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The Effect of Fasting on the Interaction between Taste Perception and Metabolic Regulation

A thesis presented in partial fulfilment of the requirements for the degree of Master of Food Technology at Massey University

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ABSTRACT

Taste perception, via reception of tastants and endocrine signalling within the tongue, plays a key role in consumer acceptance and sensory evaluation of foods. Taste perception triggers hormones that are crucial in the control energy balance and appetite exerts a strong effect on food intake, satiety and metabolic regulation. Due to the complex interaction of genetic, biological and psychological factors, the influence of fasting on the relationship between taste perception and associated metabolic parameters remains to be explored.

The present study investigated the effect of fasting on interaction between taste perception and metabolic regulation through three main objectives. The first objective was to explore the relationship between the bitter taste sensitivity and the fatty acid taste sensitivity. Forty healthy male adults were classified into three taster groups based on their sensitivity to bitter agent 6-N-2-propylthiouracil (PROP): nontasters (n=10), medium tasters (n=20) and supertasters (n=10). The groups were also confirmed with fungiform papillae densities. However, no significant correlation was observed between PROP status and fungiform papillae densities. Also, results showed neither PROP status nor the fungiform papillae density associated with fatty acid thresholds.

The second objective was to investigate the effect of overnight fasting or meal consumption on sweet and fatty acid taste perception. Detection thresholds for sucrose and linoleic acid were measured by using ASTM method during fasted and satiated state. The result showed increases in sucrose detection thresholds under the both fasted state and satiated state. The linoleic acid thresholds increased after meal consumption and reduced after prolonged fasting.

This led to a further investigation on the last objective- the role of key plasma metabolites on fatty acid taste perception in fasting and satiated states. The results indicated that neither the effect of metabolic status on fatty acids thresholds nor relationships between fatty acid thresholds and blood metabolic parameters were observed. Furthermore, there was no significant difference in blood metabolites across

PROP taster group, which means that PROP classification cannot be considered as a predictor to the blood metabolites.

In conclusion, the present study provides evidence suggesting that PROP sensitivity cannot predict fatty acid taste sensitivity and metabolic status has no effect on fat taste perception. In addition, blood metabolites do not show any difference among PROP taster group and any relationship with taste perception either.

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LIST OF ABBREVIATIONS

3-AFC	3-alternative forced choice
ANOVA	Repeated-measure analysis of variance
APC	Aerobic plate count
ASTM	American Society for Testing and Materials
B trial	Breakfast being provided trial
B1	The session before breakfast in B trial
B2	The session after breakfast in B trial
BMI	Body mass index
CCK	Cholecystokinin
CD36	Cluster of differentiation 36
CGRP	Calcitonin-gene related peptide
DRK	Delayed rectifying potassium channels
EDTA	Ethylenediaminetetraacetic acid
FPG	Fasting plasma glucose
GC	Gas chromatography
GLP-1	Glucagon-like peptide-1
GPCR	G protein-coupled receptors
HDL-C	High density lipoprotein cholesterol
LA	Linoleic acid
LMS	Labeled magnitude scale
MT	Medium- taster
NB trial	No breakfast trial
NB1	the session before break in NB trial
NB2	the session after break in NB trial
NEFA	Non-esterified fatty acid
NT	Non-taster
PPG	Postprandial plasma glucose
PROP	6-N-2- propylthiouracil
PTC	Phenylthiocarbamide
PYY	Peptide YY
SEM	Standard error of mean

ST	Super-taster
TC	Total Cholesterol
TG	Triglyceride

1 Introduction

It is widely accepted that the human tongue can detect 5 basic tastes (i.e. sweet, bitter, sour, salty and umami). However, over the last 20 years, accumulating evidence suggests that the list should include a new taste perception for fat or more specifically, fatty acids.

Fatty acid taste perception remains controversial due to the difficulty of isolating the fatty acid taste perception from other senses, such as mouth coating, texture and aroma. Psychophysical studies support that humans are able to detect the fatty acid taste in the oral cavity even when non-gustatory cues are minimized, suggesting a true 'taste' component to the fatty acid taste perception (Chale-Rush *et al*., 2007; Mattes, 2008 and 2009; Stewart *et al*., 2011). Labban *et al*. (2005) and Mattes (2005) report a relationship between fatty acid taste and other oral sensations, such as salty, sour and creamy taste. Some researchers believe that the bitterness Phenylthiocarbamide (PTC) and 6-N-2- propylthiouracil (PROP) sensitive phenotype is strongly associated with fat taste perception and PROP supertasters have slightly but not significantly lower body mass index (BMI) than medium tasters or nontasters (Tepper and Nurse, 1998; Tepper, 1998; Stewart *et al*., 2007). Individual differences in taste intensity are, in part, genetically determined and these affect food preferences and consumption. The taste perception informs the body about the quality of ingested foods, and controls food and energy intake by triggering hormones that are crucial in the control of energy balance and appetite. Whether the fatty acid taste perception relates to fasting, body phenotype and/or blood metabolites, such as glucose, lipid profile and free fatty acid, remains to be determined. Moreover, positive relationships have been observed between the ability to detect oral fatty acids with habitual dietary intakes and body phenotype, including gender, age and BMI (Stewart *et al*., 2007). However, these results are also challenged by other studies suggesting no link between fatty acid taste and bitterness sensitivity (Drewnowski *et al*., 1998; kamphuis *et al*., 2001). Yackinous and Guinard (2000) reported there was no relationship between PROP taster status and the perception of fat. Furthermore, even though PROP supertasters clearly have higher taste papillae density, they did not perceive other taste stimuli stronger than the PROP nontasters or medium tasters. Whether individuals are sensitive to the fatty acid taste perception on the basis of being

relatively insensitive to bitterness or whether the fatty acid taste perception is influenced by physiological characteristic, such as body phenotype, remain unanswered.

The major objectives of the research were:

1) to investigate the relationship between the bitter (PROP) taste sensitivity and the fatty acid taste sensitivity.

2) to explore the effect of overnight fasting or a meal consumption on sweet and fatty acid taste perception.

3) to investigate the relationship between body phenotype and fatty acid taste perception – to determine the role of key plasma metabolites on fatty acid taste perception in fasting and postprandial states.

The main hypotheses of the research were:

1. Fatty acid taste perception is influenced by body phenotype, having a positive association with PROP status.
2. Altered metabolic marker concentrations can be indicators of fatty acid taste sensitivity.
3. The influence of overnight fasting on concentrations of blood metabolites is associated with changes in oral sensory sensitivities (i.e. sweet, bitter and fatty acid taste).

To test these hypotheses, a randomised, single-blind, cross-over study was conducted with 40 healthy male subjects (18-50 year olds). Sensory trials were performed using sweet and fatty acid solutions at two different sessions in an overnight fasted state. Two main trials included: a “breakfast trial” and a “no-breakfast (fasting) trial” to study the effect of a meal on taste perception. Blood samples and anthropometric measurements were obtained. Analysis of sensory data and measurement of metabolic markers of carbohydrate and lipid metabolism were performed to define their relationship with taste perception.

2 Literature review

2.1 Fat perception

2.1.1 Dietary fat

To understand the orosensory properties and metabolic influences of dietary fat, it is helpful to know about fatty acids (FAs) and dietary fats.

Chemically, food lipids are generally referred to as fats (solid) and oils (liquid), containing a variety of fatty acid compositions as shown in Table 1. Most vegetable oils, especially those from oilseeds, are highly unsaturated and most animal products are rich in saturated fatty acids.

Dietary fat is the fat consumed in plant and animal products mainly consisting of triglycerides (TG). TG is an ester derived from glycerol and three fatty acids. The three fatty acids are attached to a glycerol molecule and differ in different triglycerides (Figure 1). When they are not attached to other molecules, they are known as "free" fatty acids.

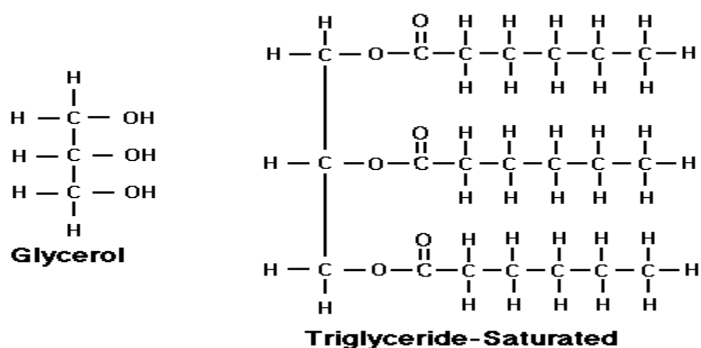


Figure 1 Chemical structure of glycerol and saturated triglycerides

Fatty acids contain a number of carbons in a straight chain from 4 to 28. Some have double bonds and are known as unsaturated and some without double bonds are known as saturated (Figure 2). The length of the hydrocarbon chains and the type of bond determine the physical properties of TG.

Linoleic acid (LA) is an unsaturated n-6 fatty acid with an 18-carbon chain and two *cis* double bonds; the first double bond being located at the sixth carbon from the methyl end. It belongs to one of the two families of essential fatty acids that cannot be synthesized from other food components. It is abundant in many vegetable oils, comprising over half (by weight) of safflower, sunflower, and corn oils (Table 1). LA has been chosen in the present study not only because of its importance for human health but also because it has been used successfully to determine fatty taste thresholds, showing its gustatory response in humans and animal studies (Grosch and Laskawy, 1984).

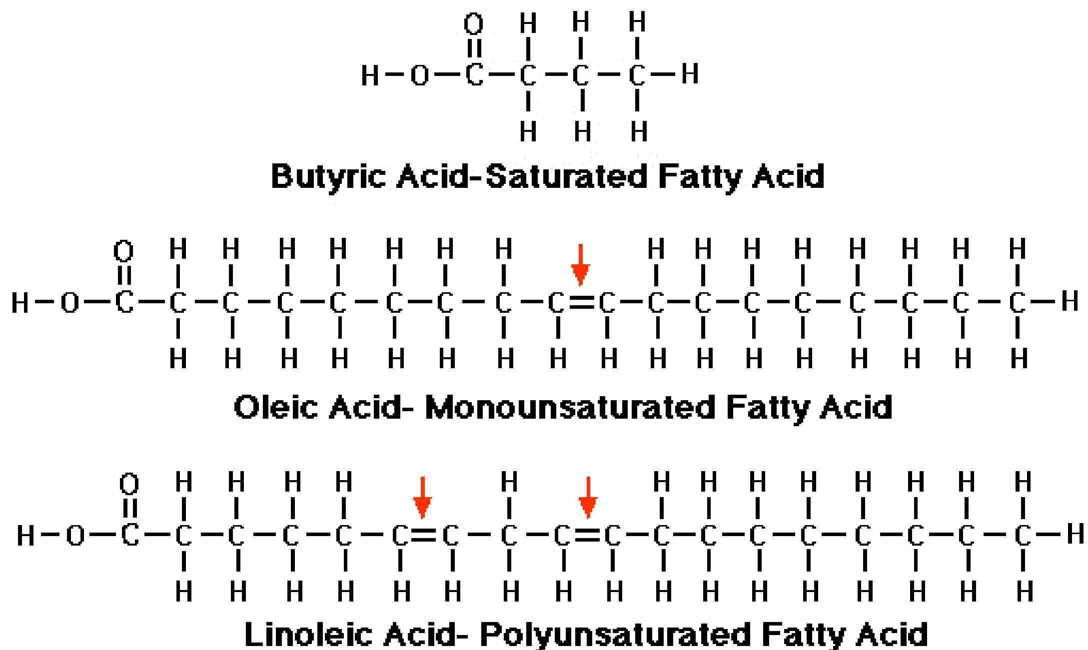


Figure 2 Chemical structures of fatty acids

Table 1 Fatty acid composition (w %) of some common edible fats and oils

	Saturated					Mono unsaturated	Poly unsaturated
Oil or Fat	**Capric Acid C10:0**	**Lauric Acid C12:0**	**Myristic Acid C14:0**	**Palmitic Acid C16:0**	**Stearic Acid C18:0**	**Oleic Acid C18:1**	**Linoleic Acid (ω6) C18:2**
Almond Oil	-	-	-	7	2	69	17
Butterfat (cow)	3	3	11	27	12	29	2
Canola Oil	-	-	-	4	2	62	22
Cocoa Butter	-	-	-	25	38	32	3
Coconut Oil	6	47	18	9	3	6	2
Corn Oil	-	-	-	11	2	28	58
Flaxseed Oil	-	-	-	3	7	21	16
Grape seed Oil	-	-	-	8	4	15	73
Lard (Pork fat)	-	-	2	26	14	44	10
Olive Oil	-	-	-	13	3	71	10
Palm Oil	-	-	1	45	4	40	10
Peanut Oil	-	-	-	11	2	48	32
Safflower Oil*	-	-	-	7	2	13	78
Sesame Oil	-	-	-	9	4	41	45
Soybean Oil	-	-	-	11	4	24	54
Sunflower Oil*	-	-	-	7	5	19	68
Walnut Oil	-	-	-	11	5	28	51

Unsaturated fatty acids (USFA) are easily oxidized and the oxidised products are believed to impart taste and flavours described as bitter, sour, astringent or soapy. This may influence the discrimination of oral sensitivity (Forss, 1972).

2.1.2 How is fat detected?

Compared to bitter and sweet taste perception, fat perception is more difficult to study due to its complicated stimulating process involving gustatory, textural and olfactory cues and various physical forms (e.g. liquids, solids, semi-solids).

2.1.2.1 Texture

It is widely accepted that oral texture is the main attribute for dietary fat taste perception. Perception of the creamy texture of oil-in-water emulsions such as milks and creams as well as the texture of oil is related to viscosity, lubricity, cohesiveness and mouth coating properties. These characteristics have been always quantified by

laboratory measurements and correlated with people's sensory ratings (Drewnowski and Greenwood, 1983; Duffy *et al*., 1996; Weenen *et al*., 2005).

It is known that fat content of a food is able to be rated by manually tactile stimulation or oral stimulation. In early 1987, an oiliness judgement study conducted by Mela *et al*. (1987) suggested that when the samples are presented orally, and when the samples are rubbed between two fingers, only textual properties can be perceived.

In order to develop a sensory profile of milk with varying fat content, a free-choice profiling study was conducted and found that the differences between samples are mostly described with textural terms relating to viscosity. This may be because terms for the olfactory and taste components of fats were not familiar to subjects (Kirkmeyer and Tepper, 2003). In another related study, 'thickness' was the only significant adjective rating discriminated among dairy products, although other attributes, such as cohesiveness and lubricity, were important for some subjects. Using nose clips to prevent olfactory cues and an opaque cup to prevent visual cues did not change the ratings of thickness.

It has been reported that 0.5% to 36% of fat content in fluid dairy products are perceived through viscosity while other properties play a smaller role in the taste perception (Mela, 1988). However, some other data suggest that lubricity could be another relevant tactile attribute besides viscosity (Ramirez, 1994; Verhagen *et al*., 2003). Ramirez (1994) found in an animal study that lubricity is more important than viscosity in discriminating triglyceride oil, silicone oil and mineral oil. It remains unclear how the fat globules in a fatty food contribute to the fat perception.

Also, texture is not the only attribute accounted for fat detection. For example, increase in temperature affects the texture of fats but has no effect on perceived fat content. Mela *et al*. (1994a) found that oral food temperature changing from 20 C ° to 36° C had no effect on fat ratings in oil-in-water emulsions. This finding is interesting because in the food industry, melting and the relating phenomena, such as degree of saturation, are considered to cause variations in fat perception.

2.1.2.2 Olfaction

Studies in mice and rats have provided evidence for olfactory contribution to fat taste perception, indicating odour cues play a role in discrimination amongst different types of fats. Furthermore, the olfactory input is able to reduce the high fat food preference in mice (Kinney *et al*., 1996).

Previous studies have provided conflicting evidence for olfactory contribution to human fat discrimination. In some studies, blocking the olfactory inputs did not alter performance on fat detection (Mela and Christensen, 1987), whereas in other studies the detection thresholds were found to be higher without the olfactory cues (Schiffman *et al*., 1998).

Schiffman *et al*. (1998) has revealed an important role of olfactory cues in taste perception by showing impaired taste detection to fatty acid solutions after elimination of olfactory inputs. However, in another study, elimination of odour cues had no effect on detection thresholds for medium-chain TG, soybean oil, and mineral oil. Furthermore, no difference was found in the fat content, either with or without olfactory inputs (Mela and Christensen, 1988).

