THE EFFECT OF FOOD FLAVOUR ON HUMAN APPETITE AND EATING BEHAVIOUR

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ABRIEVICATION

BMI	Body Mass Index
BMR	Basal Metabolic Rate
GI	Gastrointestinal
TR	Taste Receptor
CNS	Central Nervous System
NTS	Nucleus of the Solitary Tract
ACC	Anterior Cingulate Cortex
OFC	Orbitofrontal Cortex
LHA	Lateral Hypothalamus (satiety centre)
NTS	Nucleus of the Solitary Tract
GLP-1	Glucagon-like Peptride-1
PYY	Peptide YY
CMC	Carboxymethyl Cellulose
SSS	Sensory-Specific Satiety
SSA	Sensory-Specific Appetite

Abstract

Overconsumption of foods is thought to be one of the main causes of the rising number of global obesity. This thesis aims to investigate the role of food flavour in human appetite and eating behaviour through three studies.

The first study investigated whether the sweetness intensity of a milkshake affected *ad libitum* intake of the milkshake and sensory-specific satiety (SSS). In a crossover single-blinded design, 24 participants consumed *ad libitum* high, ideal and low sweetness (HS, IS or LS) milkshakes over three visits. After milkshake intake, participants consumed *ad libitum* one, or both of a sweet and a savoury snack. All milkshake consumption was similar, suggesting that the sweetness intensity did not affect the *ad libitum* intake of the milkshake. After intake of all sweet milkshakes, ratings of desire for something sweet decreased, and subsequent savoury snacks were consumed more than subsequent sweet snacks. The sweetness intensity of milkshakes did not affect the change in the desire for something sweet or the subsequent snack intake. Ratings of desire for something savoury increased after the intake of HS milkshake and were higher than the ratings collected following the intake of IS milkshake. Therefore, this study suggested that a sweeter milkshake did not affect the magnitude of SSS for sweet foods, but increased a stronger sensory-specific appetite (SSA) for savoury foods.

The second study examined the effects of aroma, taste and their interaction on subjective appetite sensation and subsequent lunch intake. In a crossover design, 26 females consumed 1 of the 4 test drinks as a preload: 1) water; 2) strawberry aroma in water; 3) sucrose and citric acid in water; 4) strawberry aroma, sucrose and citric acid in water. The subsequent lunch intake did not differ after all drink preloads. The drink with only aroma or only taste were not different from water in affecting appetite sensation. A drink with both aroma and taste reduced hunger ratings greater than water or a drink with only taste or aroma, during 15 min drinking and up to 30 min post drinking. Meanwhile, the drink with

both taste and aroma was the highest in perceived flavour intensity. This suggests enhancing flavour perception of a drink through aroma-taste cross-modal interaction can increase the satiating effect of a drink.

The third study investigated effects of sweetness, thickness and caramel flavour perception of custards on expected satiation and expected satiety of the custards. 90 participants (65 females, 25 males) tasted 18 custard samples over two sessions. Ingredients of custards were different only in the concentrations of caramel aroma, Truvia sweetener and carboxymethyl cellulose (CMC), based on an experimental design. Thickness enhanced both expected satiation and expected satiety. Sweetness enhanced expected satiation but not expected satiety. Caramel flavour did not affect expected satiation or expected satiety. The cognitive expectation on satiation and satiety has previously been shown to determine self-selected portion size. Therefore, the current study suggests that manipulating sweetness and thickness perception of a food without changing its energy content might help portion size control, via manipulating consumers' cognitive expectation of the food.

In conclusion, manipulating food flavour is a promising area to explore with the respect to hunger suppression and fullness enhancing, limiting the intake of eaten foods while promoting intake of other foods via SSS or SSA, and contributing to the cognitive control of portion size. Therefore, manipulation food flavour might be helpful for appetite control and supporting an energy-restrict diet; however, it seems challenging to reduce actual food energy intake through manipulating only the flavour properties of foods.

Chapter 1. General Introduction

1.1 Overweight and Obesity

1.1.1 Health risk and economic impact

The increase in the human race classified as overweight is a rising global issue. Around 39% of the world adult population are overweight (BMI ≥ 25 kg/m^2), of which 13% are obese (BMI ≥30 kg/m^2) (WHO, 2016b). This has significant health implications, resulting in increased risk of type-2 diabetes, cardiovascular diseases, ischemic stroke, cancer and mortality (WHO, 2016b). Excessive weight is also associated with mental health disorders including low self-esteem and depression (Muzy et al., 2013). The rising overweight and obesity place an increased economic burden. Globally, overweight and obesity account for approximately 9.1% of a country's total healthcare expenditure (Withrow and Alter, 2011). In the UK, the estimated direct NHS costs associated with overweight and obesity are £6.3 billion, and indirect costs are £27 billion by 2015 (NHS, 2010). Despite many efforts to address and prevent the problem, the rate of overweight and obesity is still increasing each year. Therefore, there is an urgent need to discover novel solutions to tackle this issue. This thesis aims to explore whether managing food flavour might help weight management through its effect on appetite and eating behaviour.

1.1.2 Definition and classification

Overweight and obesity is the condition referring to a person with excessive body fat accumulation that leads to a risk to health (WHO, 2016c). The Body Mass Index (BMI), calculated as the weight (kg) divided by the square of height (m^2), is the most commonly used approach to classifying overweight and obesity in adults (Deurenberg et al., 1991). BMI provides a useful indication of the negative health consequences in relation to weight, for the majority of the population (Deurenberg et al., 1991). The World Health Organization (WHO) recommends a healthy BMI range of 18.5 to 24.9 kg/m^2 and classifies overweight as a BMI over 25

kg/m^2 and obese as a BMI above 30 kg/m^2 (WHO, 2016c). However, BMI does not always correlate with body fat content to the same degree for different individuals or populations, such as athletes (Forbes and Reina, 1970). Athletes can have a BMI above 25 kg/m^2, not because of excessive fat, but rather due to their high body muscle content. The waist circumference or the waist-hip ratio is recommended as an additional method to indicate the distribution of body (WHO, 2008).

1.1.3 Causes

Fundamentally, overweight and obesity is caused by long-term positive energy balance between energy intake and energy expenditure (Hill et al., 2012). Energy intake results from the consumption of foods and drinks, while energy expenditure is the energy used by the body, which includes basal metabolic rate (BMR), diet-induced thermogenesis and physical activity (Bomer et al., 2015). The first law of thermodynamics states that energy is never created nor destroyed but rather transferred between different forms. Energy balance in the human body can be defined by the following equation (Frayn, 2010):

Energy intake (food) = Energy expended (heat + work) + Energy stored

The relationship between energy intake and expenditure dictates whether the weight is stable, lost, or gained. Positive energy balance (intake>expenditure) results in the transformation of the excessive energy intake into body stores, mainly adipose tissue. Adipose tissue is made of 75% fat and 25% non-fat tissue (3/4 water and 1/4 protein) (Gandy and Garrow, 2007).

Globally, there has been a reduction in physical activity due to changes in lifestyle (WHO, 2016a). Decreased energy expenditure is believed to contribute to the rising number of overweight and obese. Concurrently, increased intake of highly palatable and energy-dense foods, resulting in excessive energy intake, has contributed to an excessive weight gain in

many developed countries such as the USA (Le Page, 2016, Trude et al., 2016, Swinburn et al., 2009).

1.1.4 Control of energy intake as a solution

A reduced energy intake, which results in a negative energy balance, can be an effective way in reducing body weight (Hill et al., 2012, Herrmann et al., 2015, Westerterp-Plantenga et al., 1998). Even a small reduction in body weight had been shown to reduce the risk of developing physical and emotional diseases for the obese individuals (Goldstein, 1992). The food industry can potentially play a crucial part in supporting an appropriate diet, by offering a range of commercial options with energy reduction (Verduin et al., 2005). Recently, there has been a rising interest in developing satiation- and satiety-enhancing food products. Such foods can suppress hunger sensation and increase fullness for longer, and hence may help intake control and support weight management.

