 1991) was used in the training of the panel. There are several different methods of descriptive analysis available but generic descriptive analysis still remains the best to combine different approaches and is frequently employed during practical applications. One group of participants consisting out of 16 judges (8 female and 8 male) between 20 and 50 years (with 1-2 years of experience on descriptive sensory panels) were used for the descriptive analysis of the 36 grapefruit-like beverages. Training of a experienced panel consisted of two training sessions of two hours each, for descriptive attribute and methodology development. Considering the economic and time-consuming aspects of training assessor panels for descriptive analysis, the training for this study was quick and effective. The descriptive analysis process consisted of six sessions of 12 beverages per session and evaluated in duplicate. Reference standards used to define the sensory descriptors were present during training and evaluation sessions but were only used when a problem with a particular term was identified. A total of 21 attributes were generated to characterize the appearance, aroma, flavour and after taste of the grapefruit-like beverages and were evaluated on a structured nine-point horizontal line scale (10 cm). Geel, Kinnear and De Kock (2005) reported a descriptive sensory evaluation of 11 commercially available instant coffees using the generic descriptive evaluation method as reported by Einstein (1991). The coffee was evaluated in triplicate by a trained panel of 12 participants, using 29 descriptors. The scoring of the perceived intensity was also made on an unstructured 10 cm line.

Hedonic assessment is the economical and ideal method to find out the influence in variations in the composition of the beverages and is done by consumers. Ninety-six black African females aged 18-24 years took part in the hedonic study on the twelve most diverse grapefruit-like beverages using the Simplified Labeled Affective Magnitude (SLAM) scale (Lawless, Sinopoli and Chapman, 2010). A drawback of this consumer test is that only 12 of the total of 36 beverages could be evaluated. Bitter/sweet had to be evaluated at the same time in the subgroup of 12 beverages. This may raise the question of how one can be sure if it is bitter or sweet that affects the liking of the beverages. The research budget made provision for the inclusion of a relatively small subset of consumers i.e. 100. Luckow and Delahunty (2004) reported 100 consumers participated in a consumer acceptance test of orange juice containing functional ingredients. It was decided to use African females due to the underrepresentation of the African population in similar studies. Considering the limitation of the research budget, it was also decided to focus on one gender group first. This study covered a specific age group who form a small part of the total South African population and who may have different preferences for grapefruit juice compared to other subgroups. Sensory-processing abilities are known to deteriorate in the elderly. The preferences of younger consumers may also differ due to various external factors. There is some evidence that bitterness perception varies over the lifespan, and this variation over time may be partially under hormonal control and/or regulation because changes occur in both puberty and pregnancy (in the case of females) (Duffy *et al*., 2010). With time, individuals may also learn to like initially aversive oral sensations, such as the bitterness of grapefruit. Variation in taste receptor genes influences taste sensitivity of children and adults. In addition to genes and age, other factors for example culture also contribute to taste preferences. In order to work only on an average customer group of the population, it was decided to work on females, aged 18-24 years.

Both internal and external preference mapping were used to relate the sensory characteristics of the 12 grapefruit-like beverages to consumer liking responses. Partial Least Square (PLS) mapping which is also a kind of preference mapping was used to determine the positive and negative drivers of colour, aroma and flavour liking, and also to predict consumer liking of the 24 samples that were profiled by the descriptive sensory panel, but not evaluated by the consumers. The 'Uncertainty test' which is a feature of specific software (The Unscrambler, CAMO) was not applied in the PLS in order to find out the variables significantly associated. In this analysis, we have run the PLS regressions in SIMCA-P software (Umetrics) and therefore we used the corresponding so-called 'VIP' feature for variable selection. The VIP values summarise the overall contribution of each X-variable to the PLS model, summed over all components and weighted according to the Y variable accounted for each component [www.umetrics.com (accessed March 2019)].

Traditional sensory methods are used to relate consumer and sensory data (e.g. the regression method). By contrast, preference mapping techniques examine the preference of each consumer. The internal preference mapping analyzes the hedonic ratings by the 96 customers for the 12 grapefruit-like beverages by principal component analysis of the covariance matrix, and provides a summary of the main preference directions. External preference mapping regresses the preference or liking of each consumer onto the first two components of the PCA of the products' sensory characteristics (derived from the descriptive analysis). Ward's hierarchical cluster analysis was used to identify clusters of consumers based on similar preference rating scores.

A variation exists in the ability of individuals to detect bitterness, and people can be categorized by propylthiouracil (PROP) sensitivity as either super, medium or non-tasters. The paper disc method (Zhao *et al*., 2003) was used in this study to characterize PROP non-tasters, medium tasters and supertasters. The use of the paper disc method is simple, reliable and convenient. Several other psychophysical methods are available, but can be time and labour intensive, and require subjects to taste many samples and like other general screening methods (Lawless, 1980), they cannot distinguish medium tasters from supertasters. Sodium chloride (NaCl) is used as a reference standard in the paper disc method since taste sensitivity to this compound does not vary by PROP taster status (Kirkmeyer and Tepper, 2003; Zhao *et al*., 2003). It is based on the rationale that non-tasters give much lower ratings to PROP than to NaCl and supertasters give much higher ratings to PROP than to NaCl. Thus, if a subject gives a borderline rating to PROP, the NaCl rating is used to help clarify the classification. Participants rated the intensity of the sensation from tasting each disc using a 100 mm labelled magnitude scale (LMS), with label descriptors placed at quasi-logarithmic intervals along the length of the scale from 'barely detectable' (at the bottom of scale, 0 mm) to 'strongest imaginable' (at the top of the scale, 100 mm) (Zhao *et al*., 2003). Green, Dalton, Cowart, Shaffer, Rankin and Higgins (1996) reported LMS as continuous line scales on which the location of verbal descriptors is based on their semantic magnitudes as empirically determined via magnitude estimation (ME). Green *et al*. (1996) reported further that the features and properties of these scales are derived and validated using ratio scaling (i.e. ME), they can be assumed to yield ratio-level data equivalent to ME. Because they are further bounded by 'no sensation' and 'strongest (or maximal) imaginable sensation' on each end, they enable comparison of individual and group differences within the context of the full range of perceived intensities. In addition, because the positions of their semantic labels have been empirically determined, they provide meaningful semantic information about the subjective experience. It is based on the assumption that subjects are able to make numerical judgements in direct proportion to sensory magnitude. Because there is no way to test the assumption in an absolute sense, some researchers have questioned the validity of the method (Anderson and Wegener, 1982). However, strong evidence in support of LMS has come from studies in vision, hearing, and touch that found additivity of sensation magnitude for pairs of independent stimuli (Balanowski, 1987).

In this study, saliva samples have been taken from the participants to be used to extract DNA using the Oragene OG-500 prepIT.L2P5 DNA extraction kit (DNA Genotek). The protocol provided by the company was used. DNA for genotyping studies can be produced from virtually any tissue. Traditionally, large scale genotyping projects have used DNA derived from whole blood (Mangold, Payne, Ma, Chen and Li, 2008). Over the past several years studies report extracting human DNA from cells from the cheek or saliva using swabs for genotyping studies (Menella, Pepino and Reed, 2005; Joseph, Reed and Mennela, 2015). As blood harvesting is an invasive method that should be performed by trained personnel and in designated facilities, alternative DNA sources are increasingly considered. Saliva represents an important choice, and this is reflected by the growing number of commercially available saliva collecting kits. Besides being extremely easy to use, ship and store, such kits include various stabilizers and hence offer the advantage of remarkable stability of the product. However in the absence of stabilizers, the samples' stability is limited, and should not be stored (Garbieri, Brozoski, Dionisio, Santos and Das Neves, 2017). A disadvantage is the cost of the analysis since the use of the assay can be very expensive. The set of genes that encode the known proteins that function as bitter receptors, were reviewed. The genetic variation in both bitter genes, *TAS2R38* and *TAS2R19* were investigated by genotyping their alleles. This study specifically focused on a group of young South African non-caucasian females to focus the scope of investigation. Bitter sensitivity is present most often in females, showing a higher supertaster status for women than men (Teppers and Nursea, 1997). Females were chosen, in the age group 18-24 years, because older women are more educated on the health benefits of consuming fruit and vegetables, caring for children and are familiar with preparing and serving healthy food options for their families. The perception of bitter intensity in older persons declines with age and may influence (usually positively) the liking of bitterness in beverages (Drewnowski, 2001).