Furthermore, the route of olfactory stimulation may be important for odour discrimination. In a laboratory olfactory test, odours are usually presented orthonasally, whereas in taste test, the primary source of odours is retronasal. Therefore, odour perception may vary by these different routes (Halpern, 2000). Additionally, chemistry reactions, for example fat oxidation, can also affect odour perception. It is known that natural oils have pleasant odours when they are still fresh but the rancid oils generate unpleasant odours. It is the decomposition productions of TGs rather than the TGs themselves, which is the main source of the odour.

2.1.2.3 Taste

Many animal studies have reported the fat taste perception with dietary fat intake based on oral detection of free fatty acids (Gilbertson *et al*., 1997 and 1998). Polyunsaturated fatty acids are able to inhibit delayed rectifying potassium channels (DRK+) at taste cells in rats. This inhibition is greater in rats preferring carbohydrates

than those preferring fats, showing an ingestion-inhibition effect of free fatty acids (Gilbertson *et al*., 1998). However, there is no evidence showing the similar ingestion-inhibition signal by free fatty acids observed in humans. Also based on animal models, some reports have revealed a potential fatty acid taste receptor called Cluster of Differentiation 36 (CD36). The receptor was found in mice, and may exist in humans and impact on food preference (Abumrad, 2005; Gaillard *et al*., 2008). Fukuwatari (2003) hypothesized that the fatty acid taste receptor CD36 is localized in lingual taste buds and participates in the oral recognition of fat. Laugerette (2005) and Pepino *et al*. (2011) have reported that CD36 is found in human circumvallate taste buds and it is also co-localized with gustducin, a taste signalling protein related to basic taste perception. These findings may provide evidence revealing the relationships between fat taste perception and basic taste perception.

Individual differences in taste intensity are genetically determined and have an effect on food preferences and consumption (Drewnowski *et al*., 1997; Glanz *et al*., 1998). Several studies have correlated the genetic ability to taste the bitter compound 6-N-2-propylthiouracil (PROP) with fatty food preference (Bartoshuk *et al*., 1994; Tepper and Nurse, 1997 and 1998). According to Tepper (1998), healthy subjects were classified as nontasters (NTs), medium tasters (MTs), or supertasters (STs) of PROP and evaluated fat content and liking of salad dressings with high fat content (40% fat) and low fat content (10% fat). The result showed that the NTs chose the high-fat salad dressing in preference to the low-fat dressing, while MTs and STs liked the low-fat dressing. It was possible that MTs and STs were highly sensitive to fat content and considered the high-fat dressing to be too oily, resulting in their lack of preference. However, this study did not explain what factors drove the NTs' liking for the salad dressing high in fat.

Conflicting relationships between PROP taster ability and fat taste perception have been found to date. Two studies by Drewnowski (1998) and Guinard (1999) have failed to find a correlation between PROP taste sensitivity and fat perception. Kissileff *et al*. (2001) found that PROP tasters, who were more sensitive to oral taste stimuli than NTs (Bartoshuk *et al*., 1994), could discriminate the free fatty acids content in a high-fat food. In addition, some studies suggested that fat sensitivity is

more likely to be associated with increased fungiform papillae density (Lim *et al*., 2008; Yackinous and Guinard, 2000).

Yackinous and Guinard (2000) did not find any relationship between PROP taster status and the perception of fat. Even though STs had higher taste papillae densities than that of the NTs or MTs, no significant difference in taste perception was found. However, the significant difference between PROP taste groups was found in tactile sensitivity, and Yackinous and Guinard (2000) proposed a 'general sensory ability', including tastes, irritants and tactile perception.

Mattes (2007) documented that fatty acids promoted the perceptions to other taste compounds such as sodium chloride (NaCl), citric acid and caffeine but not to sucrose. Eight samples were assessed in duplicate by using an ascending, three-alternative forced choice (3-AFC) procedure. The samples were separated into two groups. One was made by adding linoleic acid to the solutions with different tastants (NaCl, sucrose, citric acid and caffeine). The other group consisted of the solutions with added tastants alone. The data showed recognition thresholds were significantly higher for the citric acid and caffeine solutions with added fatty acids in comparison with no fatty acids samples. The recognition thresholds for sweet, sour, and salty solutions with fatty acids were reduced or unchanged. According to his previous observation suggesting that fatty acids can be detected alone in humans (Mattes, 2005), it is possible that fatty acids could act as primary stimuli rather than general modulators of taste cells.

However, there were limitations to the study by Mattes (2005). Firstly, only four prototypical tastants were used and they were not fully representative of all the taste stimuli presented in our daily life. Secondly, the results were not consistent with the findings on the enhancement effect with saccharin reported by Gilbertson (2005). This may be due to Gilbertson using saccharin in animal model and Mattes (2005) using sucrose in humans. There may be different transduction pathways for nutritive and non-nutritive sweet agents and different fatty acids sensing mechanisms between humans and animals.

Stewart *et al*. (2007) investigated the significance of oral fat sensitivity and its putative influence on fat intake, fat perception and BMI in human subjects. A group of subjects were classified as hypo- or hypersensitive tasters by their oral fatty acid sensitivity to oleic acid, which is different from the commonly used PROP status classification. Subjects were screened, and triplicate triangle tests were used in which they were presented with three samples per set, consisting of two control samples and one fatty acid sample. Individuals who successfully identified the fatty acid sample three times correct in a row were classified as hypersensitive subjects and those who failed were classified as hyposensitive subjects.

Their results agreed with the work done by Mattes (2005) which showed that fatty acids can be sensed over a range of concentrations by human subjects. However, Stewart *et al*. (2007) has also failed to find the relationship between oral fatty acids sensitivity and prototypical tastants. The negative relationship between fat sensitivity and energy consumption and BMI suggested that oral sensitivity to fatty acids may influence habitual fat consumption and be associated with the perception of fat in foods and could affect body weight (Stewart *et al*., 2007).

LA has a taste threshold of 1–2 mg/ml (Grosch and Laskawy, 1984), which is similar to the free fatty acid content (3–6 mg/g fat) of dairy products (Lin *et al*., 1995). Hence, it seems likely that humans are able to perceive fatty acids even in the mixed fat foods and describe them as bitter-burning taste, sour taste or somewhat warming taste (Schiffman and Dackis, 1975; Grosch and Laskawy, 1984). Therefore, when fatty acids exist (low concentration) in foods, they could contribute to the reported ‘‘fatty taste’’ (Schiffman *et al*., 1998).

2.2 Methods for testing taste threshold

In 1927, Thurstone developed a theory named Thurstone's Law of Categorical Judgement. In his study, he proposed a model that illustrated and explained variations in individual's response even to the same stimulus. This decision making model shows that when an individual receives a specific stimulus concentration, a so-called 'discriminal process' is elicited with a consequent response behaviour. The response of a subject varies at the same concentration every time it is presented. As a result of

environmental or mental differences, the same subject could go through various discriminal processes, leading to the varying responses for that same stimulus concentration. The responses are shown in a certain range, called discriminal dispersion. If the range is normally distributed, the distribution represents the probability of responses to different stimulus concentrations. It means when two stimuli with different concentrations are presented, the intensity perceived are different and represented by two normal distributions (Thurstone, 1927).

This decision making procedure is also applied in a signal detection theory (Green and Swets, 1966). Brown (1974) proposes an R-index by using a series of rating and ranking tasks based on the signal detection theory. In the rating task, a subject is presented with a sample and asked to identify the sample as a signal or a noise. The response is determined by selecting one from a group of categories, including signal sure, signal unsure, noise sure, and noise unsure. The ranking test is similar to the rating test, where the subject is asked to rank samples according to signal strength (Brown, 1974).

The signal detection rating method (R-index) has been used to determine taste thresholds for sucrose (McFadden *et al.*, 1971) and NaCl (O'Mahony, 1972). The results of these two studies are reportedly comparable to thresholds reported in past studies but little research has directly compared threshold values obtained from different methods. In addition to the R-index method, American Society of Testing and Materials (ASTM) method of ascending limits has been widely used in many other studies.

As the R-index method was applied in salt and sweet threshold determination, Robinson *et al*. (2005) compared bitter taste thresholds by using the R-index method and the ASTM method of ascending limits test. They found that only 9 out of 32 subjects successfully found differences in the ascending limits test while there were 11 out of 32 subjects in the R-index test. It means that a smaller percentage of subjects found differences at or below certain caffeine concentrations when using the ascending limits test (28%) compared to the R-index test (34%). The thresholds compared between the two methods were detection thresholds and the R-index method generated lower mean threshold value than the mean threshold value

determined by the method of limits. However, the differences between thresholds from the two methods could be affected by the sample size. In the R-index rating method, a subject is presented with five replicates of the noise and the signal for each stimulus and a subject received five replicates of the seven caffeine samples with different concentrations in the method of limits. Therefore, a subject received 105 samples in the method of limits, which was more than the samples (40 samples) presented in the R-index test. Therefore, the increased occurrence of stimulus may produce a higher, less sensitive threshold value, which is consistent with previous findings (Pangborn, 1959).

2.3 Stopping rules of ASTM method

In the early days, the method of limits was widely used for measuring thresholds to get around several problems, such as fatigue of sensory adaption caused by the descending series or the error of anticipation determination for taste and smells recently. The difficulty of the test is that the resulting threshold is influenced in part by the sample size, including the number of target and the blank stimulus, which increases the difficulty in discrimination resulting in a higher estimate threshold.

The ascending series of 3-alternative forced choice (3-AFC) test given in the ASTM procedures E-769 is a typical example of such a procedure. The 3-AFC procedure is derived from triangle test developed by Dravieks *et al*. (1970) with a wide application in flavour science. In the method, the subject is required to choose the one target stimulus containing the taste or smell of interest from a trial containing two blank or diluent samples. In some circumstances, a warm-up example may be given at a suprathreshold concentration to provide familiarity with the taste. However, this sort of warm-up examples is cancelled to avoid adaption and fatigue effects (Lawless and Heymann, 1999).

The ASTM method E-679 sets up two elimination rules for the method cited as the following: "the judgements are completed when the panellist either (1) completes the evaluation of all sets of the scale, or (2) reaches a set wherein the test sample is

correctly identified, then continues to choose correctly in higher concentration test sample sets."

The first elimination rule specifies a very specific probability that it is possible for some NTs to miss all the targets of the whole scale. As they miss the last target at the highest concentration, the threshold is obtained based on the geometric mean of the highest concentration and the next theoretical higher concentration calculated by the constant sample interval. Similarly, subjects who correctly indentify all the targets, the threshold is the geometric mean of the first concentration and the previous concentration before that in hypothetical series. Although these two rules are reasonable, there might have effects on thresholds as they are not based on actual data but on some hypothetical concentrations.

The second stopping rule requires a point above which subjects could correctly finish the whole concentration scale. There are two ways to approach it. The first one is that subjects complete every trial until the last one of the scale. The last missed concentration is the stopping point and is calculated by the geometric mean with the next correct concentration. However, there are several shortcomings. Firstly, the possibility that subjects can get correct answers by guessing should be considered (Lawless, 2010). There is a 33% chance that the next correct answer occurs by guessing. In this case, the best-estimate threshold value obtained could indicate a higher sensitivity of a participant to the stimuli. As is known, the 3-AFC procedure combines the triangle test with a discrimination test. It requires the subjects to give proof of detection by discriminating the target stimulus from the background level. However, as the forced-choice element is introduced, there is a remaining issue of how to deal with the chance of guessing the answer correctly. Secondly, as the last missed concentration value is used as the stopping point, it is possible that a subject could miss the target at a higher concentration level after a correct answer at a lower level but the correct answer is discounted because thresholds calculation requires a completely correct series above that concentration. Moreover, a subject who is highly sensitive to stimuli could detect at lower levels in the series, or miss the target at higher levels above his or her threshold due to fatigue or adaptation. Steven *et al.* (1988) noted this phenomenon in 1988. They used ascending forced choice method in

their extensive repeated testing and found that it is common for subjects to 'get the signal' on one trial and temporarily lose it on the next. The second approach is to set up a cut-off point at which subjects identify the target with certainty and then finish the procedure. The geometric mean is obtained base on the correct concentration and the previous missed concentration (Stewart *et al*., 2007; Mattes, 2009a and 2009b).

Mattes (2009a) used an ascending, 3-AFC procedure to measure oral detection threshold for free fatty acids on 3 tongue sites. Stimuli were placed on one side of the tongue and a blank without fatty acids, on the other side. Subjects were asked to indicate which side received the stimulus. The procedure continued until subjects gave 3 consecutive correct identifications. Mattes also used the same procedure in another study for identifying oral detection for short- medium- and long- chain free fatty acids (Mattes, 2009b). The samples were presented in ascending order and the presentation of the same concentration was presented after a correct identification while a higher concentration sample was given after an incorrect identification. Detection thresholds were defined as the lowest concentrations where three consecutive correct answers occurred. Chale-rush *et al*. (2007) used the same procedure in two of their studies and the procedures stopped when the participant correctly identified the stimulus on 3 successive trials.

The method is also applicable to a recognition threshold. For recognition, the participant is asked to identify the specific flavour in a sample rather than just to identify the odd sample. Robinson *et al*., (2005) measured the detention and the recognition threshold by using the method of limits. The lowest concentration where the subject correctly discriminated the odd sample was determined as the detection threshold while the lowest concentration where the subject must correctly identify the stimulus was determined as the recognition threshold. Therefore, the mean recognition threshold was greater than the mean detection threshold, which is consistent with the previous findings (Lawless and Heymann, 1999).

However, the thresholds obtained from the method of limits are not absolutely definitive and can be affected by many encountered problems, including taste adaptation, habituation or anticipation and fatigue when responding to the large

number of samples (Lawless and Heymann, 1999). The subject could adapt to the stimulus and miss the odd sample even if it became more obvious at higher concentrations. Also, a subject might be biased to follow the same responding pattern as the previous presentation, which is known as habituation. Furthermore, anticipation could occur when subjects realize the change in pattern of stimulus intensity. It is possible that subjects could be fatigued when they received a large group of samples during the method of limits. In addition, physiological effect should be considered. For example, it is possible that an incorrect identification could be obtained by either taste blind or lack of confidence. As a result, the method of limits is best conceived of as a detection task.

2.4 PROP taste status and classification methods

2.4.1 Methods for PROP classification

The bitter compound PTC was used widely in many earlier studies, including Hartmann's ascending (Hartmann, 1939) series method and classic Harris-Kalmus method (Harris and Kalmus, 1949). Lawless (Lawless, 1980) developed a simple screening method involving tasting a suprathreshold (above threshold) of PROP solution, which was used for screening adults and children (Keller *et al*., 1999; Mela, 1990), but only non-taster group and taster group were generated which limits its applications in many studies. Lawless also suggested the substitution of PROP for PTC, considering the sulphurous taste and toxicity issues of PTC. Based on Lawless's study, many modified threshold techniques have been developed. However, these methods are time intensive and involve a lot of samples because suprathreshold intensity can not be predicted by threshold determination alone and the two thresholds are always combined together for PROP intensity ratings. Furthermore, like the Lawless screening method, thresholds methods cannot distinguish MTs from STs.

The method which further divided PROP tasters into MTs and STs was developed by Bartoshuk *et al* (Bartoshuk *et al*., 1994). Considering the lack of criterion of suprathreshold method for dividing MTs and STs, Bartoshuk defined a 'PROP ratio' to separate the two groups. In this method, subjects rate the intensity of five PROP and NaCl concentrations on a magnitude estimation scale. The PROP ratio represents

the relative PROP intensity compared to NaCl across the two highest concentrations. Hence, the PROP ratio is calculated as:

$$\text{PROP ratio} = (PROP_4 / NaCl_4 + PROP_5 / NaCl_5) / 2$$

where $PROP_4$ and $PROP_5$ represent the perceived intensities of the fourth and the fifth PROP concentrations, and $NaCl_4$ and $NaCl_5$ represent the fourth and the fifth matching concentrations of NaCl. Then, Bartoshuk used a PROP ratio of 1.2 as a criterion to distinguish STs from MTs. Drewnowski *et al*. (1998, 1997 and 2000) have used the Bartoshuk method to calculate the PROP ratio, but the cut-off scores varied from 1.6 to 2.5. Although different cut-off scores were used in different studies, it had no effect on the relationship between PROP taster groups. Therefore, STs who are highly sensitive to PROP solutions have given higher intensity ratings to PROP than to NaCl while NTs who are highly insensitive to taste stimuli have shown an opposite pattern in PROP and NaCl intensity ratings. The intensity ratings given by MTs are similar between PROP and NaCl samples (Bartoshuk, 1994; Drewnowski *et al*., 1998, 1997 and 2000; Tepper and Nurse, 1997).