This thesis hypothesized that food flavour might be helpful for weight management through its potential influence on appetite sensation (satiation and satiety), food intake, sensory-specific satiety and cognitive belief of foods (expected satiation and expected satiety). The introduction Chapter 1 will first explore the basic physiological mechanisms of the human body with respect to appetite regulation and food intake control, followed by psychological mechanisms including sensory-specific satiety and expected satiation or satiety in sections 1.5 and 1.6. Important food factors that influence appetite will be summarised in section 1.7. A brief overview of the physiology of flavour modalities (gustatory, olfactory and oral somatosensory), and their multimodal interaction on food flavour perception will be summarised in section 1.8. Last but not the least, a literature review of current findings on the role of flavour in appetite regulation and eating behaviour will be outlined. Knowledge gaps and research opportunities will be highlighted.

1.2 Eating behaviour and appetite (concepts)

1.2.1 Eating behaviour

While the need for energy expenditure is continuous, food intake occurs as discontinued eating episodes (Blundell and Burley, 1987). **Eating behaviour** is any action of a living creature related to the procurement of foods and nutrients. Eating behaviours are motivated behaviours (Epstein 1982) which are motivated by biological needs, psychological desire for pleasure and comfort, and it is influenced by economic, social and cultural aspects (Mela, 1996). The motivation for feeding behaviour can be explained by a general term ‘appetite’.

1.2.2 Appetite

The definition of appetite in the literature is inconsistent. This thesis used a broad definition of appetite. **Appetite** is defined as ‘a desire or motive derived from a biologic or psychological need for food’ (Dictionary, 2012). The term appetite can be used in relation to the whole area of food selection, preference, intake and motivation (Blundell et al., 2010).

A few motivational terms can describe the appetitive status in different dimensions including hunger, fullness, satiation and satiety. **Hunger** is ‘the discomfort, weakness, or pain caused by a prolonged lack of food’ (Oxford Dictionaries, 2016), and it is the conscious sensation reflecting the drive to eat (Blundell et al., 2010). On the contrary, **fullness** is the state of having eaten enough or more than enough and feeling full up (Oxford Dictionaries, 2016).

Satiation is ‘the state of being satisfactorily full and unable to take on more’ (The American Heritage Medical Dictionary, 2007). **Satiation** occurs when the appetite for food has been satisfied and leads to the termination of an eating episode (Benelam, 2009). **Satiety** describes the feeling of fullness and satisfaction that persists after the eating period, which may suppress the further intake of food until the feeling of hunger returns (Benelam,

2009). Satiation and satiety have both physiological and psychological dimensions. In comparison, hunger sends signals to trigger and encourage intake, while satiety and satiation inhibit eating.

1.3 The 'Satiety Cascade'

The 'satiety cascade' was first proposed by Blundell (1987). A modified version of the cascade (Blundell et al., 2010) can be used as a framework for studying the influence of food-related factors on satiation and satiety (**Figure 1-1**). It outlines the various signals and processes that lead to the termination of an eating episode (satiation), and the processes that further suppress appetite and intake after eating until hunger returns (satiety) (Blundell et al., 1987, Blundell and Bellisle, 2013, Harrold et al., 2012). Both physiological and psychological factors contribute to the satiation and satiety process.

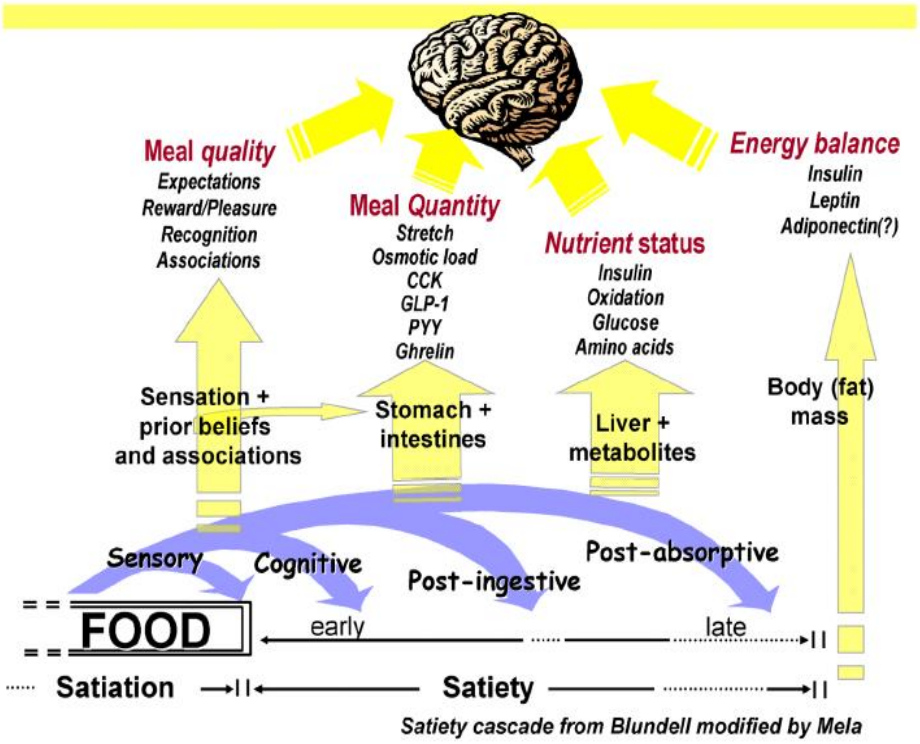

Figure 1-1 Satiety cascade (Blundell et al., 2010)

At the pre-prandial (before meal) stage, internal hunger signals contribute to the initiation of food intake. **Sensory information** about the food including taste, flavour, and texture, visual and auditory cues may also enhance hunger and stimulate intake in the pre-prandial and early prandial phases. Towards the end of an eating event, sensory cues may be one of the satiation cues that contribute to the termination of the meal. Sensory cues may potentially induce satiety signals that influence time to next meal and subsequence intake through both psychological and physiological mechanisms (Blundell et al., 1996, Blundell and Bellisle, 2013).

Cognitive factors such as the beliefs, expectation or association of the foods, conscious control over the amount eaten, awareness of the ambient environment, or memories about the previous eating experiences could also affect the eating behaviours before, during and after the meal (Blundell and Bellisle, 2013). The sensory properties of food may also evoke different cognitive responses that affect eating behaviours.

Post-digestive signals from the gastrointestinal (GI) tract and various hormonal signals contribute to the termination of eating and inhibiting of further eating (Bellisle and Blundell, 2013).

Digested nutrients are absorbed in the small intestine, and are transported in the blood or lymph circulation. The digestion products of carbohydrate, protein and lipids are monosaccharides, amino acids and triacylglycerol with limited fatty acids. These **post-absorptive** (after meal) **signals** have been found to influence appetite and energy intake (Morton et al., 2006). At the end of the post-absorptive phase, hunger returns and another eating event may follow.

Long-term control of energy balance is affected by the adipose tissue and pancreas with the release of leptin and insulin hormones (Niswender, 2008).

While the proposed ‘satiety cascade’ model is helpful in providing a general framework for understanding the underlying processes and

mechanisms of appetite and food intake control, studies should not be limited by this model. For example, the role of the cognitive factors was limited to the 'early satiety phase', but cognitive beliefs about food has been shown to have a prolonged impact on satiety up to 3 hours after the meal (Brunstrom et al., 2011). Similarly, the time effect of the sensory factors may persist into the post-digestive stage.

Understanding the physiological mechanisms involved in managing appetite regulation and food intake, and eating behaviour will provide potential targets for the design of food-related cues to influence satiation and satiety. Therefore, physiological mechanisms for appetite regulation are outlined in the following section 1.4.