Understanding flavour integration is essential to understand consumers' choices. Any sensory input for example taste, smell, vision, hearing, touch, can potentially influence food perception and preference (Spence *et al*., 2014). Bennett, Zhou and Hayes (2012) reported that bitter suppression occurs at the peripheral level through inhibition of taste receptors, the central cognitive level through the perception of bitters mixed with other tastes or olfactory flavourings, or physically blocking the bitter stimulus from reaching receptors, as with fat or cyclodextrin. The sodium cation from sodium salts suppresses bitterness of aqueous pharmaceuticals (Menella, Pepino and Beauchamp, 2003), even without adding a salty taste, which suggests peripheral inhibition at the receptor level (Keast and Breslin, 2005). Sodium acetate (NaAc) has been shown to be effective at blocking the bitterness of aqueous pharmaceuticals (Keast and Breslin, 2002). The research studied the effect of varying the bitterness, sweetness and aroma intensity as well as the colour of grapefruit-like beverages on sensory properties and consumer liking. It is important to understand how and where interactions between senses occur as they often impact on the perception of flavour, as well as other attributes, affecting the key sensory profiles of products. The mechanisms proposed to explain the mutual influence of aroma and taste when perceived together can occur at physico-chemical, physiological or psychological levels. For flavour perception to occur, the chemicals responsible for flavour perception must be released from the food matrix and transported to the flavour receptors in the mouth and nose (Figure 16). The grapefruit-like beverage samples were kept at 14°C until served. This is the temperature at which grapefruit juice is typically consumed.

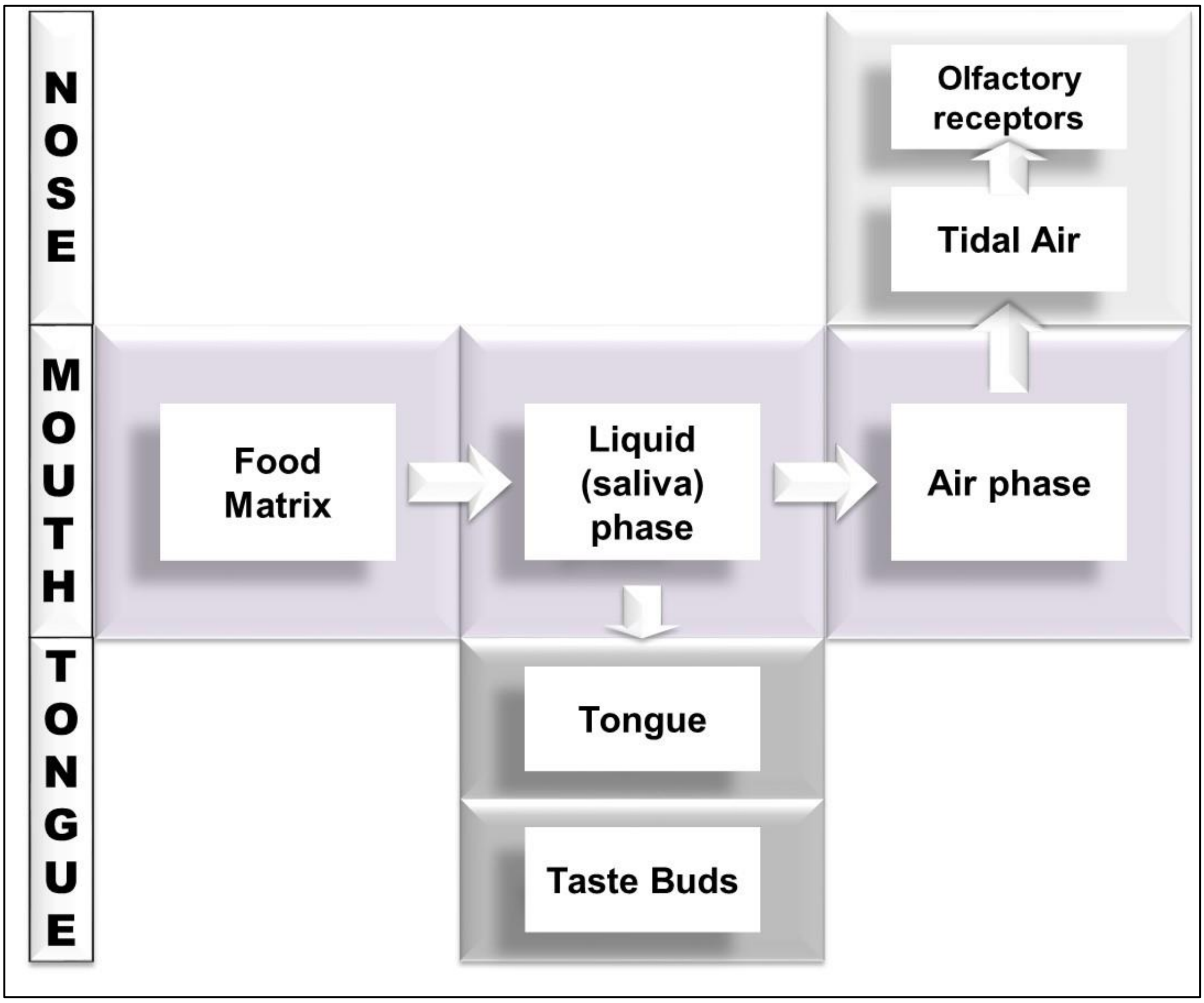

Figure 16 Schematic representation of flavour release in vivo and subsequent flavour transport to the receptors in the mouth and nose (Taylor, 2002)

The physical interaction of temperature with odour and the sensory interaction of temperature with taste as seen in Figure 17 both lead to one obvious practical conclusion; samples should be evaluated at the temperatures at which they will be used (Delwiche, 2004).

The overall process is governed by the properties of the flavour compounds, the nature of the food matrix and the physiological condition of the mouth, nose and throat during consumption of the food. Furthermore, individuals vary in their rate of breathing, swallowing and salivation, which affects the transport of flavours from the saliva phase to receptors on the tongue and in the nose (Taylor, 2002). The characteristics of saliva are greatly influenced by age (Xu, Laguna and Sarkar, 2019). Age-related salivary disorders such as hyposalivation can alter the way saliva interacts with foods and perception (Muǹoz-Gonzalez, Brule, Feron and Canon, 2019). Biomarkers present in saliva can be used to differentiate individuals (Lucas, Barbosa, Castelo and Gaviao, 2019). The quantification of salivary differences/simalarities between different consumer groups can provide useful information for the design of products targeting specific markets (Mosca, Stieger, Neyraud, Brignot, Van de Wiel and Chen, 2019). Different types of interaction between saliva and food components are presented by Perez-Jimenez, Rocha-Alcubilla and Poza-Bayon (2019) as enzymatic breakdown of aroma compounds, precipitation of proteins (Carpenter, Cleaver, Blakeley, Hasbullah, Houghton and Gardner, 2019), or even saliva as an effective emulsifier for oral emulsification of oil/fat (Glumac, Qin, Chen and Ritzoulis, 2019).

Tastes can increase the apparent intensity of aromas; conversely, the perceived intensity of tastes is increased when we taste flavoured solutions, especially when there is a logical association between them, such as between sweetness and fruitiness (Noble, 1996).