Tepper *et al*. (2001) developed two brief screening methods by modifying the Bartoshuk method (1994) which originally covered five concentrations of PROP and NaCl. The procedure was simplified to three concentrations or to one concentration of each stimulus, named three-solution test and one-solution test, respectively. The two modified tests are reportedly reproducible and as valid as the original Bartoshuk procedure. In the three-solution test, three concentrations of PROP solutions (0.032, 0.32, and 3.2 mmol/l) and three concentrations of NaCl solutions (0.01, 0.1 and 1.0 mol/l) are evaluated, and subjects are categorised into three taster groups by visual comparison of the taste function for PROP and NaCl (Bartoshuk,1994). In the one-solution test, the medium concentration of PROP (0.32 mmol/l) and NaCl (0.1 mmol/l) are presented to subjects and the cut-off score used for group classification are determined and confirmed by calculating the 95% confidence interval around the group means for PROP intensity.

Finally, a screening method using filter paper strips impregnated with PTC/PROP was described many years ago (Blakeslee and Fox, 1932). Although this method could produce classification errors for the lack of a reference standard (e.g. NaCl), the simplicity of paper tests has fostered interest and further investigation into this

technique (DiCarlo and Powers, 1998; Intranuovo and Powers, 1998; Reed *et al.*, 1999; Prescott and Swain-Campbell, 2000). For this reason, a modified filter paper screening test developed by Zhao *et al.* (2003) has become popular in recent years. Subject was presented with filter paper squares impregnated with PROP or NaCl and rated the intensity on a Labelled Magnitude Scale (LMS) (Appendix 1). This paper disk method has used the same one-solution method procedure described above. Compared to the three solutions test, the high degree of association has proven that this paper disk method is reliable and valid. PROP status is also associated with responsiveness to other sensory cues.

2.4.2 The influence of PROP status on food preference and creaminess

Prototypical tastants (Bartoshuk *et al.*, 1998; Prescott *et al.*, 2001; Bajec and Pickering, 2008a; Hayes *et al.*, 2008; Tepper *et al.*, 2009), irritation from ethanol (Bartoshuk *et al.*, 1993 and 1994; Prescott and Swain-Campbell, 2000; Duffy *et al.*, 2004) and the tactile sensation of astringency (Pickering *et al.*, 2006; Bajec and Pickering, 2008a), evidence also indicates that STs perceive more intense retronasal aroma as well as thermal stimuli than NTs and MTs (Bajec and Pickering, 2008a).

It seems likely that the responsiveness of STs to taste stimuli in solutions is similar to the responsiveness to the same taste qualities in food. So that STs perceive bitterness, (Lanier *et al.*, 2004; Sandell and Breslin, 2006; Dineharte *et al.*, 2006; Zhao and Tepper, 2007; Tepper *et al.*, 2009), sourness (Prescott *et al.*, 2004), astringency (Pickering *et al.*, 2004 and 2006), saltiness (Sullivan *et al.*, 2007), sweetness (Duffy *et al.*, 2003; Hayes and Duffy, 2007), and creaminess (Tepper and Nurse, 1997; Hayes and Duffy, 2007) in foods more intensely than MTs and NTs.

It has been reported that complex mixtures have greater potential to increase or decrease specific sensations. For example, in sucrose-fat mixtures, increasing sucrose concentration suppresses fat perception in solids (Drewnowski and Schwartz, 1990; Guinard and Mazzucchelli, 1999). Whereas in liquids, increasing fat content could suppress sweet taste perception (Drewnowski *et al.*, 1987and 1989). Also, the sweet taste perception is suppressed when viscosity and tactile inputs increase (Calvino *et al.*, 1993; Hollowood *et al.*, 2002). A competing taste/tactile theory was used to

explain the suppression, as more sucrose is added, the increased sweet input may first off-set and eventually overtake the suppressive tactile effect. However, this explanation has been challenged by another study reporting that at the same sucrose concentration, no difference was found in sweet intensity ratings between the milk samples and the water-based samples (Hayes and Duffy, 2007).

As creaminess is always considered as 'thickness' and related to tactile perceptions, it has been reported that fungiform papillae density is related to taste and tactile perception (Lim *et al.*, 2008). In addition to taste buds, fungiform papillae also contain a number of trigeminal nerves (Farbman and Mbiene, 1991; Whitehead and Kachele, 1994) which are considered to be responsible for tactile perception. Essick *et al.* (2003) found fungiform papillae number is highly correlated to tactile acuity on the tip of the tongue in women. Findings on PROP bitterness also support this assumption. PROP has been suggested as a predictor of creaminess and the link between them is attributed to the fungiform papillae density, not PROP genotype. For example, Tepper and Nurse (1997) reported that MTs and STs could discriminate different fat content in salad dressings, whereas NTs could not. However, recent studies also indicate that the relationship between PROP bitterness and fat perception is weak and the weak link is detected only in samples with over 35% fat content (Tepper and Nurse, 1997; Hayes and Duffy, 2007).

In addition, Lim *et al.* (2008) found that no perceived taste stimuli intensity, including PROP, was related to the perceived creaminess of the milk. This finding is in agreement with the results of other studies (Drewnowski *et al.*, 1998; Yackinous and Guinard, 2001) and against the suggestion that PROP sensitivity could be used as an indicator of general taste or tactile perception. In addition, Mela (1988) reported that the difference in viscosity resulting from fat content is related to different creamy sensations. Similarly, several studies support the taste contribution of creamy (Tepper and Kuang, 1996; Richardson-Harman *et al.*, 2000; Richardson-Harman *et al.*, 2000; Weenen *et al.*, 2005; Hayes and Duffy, 2007). These studies indicate that creamy detection may not be a tactile perception and it is different from fat perception. Another study suggested subtle differences between 'fat' and 'creamy' by comparing

the difference in slopes and intercepts of two power functions generated from the rating of these two concepts (Drewnowski and Greenwood,1983).

2.5 Body phenotype and blood metabolites

2.5.1 The relations between taste perception and BMI

It is well known that taste perception may influence eating behaviour and hence body mass. There are a number of studies that have related BMI to sweet taste perception (Drewnowski *et al.*, 1985; Tepper and Seldner, 1999; Salbe *et al.*, 2004) and bitter taste perception (Tepper and Ullrich, 2002; Goldstein *et al.*, 2005; Duffy, 2007; Tepper *et al.*, 2008), but little is known about the relationships between the perception of savoury tastes, such as salt and glutamate (umami), and BMI (Donaldson *et al.*, 2009).

A recent study reported that overweight people may not only show less sensitivity to sweet taste but also have greater sweet liking than normal-weight people (Bartoshuk *et al.*, 2006).

However, some other studies have shown conflicting findings that subjects with high BMI perceive sweet foods less pleasant than subjects with low BMI (Felsted *et al.*, 2007). Also, no difference in sweet taste perception across BMI groups was observed by Anderson (1995). The inconsistencies between studies could be attributed to different psychophysical techniques used for sweet taste determination (Bartoshuk *et al.*, 2006). Further, the relationship between BMI and sucrose taste perception have been investigated with fat content in sweet food as the increased weight gain is more likely to occur when diets are high in both fat and sucrose (Drewnowski *et al.*, 1982; Simchen *et al.*, 2006). It has been suggested that compared to sweet perception or preference, fat preference has a greater effect on body mass. For example, obese women preferred foods less sweet but higher in fat (Drewnowski *et al.*, 1985). Therefore, as there is a link between sweet and fat preferences in obese people, it is possible that the greater fat pleasurable response found in obese people could be enhanced when food contains both fat and sugar (Drewnowski, 1983).

Bitter taste perception, especially PROP taste sensitivity, has also been widely studied in relation to body mass. It is well known that STs with greater number of taste buds on the tongue could render bitter foods such as broccoli, brussels sprouts, and grapefruit unpalatable (Logue, 1985). Therefore, it is expected that STs avoid fruit and vegetables in preference for energy-dense fatty and sugary foods, and they may gain weight as a result. However, STs had slightly but not significantly lower body weights than MTs or NTs among male subjects (Tepper and Nurse, 1998). Sitton and Sullivan (2007) reported the same effect for college-aged subjects that STs with the highest numbers of taste buds had the lowest BMI as they consume less food. They also explained this observation by the experience of greater intensity of flavours producing satiety more quickly. If high fat diets produce more rapid feelings of satiety than low-fat diets; this could explain their consumption of fewer calories. On the other hand, according to the study by Tepper and Nurse (1998), the NTs chose the high-fat salad dressing in preference to the low-fat dressing, while MTs and STs preferred to the low-fat salad dressing. Therefore, the increased weight gain in NTs could be explained by long time consumption of high-fat foods due to their greater liking of fat. This relationship was also observed in women. Tepper and Ullrich (2002) found that middle-aged female STs actually had lower BMI than that of the NTs or MTs. Gretchen *et al*. (2005) reported similar results that BMI is higher in NT women than in ST women (BMI 29.7 vs 23.5). They suggested an explanation that in addition to avoiding fruit and vegetables, STs also shunned foods high in fat and sugar. However, this explanation has been challenged by others. The literature on relationships between PROP taster status and BMI is contradictory. Some studies have reported no relation was found between BMI and taster status in women (Kaminski *et al*., 2000; Timpson *et al*., 2005; Yackinous and Guinard, 2002; Drewnowski *et al*., 2007). It is assumed that the difference between studies is a result of factors affecting eating behaviour and BMI, such as dietary restraint (Tepper and Ullrich, 2002).

2.5.2 Taste perception and hormones

There is growing evidence that sweet taste perception can be modulated by hormones or other factors affecting receptors presented in the peripheral gustatory system. The hormone leptin has been proposed to influence sweet taste sensitivity and the leptin receptor has been proposed to suppress sweet taste responses in sweet-sensitive receptor cells (Kawai *et al*., 2000; Shigemura *et al*., 2004; Nakamura *et al*., 2008).

Furthermore, diurnal changes in circulating leptin levels in both rats and humans, have been linked with diurnal patterns of sweet thresholds suggesting that leptin may act as a modulator for sweet taste sensitivity. This diurnal variation in recognition thresholds is sweet-taste selective, including sucrose, glucose and saccharin (Nakamura *et al.*, 2008). A series of animal studies have shown that Glucagon-like peptide-1 (GLP-1), which influences glucose transport, metabolism and homeostasis (Rehfeld, 1998), has an effect on maintaining or enhancing sweet taste sensitivity (Shin *et al.*, 2008). Thus, leptin regulates the consumption and palatability of sweet food in mice by altering peripheral sweet taste sensitivity (Jyotaki *et al.*, 2010).

In addition to sweetness, fatty acids have been recognized as important cell signalling molecules (Mattes, 2009b). They modulate series of physiological processes through binding to G protein-coupled receptors (GPCR). Dietary fat is believed to influence food intake through satiety hormones. When dietary fat is in the mouth, its palpability may promote the desire for eating and when it is presented in the intestines, it may reduce appetite by release of satiety hormones like Cholecystokinin (CCK), PYY (Peptide YY) and GLP-1 alter through altering gustatory coding to terminate food intake (Itoh *et al.*, 2003; Hirasawa *et al.*, 2005; Milligan *et al.*, 2006).

2.5.3 The relationship between oral stimulation and blood metabolites

The blood metabolic parameters may be influenced by oral taste stimulation. Multiple studies have reported that oral exposure to fatty acids alters postprandial lipid concentrations (Mattes, 2001, 2002 and 2010; Robertson *et al.*, 2003; Chavez-Jauregui *et al.*, 2010; Heath *et al.*, 2004). Tactile and olfactory cues may contribute to dietary fat taste, but they are not able to trigger changes of TG. An olfactory stimulation study found that olfaction alone is not effective to change TG concentrations in blood by comparing differences in serum TG concentration under the condition of including or excluding olfactory inputs (Mattes, 2001). No effect of carbohydrate as an effective oral stimulus on circulating TG concentrations has been found while fat has been reported to associate with increased circulating TG (Mattes, 1996, 2001 and 2002; Crystal *et al.*, 2006). Various fat substitutes giving the same textural properties, like mouth-feel, did not elicit the serum TG concentration (Mattes, 2001). The plasma TG response is greater in full-fat foods rather than fat-free foods

(Chavez-Jauregui *et al*., 2010; Mattes, 1996; Mattes, 2009b and 2010). Whether fatty acids are adequate stimuli to increase TG or not still remains unclear in humans. Limited studies have investigated the relationship between them, suggesting that monounsaturated fatty acids (i.e. oleic acid) may be less effective than polyunsaturated free fatty acids (i.e. linoleic acid) as oral stimuli (Tittelbach and Mattes, 2001; Crystal *et al*.,2006). The influence of subject characteristics, such as age, sex, PROP taster status and BMI, on TG responses to oral fat exposure have been explored (Mattes, 2009). The results have shown that PROP status and gender have no significant effects on TG response and BMI is related to the TG peak concentration after oral fat stimulation.

Robertson *et al*. (2002) used a modified sham feeding study to investigate the effect of oral stimulation 1h before lipid ingestion on the concentration of postprandial TG. After an oral fat stimulation, serum TG concentrations remained elevated over baseline for 5–8 h (Mattes, 1996, 2001, 2009b and 2010; Heath *et al*., 2004). Thus, suggesting that the oral exposure to fat in our daily life is a long lasting effect. In the present study, linoleic acid was used as taste stimuli and mineral oil was used as a mask agent. Although the concentrations of them were low, it is uncertain if either or both of them are effective to trigger the TG rise.

2.5.4 The effect of metabolic status on blood metabolites

As the blood metabolites (e.g. glucose, TG, and TC) concentration flux could be considered as a quality control check, the effect of metabolic status should be considered. It is known that some blood metabolic parameters, especially plasma glucose, have remarkable fluctuation corresponding to fasting or satiated state.

In healthy individuals, the postprandial blood glucose concentration is normally within the range of 4–5.5 mmol/l, but arterial concentrations vary from about 3.5 mmol/l after exercise to 9 mmol/l after a meal (Casey, 2003). Postprandial plasma glucose (PPG) levels peak around 1 hour after a meal and return to basal levels within 2 to 3 hours (American Diabetes Association, 2001). PPG is affected by a series of

hormones through the opposing actions. The relative concentration of each hormone in the blood at a given time determines their net effects. Some (e.g. insulin) encourage the clearance of glucose from the plasma, while others, (e.g. glucagon, growth hormone) stimulate the production and release of glucose into the blood. In addition, some other factors also affect PPG concentration, including physical activity, and meal composition; for instance, ingestion of carbohydrates contributes substantially more to PPG than proteins or fats (Buse, 2003). Further, the magnitude of PPG excursion might be predicted by fasting plasma glucose (FPG) levels (i.e., the higher the FPG level, the higher the PPG level) and low FPG was associated with low BMI and low cholesterol (TC) (Wei *et al.*, 2000).

It is known that fasting represents the opposite extreme of overeating. Under the satiated condition, the oxidation of endogenous fat stores is able to meet the body energy requirements (Owen *et al.*, 1998). However, considering the satiated state after a typical overnight fast, the energy supply process is rapidly moved from predominantly fat oxidation to carbohydrate oxidation (Flatt *et al.*, 1985). Furthermore, as prolonged fasting represents an even greater perturbation in energy status, the oxidation of endogenous fat stores is with an even greater dependence due to the almost complete utilization of glycogen storage during extended fasting (Owen *et al.*, 1998). It is interesting that ingestion of glucose after prolonged fasting leads to a decreased carbohydrate oxidation. Therefore, corresponding to the different energy supplying process, many circulating parameters are significantly elevated after a prolonged fast, including NEFA, glycerol, and ketone (Wolfe *et al.*, 1987; Owen *et al.*, 1998).

2.5.5 The influence of diet composition on metabolic responses

Meal consumption has significant effect on certain blood metabolite concentrations but the metabolic responses differ with diet composition. As a standard breakfast is offered in the present study comprising of carbohydrate and dietary fat, their effects on blood results should be considered.

It is known that diet composition has an effect on the production and clearance of lipoproteins as well as remnants derived lipoprotein (Cohen *et al*., 1988; Dubois *et al*., 1994 and 1998; Zampelas *et al*., 1994). For example, a diet low in fat and high in carbohydrate was reportedly associated with fasting hypertriglyceridemia and with a decrease in high density lipoprotein cholesterol (HDL-C) concentrations in humans (Cominacini *et al*., 1988).

Pedersen *et al*. (1999) evaluated the response to two consecutive solid test meals with different dietary FAs. The study investigated the effect of dietary fatty acid composition on postprandial lipid, glucose, insulin, and NEFA responses after intake of two consecutive solid test meals containing grape seed oil, sunflower oil or palm oil, with or without a glucose drink. The results showed that consumption of glucose with a mixed meal lowered the immediate TG and NEFA concentrations compared with the same meal without glucose, irrespective of the type of oil, consistent with an early study in which addition of glucose to a mixed meal containing dairy fat resulted in suppression of postprandial lipaemia (Albrink *et al*., 1958; Cohen and Berger, 1990). The fatty acid composition of the meal had no significant effect on the lipid and lipoprotein responses but it significantly affected the insulin responses. Further, the composition of the first meal did not affect the metabolic response to the second meal.