1.4 Physiological mechanisms for appetite regulation

There are two interactive physiological mechanisms for appetite regulation. One is the gastrointestinal tract (GIT) mechanism, while the other is the brain mechanism (Cegla et al., 2010). At the GIT level, the ingestion of foods evokes satiation and satiety by two main types of signals, GI distention and release of hormonal peptides (Cummings and Overduin, 2007). These signals from the GI tract are transmitted to the brain via both the nerve systems (vagal nerve and the spinal afferent system) and circulation (Dockray and Burdyga, 2011).

1.4.1 Gastrointestinal mechanisms

Mechanoreceptors and chemoreceptors located on the gastrointestinal tract send physical and chemical signals to the central nervous system in order to regulate appetite and control intake (Harrold et al., 2012). The gastric satiation signals are primarily from gastric distention (mechanical) while the intestinal satiation signals are mostly from the effect of chemical nutrients with a small part from the intestinal distension (Powley and Phillips, 2004).

1.4.1.1 Gastric signals

The stomach itself promotes satiation by signalling gastric distension to the brain, and the brain therefore may send inhibiting signals to delay the gastric emptying of food (Cummings and Overduin, 2007). Mechanoreceptors in the stomach wall detect changes in the stretch tension and volume of the stomach caused by the amount of food present (Harrold et al., 2012). These receptors communicate this information about gastric distension to the brain via the vagal nerve and spinal sensory nerves (Ritter, 2004). Gastric distension seems to promote satiation independently of the nutritional composition of the food (Phillips and Powley, 2000).

Ghrelin, a gut hormone, produced mainly by the endocrine cells of the stomach, and proximal small intestine, stimulates appetite and promotes food intake (Cheng et al., 2010). Ghrelin receptors are located in the hypothalamus, heart, pancreas, intestinal, lung and adipose tissue (Kaiya et al., 2014). Ghrelin actions may be mediated by vagal nerve or directly via ghrelin receptors in the brain (Cummings, 2006). Plasma ghrelin levels rise during fasting and drop quickly on feeding within 1 hour (Callahan et al., 2003, Cummings and Shannon, 2003, Tschop et al., 2001). During consumption, the plasma ghrelin levels decrease in relation to the decrease in subjective hunger sensation (Cummings et al., 2004).

1.4.1.2 Intestinal signals

Like the stomach, there are receptors located on the small intestinal wall that detect stretch signals and send them to the brain via vagal afferent nerve (Harrold et al., 2012). The chemoreceptors in the intestine can detect the nutritional composition of the food and the absorption information of the nutrients, then relay this information to the brain and signal satiation (Harrold et al., 2012). Infusion of protein, carbohydrates and fat directly into the small intestine enhances satiation (Ritter, 2004).

The primary intestinal satiating signal is the release of gut peptides (hormone) from the Enteroendocrine cells (Kairupan et al., 2016). These hormonal signals from the intestine are ‘episodic signals’ of satiation (or satiety) which occur in episodes of eating and are involved in short-term appetite regulation (Benelam, 2009). Together with the GI distension, hormonal signals increase the perception of fullness, contributing to meal termination (Cummings and Overduin, 2007).

1.4.1.3 Intestinal hormonal signals

1.4.1.3.1 Cholecystokinin (CCK)

Cholecystokinin (CCK) is released from the endocrine L-cells in the beginning and middle part of small intestine (Roth et al., 1992). CCK is released in response to intake of nutrient, and is decreased gradually after consumption (Kairupan et al., 2016). CCK modifies short-term satiation, but not for long-term weight control (Kairupan et al., 2016). CCK can act directly to the brain hypothalamus which is believed to be the homeostatic energy control centre (Akieda-Asai et al., 2014). In addition, CCK mediates its effects on satiation primarily via the vagal nerve, which was supported by the fact that CCK receptors are expressed in the vagal afferent neurons (Beglinger et al., 2001, Moriarty et al., 1997). Peripheral administration of CCK results in a delayed gastric emptying and a reduction in meal intake in humans (Kuyumcu et al., 2013, Muurahainen et al., 1988, Tenk et al., 2014).

1.4.1.3.2 Glucagon-like petide-1 (GLP-1)

Glucagon-like petide-1 (GLP-1) is produced mainly in the intestine, the pancreas and the brain, in response to glucose ingestion (Kairupan et al., 2016, Zemankova et al., 2015a), and also to high protein food intake (van der Klaauw et al., 2013). GLP-1 acts through GLP-1 receptors in the pancreas, brainstem, hypothalamus and vagal nerves (Imeryuz et al., 1997, Parkinson et al., 2009, Tomlinson et al., 2016). GLP-1 is believed to contribute to the inhibition of the gastric motility and emptying (Imeryuz et

al., 1997, Tong and D'Alessio, 2014). Peripheral and central administration of GLP-1 has been found to suppress hunger, enhance fullness and reduce intake in humans (Shah and Vella, 2014). GLP-1 also plays an important part in glucose homeostasis, by inducing the release of glucose-dependent insulin, and reducing secretion of glucagon (Drucker, 2006, Zemankova et al., 2015b).

1.4.1.3.3 Peptide YY (PYY)

Peptide YY (PYY) is produced by the L-cells in the ileum, and is increased along the intestine, and reaches its highest concentration in colon and rectum (Kairupan et al., 2016). PYY mediates its effect on satiety mainly by binding to the Y2 receptors in the hypothalamus (Abbott et al., 2005). PYY enhances satiety, delays gastric emptying and intestinal transport (Benelam, 2009). PYY is co-secreted with GLP-1 and both are released into the circulation in proportion to energy consumption (Adrian et al., 1985). Plasma PYY increases following food consumption, reaches a peak level 1-2 hours after, and it remains above the baseline for 6 hours (Kairupan et al., 2016, Little et al., 2014). The macronutrients of the meal influence the PYY release: protein stimulates higher release than carbohydrates and lipids (Batterham et al., 2006). Intravenous administration of PYY decreased food intake in humans (Batterham et al., 2002, Batterham et al., 2003).

1.4.2 Long-term mechanisms

‘Tonic signals’ of satiety indicate the energy storage in the body to the brain, allowing the long-term regulation of energy intake and expenditure and body weight control (Benelam, 2009). Liver, pancreas and adipose tissue are the organs implicating the energy storage in the body (Mohamed-Ali et al., 1998).

1.4.2.1 Adipose tissue and leptin

The adipose tissue synthesises the peptide hormone leptin. Blood leptin levels are in proportion to the amount of adipose tissue and BMI, and are decreased by weight loss (Ben Slama et al., 2015, Houde et al., 2015). Leptin may act directly on the hypothalamus of the brain to reduce food intake and increase energy expenditure (Friedman, 2015). Leptin levels influence the sensitivity of short-term signals of CCK and GLP-1 (de Graaf et al., 2004).

1.4.2.2 Pancreas: insulin

Insulin, produced by the ß cells of the pancreas, is thought to influence long-term energy balance. Insulin secretion rises rapidly following food intake and acts to regulate blood glucose levels (Polonsky et al., 1988). Administration of insulin results in a reduction in food intake and body weight (Ikeda et al., 1986). In the long term, blood insulin level is increased with increasing adipose tissues, suggesting insulin resistance in obese population (Benelam, 2009).

1.4.3 The brain mechanisms

1.4.3.1 Hypothalamus: centre for energy homeostasis

The human brain is composed of four basic structures: the cerebrum, cerebellum, diencephalon, and brainstem. The diencephalon embodies the thalamus and hypothalamus. The hypothalamus is the brain's interface with the hormonal and autonomic systems that control the internal energy homeostasis (Carpenter, 2003). The hypothalamus has long been implicated as a centre for appetite and intake control. Electrical stimulation of the ventromedial hypothalamus (VMH) increased food intake while electrical stimulation of the lateral hypothalamic area (LHA) decreased food intake in rats (Anand and Brobeck, 1951). It has been suggested that

the hypothalamus controls both the hunger centre (in VMH) and satiety centre (in LHA) in humans (Harrold et al., 2012).