Taste-aroma interactions are more probably a function of cognition (congruency), occurring at the central processing level rather than at a receptor level (Noble, 1996). Lim *et al*. (2014) however report that congruency plays an important role, although to different degrees, in both types of taste-odour interactions: retronasal odour referral to the mouth and retronasal odour enhancement by taste. Taste-odour congruency is necessary but not a sufficient condition for retronasal odour enhancement by taste and it showed that sucrose, a congruent tastetant for citral and coffee odour, significantly enhanced the perceived intensities of both test odours. Citric acid and caffeine, however failed to enhanced the odour of citral and coffee although the sour taste of citric acid and the bitter taste of caffeine were rated as quite congruent with citral and coffee odour, respectively. Hewson, Hollowood, Chandra and Hort (2008) reported an increase in flavour perception in a model citrus flavoured beverage on the addition of sugars (glucose and fructose), although, interestingly, glucose showed a different profile to fructose despite equi-sweet levels being used. It provides evidence for taste-aroma interactions within that citrus-flavoured system which are not due to physico-chemical interactions within the beverage matrix. That study has also uncovered apparent differences between effects of fructose and glucose on flavour perception, again not due to alterations in physical factors, and raises the intriguing possibility of different receptors/receptor mechanism between the two monosaccharides. However, in the study on grapefruit beverages reported here, varying the bitterness of the grapefruit-like beverages did not have a significant effect on any of the aroma attributes. In this study sweetness of the beverages had a significant effect only on the soapy aroma of the grapefruit-like beverages. There is a possibility that it could have reminded the evaluator of scented soap (e.g. dishwashing liquid) with a possible citrus fragrance. Both Lim *et al*. (2014) and Green *et al*. (2012) reported that sucrose, but not other taste stimuli, can significantly enhance the perceived intensity of various sweet congruent food odours including citral aroma.

Taste and smell configurations resulting from referral are frequent, almost as if the combination of the two is so anticipated that it is difficult to perceive one without perceiving the other. The bitterness of the grapefruit-like beverages had a significant effect on other flavour attributes, astringent, sweet, sour, bitter and grapefruit flavour and bitter after taste. Taste perception occurs during gustation as seen in Figure 17 as bitter taste-active compounds (naringin) in the grapefruit-like beverages stimulate taste receptors (G-protein) on the tongue. The information from these receptors gets transmitted to the brain, where it is processed and integrated. Negative drivers of flavour liking were soapy aroma, bitter after taste and sour flavour. Sucrose intensity had a significant effect on sour, sweet, bitter, astringent and grapefruit flavour and bitter after taste of the grapefruit-like beverages. Beckett *et al.* (2014) reported that sucrose had a masking effect on the bitter taste of sinigrin, goitrin and quinine. Adding a sweet-tasting compound suppresses bitterness via central integration of the mixture of taste qualities (i.e. mixture suppression) (Kroeze and Bartoshuk, 1985). Menella *et al*. (2015) did a study of the effect of sucrose on different bitter compounds, urea, caffeine, denatonium benzoate, propylthiouracil (PROP), and quinine to see if it will reduce their bitterness. The study showed that sucrose suppressed bitterness to different extents for each particular bitter compound, but for some bitter agents the effect of sucrose was marked; for instance, it substantially lowered bitterness ratings of caffeine. In adults, sucrose was effective in reducing the bitterness ratings from moderate to weak for all compounds tested, but those individuals with the sensitive form of the sweet receptor reported a greater reduction of bitterness for caffeine and quinine. There was variation among people and among bitter agents in the ability of sucrose to suppress bitterness, for some people and for some compounds, sucrose is an unequivocally effective masker of bitter taste. Psychophysical studies in adults suggest that the mode of action for bitterness suppression differs between sugars and salts. Sodium salts appear to suppress bitter taste at the periphery (receptor level), and this suppression is compound specific (Narukawa, Tsujitani, Ueno, Nakano-Ooka, Miyamoto, Sawano and Hayashi, 2012). Sugars, on the other hand, act along the central gustatory pathway (cognitive level) and have been shown to suppress the bitterness of a range of bitter agents in adults (Keast, Canty and Breslin, 2004). When sucrose is added to fruit juices, not only are the perceived level of bitterness and sourness reduced (as was found in this research) but the sweet odour intensity rating also changes (although this was not found here) (Von Sydow *et al*., 1974).

The research showed a decrease in both bitterness and astringency in the grapefruit-like beverages with an increase in aroma concentration. The perceived intensities of tastes are increased when humans taste flavoured solutions, especially if there is a logical association between them, such as between sweetness and fruitiness-fruits are often sweet (Noble, 1996). Oladokun, James, Cowly, Dehrmann, Smart, Hort and Cook (2017) reported an increase in perceived bitterness intensity, and astringency after the adition of Hersbrucker hop aroma extract in three distinctive hop beer varieties due to some level of congruency in the resultant taste-aroma interaction (Hersbrucker, East Kent Goldings, Zeus). Sáenz-Navajas *et al.* (2010) reported a decrease in bitterness and astringency as well as an increase in sweetness with the addition of volatile fruity extracts to 'reconstituted wines'.

It was also clear in the research on the liking of grapefruit-like beverages that aroma concentration had a significant effect on the majority of the sensory attributes. While it is only natural to think of taste (i.e. gustation) as playing a key role in multisensory flavour perception, the majority of researchers agree that it is the sense of smell (or olfactory) that actually contributes to the flavour information we experience (Spence, 2015). As expected the aroma compounds used in the grapefruit-like beverages (containing a citral component), had a significant effect on the intensity of the citrus aroma attribute. Hewson *et al.* (2008) highlighted an additive effect of sweet components with citral and/or limonene volatiles having a 'citrus'-like aroma.

Colour of the beverages had a significant effect on the perception of overall and grapefruit aroma intensities, deteriorated/rotten, muddy/mouldy, fruity and sweet aroma intensity. Odour identification is lessened when odours are presented without colour cues or when they are paired with inappropriate colours (Blackwell, 1995). Zellner and Kautz (1990) suggested that the colour-induced olfactory enhancement seen with solutions smelled orthonasally might be the result of a conditioned olfactory percept caused by the colour. The orthonasal colour-induced odour enhancement might thus be due to a combination of the actual odour the subject experienced from smelling the solution and the colour-induced conditioned percept caused by the previous pairing of particular colours with different odours (most red-coloured beverages are fruit odours). Morrot, Brochet and Dubourdieu (2001) found that when white wine was coloured red, individuals tended to describe the wine with more red wine odour terms instead of using white wine odour terms. Blackwell (1995) reported in a study that visual cues significantly influenced odour assessment of six fruit solutions. Colour also had a significant effect on grapefruit flavour intensity with the highest value perceived in the red coloured beverages. The Star Ruby variety with a red colour is the most planted grapefruit variety in South Africa and it is possible that the panelists are most familiar with red grapefruit. Roth, Radle, Gifford and Clydesdale (1988) altered the relationship of green and yellow colours in lemon and lime flavoured sucrose solutions and found that these colour changes had an impact on sweetness ratings. Grapefruit-like beverages with a red colour were preferred by consumers over those with yellow colour. It could be expected since the majority of consumers were PROP tasters (33 % super tasters and 53 % medium tasters) and one can expect that they would prefer red coloured beverages as these were more associated with sweetness. However, there were no significant drivers for colour liking. The participants perceived the red samples as sweeter with low aroma. Maga (1974) reported the same in a study colouring a solution red resulted in a significant lowering of participant's sensitivity to bitter taste, while the addition of yellow and green colouring had no such effect. Spence *et al*. (2010) reported that red colour decreased the perception of bitter taste sensitivity. The research showed a perceived decrease in both bitterness and astringency perception in the grapefruit-like beverages with an increase in the aroma.

The low naringin/high sucrose samples were preferred over the high naringin/low sucrose samples. Fellers *et al.* (1988) reported similar results and found with an increase in the ratio of °Brix/acidity of reconstituted grapefruit juice the consumer perception of sweetness increased and bitterness and aroma decreased. Bitterness was seen as a reason for dislike of grapefruit-like beverages. Lanier *et al.* (2005) also found in a study where participants tasted grapefruit juice in the laboratory, the sweetness was positively associated with liking, whereas bitterness was negatively associated with liking.