The FA composition of the diet also affects the glucose and insulin responses after meals (Rasmussen *et al*., 1996; Joannic *et al*., 1997). As insulin plays a central role in lipid metabolism, carbohydrate metabolism and lipid metabolism are strongly interrelated. In a study of eight subjects, they found that glucose and insulin responses after 30 min were significantly lower after meals containing polyunsaturated fatty acids than after meals containing monounsaturated fatty acids. Rasmussen *et al*. (1996) showed that the release of insulin was stimulated by butter but not olive oil. The authors explained this result in part by differences in fatty acid chain length. In addition, the amount of fat in a meal seems to be able to affect glucose and insulin responses as well.

Besides fats, carbohydrate composition has been demonstrated to influence postprandial lipaemia. Addition of glucose and soluble fibre to a meal resulted in lower postprandial lipoprotein concentrations (Cohen *et al*., 1988; Cohen and Berger, 1990; Cara *et al*., 1992; Sandstrom *et al*., 1994; Dubois *et al*., 1995). The effect of glucose is thought to be mediated in part by the stimulation of lipoprotein lipase by insulin, leading to increased catabolism of TG in TG-rich lipoproteins and thereby reduction of plasma TG concentrations. The lipid-lowering effect of some fibres is thought to be, in part, a consequence of decreased fat and cholesterol absorption in the small intestine (Lairon, 1996). Furthermore, added glucose might also reduce postprandial lipaemia via delayed gastric emptying (Cohen and Berger, 1990). However, not all studies find that addition of glucose to fat-containing meals results in less postprandial lipaemia (Mann *et al*., 1971; Nicholls and Cohen, 1985; Cohen and Schall, 1988).

It is well known that the concentration of NEFA in plasma increase with fasting and decrease after a meal, but there is no reported effect of fatty acid composition in the meal on the changes of plasma NEFA concentration (Pedersen *et al*., 1999). The addition of glucose to the meal led to significantly lower NEFA values, which may be explained by enhanced suppression of hormone-sensitive lipase activity by insulin (Frayn *et al*., 1994).

The range of plasma NEFA availability is possible because of the exquisite sensitivity of adipose tissue lipolysis to both insulin (Jensen *et al*., 1989) and catecholamines (Galster *et al*., 1981). Small changes in plasma insulin concentrations have dramatic effects on adipose tissue lipolysis, and therefore NEFA availability. The insulin dose response characteristics of NEFA changes were measured in normal humans (Jensen *et al*., 1989). Thus, overnight postabsorptive plasma insulin concentrations are a significant restraint to basal NEFA flux. Maximal suppression of NEFA flux in normal humans occurs after the ingestion of a small, carbohydrate-containing meal (Marker *et al*., 1991). Catecholamines are other important stimulators of NEFA concentrations under pressure or during exercise (Wolfe *et al*., 1987; Marker *et al*., 1991). In addition, growth hormone and cortisol also stimulate lipolysis, but appear to be much less potent than catecholamines (Boyle *et al*., 1992).

2.6 Conclusions

The literature suggests that dietary fat includes the fat consumed in plant and animal products and high levels of dietary fat may encourage overeating, leading to weight gain. Compared to prototypical taste, fat taste perception is more complex involving gustatory, textural and olfactory inputs. Currently, based on many animal and human studies, it has been reported that free fatty acids can serve as taste signals and related to fat perception. Now, a number of putative receptor for fatty acid has been proposed, including DRK, CD36 and GPCR. A cell diffusion mechanism has also been suggested as co-existing with receptor-medicated transductions. Many studies have been performed on the existence of fatty acid taste and the relationships among fat perception and other taste qualities; BMI and food preferences in either mixed foods or homogenized emulsions. However, the results remain contradictory and none of them investigates the effect of fasting or satiated state on the threshold changes. 3-AFC test from ASTM standard has been widely used in many studies and proven to be a reliable and valid method for measuring sensory thresholds.

It is known that PROP status may be related to other prototypical taste qualities, which also may affect body weight and food preference. However, whether PROP sensitivity is associated with fat perception or not is still a debate. Some researchers believed that the bitterness sensitivity seems to be associated with fungiform papillae density but the taste papillae density is not able to predict PROP taste status. The classic PROP status classification method was developed by Bartoshuk in 1994 and recently other tests modified from Bartoshuk's tests have been used in many studies, including the three-solution test.

It is known that taste perception triggers hormones that are crucial in the control of energy balance and appetite to exert a strong effect on food intake, satiety and metabolic regulation. For example, oral exposure to dietary fat and FAs may trigger plasma TG rise, so it is possible that oral detection of dietary fats and FAs is able to be assessed through a change of TG. In addition, the changes of plasma metabolites parameters are related to the metabolic status or the dietary composition.

Therefore, this study tests the hypothesis that fatty acid taste perception could be influenced by body phenotype or predicted by PROP status. In addition, the relationship between taste perception and blood metabolites is also investigated. Furthermore, the influence of metabolic states (overnight fasting and satiated) on concentrations of blood metabolites is associated with changes in oral sensory perception.

3 Materials and Methods

3.1 Study outline

The overview of the trial is shown in Figure 3 and Figure 4. The project included three visits. In the first visit, a preliminary test was carried out for screening and classification of subjects based on their perception of PROP using a standard published protocol (Tepper *et al*., 2001). Anthropometric measurements were taken at the end of the preliminary test.

In the second and third visits, subjects were randomly assigned into 2 groups of equal numbers following a crossover study design. The second and third visit was run after an overnight fast. For the second visit, following a brief introduction of the procedures, 15 ml of blood was collected in the fasted state. Sensory test I using the 3-AFC procedure (as described in Section 3.4) to determine the detection thresholds of subjects to sweet taste and fatty acid taste was then carried out. When the sensory test was finished subjects either consumed a full breakfast (approximately 800kcal) over 30 min (B trial) had a 30 min break (NB trial)). For both trials, subjects waited 1 hr before a further 15 ml blood sample was taken followed by sensory test II (using the same sensory test as in the sensory test I). Fungiform papilla counting was completed during the rest period in the NB trial.

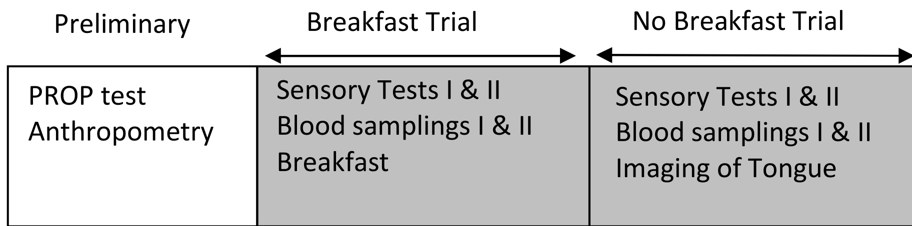


Figure 3 Overview of the study involving 3 visits

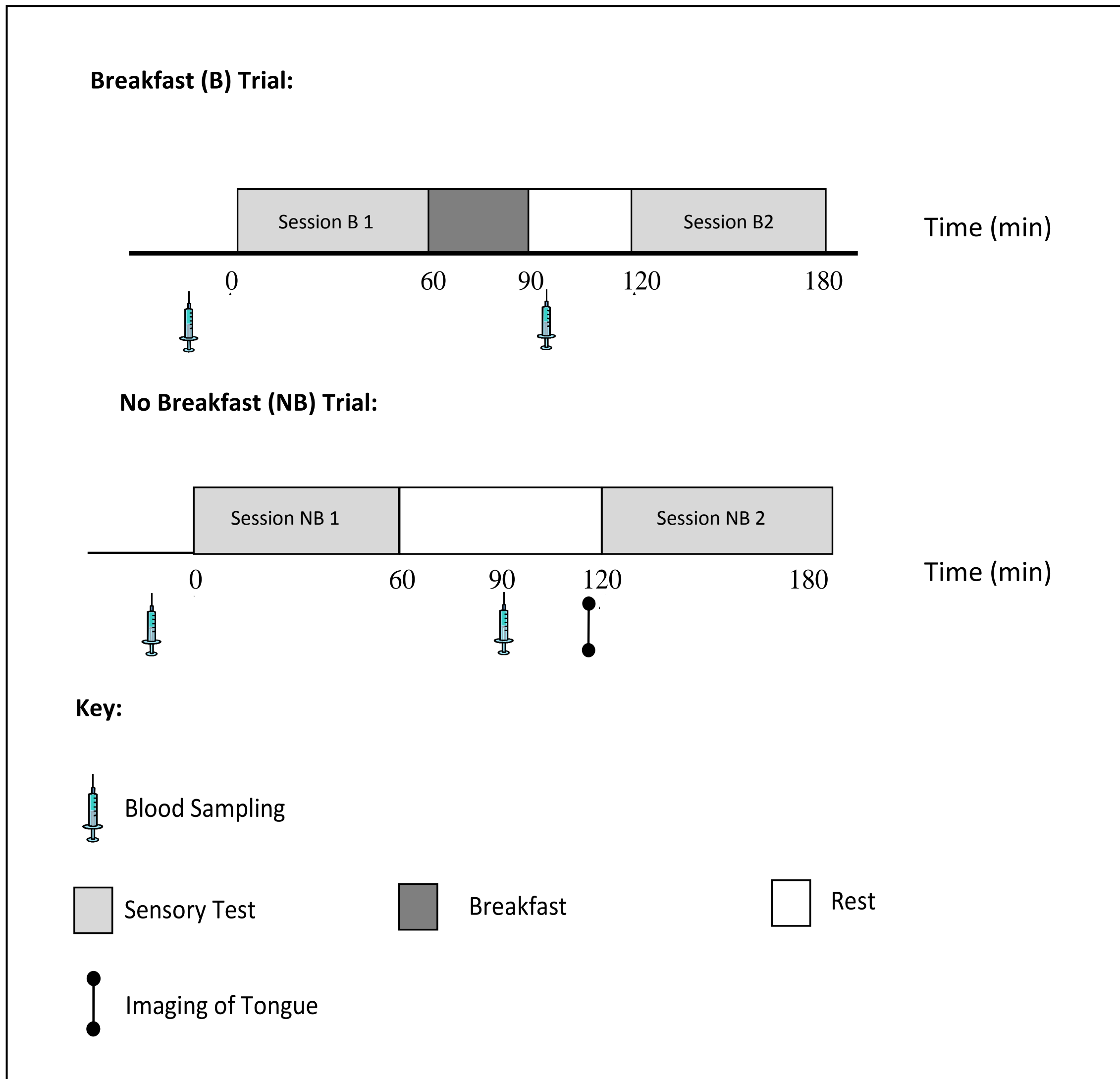


Figure 4 Detailed diagrams showing trials

3.2 Subjects

Forty men were recruited by advertisement within the Greater Auckland area. Exclusion criteria included smokers, allergic to the test materials, taking prescription medication or recreational drugs, or have a clinical cause for a dry mouth. Body weight, height and waist/hip ratio were measured and BMI (kg/m^2) was calculated as body weight (kg) divided by height (m) squared. Furthermore, all subjects completed a PROP status test to divide them into nontasters, normal tasters and supertasters (Tepper *et al*., 2001). All subjects gave their written informed consent. The study was approved by the Massey University Human Ethics Committee (Southern A, Application-10/81).

Power analysis was conducted to determine an appropriate sample size to achieve adequate power. Using data generated by a previous study (Chale-Rush *et al*., 2007) and an α 0·05 and a 20% β (20% power), it was calculated that a sample size of 39 would be required.

3.3 Linoleic acid (LA) sample

Linoleic acid was chosen for taste tests as it has been used successfully to determine thresholds for fatty acid taste in humans. Food grade linoleic acid (Sigma-Aldrich, New Zealand) was added into gum acacia prepared solutions. All preparations were mixed with 5% (w/v) gum acacia (Ingrediantstop. New Zealand) and 5% (w/v) food grade liquid paraffin (Sigma-Aldrich, New Zealand) to produce perceptually identical texture attributes, including viscosity and lubricity between linoleic acid and control samples. To prevent oxidation of LA during testing, 0.01% (w/v) ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich, New Zealand) was added. Food grade linoleic acid (Sigma) was stored under nitrogen below -8 °C and was used without further purification. It was added at varying concentrations (0.01 mM – 0.1 mM) to prepared gum acacia solutions.

To ensure test samples were stable and that LA did not separate from other components; samples were homogenized for 4 cycles at 20/200 bars using a high-

pressure homogenizer (model: APV-2000; APV manufacturing, Poland). Control samples were prepared in the same way, but without fatty acids added.

Dilutions of each sample were prepared one day prior to sensory testing. Each sample was provided in a 3-digit random number labelled 30ml clear portion cup containing 10 ml of sample at room temperature (22°C).

3.4 Chemical analysis

A series of chemical tests were conducted to investigate the properties and ensure better emulsions before the main trial. In order to eliminate the textural variability among samples, droplet size, viscosity, fatty acid oxidation and physicochemical stability were conducted to investigate the physicochemical characteristics of the samples. Microbiological tests were conducted for the safe consumption of samples.

3.4.1 Emulsion preparation and homogenization techniques

In previous studies, there were three different homogenization techniques used for producing emulsions. In order to investigate the differences between the methods, triplicate samples were prepared as described in Table 2 and processed by A) Ultra sonic water bath for 30 minutes, B) Branson sonifier cell disruptor for 2 minutes (model: S-150D; Danbury, CT) with probe amplitude setting at 45%, C) High-pressure homogenizer for 4 cycles at 20/200 bars using a APV-2000. All samples were prepared in polypropylene containers.

Table 2 Compositions and emulsifying Processers of each obtained sample

Sample	% Gum acacia (w/v)	% Mineral oil (w/v)	% EDTA (w/v)	% Linoleic acid (w/v)	Emulsifying Processer
A	5	5	0.01	--	Ultrasonic water bath
B	5	5	0.01	1	Ultrasonic water bath
C	5	6	0.01	--	Sonifier cell disruptor
D	5	4	0.01	1	Sonifier cell disruptor
E	5	4	0.01	--	High-pressure homogeniser
F	5	4	0.01	1	High-pressure homogeniser

3.4.2 Emulsion particle size and physicochemical stability

To investigate the effects of different homogenization techniques on the emulsions, the particle size distribution and micrograph observation were performed. Samples were prepared as described in Section 3.4.1.

Emulsion droplet size was determined using a Malvern Mastersizer MS2000 laser light-scattering analyser with absorption parameter value of 0.01 and refractive index ratio of 1.46. A light microscopy was examined and photographed immediately after homogenization in duplicate under bright field illumination with 40× or 100× objective lens on a Zeiss Photo-Microscope.

To observe the variation of particle size during the short-time storage and different homogenisation treatments, photos were taken by a digital camera immediately after homogenization and after an overnight stand. The emulsions were stored in polypropylene tubes in the fridge (4°C).

3.4.3 Viscosity and fatty acid oxidation

To ensure that addition of FAs do not affect the physicochemical characteristics of the emulsions, the viscosity of the samples were measured. Samples were prepared as described in Section 3.4.1 but only with the high-pressure homogenizer. Apparent viscosity of each sample was determined in duplicate at a shear rate of 1-100 s^{-1} using a stress controlled rheometer (TA AR550 Rheometer) equipped with a cone and plate

(6º/20mm) geometry at a room temperature of 25°C. To ensure no oxidized products were produced during the sample preparation procedure, gas chromatography analysis was also performed.

3.4.4 PROP taster status

PROP taster status of the subjects was identified by their PROP/sodium chloride (NaCl) ratio using the 3-solution test (Rankin *et al*., 2004). Sample concentrations used were PROP (3.2×10^{-5}M, 3.2×10^{-4} M and 3.2×10^{-3} M) and NaCl (0.01 M, 0.1 M and 1.0 M). The procedure was used to generate suprathreshold taste intensities. Since PROP taster status does not influence intensity judgements for NaCl, solutions of NaCl were served as a standard (Bartoshuk *et al*., 1994). The intensity of the taste was recorded on a labelled magnitude scale (LMS). The LMS is a quasilogarithmic scale with label descriptors anchored at the lower end with the phrase ‘‘barely detectable’’ and at the high end with the phrase ‘‘strongest imaginable’’ (Green *et al*., 1993). The scale is presented in appendix 1.

During the test, NaCl solutions were presented in a random order, followed by three randomized PROP solutions. Subjects rinsed their mouths with distilled water before they began tasting the test solutions and between each test solution. They were required to place the whole sample in the mouth (10 ml), expectorate it and rate its intensity by making a single mark on the LMS scale.