A number of neuropeptides have been identified as being involved in appetite regulations in the hypothalamus in relation to hormonal signals. Administration of neuropeptides α melanocyte stimulating hormone (αMSH) and cocaine amphetamine regulated transcript (CART) inhibit intake (Rossi et al., 1998, Kristensen, 1999, Nagelova et al., 2014). αMSH and CART are stimulated by leptin or CCK (Akieda-Asai et al., 2014). In contrast, administration of neuropeptide Y (NPY) and agouti gene related peptide (AgRP) increased food intake (Gehlert, 1999, Rossi et al., 1998, Zhong et al., 2013). NPY and AgRP are stimulated by ghrelin but inhibited by GLP-1, PYY, insulin and leptin (Stanley et al., 1986, Baver et al., 2014).

1.4.3.2 Brainstem

The brainstem connects the spinal cord with the brain and receives gut information via the vagal nerve, including information about gastric distension, volume and the presence of nutrients (Sawchenko, 1983, Schwartz et al., 1993). The brainstem is connected with the hypothalamus, and they interact to affect energy homeostasis (Ahima and Antwi, 2008).

1.4.3.3 Central hedonic reward system

Humans often continue feeding beyond satiation or with the absence of homeostatic hunger. One of the reasons is that feeding behaviour is also mediated by the hedonic reward system, in addition to the energy homeostasis system (Harrold et al., 2012). The energy homeostasis system refers to the internal physiological control of energy intake, while the hedonic reward system (‘non-homeostasis’ system) stimulates food intake via processing hedonic responses towards the external food (Dalton and Finlayson, 2013).

The orbitofrontal cortex (OFC) is considered to be involved in the subjective pleasantness of the food and sensory-specific satiety (Rolls,

2012). The hedonic reward system is complex, and it is thought to interact with the homeostatic system to affect satiation and food intake (Benelam, 2009). The hedonic system has been found to mediate intake by the release of opioid and endocannabinoids. The endogenous opioid system promotes the over-consumption of highly palatable (liking) food possibly by delaying or blunting the satiety systems (Katsuura et al., 2011). Endocannabinoids stimulate the intake of palatable food possibly by increasing the desire to eat (wanting) and blocking satiation signals to terminate intake (Woods, 2007). With easy access to abundant highly-palatable food, the hedonic motivator for food intake can override the homeostatic system control, resulting in a risk for over-consumption and overweight (Blundell and Finlayson, 2004).

1.4.3.4 Other brain function in appetite control

The cerebral cortex is the outer lay of the cerebrum that is composed of grey matter. Different areas of the cerebral cortex are involved in different functions including perception, awareness, language, thought, memory, attention and consciousness (Marciani et al., 2010)

Since eating behaviours in human are affected by many factors, including internal homeostasis and external environment, physiological and psychological factors, it is believed that many areas of the cortex are involved in affecting feeding behaviours (Harrold et al., 2012). However, there is limited knowledge about how different cortex areas are involved in affecting feeding behaviours. The limbic system lies underneath the cortex including the amygdala, hippocampus, and cingulate gyrus. The limbic system connects with the hypothalamus, the olfactory areas and many other regains of the brain. The limbic system appears to be concerned with motivation, emotion and memory relating to chemical stimuli including taste stimuli (Carpenter, 2003). The amygdala also plays a key role in pleasure and reward from taste or odour and is important in food intake control (Zald and Pardo, 1997, Marciani et al., 2010).

1.5 Sensory-specific satiety (SSS) or satiation

1.5.1 Definition

When food is consumed, the desire to eat (wanting) and the pleasantness (liking) of the food consumed decreases, while the desire to eat and the pleasantness of non-consumed food decreases less or remains unchanged (Rolls et al., 1981, Rolls, 1986). This phenomenon is called **sensory-specific satiety (SSS) or satiation** and is characterized as 'a decreased hedonic-reward response to the sensory properties of a particular food as it is eaten' (Rolls, 1986). For example, following the intake of a hard food, the desire for hard foods decreased, while the desire for soft foods stayed unchanged (Guinard and Brun, 1998).

Sensory-specific satiation describes the decrease in liking and wanting during an eating event, which is one of many factors that contribute to the termination of a meal (Hetherington and Havermans, 2013). Sensory-specific satiety describes a prolonged decrease in liking and wanting after consumption, which may affect food choice and subsequent food intake (Hetherington, et al., 1989). Sensory-specific satiation or satiety limits intake of a food that is highly liked, and promotes intake of a variety of foods. It might be highly biologically adaptive in reducing nutrient deficiency and promoting nutrient balance (Hetherington and Havermans, 2013).

1.5.2 Mechanisms

Sensory-specific satiation happens as rapidly as two minutes after starting consumption, which suggests that it can work independently without the feedback from digestion and post-absorption (Hetherington et al., 1989, Weenen et al., 2005). Sensory-specific satiety is most intense immediately after eating to satiation, stays relatively strong for 2 hours after consumption, and may still have minor effects more than 24 hours after consumption (Weenen et al., 2005).

The characterisation of sensory-specific satiety or satiation has two elements: liking and wanting. Liking is the pleasure from the oral sensory exposure of eating food, while wanting (desire) refers to the intrinsic motivation to eat food (Mela, 2006). The orbitofrontal cortex (OFC) has been proposed as being involved in the subjective pleasantness of a food (Rolls, 2012). For taste stimuli, sensory-specific satiation or satiety can be characterised as the decrease in the pleasantness of the stimuli. Thus, sensory-specific satiation or satiety is a result of changes in taste processing (Hetherington and Havermans, 2013). The intensity and recognition of taste stimuli are processed in the primary taste cortex (the anterior insula and post-central gyrus) (Rolls, 2012). The hedonic response to the taste or flavour stimuli is processed in the secondary taste cortex (the OFC) (Rolls, 2012). When food is eaten to satiation, the signals in the OFC, the pleasantness centre, were reduced specifically to the eaten food while the signals in the primary taste cortex remained unchanged (Rolls and Grabenhorst, 2008, Rolls, 2012). This was supported by behaviour studies that showed the pleasantness of foods eaten reduced, while the perceived intensity of the taste or flavour of the consumed foods stayed stable (Rolls and Rolls, 1997, Rolls et al., 1983). Clearly, sensory-specific satiation or satiety is not a result of sensory adaption or fatigue (changes in the sensory sensitivity). Instead, it is suggested that sensory-specific satiation or satiety can be partly explained by response habituation (Rolls et al., 1981).

The relationship between liking and wanting is less well understood, but it would appear that liking and wanting mechanisms can work together as well as separately (Finlayson et al., 2007). Liking affects wanting but wanting seems to involve more neural pathways than liking (Berridge, 1996). Liking has been found to be mediated by the release of the endogenous opioid neurotransmitters while wanting is controlled by the release of endocannabinoids (Woods, 2007, Berridge, 1996). Wanting corresponds to changes in desire to eat. Berridge (1996) hypothesised that wanting is the consequence of the brain assigning value to an eating

episode. Wanting is influenced by both sensory and cognitive inputs. It is a desirable entity rather than needing (hunger).

1.6 Expected satiation/satiety

In humans, food intake is not only controlled by our feelings of hunger and satiation within an eating event, rather, we learn to control our meal size through cognitive activities such as decisions about meal planning made even before eating (Brunstrom, 2011). Until now, little is known about the cognitive control of food intake (Brunstrom et al., 2010a). Brunstrom and Rogers (2009) identified that our expectation of the satiating capacities of food, as an important cognitive process, which determines our pre-meal decisions about our meal size (Brunstrom and Rogers, 2009).

1.6.1 Definition

Expected satiation is defined as the relative satiation (feeling of fullness) that a person expects from a food during consumption; expected satiety refers to the magnitude to which a food is expected to stop a person feeling hunger between meal intervals (Brunstrom and Rogers, 2009). Brunstrom and his colleagues have developed the ‘method of constant stimuli’ (Brunstrom et al., 2008b) and later the ‘method of adjustment’ (Brunstrom and Rogers, 2009) to compare the expected satiation and expected satiety of different foods on a calorie-to-calorie basis.