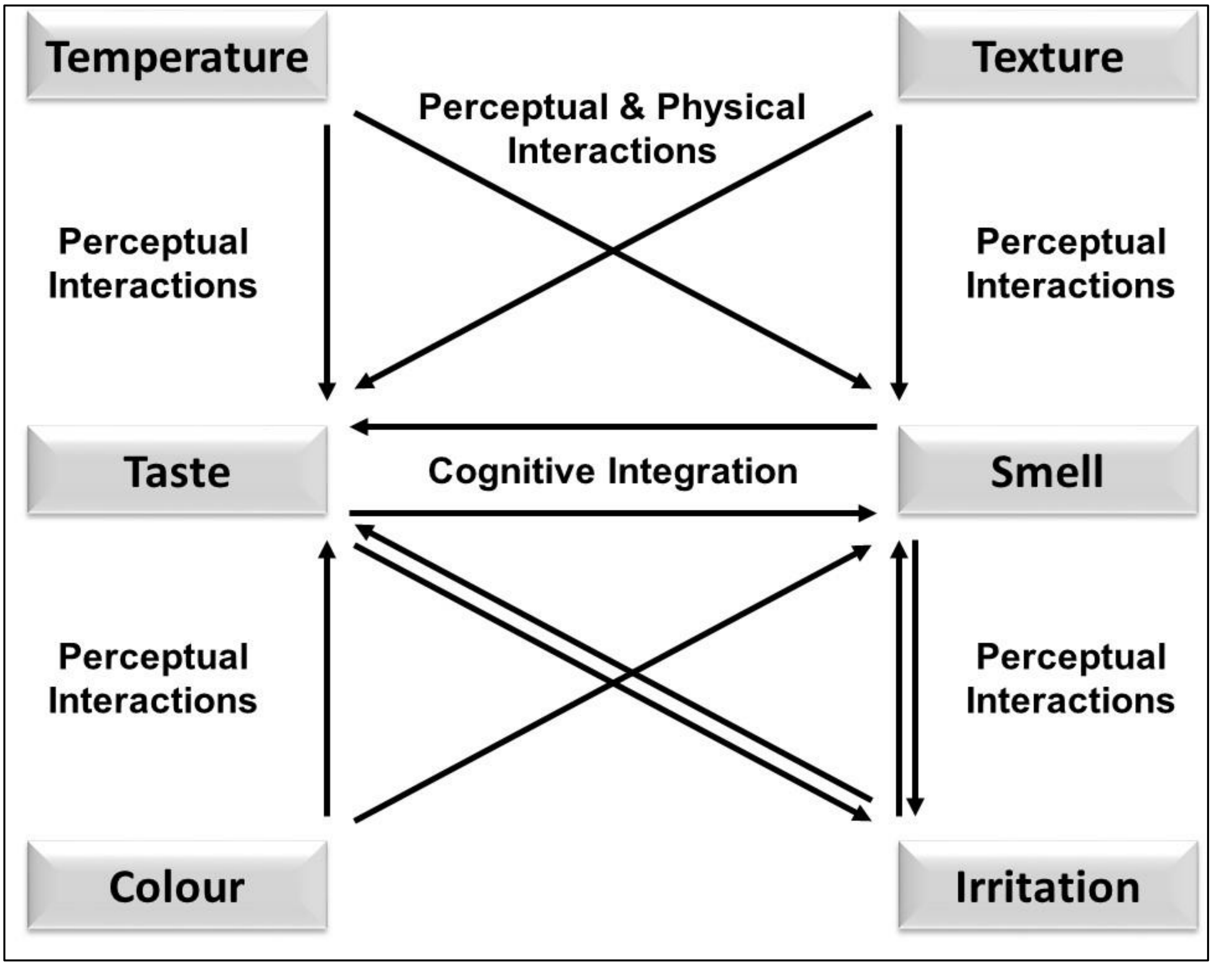

Figure 17 Summary of perceptual interactions evoked during ingestion. Arrowhead indicates a modality that has been demonstrated to interact with another modality (Delwiche, 2004)

Some bitterness in processed grapefruit products is acceptable to consumers but excessive bitterness is one of the major consumer objections to such products as pointed out by Bell (1955) and Birdsall (1955) and confirmed here.

Genetics plays a significant role in the perception of different taste qualities, accounting for over 30 % of the variance in sweetness, sourness, and bitterness (Knaapila *et al*., 2012; Hwang, Zhu, Breslin and Reed, 2015). Polymorphisms in the bitter taste receptor gene *TAS2R38* alter the ability to sense the intensity of bitterness of PROP and genetic variation in sensitivity towards PROP may affect food preferences (Deshaware and Singhal, 2017). PROP is extremely bitter for some, while others will perceive little or no bitterness (Bartoshuk *et al*., 1994; Tepper *et al*., 2009). In this study, 96 individuals were classified according to their PROP taster status. Contrary to expectation, a higher intensity rating was recorded for the disc with NaCl by supertasters compared to the other two taster groups. The concentration of the NaCl on the paper discs was not measured post application which is a limitation and should be a suggestion for future studies. The PROP taster status distribution of the group in this research was 26 % supertasters, 65 % tasters and 9 % non-tasters. The frequency of non-tasters varies greatly among populations around the globe from as low as 7 % to more than 40 % and depends on race and ethnicity (Guo and Reed, 2001). The non-tasters in this all-female South African population group was much lower than the expected frequency obtained in previous studies of approximately 31 % found in a population in the United State consisting of 23 adult males and 39 females (Zhao *et al*., 2003) and another study in the United States which found 29 % non-tasters out of 114 adult females (Yackinous and Guinard, 2002). Fifty-four women, aged 18-30 years, consisting out of 75 % Caucasian, 18.5 % Asian/pacific islander, 1.9 % African-American, 1.9 % native American and 1.9 % listed as other were classified as 39 % non-tasters and 61 % tasters using a PROP-saturated filter paper as well as PROP solutions. A high degree of correlation was found between the two methods (Ly and Drewnowski, 2001). Firty-two women, age 18-45 years, consisting out of mostly Caucasians (78 %). The remaining subjects were Hispanic (10 %), African-American (6 %), and Asian (6 %) were classified as 44 % non-tasters and 56 % supertasters (Tepper, Neilland, Ullrich, Koelliker and Belzer, 2011) using the filter paper method developed by Zhao *et al*., 2003. Medium tasters were excluded by using a technique by (Zhao and Tepper, 2007). Another study indicated, the distribution of tasters and non-tasters among white Caucasians in North America was reported as bimodal, with the majority of individuals (approximately 70 %) identified as 'tasters' (Tepper, 1998; Bartoshuk *et al*., 1994). The supertaster population i.e. participants containing the highest density of taste papillae on their tongues, are mostly found amongst women (Drewnowski, 2001). Robino, Mezzavilla, Pirastu, Dognini, Tepper and Gasparini (2014) did a study in six different populations of the Caucasus region at the border of Europe and Asia as well as in Central Asia and differences in the distribution of PROP phenotypes across populations were detected, with a higher frequency of supertasters in Tajikistan (31.3 %) and Armenia (39.0 %) and a higher frequency of non-tasters in Georgia (50.9 %).

Future research should include a larger group of South African population consisting of different groups of men and women. The studies can include determining the density of the fungiform taste papillae on the anterior surface of the tongue to determine if supertasters have a greater density of the papillae. Non-tasters and medium tasters in this study who were less sensitive to the bitterness of PROP liked the flavour of the grapefruit-like beverages more than the supertasters. Previous research showed those who perceive PROP as more bitter also perceive a wide range of compounds such as grapefruit juice and coffee (Lanier *et al*., 2005) and brassica vegetables (Drewnowski and Gomez-Carneros, 2000; Gorovic, Afzal, Tjønneland, Overvad, Vogel and Albrechtsen, 2011) as more bitter and less acceptable (Dinehart *et al*., 2006). Akella *et al*. (1997) reported that PROP tasters rated naringin (from grapefruit) solutions and infusions of Japanese green tea as more bitter than did non-tasters and liked them less and reported lower preferences for grapefruit juice. Contrary to expectation non-tasters in this study did not like beverages with varying bitterness similarly. Bitter/sweet compounds had to be evaluated at the same time in the subgroup of 12 beverages. When naringin was decreased, sucrose was increased. Beverages with a higher bitterness tasted less sweet, while the opposite is also true for beverages with less bitterness having a higher sweet taste. It is therefore likely that the liking of the non-tasters is based on sweetness and not bitterness given that these individuals are not sensitive to differences in bitterness.