3.4.5 LA and sucrose detection thresholds

Before the main trial, samples were tested for the aerobic plate count test to make sure that they were safe for sensory testing. In the main trial, threshold experiments were performed in aqueous solutions (sucrose test) and in emulsions (fatty acid test). Each trial (depicted in Fig 4) consisted of two replicates of each test. Aqueous tests included ten sets of sucrose samples. Emulsion tests included eleven sets of linoleic acid samples. Each set of three samples consisted of two controls (distilled water for the aqueous test or the appropriate “tastant-free” emulsion for the emulsion test) and a target.

Emulsion composition and preparation was described in Section 3.4.1 and the aqueous sucrose solutions were made by adding sucrose into distilled water. The sucrose and LA concentrations are shown in Table 3.

Table 3 Concentrations used for the determination of detection thresholds

	1	2	3	4	5	6	7	8	9	10	11
sucrose (mM)	0.1	0.2	0.4	0.8	1.6	3.2	6.4	12.8	25.6	51.2	
Linoleic acid (mM)	0.50	0.75	1.14	1.71	2.57	3.85	5.78	8.66	13.01	19.52	29.28

The subjects evaluated samples in a sensory booth and were each given as much water as desired for rinsing between samples. To eliminate additional odour and visual cues, nose clips were used and all tests were conducted under red lights (Chale-Rush *et al*., 2007). The detection thresholds were measured by using the 3-AFC test. Each subject was given a set of three randomly ordered samples consisting of two cups containing the control and a single cup containing the target stimulus, in ascending order from the lowest to the highest concentration. Subjects were asked to rinse their mouths with water before beginning the task and between each sample set. The trial was finished when a subject correctly identified the fatty acid sample from the other two in the set in three consecutive samples. The chance of correctly guessing the sample with added fatty acid three consecutive times in a triangle test is 3.7%. Detection thresholds were calculated following the guidelines of ASTM E679.

3.5 Breakfast consumption during the test

The same breakfast (the same meal and the same amount) was served to all subjects and they were asked to eat as much as they could, for the breakfast trial.

The breakfast was weighed before and after the consumption to calculate the macronutrient intake (as some subjects may not finish the provided breakfast). The breakfast menu was designed with the support of a dietician, and details of the breakfast nutritional information are presented in Table 4.

The breakfast included wholemeal bread (2 medium slices), ham (100g), tasty cheese (40g), butter (9g), muesli (100g), salad (100g), banana, mandarin, milk (1.5cup).

Table 4 Nutrition information of the breakfast

Nutrient	Per meal
Energy (kJ)	5307.28
Protein (g)	53.84
Total fat (g)	45.71
Carbohydrate (g)	162.16

3.6 Fungiform papillae counts

Initial trials were carried out with different concentrations (0.01g/ml, 0.05g/ml and 0.1g/ml) of food dye - Brilliant Blue solutions (Sigma-Aldrich, New Zealand). The images were recorded and compared, in order to get the most appropriate colouring concentration at which the colouring is adsorbed to the fungiform papillae.

Subjects rinsed their mouths with distilled water. They were then asked to dry the tongue with a piece of filter paper and then extend their tongues as far as possible, holding it steady with the lips. Then a 5 mm diameter circular piece of filter paper that contained blue food dye was placed at the tip of the anterior part of the left side of the tongue, close to the midline of the tongue. Two or three images of the tongue were recorded with a Nikon Coolpix L22 digital camera (7.0 megapixels). Fungiform papillae were counted by observation.

3.7 Data analysis

Data were analysed with SPSS, version 17.00 (SPSS, Inc., Chicago, IL, USA). Normal distribution of data was investigated by using Kolmogorov-Smirnov test. The level of significance was accepted as $P < 0.05$.

3.7.1 PROP classification

When normal distribution was determined, the data was examined using a repeated-measure analysis of variance (ANOVA) with concentration levels and stimuli type (PROP or NaCl) as within-group factors, taster groups (NT, MT and ST) as between group factors. Mauchly's test of sphericity was used to determine whether the assumption of sphericity was being violated. When this did occur, the Huynh-Feldt correction was applied. Data are presented as Mean ± SEM.

3.7.2 Fungiform Papillae counts

As data were normally distributed, one-way ANOVA and *post-hoc* test revealed the differences in papillae densities among PROP taster groups. Pearson's test was used for association between papillae densities and body phenotypes. Data are presented as Mean ± SEM.

3.7.3 Body phenotypes

As data were normally distributed, a one-way ANOVA was used to determine differences in body phenotypes across PROP status groups. Also, Tukey test was used as *post-hoc* test. Pearson's correlation coefficient (*r*) was used to investigate the relationship between variables. Data are presented as mean ± SEM.

3.7.4 Main trials

Because of a lack of normality, Wilcoxon Signed-rank test was used to test differences in threshold values among sessions (B1, the session before breakfast; B2, the session after breakfast; NB1, the session before break and NB2, the session after break). Kruskal-Wallis test was used for the differences in threshold values among

PROP taster groups. When significant differences were shown, Mann-Whitney test using Bonferroni adjustment was conducted to ascertain where the differences lay. Spearmen's correlation coefficient (r^2) was used to investigate the relationship between sensory thresholds and other variables. Data are presented as the median [25% percentile, 75% percentile].

Two out of five blood metabolite parameters were found to be normally distributed. One-way ANOVA and *post-hoc* were performed to investigate the statistical significance of differences in plasma TC and HDL-C among PROP taster groups and a paired sample T-test with Bonferroni adjustment was used for differences within taster groups. Data are presented as Mean ± SEM. In addition, for non-normally distributed data, Kruskal-Wallis test was carried out to explore differences in plasma glucose, TG and NEFA among PROP taster groups. When significant differences were shown, Wilcoxon Signed-rank test were conducted to ascertain where the differences lay. Spearmen's test was used for relationships between sensory thresholds and blood metabolites. Data are presented as Mean ± SEM (TC and HDL-TC).

4 Results and Discussion

4.1 Chemical analysis

4.1.1 Emulsion stabilization and micrograph observation

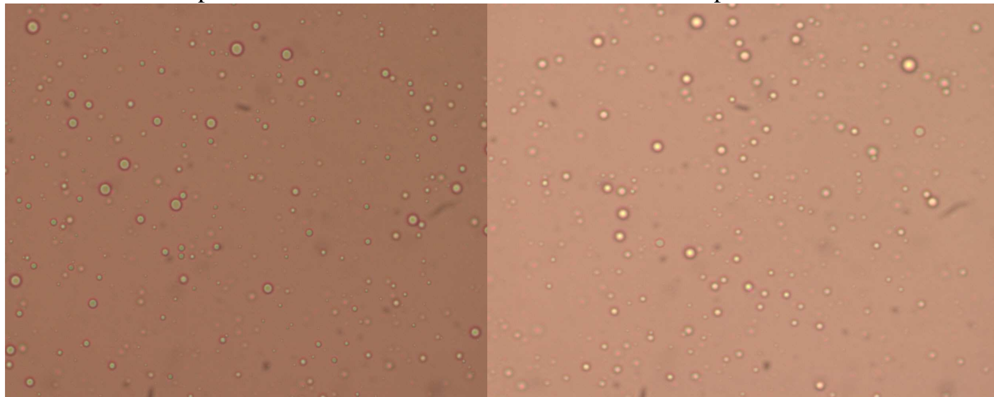
There are three homogenization techniques available for making emulsions. In order to reduce the textural variability of samples, well homogenised and better stable emulsions were required for the main trials. Emulsion observation was carried out to determine the physicochemical characteristics of each emulsion.

Figure 5 Top: Appearance of emulsions (from left to right; acacia solution with LA before any homogenization techniques; A, sample without LA by ultrasonic water bath ; B, stimulus sample with LA by ultrasonic water bath; C sample without LA by probe sonicator (sonifier cell disrupter); D, stimulus sample with LA by probe sonicator (sonifier cell disrupter); E, sample without LA by homogeniser; F, stimulus sample with LA by homogeniser.). Bottom: Appearance of sample C, D, E and F after 12 h

Photos of the emulsions were taken immediately after the homogenization and after 12 hour storage at 4°C (Figure 5). Samples A and B, which were made by the ultrasonic water bath, showed obvious differences in appearance and were more transparent than the others. Also, there were foam layers on the top of these two emulsions, indicating a poor homogenised emulsion. The other four samples looked identical in appearance as they were all white opaque solutions. For this reason, it was clear that samples C-F had smaller particles than samples A and B, suggesting that the sonifier cell disruptor and the high-pressure homogeniser are able to produce better emulsions than the ultrasonic water bath. After 12h storage in the fridge, the appearance of the samples had changed. There were thicker flocculation layers on samples D and E compared to samples F and G. In addition, samples F and G appeared more opaque, suggesting a better homogenized state. Therefore, it seemed that high pressure homogeniser generated a better homogenized and stable emulsion than the other methods.

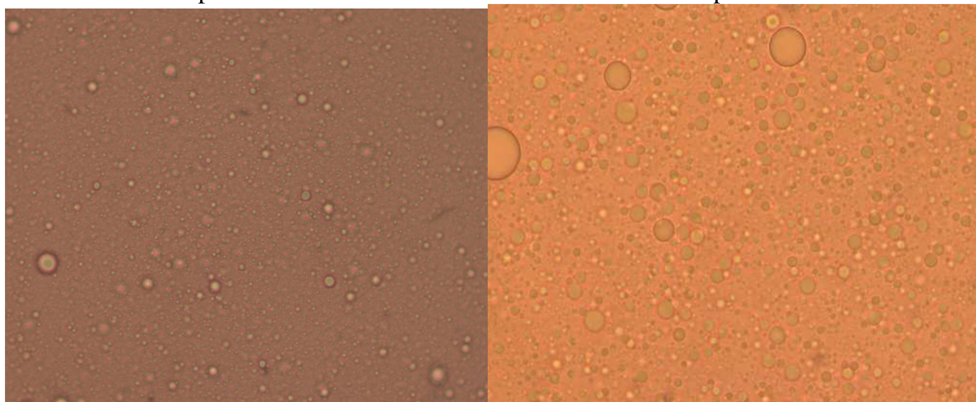
Water bath (x 40 magnification)

Sample A without LA Sample B with 1% LA



Sonicator(x 100 magnification)

Sample C without LA Sample D with 1% LA



High-pressure homogeniser (x 100 magnification)

Sample E without LA Sample F with 1% LA

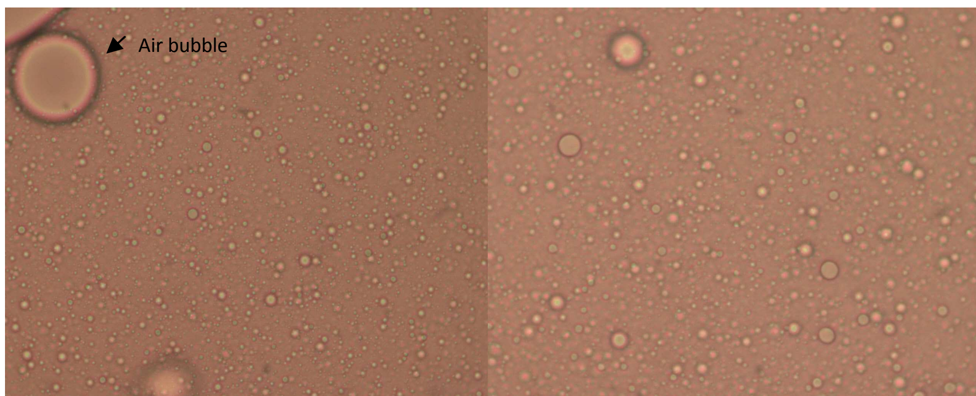


Figure 6 Micrographs of emulsions produced by different emulsifying processor before overnight stand (Sample compositions are depicted in Table 2)

A light microscopy was used to examine the samples with 100× objective lens and photographs were taken immediately after homogenization. Although we could not measure the particle diameters from the image, Figure 6 clearly shows that the sonifier cell disrupter and high-pressure homogenizer produced smaller droplets and well dispersed emulsions compared to that of the ultrasonic water bath.

4.1.2 Particle size distribution

Technically, the samples were required to be analyzed immediately after homogenization. However, due to the absence of equipment, all samples were sent to Palmerston North for analysis which extended the time before analysis to 24 hours. As a result, the changed emulsion system may have affected the accuracy of the results.

The average particle size was characterized by the mean droplet diameter, which was used to monitor changes in the droplet-size distribution of the emulsions. The average droplet values of each sample are shown in Table 5 below.

Table 5 Average particle size of each sample

ID	Sample	Emulsifying Processer	Mean droplet diameter (μm)
1	Without linoleic acid	Ultrasonic water bath	101.1
2	With 1% w/v linoleic acid	Ultrasonic water bath	34.8
3	Without linoleic acid	Sonifier cell disruptor	13.8
4	With 1% w/v linoleic acid	Sonifier cell disruptor	5.6
5	Without linoleic acid	High-pressure homogeniser	6.3
6	With 1% w/v linoleic acid	High-pressure homogeniser	0.6

Samples 1, 3, 5 were the non-LA samples and 2, 4, 6 were stimulus samples with 1% w/v LA added. As seen in table 5, the high-pressure homogeniser generated the emulsions containing the smallest particle size. Also, the addition of LA depressed the flocculation and resulted in much smaller droplets.

Previous results on the effects of droplet size on flavour detection have shown that the tactile response was influenced with tastant containing emulsions with droplet sizes

varying between 1.0 and 5.5 µm (Nakaya *et al*., 2006). Conversely, the tactile response was not affected in unflavoured emulsions with particle sizes ranging from 0.5 to 6.0 µm (Akhtar *et al*., 2004; Vingerhoeds *et al*., 2008). Akhtar *et al*. (2004) reported that subjects were unable to discriminate between emulsions of different droplet size in the range of 0.5-2.3 µm. They also suggested that the rheological characteristics are insensitive to the droplet size distribution. this agrees with previous work conducted by Tyle (1993), concluding that consumers are only able to detect food particles at least as small as 5 µm. Unfortunately, due to the inaccuracy of data in the present study, it is unsure that the difference between the no fatty acid sample E and stimulus sample F has any effect on the taster perception or not.

According to the results above, the high-pressure homogeniser was selected as the emulsion processer as it made well-homogenized emulsions having identical appearance and the smallest particle size which will have the least effect on non-gustatory cues.

4.1.3 Apparent viscosity analysis

To ensure that fatty acid addition did not affect the physicochemical characteristics of the emulsions, the viscosity of samples at two different concentrations were determined and compared to the emulsions without fatty acid (controls). The two tastant samples contained 0.079 % w/v and 0.179 % w/v LA respectively. Samples were measured immediately after homogenization in duplicate. The two concentrations were selected from preliminary tests identifying two different threshold levels at which were correctly identified by a non taster and a medium taster. All the emulsions were tested at shear-rates of 1-100 s^{-1} at 25°C. Viscosity profiles for the emulsions are shown in Figure 7.