1.6.2 Expectation and energy intake

High palatability is thought to promote the selection of a large meal size, contributing to the overconsumption and overweight (Drewnowski, 1998, Johnson and Wardle, 2014, Yeomans, 1998). However, our decision about meal size is not only motivated by a desire to enjoy the hedonic sensations of a food, but also motivated simply by a need to replete nutrients and energy and stave off hunger (Brunstrom, 2011).

Brunstrom and Shakeshaft (2009) correlated the measures of liking, expected satiety and ideal portion size of 8 different snacks (Brunstrom and Shakeshaft, 2009). Both expected satiety and liking predicted the ideal portion size (Brunstrom and Shakeshaft, 2009). In another study by Brunstrom and Rogers (2009), the role of expected satiation and the role of palatability on subjects' ideal portion size were compared across a wider range of 17 different foods (Brunstrom and Rogers, 2009). In this case, expected satiation (r=-0.80) predicted the ideal portion size remarkable better than palatability (r=0.06) (Brunstrom and Rogers, 2009). High expected satiation was correlated with smaller selected portion size (kcal); and low energy density foods tend to have high expected satiation (Brunstrom and Rogers, 2009). Interestingly, the 17 different foods varied remarkably in their expected satiation (about 4 fold), but their differences in palatability were modest (Brunstrom and Rogers, 2009). It was probably a genuine reflection of the real-world experience of food characteristics. Most foods in the market are comparably palatable to meet consumers' hedonic demand, and the difference in expected satiation outweighs differences in palatability (Brunstrom, 2011). Therefore, this challenges the relative importance of palatability in the meal-size selection and suggests that expected satiation determines real-world self-selection of portion size (Brunstrom and Rogers, 2009).

Brunstrom proposes that the actual intake of a meal is likely to correlate positively with the portion of the meal a person selects (Brunstrom, 2011). It is supported by a study by Rolls et al. (2007) who reported that large portion sizes promoted higher energy intake (Rolls et al., 2004b). In a recent study by Fay et al. (2011), 764 subjects from the south-west of England reported their previous meal experience via an internet-based questionnaire (Fay et al., 2011). Plate cleaning happened remarkably at 91% of meals and was highly correlated with pre-meal planning (92% of meals) (Fay et al., 2011). The consumption amount of a meal appears to depend highly on the amount served or planned (Brunstrom, 2011). The amount eaten was resistant to deviation from the pre-planned meal size:

28% of subjects continued eating beyond feeling satiation, and 57% reported that more could be eaten at the end of the meal (Fay et al., 2011). Together, these results suggested cognitive expectations for satiation and satiety are important factors determining the amount of energy we consume.

1.6.3 Expectation and appetite sensations

In addition, expected satiety was reported to influence the actual satiety of a food (Brunstrom et al., 2011). In a study by Brunstrom et al. (2011), 32 subjects were asked to consume an identical fruit smoothie and report their subjective appetite feelings for 3 hours after consumption (Brunstrom et al., 2011). Before consuming the smoothies, half of the subjects were shown a small portion of the fruit (group 1) and the other half of the subjects were shown a large portion of the fruit (group 2) (Brunstrom et al., 2011). Interestingly, the expected satiety of the same fruit smoothie was rated higher by group 2 than group 1. Participants in the group 2 feel less hungry (-10% to -15% reduction) and more full (+10% increase) after consuming the same amount of fruit smoothie (Brunstrom et al., 2011). This indicates that expected satiety about a food could affect the actual feeling of hunger and fullness after consumption without changing the properties of the food. One possible explanation is that cognitive expectation on satiety might influence a set of physiological responses including the gastric emptying (Brunstrom et al., 2011). Another possibility is that expected satiety influences actual satiety of the smoothie via memory processes (Brunstrom et al., 2011).

1.6.4 Factors affecting expected satiation and satiety

Energy density is one of the most important determinates of expected satiation and satiety (Brunstrom and Rogers, 2009, Brunstrom et al., 2008b). Low energy density is associated with a high expectation on satiety and satiation across different foods when compared on a calorie-to-calorie basis (Brunstrom and Rogers, 2009, Brunstrom et al., 2008b). For

example, 894 kcal of cashew nuts (high energy density) was expected to be equally satiating by subjects as 200 kcal of pasta (low energy density) (Brunstrom et al., 2008b). Perceived volume positively correlated with the expected satiation of different foods, possibly through affecting the perceived energy density (Brunstrom et al., 2010a).

A food in a heavier container was expected to be more satiating than a food in a visually identical but lighter container, which was potentially because that the perceived energy density of the food was increased with increased weight of the container (Piqueras-Fiszman and Spence, 2012).

Forde et al. (2013) explored the role of the oral processing characteristics of solid foods in expected satiation (Forde et al., 2013). Foods that were chewier, consumed in smaller bites, and therefore had longer oral exposure time, were expected to be more satiating (Forde et al., 2013).

1.6.5 Expectation and associative learning

It seems that people find it effortless to express their expectations of the satiating capacities of food, suggesting that decisions on such expectations occur habitually, and are learned from everyday eating experience (Brunstrom, 2011). Brunstrom suggested that expected satiation and expected satiety are based on learned association. Specifically, satiation may be largely mediated by the learned association between the sensory characteristics of a food and the post-ingestion consequences of the food (Brunstrom, 2011). Several findings below support this hypothesis.

The familiarity of different foods was positively correlated with the expected satiety of the food (Brunstrom et al., 2008b). Subjects who were more familiar with a food product expected the food to be more satiating than subjects who had a lack of past eating experience of the food (Brunstrom et al., 2010b).

It appears that learning could occur even after one single experience of a novel food and that the expected satiation and satiety is not easily redefined over repeated exposure (Brunstrom, 2011, Hogenkamp et al., 2012). Hogenkamp et al. (2011) reported expected satiation of a soup did not change after repeated consumption over 5 days (Hogenkamp et al., 2012). This suggests that the unchanged expectation was due to the incapacity to relearn a pre-established association (Brunstrom, 2011). These findings provide opportunities for the manipulation of the expected satiation and satiety of already familiar food products to sustainable reduction of the energy intake.

1.7 Food-related factors that influence appetite

1.7.1 Macronutrients

The macronutrient composition of food contributes to the control of appetite and food intake. Some studies have shown that protein has a stronger satiating power than fat and carbohydrate with equal energy. Higher protein diet increased ratings of satiety and decreased subsequent energy intake compared to lower protein diet (Halton and Hu, 2004).The effect of protein on satiety and satiation depends on the types of protein. For example, fish protein has higher satiating power than beef protein (Borzoei et al., 2006).

1.7.2 Energy density

Energy density is the amount of energy in a given weight or volume (kcal/g or ml/g) (Benelam, 2009). Among many characteristics of food, energy density seems to be one of the most important predictors of food intake (Benelam, 2009). Human subjects have been found to consume a similar weight of food daily, rather than the similar energy of food (Rolls et al., 2000). A number of studies reported that energy intake is increased with the high energy density of the foods eaten (Bell et al., 1998, Fisher et al., 2007, Rolls, 2009a). Increasing the volume of a drink by incorporating air

while the energy content kept constant has been shown to reduce subsequent food intake (Rolls et al., 2000). Satiation and food intake might be determined mostly by energy density of a food even more than by its energy content and macronutrient composition (Brunstrom, 2011).

1.7.3 Sensory perception of food

Sensory perception of food, such as the taste, smell, texture, temperature, appearance and oral-processing sounds of food might affect appetite, satiation, and satiety at the pre-prandial phase, prandial phase and even at early post post-prandial phase (Blundell et al. 2010). At the early stage of a meal, especially at a high hunger phase, sensory signals may activate the reward centre in the brain, encourage energy intake (positive feedback). The ongoing sensory information generates satiation signals that will soon override the reward signals at the later stage of eating and contribute to the termination of energy intake (negative feedback).