Unfortunately, this is the biggest limitation of this study. In an ideal study, one factor need to be kept constant for example sucrose concentration (sweetness) and then only naringin concentration (bitterness) is varied. Since there was a bitter sweet interaction in the current study and only bitter genes were looked at, it may be interesting to include the sweet gene TAS1R2/TAS1R3 in similar future studies. All groups preferred the high bitter beverages less than the low and medium bitter beverages. It was expected that the supertasters would have liked the high bitter beverages less than the medium tasters and non tasters. Guadagni, Maier and Turnbaugh (1974) reported that natural sugars in orange juice had the effect of raising the detection threshold for naringin and reduce the bitterness. By raising the threshold it will be more difficult to taste naringin and drinks will be more acceptable to medium tasters and supertasters. Individuals more sensitive to the bitterness of PROP have heightened perception of sweet tastes from sucrose (Lucchina, Curtis, Putman, Drewnowski, Prutkin and Bartoshuk, 1998). It is therefore likely that the liking of the supertasters is based on both bitterness and sweetness and that they would also be more sensitive for high sweetness.

The *TAS2R* gene family (Chandrashekar *et al.*, 2000) bind structurally to different molecules which elicit bitterness. Bitter compounds encompass a variety of structurally diverse molecules found ubiquitously in nature (Kim *et al.*, 2003). One of the most widely studied genes is the *TAS2R38* gene. *TAS2R38* encodes a seven transmembrane G protein-coupled receptor (Drayna, 2005). Most of the perceptual variability to PROP perception is due to genetic variation within the bitter taste receptor *TAS2R38* (Drayna, Coon, Kim, Elsner, Cromer, Otterud, Baird, Peiffer and Leppert, 2003; Bufe *et al*., 2005; Reed *et al*., 2010). It binds to the thiourea group contained in the synthetic compound PROP (Kim and Drayna, 2005). Three SNPs have been identified within the *TAS2R38* gene in this study. Previous research by Kim *et al*. (2003) and Drayna (2005) also showed three SNPs in gene nucleotide positions 145 (rs713598 C/G), 785 (rs1726866 C/T), and 886 (rs10246939 G/A). It encode for non-synonymous amino acid substitutions at positions 49 (alanine/proline, ala49pro), 262 (valine/alanine, val262ala), and 296 (isoleucine/valine, iso296val). While several allelic forms have been observed, the two most common are proline-alanine-valine (PAV) and alanine-valine-isoleucine (AVI) (Campbell *et al.*, 2012). Other haplotypes namely Alanine-Alanine-Valine (AAV), Alanine-Alanine-Isoleucine (AAI), Proline-Alanine-Isoleucine (PAI), Proline-Valine-Isoleucine (PVI) have been observed rarely (1-5 %) or in specific populations such as from Africans (Campbell *et al*., 2012), mostly in combination with PAV or AVI as heterozygotes and very infrequently as homozygotes (Hayes *et al*., 2008). PAV stands for the taster allele while AVI represents the non-taster allele. Garneau, Nuessle, Sloan, Santorico, Coughlin and Hayes (2014) reported that individuals carrying the dominant diplotype (PAV/PAV and PAV/AVI) report higher bitterness intensity towards PROP as compared to individuals carrying the homozygous recessive diplotype (AVI/AVI). PAV homozygotes exhibit greater sensitivity to PTC/PROP bitterness, while AVI homozygotes are less sensitive (Duffy *et al.*, 2004; Bufe *et al*., 2005). Heterozygotes (PAV/AVI) show intermediate bitter taste sensitivity (Caln *et al*., 2011; Hayes *et al*., 2008). This study also showed a significant effect of the *TAS2R38* rs713598 and rs1726866 genotype on the liking of the flavour of grapefruit-like beverages, which is a novel finding. Participants with *TAS2R38* rs713598 CC genotypes rated liking of the flavour of the grapefruit-like beverages significantly higher than those with CG, but not those with GG genotypes. *TAS2R38* rs1726866 participants with the CC genotype rated liking of the flavour of grapefruit-like beverages significantly higher than both those with CT or TT genotypes. Children with the bitter-sensitive genotypes (CG, GG; rs713598, ala49pro) preferred significantly higher levels of sucrose than those with the bitter-insensitive genotype both in laboratory-based measures and in reported preferences of real-world foods like cereal and beverages (Mennella *et al*., 2005). Adults with the bitter-sensitive alleles of *TAS2R38* also rated foods such as brassica vegetables (Sandell, 2006) as more bitter compared to adults with the bitter-insensitive alleles.

A SNP rs10772420 was identified in this study in a South African female population sample at position 635 in the *TAS2R19* gene. The mutation at position 635 from a cytosine to a thymine (C > T) results in a missense mutation, influencing the protein-coding amino acid from an arginine to a cysteine at position 299 (arg299cys) in the *TAS2R19* gene. The *TAS2R19* rs10772420 genotype heterozygote CT showed a lower liking for the flavour of grapefruit-like beverages than both the homozygotes CC and TT. A single nucleotide polymorphism (SNP) within the bitter receptor gene *TAS2R19* on chromosome 12, Arg299Cys SNP (rs10772420) has been associated with a differential liking for grapefruit juice (Hayes, Feeney, Nolden and McGeary, 2015). The SNP rs10772420 was previously found in a Caucasian population (Duffy *et al.*, 2009). Limonin and naringin failed to activate the Arg299 or the Cys299 variants of the *TAS2R19* receptor in vitro (Meyerhof *et al.*, 2010). SNP rs10772420 has previously been associated with dietary preferences e.g. guanine bitterness, grapefruit liking and bitterness differences among individuals (Duffy *et al.*, 2009; Hayes *et al.*, 2013). Hayes *et al.*, 2015 observed SNP-phenotype associations and self-reported liking for grapefruit juice with SNPs in *TAS2R31*. The results here are in agreement with those found in a study by Hayes *et al.* (2011). In that study, the rs10772420 SNP was also associated with responses to unsweetened grapefruit juice, with homozygotes reporting less bitterness and greater liking (Hayes *et al.*, 2011). The findings are also novel because it is the first time that the effect of taster status, *TAS2R38* and *TAS2R19* SNP genotypes on the liking of the flavour of grapefruit model beverages was described for a non-caucasian population.

The bitter taste genes *TAS2R38* and *TAS2R19* were only two of the 25 unique bitter taste genes (*TAS2Rs*) in humans. Future research could look at understanding the effect of other bitter genes and the effect of tongue papillae density on grapefruit liking. Food preferences develop with experience based on associations formed between a food flavour and the consequences of its consumption (Boesveldt, Bobowski, McCrickerd, Maitre, Sulmont-Rosse and Forde, 2018). Although the initial affective reaction to bitter is genetically mediated, this response can be modified through experience (Capaldi and Privitera, 2008). The challenge is to see and potentially promote grapefruit liking as a multi-modal experience, one that uses all senses and made strong associations with health promoting. The universality of bitter foods/beverages suggests however that their consumption is not limited to bitter insensitive individuals. Coffee, for example, is one of the world's most consumed beverages, despite the presence of caffeine and other bitter compounds. As humans, age, sensory acuity including taste, smell and texture perception often declines and this can have an impact on food perception, preferences and food intake (Boesveldt *et al.*, 2018). If the health benefits associated with grapefruit are promoted by people of all ages, its consumption might increase and will also as in the consumption of coffee not be limited to bitter insensitive individuals. The challenge is to see and potentially promote grapefruit liking as a multi-modal experience, one that uses all senses and made strong associations with health promoting benefits. In future, research can also look at the influence of grapefruit-drug interactions as well as possible taste and smell disorders on grapefruit consumption. Opportunities exist to collaborate with main consumer groupings globally to determine why grapefruit consumption is increasing among them to make the same happen in South Africa.

CHAPTER 8:
Conclusions

Varying the bitterness, sweetness, aroma intensity and colour type of a model grapefruit-like beverage have an effect on the perception of sensory properties and consumer liking of the beverage. This research developed a grapefruit research model and contributed to gathering insight into the perception of the sensory properties of grapefruit and grapefruit juice. This is of value to the grapefruit industry in order to produce products that target consumers who prefer to consume it. Based on these results, researchers could make modifications to the composition of grapefruit and processed grapefruit products to reduce the negatively liked factors and enhance the positive attributes. Consumers preferred grapefruit-like beverages with low bitterness. Bitterness was confirmed as a reason for disliking of grapefruit juice. If the consumption of grapefruit is to be increased in South Africa, research needs to focus on ways on how to ameliorate the bitterness intensity of grapefruit and/or products processed thereof. Commercially, it is the citrus industry that relies the most on debittering technologies to mask or remove the bitterness of citrus juices. As sucrose in grapefruit juice decrease, bitterness perception increase. Blending of juice batches to standardize sugar levels and naringin levels could be used for bitterness management, however has to comply with regulations.