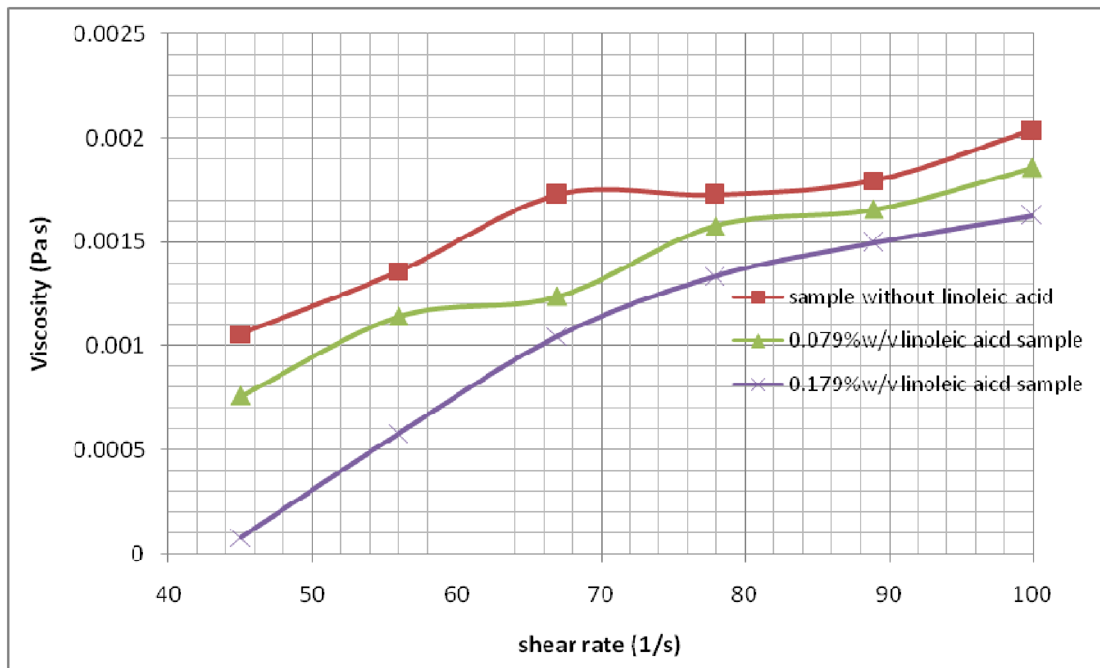


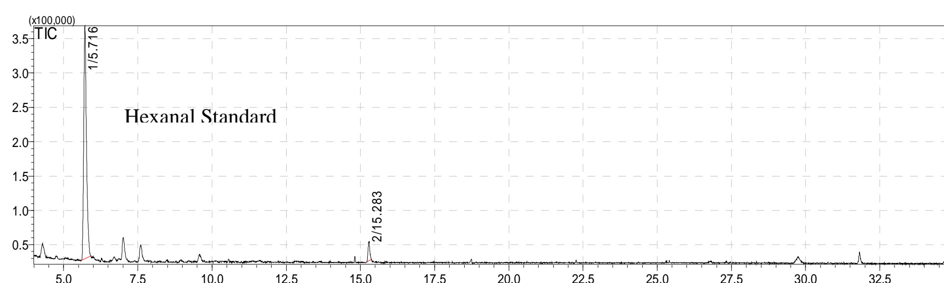
Figure 7 Viscosity profile of emulsions with varying LA concentrations

As shown in Figure 7, emulsions containing varying LA concentrations are showing similar patterns of viscosity change over the shear-rate range. The viscosity of samples decreased with increasing LA concentration, suggesting the addition of unsaturated fatty acid may influence the apparent viscosity. Recently, Chale-Rush *et al*. (2007) reported no measurable differences were observed between samples containing LA at low (0.03%) or high (1%) concentrations and the control samples (without linoleic acid) suggesting the FA did not contribute to any textural cues of the stimuli. Different sensitivities of the equipment may account for these differences. It is possible that the viscometer is less sensitive than the rheometer and unable to report the small variation between samples leading to inconsistencies between the two studies. Whether subjects can pick up these measured viscosity changes or not is yet undetermined.

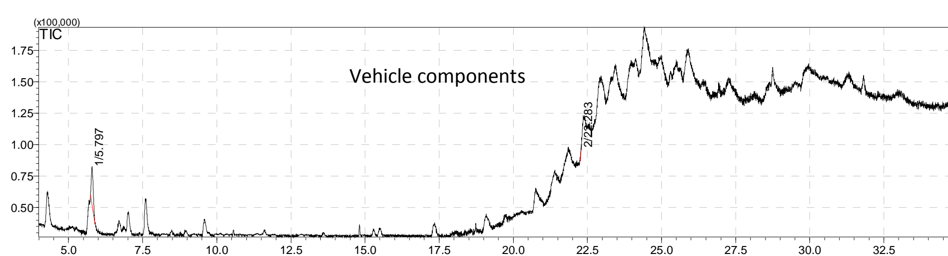
4.1.4 Fatty acid oxidation (GC analysis)

As LA is very unstable and easily oxidized when it is exposed to air and light, there is concern that the fatty acid may generate off-flavour products during the homogenization process because of the increased temperature caused by high

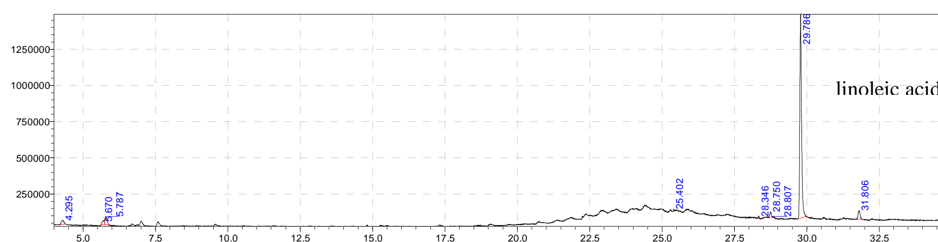
pressure. To ensure that no samples contained off-flavour products during tasting, GC analysis was carried out.



A



B



C

Figure 8 Results of GC analysis (A: Hexanal Standard; B: 'blank' sample without linoleic acid; C: stimulus sample with 0.179% w/v linoleic acid)

To determine if oxidation had occurred, a search for oxidation breakdown products via GC-MS with particular emphasis for identifying hexanal (major off flavour product) was carried out. A typical sample is presented in Figure 8 (C) and shows that for the stimulus sample with fatty acid, only LA can be found around 5.7 minutes and no other significant peaks were observed. The result suggests that linoleic acids were not oxidized during the homogenization process.

The present results indicate that the current precautions taken to minimise FFA oxidation are effective.

4.2 Preliminary test

4.2.1 PROP status classification

Results were plotted and classification was obtained by visually comparing the taster curve for PROP to that of NaCl for each subject. According to a previous study, subjects who rated NaCl higher in intensity than PROP were nontasters. Those who gave similar ratings to NaCl and PROP were medium tasters and those who rated PROP more intense than NaCl were supertasters (Bartoshuk *et al.*, 1994). However, the lack of an acceptable numeric cut-off score makes the group classification more difficult. For some very typical nontasters and supertasters, it is easy to assign them to their own groups. For example, the PROP and NaCl curves in the two graphs shown in Figure 9 are quite different from each other, strongly indicating their taster groups.

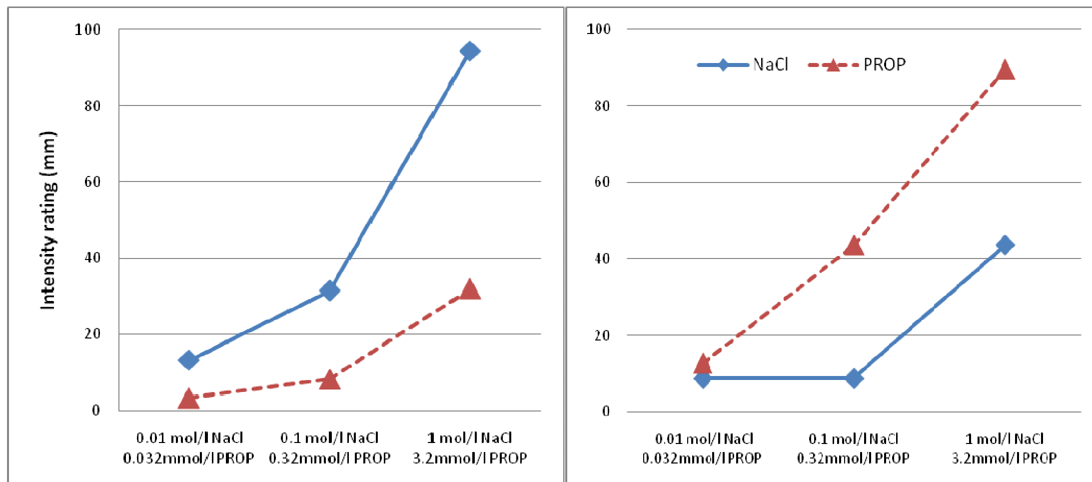


Figure 9 Intensity ratings of NaCl and PROP for a typical nontaster (left) and a typical supertaster (right).Non tasters gave higher intensity ratings to NaCl than PROP and supertasters showed an inverse pattern

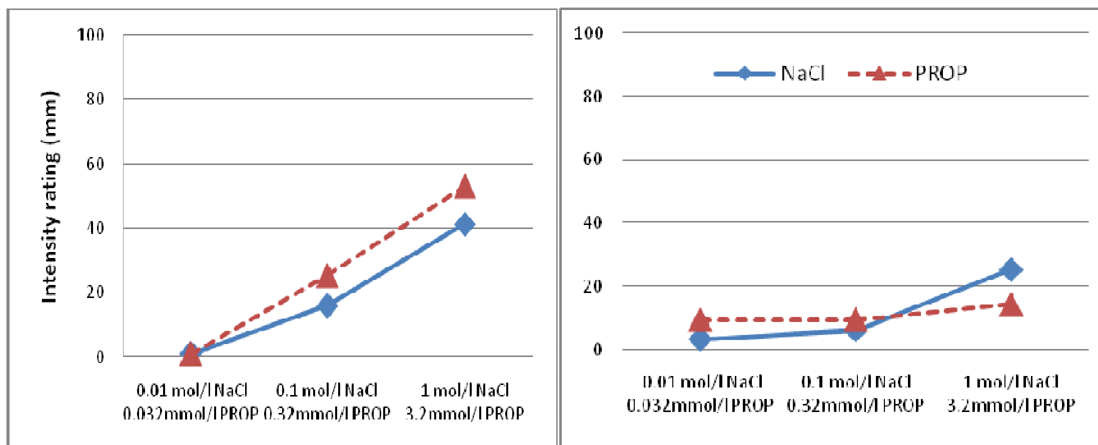


Figure 10 Intensity ratings of NaCl and PROP for unclassified subject A (left) and subject B (right)

Conversely however, as seen in Figure 10, it is difficult to classify some subjects by visual observation when the two lines of NaCl and PROP are quite closely aligned. For example, subject A could be considered as a supertaster or a medium taster due to the overlapping of the first point. Similarly, it is difficult to classify subject B because the two lines cut across each other. Therefore, the visual observation method does not pose valid and detailed criteria for classifying all subjects. However, all the subjects can be initially divided in to 3 practical groups, typical NT, typical ST and an uncertain taster groups, based on visual observation.

In order to subdivide uncertain tasters into STs and MTs, the 'PROP ratio' proposed by Bartoshuk *et al.* (1994) was used. The PROP ratio represents the relative intensity of PROP compared to NaCl across the two highest concentrations of each. Bartoshuk *et al.* (1994) used a ratio of 1.2 and other workers used a series of cutoff ratios ranging from 1.6 to 2.5.

The two highest perceived intensities of PROP and NaCl were used to calculate the ratio:

$$PROP\ ratio = (PROP_2 / NaCl_2 + PROP_3 / NaCl_3) / 2$$

where $PROP_2$ and $PROP_3$ represent the perceived intensities of the second and the third levels of PROP solutions, and $NaCl_2$ and $NaCl_3$ represent the perceived second and the third intensities of NaCl. The lower concentrations were not used because a few subjects gave zero ratings for NaCl concentrations (Bartoshuk, 1993).

According to the study of Tepper (2001), a cut-off score of 0.7 mm was used to divide medium tasters from nontasters and another line at 1.7 mm to divide medium tasters from supertasters. When all the subjects were successfully classified, the grouping was confirmed with ANOVA (Tepper, 2001).

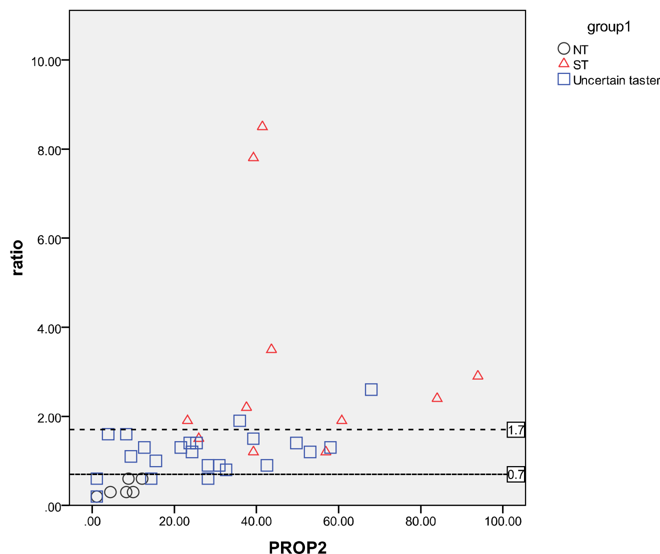


Figure 11 Scatter plot of PROP ratio vs level 2 PROP intensity ratings for 40 male subjects

Figure 11 shows that the 0.7mm cut-off line has put all the typical NTs into the NT group, including an uncertain taster with relatively high PROP intensity. The 1.7 mm cut-off line divides some typical ST-pattern subjects into the MT group, indicating even though their PROP intensities are slightly higher than NaCl intensities, they are considered as MTs. Eventually, all the subjects were classified as nontasters (n=10), medium tasters (n=20) and supertasters (n=10).

A significant three–way interaction of Taster group × Stimuli type × Concentration on the intensity ratings was found [F (4, 74) = 10.09, p<0.001]. As shown in Figure 12, NTs gave lower intensity ratings to all the concentrations of PROP than that which they gave to the corresponding concentrations of NaCl. STs gave higher intensity ratings to PROP than that which they gave to NaCl. MTs gave PROP and NaCl equivalent ratings to the two highest concentrations of NaCl and PROP. Additionally, *Post-hoc* tests with Bonferroni adjustment also showed there were significant differences among PROP taster groups ($p < 0.01$ for all tests). Figure 13 shows the

relationship between PROP and NaCl intensities within each taster group. These results are consistent with previous studies (Bartoshuk, 1993; Tepper *et al*., 2001).

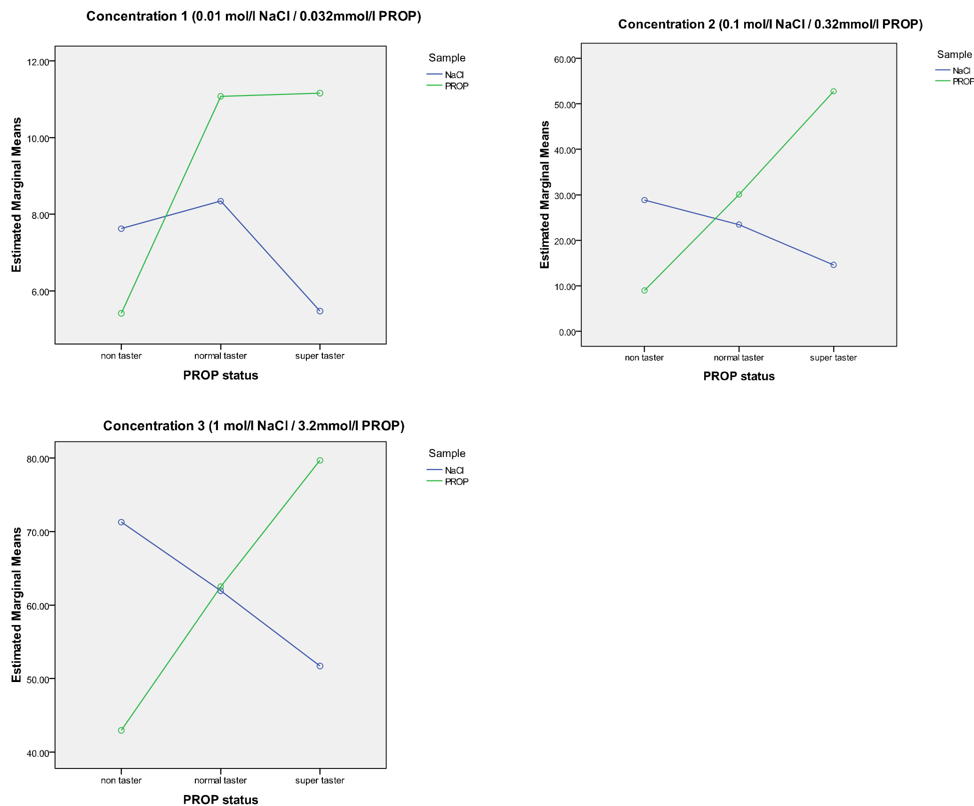


Figure 12 Interaction graphs of taster group × stimuli type for 3 concentration levels (In terms of interaction graphs, non-parallel lines indicate significant interactions)

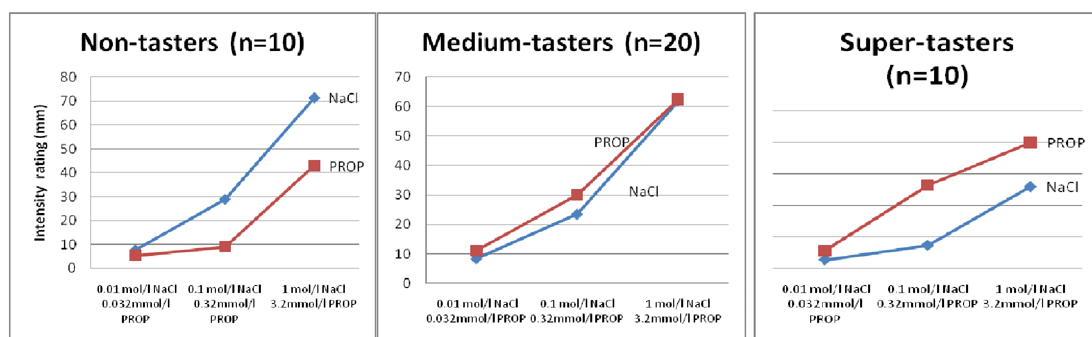


Figure 13 NaCl and PROP taste intensity ratings for nontasters, medium tasters, and supertasters

In PROP classification, some methodological design features might have contributed to the differences between studies. For example, Drewnowski *et al*. (2001) used a nine-point category scale to collect PROP intensity ratings and this scale can produce ceiling effects at the highest concentrations (i.e., subjects' judgements were

constrained by the upper endpoint of the scale), particularly for STs who are extremely sensitive to the bitterness from PROP, leading to an unusually high number of STs (40% of men and 55% of women).