1.7.3.1 Palatability

The sensory properties of foods heavily determine its palatability, and this has a direct impact on satiation and food intake (Sorensen et al., 2003). Palatability or liking of a food refers to the subjective pleasurable experience by the sensory perception of food. Palatability stimulates a positive emotional signal and increases the drive for intake by the release of opioids acting on the hedonic nerve system (Pecina et al., 2006, Berthoud and Morrison, 2008). It involves the characteristics of food and an individual's evaluation of her or his experience (Yeomans, 1998). The palatability of food can be measured by visual analogues scales in behaviour studies. In neuroimaging studies, the palatability of a stimulus can be characterised by monitoring the activation of the specific brain regions for the reward system, including the OFC (Rolls, 2012).

Increasing the palatability of a food stimulates appetite, increases meal size, duration and eating rate (Yeomans, 1998). Increasing palatability of a

food has been consistently associated with the increased the *ad libitum* intake of the food (Drewnowski, 1998). However, there was no strong evidence showing that palatability affects satiety after consumption. Increasing the palatability of a soup increased *ad libitum* intake but did not affect subjective appetite ratings after consumption (satiety) (de Graaf et al., 1999).

The palatability of foods has a strong positive correlation with the energy density of foods (Drewnowski, 1998), reflecting our innate preferences for energy-rich food (Berthoud, 2007). However, the palatability of a food can be altered by the sensory properties of the food, such as taste, flavour, texture and appearance, without changing the energy density of food. It is possible to design food with low energy density but high palatability that is both satiating and palatable (Poortvliet et al., 2007).

1.7.3.2 The extent of oral sensory exposure

The extent of oral sensory exposure plays a very important role in affecting satiation and meal termination. Important oral sensory characteristics include eating rate, bite size, oral processing duration and intensity, and perceived intensity of the oral sensory stimuli.

The duration of oral sensory exposure is an important factor determining the extent of satiation. Longer oral sensory exposure to a salty soup decreased the *ad libitum* intake of the soup (Bolhuis et al., 2011).

Higher eating rate (consumption volume per minute) is associated with increased food intake, and vice versa (Viskaal-van Dongen et al., 2011). This was possible because that higher eating rate reduced the extent of oral processing leading to shorter and less intense oral sensory exposure (Zijlstra et al., 2008). Similarly, larger bite sizes, associated with the higher eating rate, were also found to increase intake (Zijlstra et al., 2009).

In addition, increased chewing is thought to enhance satiation and short-term satiety. Chewing non-caloric sweetened gums for 15 minutes

reduced the feeling of hunger and suppressed the feeling of fullness up to 3 hours after the gums, and it also reduced subsequent snack intake, compared with non-gum conditions (Hetherington and Boyland, 2007). Chewiness reflects the structure of the food product, the length of time and amount of work required for the oral process before swallowing the food (Forde et al., 2013, Loret et al., 2011). On one hand, the total number of chews was positively correlated with oral exposure time and contributed to higher satiation (Forde et al., 2013). On the other hand, more intense chewing indicates more effort is needed for intake, which may be associated with higher energy intake via a learned association (Forde et al., 2013).

The intensity of various oral sensory stimuli has been shown to increase satiation and reduce food intake. While increasing the saltiness of a soup reduced the *ad libitum* intake of the soup (Bolhuis et al., 2011), the mechanism is not known, but increasing oral sensory stimuli intensity was thought to increase the overall oral sensory exposure. In addition, the intensity of oral sensory stimuli may affect satiation by altering the oral processing characteristics. For example, increasing aroma intensity has been associated with reduced bite sizes (de Wijk et al., 2009).

1.8 Food Flavour perception

Within recent decades, an increasing number of studies have investigated the influence of sensory perception of foods and drinks on appetite regulation and food intake. The traditional two independent research fields, nutrition, and sensory science are linked as a multidisciplinary approach to address the overweight and obesity issue (Sorensen et al., 2003). Food flavour is an important sensory cue that may affect our sensory perception and eating experience. However, the effect of food flavour on appetite regulation and eating behaviour is under investigated.

While a brief overview of the appetite regulation mechanisms in previous sections, it is also important to understand flavour perception system,

involving the gustatory, olfactory and somatosensory systems and the respective central nerve pathways (Section 1.8). Then, the current literature for investigating the links between food flavour and appetite regulation will be summarised (section 1.9).

1.8.1 The gustatory system

1.8.1.1 Taste perception in gustatory organs

During the consumption of foods, non-volatile substances (tastants) dissolve in the saliva, and are delivered to the tongue and other taste-related areas in the oral cavity including palate, pharynx, and epiglottis (Bear et al., 2001). Perception of taste is mediated by taste receptor cells (TRCs) inside taste buds (Bear et al., 2001) (**Figure 1-2c**). Activation of TRCs by chemical stimuli evokes the release of neurotransmitters via gustatory afferent axons, leading to the transmission of taste information to the gustatory cortex in the brain stem and thalamus (Figure 1-2c) (Sugita, 2006, Norgren, 1983, Bear et al., 2001).

Taste buds are mainly present inside papillae that are scattered on the surface of the tongue, but they are also located on lips, palate, pharynx, epiglottis and throat (Bear et al., 2001). There are three taste-buds-containing papillae: fungiform, foliate and vallate (or circumvallate) papillae concentrated at different locations of the tongue (Bear et al., 2001) (**Figure 1-2a**). Each papilla contains one to a few hundred taste buds. One taste bud contains 50-150 taste receptor cells (TRCs). Taste buds also contain basal cells and gustatory afferent axons. TRCs are not neurons but can form a synapse with the gustatory afferent axons near the bottom end of the taste bud. On the apical end of TRCs, thin microvilli extend to the taste pore and interact with taste stimuli. Microvilli receive chemical signals from tastants and produce transduction processes that evoke the release of neurotransmitter onto gustatory afferent axons (**Figure 1-2c**) (Bear et al., 2001, Chandrashekar et al., 2006).

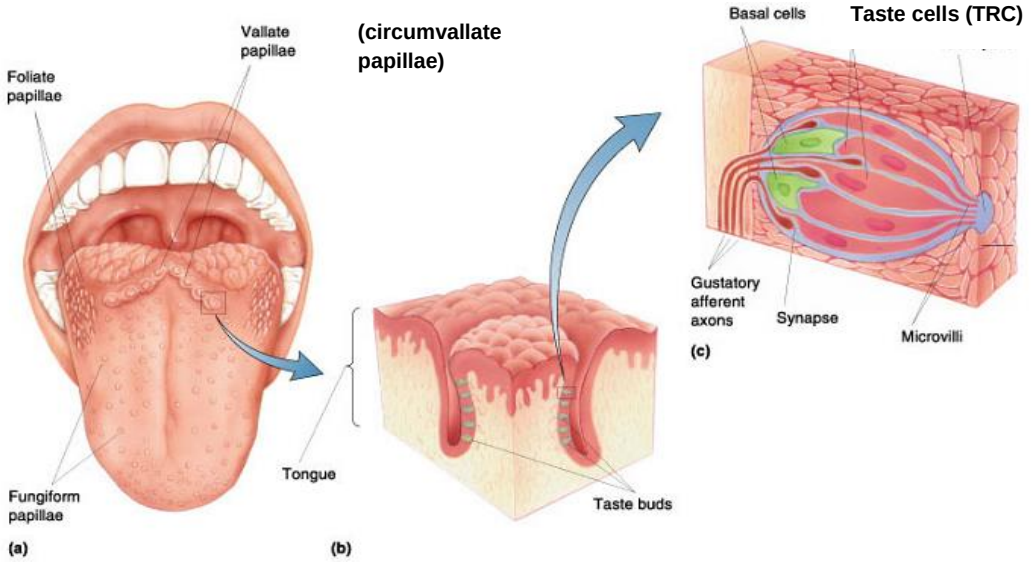

Figure 1-2 Tongue, papillae, taste buds and taste receptors (Bear et al., 2001)

1.8.1.2 Detection of five basic tastes

The gustatory system detects one of the five basic different taste qualities: sweet, umami, salty, sour and bitter (Yarmolinsky et al., 2009). A taste cell expresses only one of the few taste receptor types that selectively detect the stimuli (Yarmolinsky et al., 2009). Type 1 receptors (TR1) are heterodimers; the receptor for sweetness is composed of T1R2 and T1R3, and umami receptor contains T1R1 and T1R3 (Li et al., 2002). T2Rs recognise many bitter stimuli (Chandrashekar et al., 2000). Chemicals that taste salty and sour are detected by sodium-specific epithelial ion channels (Bachmanov and Beauchamp, 2007, Chandrashekar et al., 2010).