Interindividual differences in liking of grapefruit-like beverages can be largely explained by SNPs in *TAS2R38* and *TAS2R19,* encoding bitter taste receptor. Future research can include TAS2R31 which was also linked in literature to liking of grapefruit juice. Perception of bitter taste vary between individuals depending on genetic variations in taste receptor genes. Although the initial inborn affective reaction to bitter tasting foods and substances is genetically mediated, this response can be modified through experience. The red colour was preferred by consumers over those beverages with yellow colour, which supports other researchers findings that red colour decreased the perception of bitter taste sensitivity. The majority of consumers were PROP tasters (33 % supertasters and 53 % medium tasters) and one would expect that they would prefer red coloured beverages associated with sweetness. The insights (e.g. increasing perceived sweetness by changing the colour of grapefruit juices) can be utilized to help consumers toward healthier food behaviours and increasing grapefruit consumption. The better understanding of taster type distribution (e.g. PROP taster types) of consumers at population level could be of value for developing marketing strategies to make grapefruit more attractive to a wider range of consumers. Given the commercial opportunities associated with a better cognitive neuroscience understanding of the multisensory nature of flavour perception and the crucial individual, developmental, and cultural differences therein will assist procesors to promote grapefruit consumption. This study gives information on how grapefruit preferences could possibly be shaped by understanding differences in human sensory perception and preferences.

CHAPTER 9:
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Appendix 1:
Descriptive analysis evaluation Questionaire

Panelist Code: ______________________ Panelist Name: ______________________

WELCOME to this Tasting Session

SRD SENSORY RESEARCH DIVISION

Your Partner in Sensory Evaluation

DEPARTMENT OF FOOD SCIENCE

UNIVERSITY OF PRETORIA

To start the test, click on the Continue button below:

Question # 1 - Sample <<Sample1>>

What colour is <<Sample1>>?

- ❍ Red
- ❍ Yellow

Question # 2 - Sample <<Sample1>>

Open the lid and take short sniffs. Evaluate the aroma attributes of <<Sample1>>

Panelist Code: ______________________ Panelist Name: ______________________

Overall aroma intensity

Not intense | Very intense

Citrus aroma

Not intense | Very intense

Grapefruit aroma

Not intense | Very intense

Chemical

Not intense | Very intense

Deteriorated/Rotten

Not intense | Very intense

Question # 3 - Sample <<Sample1>>

Open the lid and take short sniffs. Evaluate the aroma attributes of <<Sample1>>

Muddy/Mouldy

Not intense | Very intense

Fruity

Not intense | Very intense

Green/Grassy

Not intense | Very intense

Panelist Code: ______________________ Panelist Name: ______________________

Peely/Peel oil

Not intense | Very intense

Soapy

Not intense | Very intense

Question # 4 - Sample <<Sample1>>

Open the lid and take short sniffs. Evaluate the aroma attributes of <<Sample1>>

Pungent

Not intense | Very intense

Woody/Spicy

Not intense | Very intense

Sweet aroma

Not intense | Very intense

Question # 5 - Sample <<Sample1>>

Take a sip and evaluate the flavour attributes of <<Sample1>>

Overall flavour intensity

Not intense | Very intense

Sour

Not intense | Very intense

Panelist Code: ______________ Panelist Name: ______________

Sweet

Not intense Very intense

Bitter

Not intense Very intense

Astringent

Not intense Very intense

Question # 6 - Sample <<Sample1>>

Take a sip and evaluate the flavour attributes of <<Sample1>>

Citrus

Not intense Very intense

Grapefruit

Not intense Very intense

Question # 7 - Sample <<Sample1>>

After swallowing, Evaluate the bitter after taste of <<Sample1>>

Bitter

Not intense Very intense

Please drink some water and eat a piece of cracker before evaluating the next product.

THANK YOU!

Appendix 2:
Online screening survey

Thank you for participating in this research project involving more than one researcher. The first activity is a survey.
The purpose of this survey is to investigate your current food consumption patterns.
This includes information on what is eaten when it is eaten and how much is eaten

PLEASE NOTE:
There are no right or wrong ansers.

To start the survey, please click NEXT

What is your age?

[ ]

Next

What is your gender?

☐ Male

☐ Female

What is your home language?

[ ]

Next

What best describes your **race/ethnicity? Do you consider yourself to be a...**

☐ Pedi/Northern Sotho	☐ Xhosa
☐ Southern Sotho	☐ Tsonga
☐ Tswana	☐ Venda
☐ Zulu	☐ Shangaan
☐ Ndebele	☐ Other (specify) ……………………..

Next

We would like to ask you about the ethnicity of your parents. These questions are optional to answer.

Next

Please indicate the group that best describes your **mother's ethnicity**. ***Mark only one***

☐ I prefer not to answer	☐ Xhosa
☐ Pedi/Northern Sotho	☐ Tsonga
☐ Southern Sotho	☐ Venda
☐ Tswana	☐ Shangaan
☐ Zulu	☐ Other (specify) ……………………..
☐ Ndebele	

Next

Indicate which of the following **best** describes your mother's educational level. ***Mark only one.***

- ☐ I prefer not to answer
- ☐ University/College
- ☐ High schoo/ Secondary school
- ☐ Primary school
- ☐ Did not go to school

Next

Please indicate the group that best describes your **father's ethnicity**. ***Mark only one***

☐ I prefer not to answer	☐ Xhosa
☐ Pedi/Northern Sotho	☐ Tsonga
☐ Southern Sotho	☐ Venda
☐ Tswana	☐ Shangaan
☐ Zulu	☐ Other (specify) ………………………
☐ Ndebele	

Next

Indicate which of the following **best** describes your father's educational level. ***Mark only one.***

- ☐ I prefer not to answer
- ☐ University/College
- ☐ High school/ Secondary school
- ☐ Primary school
- ☐ Did not go to school

Next

What is your weight? (kg)

Next

What is your height? (m)

Next

On average, how often do you participate in sport or physical activity?

- ☐ Daily
- ☐ 3-4 times per week
- ☐ 1-2 times per week
- ☐ 2-3 times per month
- ☐ Never

Next

The next two questions are about you and your health

Next

In general, how would you rate your own health? Would you say that **your health is ...**

- ☐ Excellent
- ☐ Very good
- ☐ Good
- ☐ Fair
- ☐ Poor

Next

How satisfied are you with your life?

- ☐ Very satisfied
- ☐ Somewhat satisfied
- ☐ Somewhat dissatisfied
- ☐ Very dissatisfied

Next

In this section information is needed on how you usually eat at **home** during **weekdays**. Questions regarding your home environment and how it contributes to your eating pattern are also included.

Next

How many meals do you eat a day?

Next

Do you usually eat breakfast?

☐ Yes

☐ No

Next

Please give the main reason why you eat breakfast.

Next

Please indicate to what extent the following statements apply to your parent(s)/guardian(s) and friends regarding **healthy** eating.

Next

My mother/father/guardian **cares** about eating healthy food.

- ☐ Not at all
- ☐ A little bit
- ☐ Somewhat
- ☐ Very much

Next

My mother/father/guardian **encourages me** to eat healthy food.

- ☐ Not at all
- ☐ A little bit
- ☐ Somewhat
- ☐ Very much

Next

Many of my friends **care** about healthy eating.

- ☐ Not at all
- ☐ A little bit
- ☐ Somewhat
- ☐ Very much
- ☐ I don't know

Next

Generated by Compusense at-hand

Please indicate to what extent the following statements apply to the food in your home/household.

Fruits and vegetables are always available in my home

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Vegetables are served at supper in my home.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

We have fruit juice in my home.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Milk is served at meals in my home.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

We have "junk food" in my home.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Potato chips or other salty snacks are available in my home.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Chocolates or other sweets are available in my home.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Soft/Fizzy drinks (e.g. Coke, Fanta, Sprite) are available in my home.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Please indicate how **many portions** of the following foods you **usually eat or drink a day**. Please indicate the number of portions in the space provided.