Generally, of the 40 male subjects, 10 (25%) were PROP NTs, 20 (50%) were PROP MTs and 10 (25%) were PROP STs. These portions of subjects were quite close to reported values. For example, Tepper *et al* (2001) found proportions of 25% NTs, 57% MTs and 18% STs.

4.2.2 The relationship between PROP status and Fungiform Papillae densities

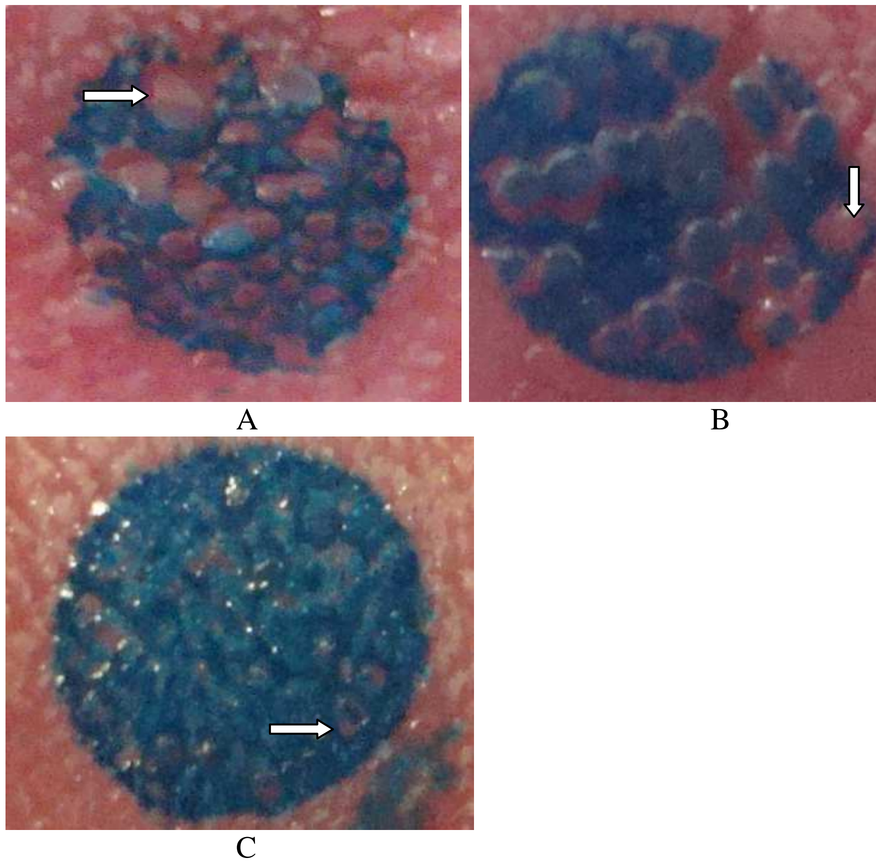


Figure 14 Fungiform papillae on human tongues (A: high papillae density of tongue; B: medium papillae density of tongue; C: low papillae density of tongue. Arrows indicate typical fungiform papillae)

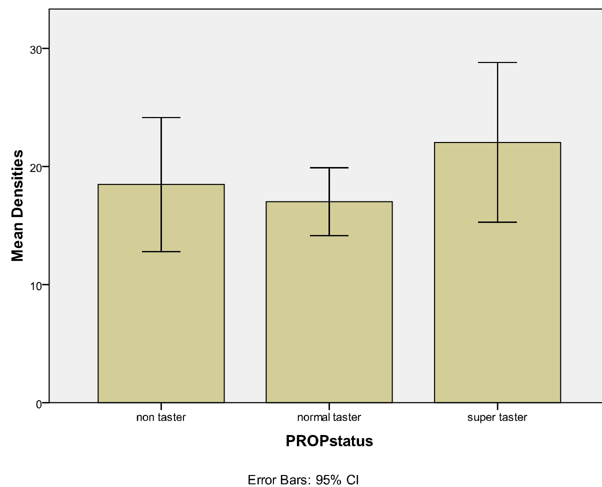


Figure 15 The density of fungiform papillae of each taster group

Figure 14 are typical photographs of the stained area of the anterior upper surface of the tongue showing high, medium and low papillae densities. Arrows indicate typical fungiform papillae. Most fungiform papillae commonly are mushroom shaped elevated structures consisting of a large head (Kullaa-Mikkonen and Sorvari, 1985; Miller, 1995; Segovia *et al*., 2002). Mean fungiform papillae densities ranged from 17 /cm^2 (MT) to 22/ cm^2 (ST) (Figure 15), and were not statistically different. Previously reported mean values based on visual observation include 47.2/ cm^2, 62.4/ cm^2, and 75.8/ cm^2 for NT, MT, and ST, respectively (Tepper and Nurse, 1997). High numbers of fungiform papillae are commonly found in STs compared with MTs and NTs (Miller and Reedy, 1990b; Bartoshuk *et al*., 1994; Tepper and Nurse, 1997; Shahbake *et al*., 2005; Yackinous and Guinard, 2001 and 2002). However such finding could not be confirmed with the present study. A reason for such difference may be that a relatively crude procedure was used to count the number of fungiform papillae from a small area on the tip of the tongue, which might have led to an underestimation of the true number of papillae. Furthermore, procedural differences are likely to be a reason for the inconsistence in the results. For example, Yackinous and Guinard (2001) investigated the front left and right sides of the tongue. Tepper and Nurse (1997) used a filter paper template on the tip of the tongue, adjacent to the middle. Shahbake *et al*. (2005) analyzed the fungiform papillae density on the left side of the tongue. Although both taste buds and taste pores are the functional units of taste perception, neither of them is visible to the naked eye. Hence, the present observations should be confirmed using more sophisticated counting methods.

In addition, aging also may have an affect on fungiform papillae density which is supported by a study comparing the difference in the density of fungiform papillae between children and adults (Segovia *et al*., 2002). The result shows that children have significantly higher fungiform papillae density than adults, leading to a possible conclusion that the number of fungiform papillae may decrease with aging.

Table 6 Spearman correlation coefficient values (r^2-value) and their significances between fungiform papillae densities and taste thresholds

	Sucrose				Fatty acid			
	Before breakfast session (B1)	After breakfast session (B2)	Before break session (NB1)	After break session (NB2)	Before breakfast session (B1)	After breakfast session (B2)	Before break session (NB1)	After break session (NB2)
Fungiform papillae density	0.201	-0.049	0.062	-0.087	-0.218	0.020	-0.221	0.056

*. Significance *P* value < 0.05

No correlation was found between the fungiform papillae density and the detection threshold for sucrose or linoleic acid in this study (Table 6). This is not consistent with prior study conducted by Zhang *et al*. (2009), which reports a negative correlation found between the density of fungiform papillae and the sweet detection threshold. Also, the present study failed to find any relationships between fatty acid thresholds and fungiform papillae densities.

4.2.3 Body phenotype and PROP status

Subject characteristics are summarized in Table 7, summarising the average age, height, weight, BMI and Waist/Hip ratio. The average age and BMI across PROP taster groups is shown in Figure 16.

Table 7 Subject characteristics

Age (Years)	Height (m)	Weight (kg)	BMI (kg/m^2)	Waist/Hip ratio
26.2±1	1.76±0	73.95±1.9	23.84±0.48	0.84±0

Mean± SEM

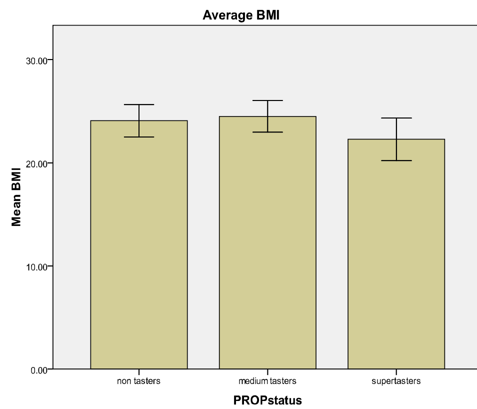


Figure 16 Mean BMI of each taster group. Graph indicates Mean ± SEM. Error bars represent 95% confidential intervals

Table 8 Pearson's correlations coefficient values (*r*-value) and their significances between body phenotype parameters

Parameters	BMI (kg/m^2)	Height (m)	Weight (kg)	Waist/Hip ratio	Age (years)
BMI (kg/m^2)		0.117	0.857*	0.263	0.372*
Height (m)			0.608*	-0.139	-0.020
Weight (kg)				0.141	0.283
Waist/Hip ratio					0.350*
Age (years)					

*. Significance *P* value < 0.05

A significant positive correlation between age and BMI, waist/hip ratio was found (Table 8). BMI and waist/hip ratio were significantly higher in older subjects, compared with younger subjects. This is consistent with previous studies (Bowen *et al.*, 2006). In addition, positive correlations were also found between BMI and weight, height and weight.

No significant differences between BMI values of the different taster groups were found ($p>0.05$). The relationship between PROP and BMI remains controversial. Several studies reported that PROP NTs who are less able to discriminate fat content in foods showed a higher acceptance of dietary fat (Hayes and Duffy, 2007; Tepper and Nurse, 1998; Keller *et al.*, 2002; Duffy and Bartoshuk, 2000; Forrai and Bankovi, 1984), and increased energy intake and greater adiposity than tasters did (Keller *et al.*,

2002; Tepper *et al*., 2010). These findings suggested a hypothesis of an inverse correlation between PROP status and BMI, which is supported by several studies (Tepper, 1999; Tepper and Ullrich, 2002; Goldstein *et al*., 2005; Tepper *et al*., 2008). However, other reports have shown no association between PROP taster status and these variables (Yackinous and Guinard, 2000; Timpson *et al*., 2005; Yackinous and Guinard, 2002; Drewnowski *et al*., 2007).

The significant relationship between BMI and age is in agreement with a previous study which revealed that among females, age was a positive predictor and PROP status was a negative predictor of BMI, while age was the only predictor of BMI in male subjects (Tepper *et al*., 2008). In addition, DNA analysis also revealed that polymorphisms at the TAS2R38 locus were not associated with BMI in either males or females (Tepper, 2008). Taking consideration of gender aside, data adds to a growing body of research suggesting that PROP status may not correlate with BMI (Dinehart *et al*., 2006; Drewnowski *et al*., 2007; Duffy and Bartoshuk, 2000; Yackinous and Guinard, 2001).

4.3 Sensory tests and metabolic analysis

4.3.1 Comparison and correlation in sensory thresholds

Table 9 Average detection thresholds for sucrose and linoleic acid of each PROP taster group and comparison of threshold values between/within taster groups during fasted and satiated states

	Sucrose (mM)					
	Before breakfast session (B1)	After breakfast session (B2)	*P* value (within groups)[2]	Before break session (NB1)	After break session (NB2)	*P* value (within groups)[2]
Total (n=40)	9.05[1.7,36.2]	13.58[3.4,18.1]	0.701	9.05[0.57,18.1]	18.1[1.13,18.1]	0.602
NT (n=10)	18.1[9.05,36.2]	9.05[0.14,18.1]	0.021*	13.28[0.92,35.86]	0.92[0.41,4.72]	0.889
MT (n=20)	4.53[1.13,36.2]	18.1[9.05,36.2]	0.586	2.1[1.4,15.94]	10.62[1.16,29.89]	0.408
ST (n=10)	13.58[0.57,18.1]	6.79[1.13,18.1]	0.959	3.14[0.61,23.91]	11.51[4.72,35.86]	0.235
P value (between groups)[1]	0.501	0.049*		0.069	0.45	
	Fatty acid (mM)					
	Before breakfast session (B1)	After breakfast session (B2)	*P* value (within groups)[2]	Before break session (NB1)	After break session (NB2)	*P* value (within groups)[2]
Total (n=40)	3.14[1.4,23.91]	4.72[0.92,29.89]	0.778	7.07[0.92,35.86]	2.62[0.77,35.86]	0.581
NT (n=10)	13.58[0.14,18.1]	18.1[1.13,18.1]	0.24	4.59[0.61,15.94]	1.16[0.61,3.14]	0.203
MT (n=20)	1.7[0.36,18.1]	6.79[0.57,18.1]	0.45	13.28[1.4,35.86]	8.85[1.16,35.86]	0.701
ST (n=10)	18.1[9.05,36.2]	18.1[9.05,18.1]	0.12	3.06[0.61,10.62]	3.84[0.41,35.86]	0.722
P value (between groups)[1]	0.72	0.17		0.31	0.14	

Median [25% Percentile, 75% Percentile]
n. number of subjects
*. Significance *P* value < 0.05
[1]. Kruskal-Wallis test was used to measure differences between NT, MT and ST group.
[2]. Wilcoxon Signed-rank test was used to measure differences within each taste group between B1and B2, NB1and NB2.

Average detection thresholds for sucrose and linoleic acids are presented in Table 9. Because of the non-normal distributions, data are presented as medians with inter-quartile ranges as the index of variance. The results show that the threshold distributions are extremely broad and some subjects do not reach a detection threshold within the range of concentrations investigated in this study.

The effect of PROP status on sensory thresholds was also investigated. A significant difference was shown in thresholds for sucrose among PROP taster groups in the B2 session (Table 9) but the difference disappeared after Bonferroni correction,

suggesting the difference was caused by a type I error, or alternatively, the variation may be too high to reach a level of statistical significance. Therefore, a conclusion can be drawn that PROP status has no effect on sensory thresholds for sucrose and linoleic acid in both B and NB trials of the present study. Additionally, the differences in thresholds within the whole subject group and within each taster group were explored. For the comparison within the whole subject group, no significant differences or correlations were found in both B and NB trial. With respect to differences within each taster group, there was only one significant difference between sucrose thresholds before breakfast and after breakfast emerging in the NT group.

The effect of metabolic status on sensory thresholds was investigated. Postprandial thresholds for sucrose were significantly higher than corresponding fasting thresholds ($p<0.05$), indicating breakfast has an effect on sucrose thresholds in nontasters while continuous fasting has no effect. Neither fasting state nor satiated state influenced fatty acid thresholds within each PROP taster group.

In the light of the methodological approach (i.e. the use of nose clips, antioxidants, gums and mineral oils) and the low fatty acid concentrations used, it is confident that the thresholds reported in the present study were based on differences in oral chemosensory sensitivity, and not on additional orosensory cues such as olfaction, irritation and texture (Chale-Rush *et al*., 2007). In addition, based on previous animal studies, specificity of sensitivity to FAs varying in saturation on different regions of the tongue have been reported (i.e., sensitivity to polyunsaturated fatty acids on the anterior tongue and monounsaturated fatty acids on posterior tongue) (Gilbertson *et al*., 1997; Hansen *et al*., 2003) Furthermore, an uneven distribution of CD36 receptor in different papillae has been observed (i.e. concentrations for circumvallate > foliate > fungiform) (Laugerette *et al*., 2005). However, the present work involved whole-mouth stimulation, so any regional differences may not have influence the results. Although all the environmental and orosensory cues are minimised, another factor should be considered. Removing the fatty acid may require more than a single water rinse because the hydrophobic nature of fatty acids likely gives them an affinity for the taste cell membrane. Failure to adequately remove the fatty acid from the membrane could produce carry-over effects during the sensory testing procedure

(Reckmeyer, 2010). In this case, even though the subjects rinsed their mouths between sample pairs, this may not have been sufficient to remove residual fatty acids.