It is now known that type 1 receptors (T1Rs) are not only present in the mouth and function in taste perception, but they are also expressed through the body (Fernstrom et al., 2012). Both T1R2 and T1R3 are present in endocrine cells of the gastrointestinal tract. They can sense the luminal sugars, facilitate glucose absorption and metabolism, and activate the release of satiety hormones especially glucagon-like peptride-1 (GLP-

1) (Dotson et al., 2010, Fernstrom et al., 2012, Jang et al., 2007, Margolskee et al., 2007).

1.8.1.3 Central taste pathway

Three cranial nerves (CV) transfer the taste information from the gustatory afferent axons to the brain. They are CN VII (facial nerve), CN IX (glossopharyngeal nerve) and CN X (the vagal nerve) (**Figure 1-3)** (Bear et al., 2001). CN VII conveys information regarding the identity and intensity of tastants from the anterior of the tongue and palate (Marciani et al., 2010). CN IX innervates the posterior of the tongue, responsive mainly to swallowing of tastants. CN X innervates taste buds on the epiglottis and pharynx (Marciani et al., 2010, Scott, 2005). These three cranial nerves unite at the nucleus of the solitary tract (NST) in the brainstem, from where they transmit taste information to the cortex via two central pathways (Scott, 2005) (**Figure 1-3**).

In the main taste pathway, taste information projects directly to the ventral posterior medial (VPM) of the thalamus and then to the primary gustatory cortex, including the anterior insula cortex and post-central gyrus (Marciani et al., 2010). The post-central gyrus has the somatosensory cortex involved in somatosensory perception (Marciani et al., 2010). The insula is believed to be associated with the conscious taste perception, including identifying the qualities of the taste substances. From insula, taste information then projects to the secondary gustatory cortex including the orbitofrontal cortex (OFC) and amygdala. The OFC not only receives signals from gustatory but also from olfactory, trigeminal, visual and auditory stimulation (Marciani et al., 2010). The OFC is thought to be involved in the processing of affective (emotional) information of taste stimuli and may play a part in hedonic responses (Zald and Pardo, 2000). It is also regulated by motivational state and responds to hunger (Del Parigi et al., 2002).

The other taste pathway transmits taste information to the NST, the nucleus of the pons, and then to the limbic system including the amygdala, hypothalamus and anterior cingulate cortex (ACC) (Marciani et al., 2010).

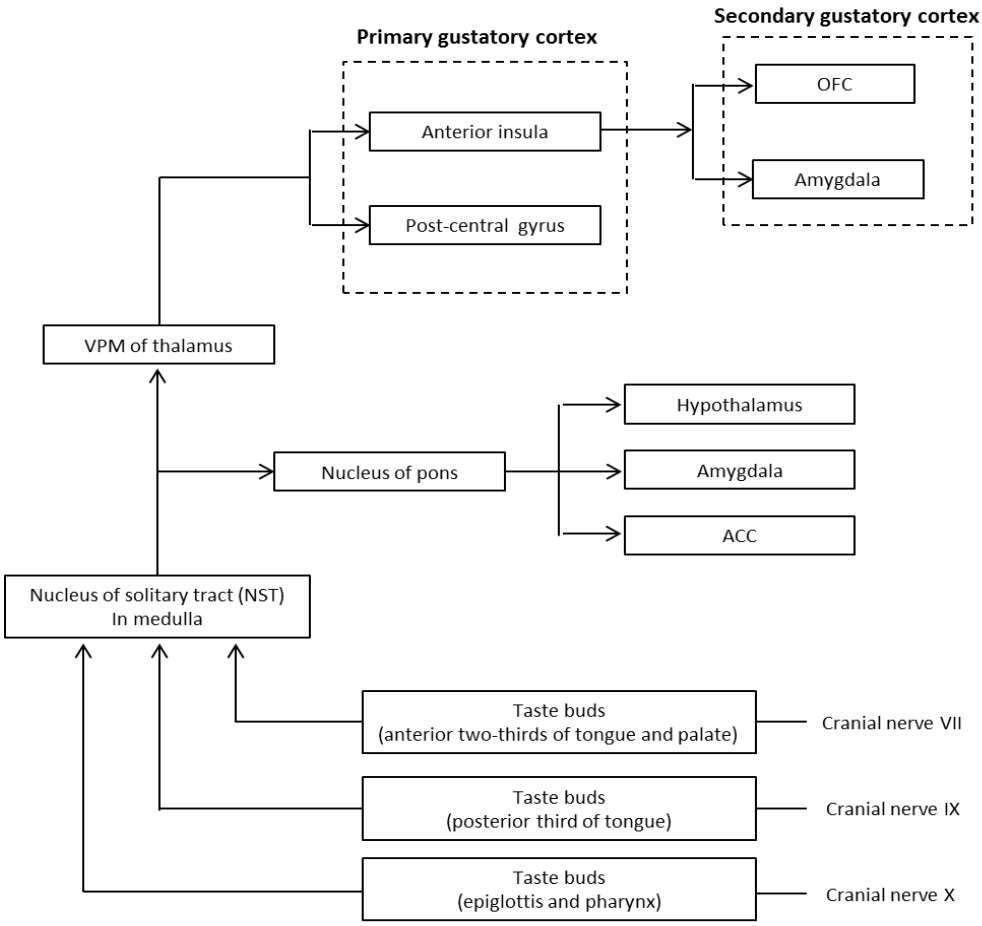

Figure 1-3 Schematic diagram of central gustatory pathways (Marciani et al., 2010)

1.8.2 The Olfactory system

1.8.2.1 Aroma perception in olfactory organs

Aroma reaches the olfactory epithelium high up in the nasal cavity through two delivery routes: orthonasally (by sniffing via the nostril) or retronasally (during consumption via the mouth to nasopharynx) (**Figure 1-4)** (Auvray and Spence, 2008). Food-related aroma volatiles can be perceived both retronasally and orthonasally, while non-food volatiles are only experienced orthonasally (Small et al., 2005). Rozin (1982) suggested that orthonasal and retronasal olfaction do not only differ in the delivery

efficiency, but they may bring about two qualitatively different perceptual experiences from the same aroma stimuli (Rozin, 1982, Auvray and Spence, 2008). Volatiles delivered orthonasally are perceived as arising from the external environment. In contrast, volatiles delivered retronasally are perceived as in the mouth in a food-related context (Rozin, 1982). Recently, Rozin's idea was supported by neuroimaging studies, which observed different neural responses towards an odour delivered via the two different routes (Small et al., 2005).