If you don't eat the food, please type "0".

Next

How many portions of meat, chicken or fish do you usually eat a day?

Next

How many portions of milk and other dairy products (e.g.cheese, yoghurt, cottage cheese) do you usually eat or drink a day?

Next

How many portions of whole grain products (e.g. brown, whole bread) do you usually eat a day?

Next

How many portions of fruit and vegetables do you usually eat a day?

Next

How many portions of fruit juice do you usually drink a day?

Next

How many portions of soft/fizzy drinks (e.g. Coke, Fanta, Sprite) do you usually drink a day?

Next

How many portions of chocolates and sweets (give number of bars, slabs or packets) do you usually eat a day?

Next

How many packets of savoury snacks (e.g. chips, popcorn, crackers, etc) do you usually eat a day?

Next

How many cups of coffee do you usually drink a day?

Next

How many cups of tea do you usually drink a day?

Next

How many teaspoons of sugar do you usually put in your coffee?

Next

How many teaspoons of sugar do you usually put in your tea?

Next

How many glasses of water do you usually drink a day?

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Home cooked food

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Take away or fast food (e.g. KFC, Nandos).

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Snacks foods (e.g. chips, chocolate, sweets, popcorn).

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Fresh fruit (e.g. oranges, bananas, apples, guava, grapes).

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Soft drinks (e.g. Coke, Sprite, Fanta).

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Fruit juice (e.g. Orange, mango, apple juice).

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Milk and other dairy products (e.g. cheese, joghurt, yogi sip).

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Vegetables and salads.

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

Please indicate how **often** you consume/use the following types of food and beverages. Indicate the number that **best represents** how **often** you eat each item.

Alcoholic beverages (e.g. beer, wine).

5-7 times per week	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

How **often** do you eat meals **away from home** (in other places than your home)?

Daily	3-4 times per week	1-2 times per week	Never
☐	☐	☐	☐

Next

If you eat **away** from home **where** do you eat **most often**? **Mark only** the food outlet where you eat **most often.**

- ☐ Street vendors
- ☐ Fast food outlets (i.e. KFC, Nando, McDonalds, Debonairs)
- ☐ Supermarkets
- ☐ Restaurants
- ☐ Other (please specify) …………………………

Next

In this section information is needed on how frequently you eat traditional foods.

Next

Do you eat the traditional food of your cultural/ethnic group?

- ☐ Yes
- ☐ No

Next

Please indicate when you usually eat your group's traditional food.

- ☐ Weekdays
- ☐ When available
- ☐ Weekend days
- ☐ Other (please specify) ……………………….
- ☐ Special occasions only

Next

Generated by Compusense at-hand

How do you feel about the traditional foods of your cultural/ethnic group? Please give your **personal opinion** in 4 to 5 lines.

Next

This section determines how **often/frequently** you eat certain foods and **how much you like** these foods.

Next

How often/frequently do you eat **spinach** and how much do you like **spinach?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **cabbage** and how much you do like **cabbage?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **broccoli** and how much do you like **broccoli?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **brussels sprouts** and how much do you like **brussels sprouts?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **green beans** and how much do you like **green beans?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **peas** and how much do you like **peas?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **butternut/pumpkin** and how much do you like butternut/pumpkin?

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **baby marrow** and how much you do like **baby marrow?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **carrots** and how much do you like **carrots?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **cauliflower** and how much do you like **cauliflower?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **mushrooms** and how much do you like **mushrooms?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **asparagus** and how much do you like **asparagus?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **lettuce** and how much do you like **lettuce?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **tomatoes** and how much do you like **tomatoes?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **green pepper** and how much do you like **green pepper?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **cucumber** and how much do you like **cucumber?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Do you know this plant?

Moroho Thepe

- ☐ Yes
- ☐ No

Next

How often/frequently do you eat **moroho thepe** and how much do you like **moroho thepe?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

Do you know this plant?

Moroho Delele

- ☐ Yes
- ☐ No

Next

How often/frequently do you eat **moroho delele** and how much do you like **moroho delele?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Do you know this plant?

Moroho Lephutsi

- ☐ Yes
- ☐ No

Next

How often/frequently do you eat **moroho lephutsi** and how much do you like **moroho lephutsi?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Do you know this plant?

Black Jack

☐	Yes
☐	No

Next

How often/frequently do you eat **Black Jack** and how much do you like **Black Jack?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

Do you know this plant?

Moroho Lerotho

- ☐ Yes
- ☐ No

Next

How often/frequently do you eat **moroho lerotho** and how much doyou like **moroho lerotho?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **potatoes** and how much do you like **potatoes?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **sweet potatoes** and how much do you like **sweet potatoes?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **beetroot** and how much do you like **beetroot?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **onions** and how much do you like **onions?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **oranges** and how much do you like **oranges?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **naartjies** and how much do you like **naartjies?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **lemons** and how much do you like **lemons?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **grapefruit** and how much do you like **grapefruit?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **yellow peaches** and how much do you like **yellow peaches?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **mangoes** and how much do you like **mangoes?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **paw paw** and how much do you like **paw paw?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **pineapple** and how much do you like **pineapple?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **plums** and how much do you like **plums?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **grapes** and how much do you like **grapes?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **bananas** and how much do you like **bananas?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **apples** and how much do you like **apples?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you eat **pears** and how much do you like **pears?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you eat **litchis** and how much do you like **litchis?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you drink **tea (e.g. Joko, Five Roses)** and how much do you like tea?

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you drink **coffee** and how much do you like **coffee?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you drink **rooibos tea** and how much do you like **rooibos tea?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you drink **mageu** and how much do you like **mageu?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you drink **fruit juices** and how much do you like **fruit juices?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you drink **soft drinks** and how much do you like **soft drinks?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you drink **ginger beer** and how much do you like **ginger beer?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you drink **commercial beer (i.e. Castle, Black Label)** and how much do you like **commercial beer?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you drink **traditional beer (bajala basesotho)** and how much do you like **traditional beer?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

How often/frequently do you drink **grapefruit juice** and how much do you like **grape fruit juice?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Generated by Compusense at-hand

How often/frequently do you drink **milk as a beverage** and how much do you like **milk as a beverage?**

Frequency

Daily	3-4 times per week	1-2 times per week	< 3 times per month	Special Occasions	Never
☐	☐	☐	☐	☐	☐

Preference

Dislike it very much	Dislike it	Neutral	Like it	Like it very much
☐	☐	☐	☐	☐

Next

Please indicate your level of **agreement or disagreement** with each of the following statements regarding food. **Mark** the appropriate number to each statement.

It is important to eat five (5) portions of fruits and vegetables every day.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Fast/junk food is low in vitamins and minerals.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

A controlled energy intake is the best method for weight maintenance and health.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

A variety of different foods should be included in one's daily diet.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Fast foods contain a lot of fat.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Foods high in fat, salt and sugar should be limited in your eating pattern.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Fast food and snacks should be eaten as a treat.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Most traditional foods are tasty.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

I am afraid to eat things that I have never eaten before.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Media (radio, television, posters and magazines) influences my food choice/what I eat.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Junk food is generally convenient (easy) to eat.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

I like to try new foods.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Even when I am busy or have limited time, I try to eat healthy food.

Strongly disagree	Disagree	Agree	Strongly agree
☐	☐	☐	☐

Next

Please type your name, surname and e-mail address. This is necessary to relate the information that you provided with the data collected in the two sessions on Thursday 3 October and Friday 4 October at the University of Pretoria. Note, your identity will be treated as strictly confidential by the research team and will not be linked to the results.