The significant difference of the recognition thresholds for sucrose observed by Zverev (2004) in fasted and in satiated conditions was not found in the present study but it is consistent with another study reporting no significant variation for sucrose thresholds in satiated and fasted states (Pasquet *et al.*, 2006). Pasquet *et al*. (2006) measured the sucrose recognition thresholds by the staircase-method with pure chemicals in water and showed the detection thresholds in fasted and satiated states were 40.1 mM (SD 7.2 mM) and 41.4 mM (SD 6.1 mM), respectively. These values are much higher than those observed in the present study and there are some possible explanations for the differences. Pasquet *et al*. measured recognition thresholds while the present study presented detection thresholds. The recognition threshold requires higher levels of stimulation than detection thresholds as subjects need to identify the nature of the tastant. Furthermore, experimental differences, such as preparation techniques and sensory test procedures could explain differences between these studies. In some studies, sucrose has been reported to be sweeter to PROP tasters than to PROP non tasters (Bartoshuk, 1979; Looy and Weingarten, 1992). In contrast, some other studies found no relations between PROP taste status and the perceived sweetness of sucrose solutions (Drewnowski *et al*., 1997; Ly and Drewnowski, 2001).

It was expected fatty acid results would be similar to those of Chale-Rush *et al*. (2007) because we used the similar fatty acid concentrations, similar preparation techniques (fatty acid added in gum vehicle then homogenized) and similar sensory test procedures (3-AFC method). Instrumental methods could not confirm a textural contribution of fatty acids from viscosity at the threshold level and visual identification has been diminished by conducting all testing under red light. However, linoleic aicd thresholds were much higher than those previously reported. Chale-Rush *et al*. (2007) reported the mean detection threshold for linoleic acid was 1.12 mM (SEM 0.285 mM) with minimal input from the olfactory, capsaicin, and viscosity-assessing tactile systems. Results are also higher than other previously reported thresholds 0.28 mM (Mattes, 2009) and 1·5 mM (Stewart *et al*., 2010). Mattes obtained thresholds after post-desensitization which could be an explanation for his

markedly lower threshold values. Also, the differences between previous reported mean values and this study's median values could also be a reason for differences.

It is hypothesised that there was a link between PROP status and oral taste perception and it was speculated that PROP status could perhaps be used as an indicator of fat sensation. However, in the present study no significant effects of PROP taster status on the perceived sweetness and fatty acid taste perception were found. The findings from previous studies have reported conflicting outcomes in terms of possible relationships between PROP status and other taste intensities. For sucrose, Bartoshuk (1979) and Looy and Weingarten (1992) have reported tasters perceive more intense sweet than nontasters while others found no relations between PROP taste status and the perceived sweetness of sucrose solutions (Drewnowski *et al.*, 1997; Ly and Drewnowski, 2001). For fatty acid taste perception, the results are in agreement with those of Drewnowski *et al.* (1998) and Guinard and Yachinous (1999) who report no association between fat perception and PROP taster status, in contrast to those of Duffy *et al.* (1996) and Tepper and Nurse (1997 and 1998). Specifically, in studies of Kamphuis *et al.* (2001 and 2003), PROP taster status was not related to linoleic acid taster status. Kamphuis *et al.* (2003) divided 24 women into linoleic acid tasters and linoleic acid nontasters based on their sensitivities to a 10μM linoleic acid solution. They investigated the relationship between linoleic acid taste perception and food intake regulation in terms of food or energy intake, or satiety and reported linoleic acid taster status was related to food intake regulation but not PROP status. Therefore this work indicates that other mechanisms not related to PROP sensitivity are affecting peoples overall sensitivity to fatty acid taste. This could be explained by the CD36 receptor and its role in fatty acid taste detection (Pepino *et al.*, 2012).

Pepino *et al.* (2012) studied the role of lingual lipase and CD36 on fat detection thresholds on 23 obese subjects because greater high-fat food preferences in obese people as compared to lean people were reported. One of their hypotheses suggested that reduced CD36 expression was associated with higher oral fat detection thresholds (i.e. lower oral sensitivity to fat). The results provided strong support that the existence of a taste component in the orosensory perception of dietary fat in obese subjects. In addition, Pepino *et al.* (2012) also reported that subjects with lower CD36

expression were less sensitive in detecting oleic acid, suggesting that CD36 genotype affected orosensory detection of fats in humans.

Combining with previous studies, Pepino *et al*. (2012) further proposed that FA detection thresholds are not related to the sweet or bitter taste perception by investigating the relationship between CD36 and alpha-gustducin. It is known that alpha-gustducin co-expressed with T2R family and T1R3, are involved in signal transduction of bitter and sweet taste, respectively (Adler *et al*., 2001; Wong *et al*., 1996). Martin *et al*. (2011) reported that alpha-gustducin expression levels in taste buds are not related to conditions of lower CD36 expression or no CD36 expression in mice, indicating that alterations in CD36 expression do not associate with changes in gustducin expression. In addition to the variations in gene expression, alpha-gustducin is not involved in fat taste signalling. Alterations in fat detection thresholds are related to the reduced CD36 expression but not the changes in gustducin expression (Love-Gregory *et al*., 2011, Ghosh *et al*., 2011) as alpha-gustducin knockout mice have decreased sensitivity for bitterness and sweetness (Wong *et al*., 1996) but the same fat preferences as those of wild type mice (Sclafani *et al*., 2007). One possible explanation suggests that the signaling mechanisms involved in CD36-mediated fat perception involve pathways distinct from those involving alpha-gustducin (Khan and Besnard, 2009). Therefore, as CD36 expression is not related to alpha-gustducin expression and CD36 does not share the same signalling pathway with alpha-gustducin, suggesting that fat taste perception is not related to the prototypical taste perception which has been confirmed by the present study.

According to the study of Nakamura *et al*. (2008), in the normal feeding condition (three meals a day), leptin concentrations started to rise before noon and peaked at night. Similar to plasma leptin levels, taste recognition thresholds for sweetness showed significant time-dependent increases. They also reported that increase in blood glucose of individuals after meals was negatively correlated with recognition of sucrose thresholds, suggesting that sweet sensitivities before meals may influence postprandial increases in glucose levels. Although leptin levels were not measured in the present study, a negative correlation between sucrose thresholds and plasma glucose changes were expected. However, the present study found, as expected, that plasma glucose levels (see Table 10) were significantly increased after breakfast, while detection sucrose thresholds did not show any differences after meal

consumption. Also, no significant relations between fasting sucrose thresholds and the postprandial increases in plasma glucose as contrasted with the Nakamura *et al*. (2008) study.

To our knowledge, this is the first study to investigate the relationship between metabolic state and fatty acid threshold values. We can conclude that metabolic state, fasting state nor a satiated state, has no effect on fatty acid threshold values. Furthermore, as shown above (Table 9), there was no apparent relationship between sweet or fatty acid taste perception and circulating blood parameters of glucose or NEFA.

4.3.2 Comparison and correlation in sensory thresholds

The present study investigated the differences in blood metabolites within taster groups as well as between groups.

Table 10 Mean concentrations of plasma metabolite parameters (glucose, TC,TG, HDL-TC and NEFA) for each PROP taster group and comparison between/within taster groups during fasted and satiated states

	Glucose (mmol/L)					
	Before breakfast session (B1)	After breakfast session (B2)	*P* value (within groups)[3]	Before break session (NB1)	After break session (NB2)	*P* value (within groups)[3]
Total (n=40)	5.27±0.07	6.06±0.19	0.00*	5.26±0.06	5.2±0.07	0.091
NT(n=10)	5.19±0.11	6.05±0.35	.025*	5.25±0.12	5.28±0.08	0.859
MT (n=20)	5.27±0.08	6.32±0.28	.002*	5.27±0.08	5.2±0.12	0.058
ST(n=10)	5.36±0.21	5.55±0.38	0.475	5.24±0.14	5.15±0.14	0.678
P value (between group)[1]	0.683	0.272		0.984	0.817	
	Cholesterol (TC) (mmol/L)					
	Before breakfast session (B1)	After breakfast session (B2)	*P* value (within groups)[4]	Before break session (NB1)	After break session (NB2)	*P* value (within groups)[4]
Total (n=40)	4.55±0.14	4.61±0.14	0.027*	4.51±0.14	4.6±0.15	0.004*
NT(n=10)	4.33±0.24	4.42±0.24	0.139	4.23±0.24	4.36±0.27	.022*
MT (n=20)	4.63±0.21	4.73±0.23	0.057	4.64±0.21	4.75±0.23	.044*
ST(n=10)	4.59±0.3	4.57±0.27	0.508	4.52±0.31	4.55±0.29	0.799
P value (between groups)2	0.69	0.68		0.49	0.56	
	Triglycerides (TG) (mmol/L)					
	Before breakfast session (B1)	After breakfast session (B2)	*P* value (within groups)[3]	Before break session (NB1)	After break session (NB2)	*P* value (within groups)[3]
Total (n=40)	1.06±0.07	1.08±0.07	0.354	1.22±0.08	1.15±0.09	0.001*
NT(n=10)	1.17±0.11	1.18±0.11	0.838	1.19±0.13	1.18±0.16	0.386
MT (n=20)	1.11±0.08	1.14±0.1	0.794	1.12±0.1	1.02±0.09	0.001*
ST(n=10)	1.29±0.19	1.2±0.16	0.059	1.44±0.24	1.38±0.24	0.415
P value (between groups)[1]	0.57	0.95		0.5	0.65	
	High density lipoprotein cholesterol (HDL-TC) (mmol/L)					
	Before breakfast session (B1)	After breakfast session (B2)	*P* value (within groups)[4]	Before break session (NB1)	After break session (NB2)	*P* value (within groups)[4]
Total (n=40)	0.99±0.03	1.01±0.03	0.261	0.98±0.04	1±0.04	0.006*
NT(n=10)	0.97±0.05	0.97±0.05	0.838	0.91±0.05	0.95±0.05	0.047*
MT (n=20)	0.98±0.05	1.01±0.05	0.823	1.01±0.06	1.03±0.06	0.121
ST(n=10)	1.05±0.08	1.04±0.07	0.878	0.98±0.06	1±0.06	0.123
P value (between groups)[2]	0.64	0.79		0.31	0.14	
	Non esterified fatty acid (NEFA) (mmol/L)					
	Before breakfast session (B1)	After breakfast session (B2)	*P* value (within groups)[3]	Before break session (NB1)	After break session (NB2)	*P* value (within groups)[3]
Total (n=40)	0.26±0.02	0.15±0.01	0.00*	0.26±0.02	0.33±0.03	0.001*
NT(n=10)	0.25±0.03	0.14±0.02	.005*	0.25±0.04	0.31±0.03	.047*
MT (n=20)	0.21±0.02	0.14±0.02	.009*	0.24±0.02	0.34±0.05	.004*
ST(n=10)	0.37±0.05	0.17±0.02	.013*	0.31±0.05	0.36±0.07	0.444
P value (between groups)[1]	0.00*	0.49		0.35	0.85	

Mean ± SEM
n. number of subjects
* Significance *P* value <0.05
[1] Kruskal-Wallis test was used to measure differences between NT, MT and ST group for plasma glucose, TG and NEFA

[2]. One-way ANOVA was used to measure differences between NT, MT and ST group for plasma TC and HDL-TC
[3]. Wilcoxon Signed-rank test was used to measure differences within each taste group between B1and B2, NB1and NB2 for plasma glucose, TG and NEFA
[4]. Paired sample T-test was used to measure differences within each taste group between B1and B2, NB1and NB2 for plasma TC and HDL-TC

From Table 10, the following trends or patterns can be described: The effect of PROP status on blood metabolites were analysed and only one significant difference was found in NEFA concentrations during the overnight fasting condition in the B trial ($p<0.05$). However, the difference disappeared after Bonferroni correction, suggesting the difference was caused by type I error. Therefore, PROP taster status does not seem to have any effect on blood metabolites.

The mean plasma glucose concentration for the group as a whole was significantly different after meal consumption as shown Table 10. The elevated postprandial glucose concentration indicates the significant effect of meal consumption on plasma sucrose concentration. For each taster group, all the postprandial glucose concentrations were increased but only the differences in NTs and MTs were significant. Therefore, the effect of meal consumption was found in NTs and MTs but not in STs.

The mean plasma NEFA concentration for the group as a whole shows a significant decrease in the breakfast trial and a significant increase in the non-breakfast trial. Within each taster group, the postprandial NEFA concentrations were significantly different from their corresponding fasting concentrations while in the NB trial, significant differences were observed in the NT and MT groups. Hence, as expected, both fasting state and satiated state have clear effects on plasma NEFA concentrations in NTs and MTs whereas STs only show significant differences in the NEFA level after breakfast.

The mean plasma TG concentrations were significantly reducted after continuous fasting within the MT group and within the whole subject group in the NB trail. For HDL-Cholesterol, the effects of the continuous fasting state were found in NTs and the whole subject group. The mean plasma TC concentration for the group as a whole showed significant increases under the condition of both fasting and satiated states. NTs and MTs have also showed significant increases after break in NB trial.

There were no significant relationships between blood metabolites and sensory thresholds; there was no apparent link between sweet or fatty acid taste perception and circulating blood parameters of glucose or NEFA. To fully test the hypothesis that altered metabolic marker concentrations may serve as indicators of sweet or fatty acid taste sensitivity, further work is required to measure circulating hormones that have previously been linked with sweet or fatty taste perception. However, the data of the present study provide an indication that the metabolic state of the subjects is consistent with the treatments of fasting and consumption of food at breakfast, and therefore provides an important quality control check i.e. subjects did fast as requested.

It is unlikely that the effect of the sensory stimuli influenced metabolic markers through ingestion of tastants. All trial subjects were instructed to expectorate the tastants to a waste cup and even if small amounts of the fatty acid or sucrose sample was ingested, the increment would be too small to account for the noted effect. Hence, ingestion of a small amount of fat or sucrose should be neligible and oral exposure to the tastants can be solely considered.

A number of basic research studies in animals lend weight to the rationale that oral taste perception of sweet or fatty acids may influence metabolic regulation. For example, studies in rats show that the degree of oral exposure to fat alters the postprandial lipid profile (Ramirez, 1985 and 1992). In addition, another study reported that oro-nasal exposure to dietary fat can influence postprandial lipid metabolism in humans (Mattes, 1996). Mattes (1996) explored the sensory attributes of high-fat oral stimuli that may influence the metabolic response and reported that the postprandial rise of plasma TG was significantly higher and of longer duration when associated with mastication of the full-fat stimulus compared with each of the approprpriate controls. Furthermore, it is well established that after oral exposure to fat, plasma TG levels rise after food consumption. Furthermore the fasting concentrations of circulating TG levels are a major determinant of the postprandial TG response after a meal (Lambert and Parks, 2012). In addition, it is known that diet composition can affect the plasma TG level. Zampelas *et al.* (1994) showed a

decreased TG concentration in plasma after meals containing large amounts of long-chain n-3 polyunsaturated fatty acids compared with a meal containing mixed oil. The fatty acid composition has a clear influence on metabolic parameters.

In the present study, only a small change in postprandial TG concentration was observed with continued fasting in the NB trial.

The concentration of plasma cholesterol after fat feeding has been measured in numerous other studies; however, changes have either been negligible in magnitude or had high variance between subjects (Barr *et al*., 1985; Tall *et al*., 1982). In addition, the HDL-cholesterol levels were inversely related to TC concentrations (Cohn *et al*., 1988). Higher fasting HDL levels tended to be associated with a postprandial decrease in total plasma cholesterol and, conversely, subjects with a lower fasting HDL level tended to show an increase in plasma cholesterol. The present results are in agreement with this idea showing a small increase in circulating concentrations of HDL-cholesterol with prolonged fasting in the NB trial.

NEFA, also known as free fatty acids (FFA), are the form in which stored body fat is transported from adipose tissue to its sites of utilization and regulation of the plasma NEFA concentration is an important path metabolic regulation during feeding and fasting. Its concentrations in normal, healthy people reach high levels after an overnight fast or during aerobic exercise and are reduced after a meal (Frayn *et al*., 1997). In the present study fasting was associated with low plasma NEFA concentrations, and NEFA levels significantly increased after breakfast. However, neither sucrose nor fatty acid taste perception appeared to be influenced by changes in circulating concentrations of NEFA during fasting or after a meal.

5 Conclusion and Recommendation

The results show that compared with water bath and sonicator, high pressure homogenizer produces a better dispersed and more stable emulsions. According to three-solution PROP test, subjects were classified in to three groups, including 25% NTs, 50 MTs and 25% STs. No significant correlations were found between fungiform papillae density and sensory thresholds, including sucrose and LA. For body phenotypes, BMI is significantly correlated with age, and in this research it did not correlate with PROP status or sensory thresholds. In addition, metabolic states did not have any effect on fatty acids thresholds and no relationship between fatty acid thresholds and blood metabolic parameters was observed. Furthermore, since no significant differences in blood metabolite concentrations were found across PROP taster group, PROP cannot be considered as a predictor to any of the blood metabolites.

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7 Appendix

1 A Labelled Magnitude Scale