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Appendix 3:
PROP Evaluation Questionaire

Panelist Code: <<Panelist_Code>> Panelist Name: <<Panelist_Name>>

WELCOME to this Tasting Session

SRD SENSORY RESEARCH DIVISION

Your Partner in Sensory Evaluation

DEPARTMENT OF FOOD SCIENCE

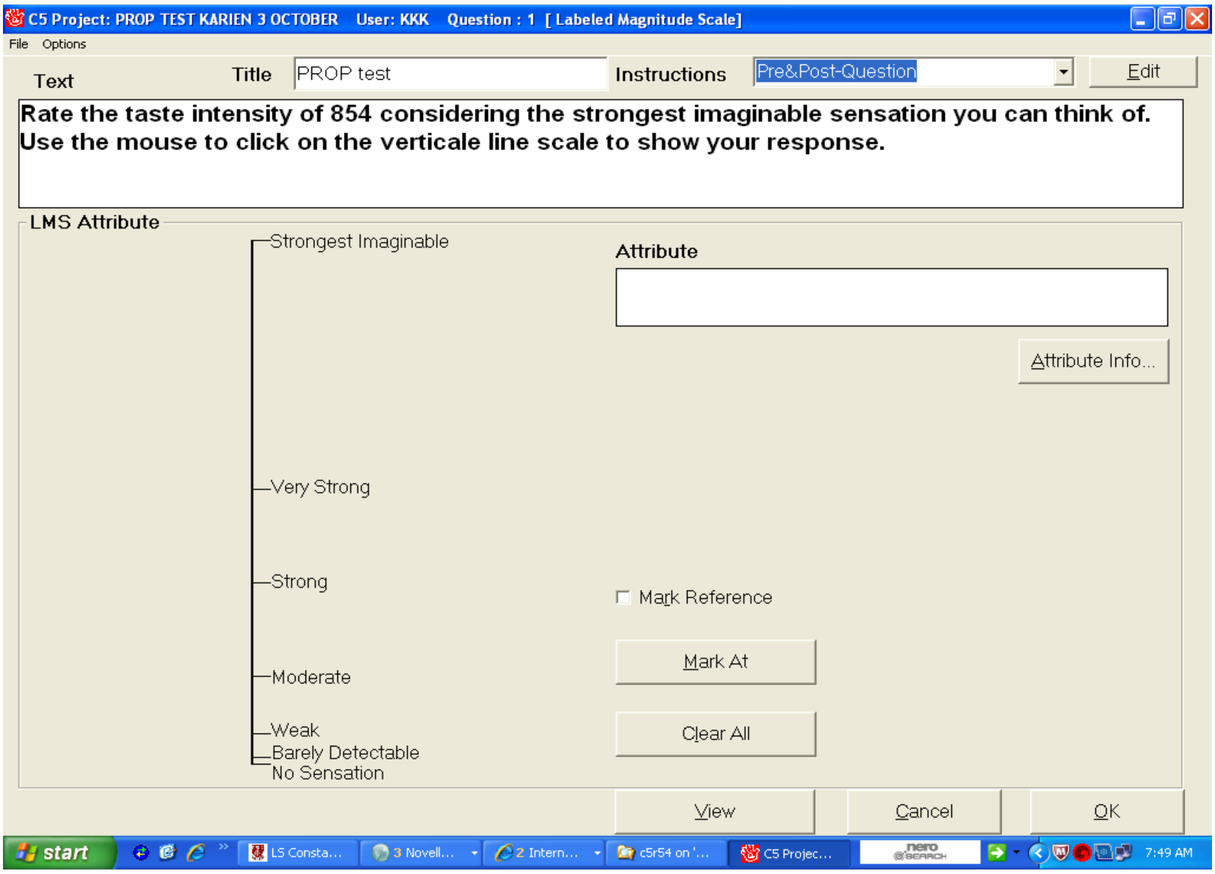
UNIVERSITY OF PRETORIA

To start the test, click on the Continue button below:

You received two filter paper discs in coded plastic bags.

You will be asked to taste the discs one by one and rate the intensity of the sensation that you perceive. The paper discs may or may not have a taste to you.

Please take a sip of water before you start.


C5 Project: PROP TEST KARIEN 3 OCTOBER   User: KKK   Question : 1 [Labeled Magnitude Scale]
File Options
Text
Title
PROP test
Instructions
Pre&Post-Question
Edit
Rate the taste intensity of 854 considering the strongest imaginable sensation you can think of.
Use the mouse to click on the verticale line scale to show your response.
LMS Attribute
Strongest Imaginable
Attribute
Attribute Info...
Very Strong
Strong
Mark Reference
Mark At
Moderate
Clear All
Weak
Barely Detectable
No Sensation
View
Cancel
OK
start
L5 Consta...
3 Novell...
2 Intern...
c5r54 on '...
C5 Projec...
7:49 AM

THANK YOU!

Appendix 4:
Consumer Evaluation and DNA (Saliva) Questionnaire

Panelist Code: ____________________ Panelist Name: ____________________

WELCOME to this Tasting Session

SRD
SENSORY RESEARCH DIVISION
Your Partner in Sensory Evaluation

DEPARTMENT OF FOOD SCIENCE
UNIVERSITY OF PRETORIA

To start the test, click on the Continue button below:

Good morning and welcome back.

Today you will be asked to do 2 things.

1. Salva collection

You will receive a plastic tube. You will be asked to spit into the tube until the amount of liquid saliva (not bubbles) reaches the fill line on the tube.

2. Fruit beverages

You will also be asked to taste and rate how much you like or dislike small portions of different fruit beverages.

You received a plastic tube.

Panelist Code: ______________________ Panelist Name: ______________________

You will be asked to spit into the tube until the amount of liquid saliva (not bubbles) reaches the fill line on the tube.

It may take some time to produce enough saliva to reach the fill line, please relax.

When done, please call the assistant to take the tube.

Do not put it down or close the lid.

Please take a sip of water and eat a piece of cream cracker.

Question # 2 - Sample <<Sample1>>

Please look at beverage <<Sample1>>, how much do you like or dislike the colour of beverage <<Sample1>>?
Use the mouse to click on the horizontal line scale to show your response.

Hedonic rating
GREATEST IMAGINABLE DISLIKE GREATEST IMAGINABLE LIKE

Question # 3 - Sample <<Sample1>>

<u>Before you taste</u>, please smell beverage <<Sample1>>, how much do you like or dislike the <u>smell/aroma</u> of beverage <<Sample1>>?
Use the mouse to click on the horizontal line scale to show your response.

Hedonic rating
GREATEST IMAGINABLE DISLIKE GREATEST IMAGINABLE LIKE

Question # 4 - Sample <<Sample1>>

Please drink beverage <<Sample1>>, how much do you like or dislike the <u>taste/flavour</u> of beverage <<Sample1>>?
Use the mouse to click on the horizontal line scale to show your response.

Hedonic rating
GREATEST IMAGINABLE DISLIKE GREATEST IMAGINABLE LIKE

Question # 5 - Sample <<Sample1>>

Did the colour match the taste of the beverage?

- ❍ Yes
- ❍ No

Please take a sip of water and eat a piece of cream cracker.

THANK YOU!

Appendix 5:
Publications

Thesis data was published in the following formats

1. Publications

a. Gous, A.G.S., Almi, V.L., Coetzee, V., De Kock, H.L. 2018. Effects of Varying the Color, Aroma, Bitter, and Sweet Levels of a Grapefruit-Like Model Beverage on the Sensory Properties and Liking of the Consumer. *Nutrients* 11, 2, 464. https://doi.org/10.3390/nu11020464.

2. Conferences

a. Gous, A.G.S., Kotze, K., Onyeoziri, I., Coetzee, V., De Kock, H.L. 2014. The effect of genetic variation in taste perception on consumer acceptance of green leafy vegetables and grapefruit. SenseAsia Asian Sensory and Consumer Research Symposium, 11-13 May 2014, SingEx, Singapore.

b. Gous, A.G.S., Almi, V.L., Coetzee, V., De Kock, H.L. 2015. Cross modal interaction of sensory properties of grapefruit-like beverages for optimization of consumer liking. 11th Pangborn Sensory Science Symposium, 23-27 August 2015, Gothenburg, Sweden.

c. Gous, A.G.S., Almi, V.L., Coetzee, V., De Kock, H.L. 2015. Cross modal interaction of sensory properties of grapefruit-like beverages for optimization of consumer liking. SAAFoST Congress, 6-9 September 2015, Tsogo Sun Elangeni, Maharani Complex, Durban, South Africa.

d. Gous, A.G.S., Almi, V.L., Coetzee, V., De Kock, H.L. 2015. Cross modal interaction of sensory properties of grapefruit-like beverages for consumer optimization of consumer liking. AfroSense, 23-26 November 2015, STIAS Conference Centre, Stellenbosch, South Africa.