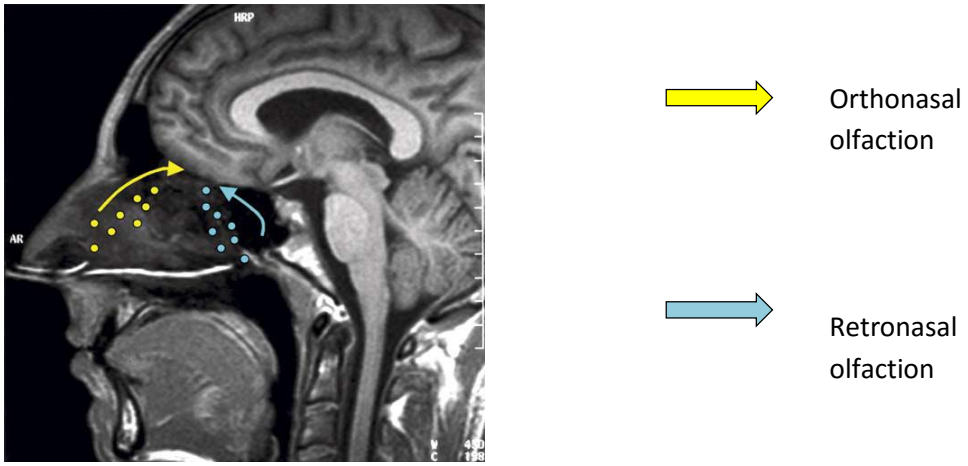

Figure 1-4 A magnetic resonance imaging (MRI) image of the human nasal cavity, demonstrating the orthonasa and retronasal delivery routes (Rolls et al., 2003)

On reaching the epithelium (**Figure 1-5**), the aromas are dissolved in the epithelial mucus film, bind to the cilia at the dendritic end of the olfactory receptor cells, activate transduction and result in the release of neurotransmitters. Volatile detection is mediated by millions of olfactory receptor neurons. Humans have approximately 350 different olfactory receptors (Buck, 2004). They are 7-transmembrane G-protein-coupled receptors (GPCR) encoded by a large multigene family (Malnic et al., 2004). It is estimated that humans can detect 10,000 to over 100,000 distinct aroma volatiles (Buck, 2004). A single olfactory receptor (neuron) recognises multiple volatiles, a single volatile is recognised by multiply receptors, but different aroma volatile compounds are recognised by different combinations of receptors (Malnic et al., 1999).

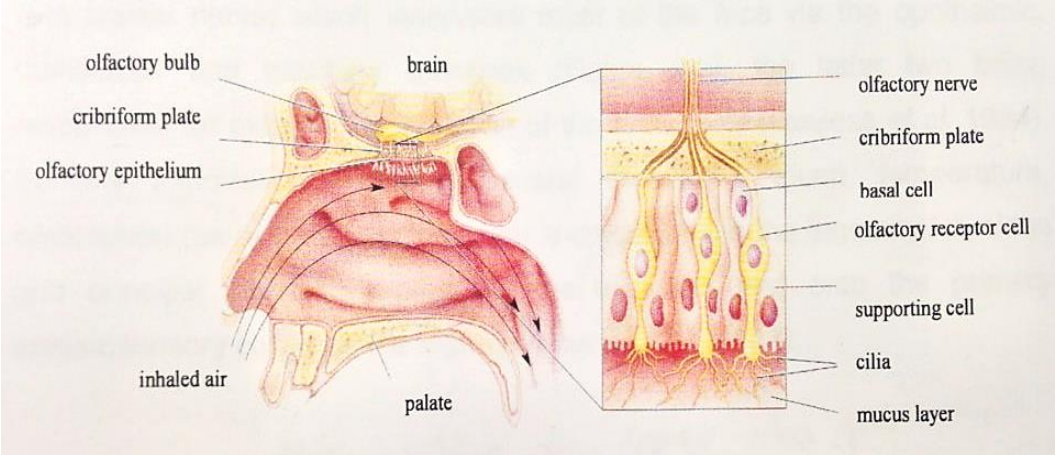

Figure 1-5 Location and structure of olfactory epithelium (Bear et al., 2001).

1.8.2.2 Central olfactory pathways

The olfactory receptor cells are neurons (Carpenter, 2003). One side of the receptors (cilia) are located within the epithelium, and the other side is fine nerve axons, which penetrate through the cribriform plate to the olfactory bulbs located just above the epithelium (Carpenter, 2003). The olfactory nerve (CN I) transmits electrical signals of aroma stimuli from the cilia in epithelium to the olfactory bulb (Marciani et al., 2006). The olfactory system is unique in that the olfactory bulb projects straight to the primary olfactory cortex and then to the thalamus and other cortex (**Figure 1-6)** (Marciani et al., 2006, Bear et al., 2001). The primary olfactory cortex (POC) is involved in conscious aroma perception and discrimination (Marciani et al., 2010).

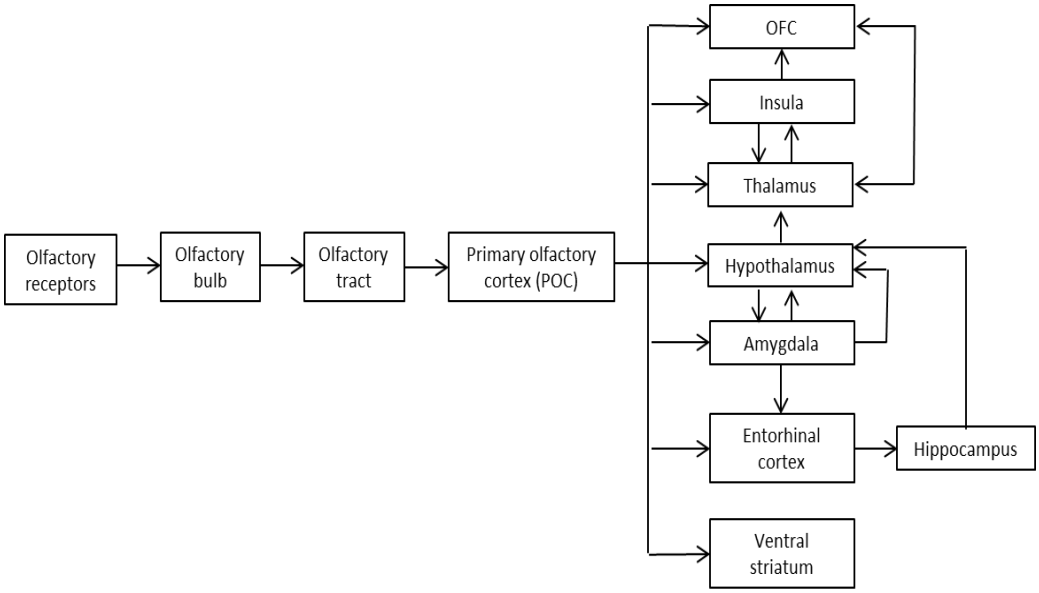

Figure 1-6 Central olfactory pathways (Marciani et al., 2010).

The POC then projects aroma information directly to the secondary olfactory cortex including OFC, insula, entorhinal cortex and ventral striatum, or via the thalamus repay, **Figure 1-6** (Marciani et al., 2010). Olfactory information also directly projects to the hypothalamus and limbic system (hippocampus and amygdala) from the POC (Marciani et al., 2010).

1.8.3 The oral somatosensory system

The somatosensory system processes sensory information concerning tactile (touch), nociception (pain), as well as temperature, texture, and consistency (thickness) of food. The somatosensory system is also stimulated by chemical substances, including menthol, alcohol, capsaicin, carbon dioxide and even high concentrations of odorants or tastants (Dessirier et al., 2000, Bryant and Mezine, 2002, Carstens et al., 2000, Brand, 2006). All of these stimuli influence our overall perception of flavour (Marciani et al., 2010, Delwiche, 2004).

1.8.3.1 Oral somatosensory central pathways

The filiform papillae are spread over the tongue and the pharynx but contain no taste buds, and it is involved in the somatosensory system (Smith and Margolskee, 2001). Somatosensory signals from the posterior of the tongue and the pharynx are transferred to by the NST by the glossopharyngeal nerve (CN IX) and the vagal nerve (CN X) (Carpenter, 2003). From the NST, the taste information is then transmitted to the VMP of the thalamus (**Figure 1-7**). Oral sensory signals from the nasal cavity and anterior of the tongue are transmitted by the trigeminal nerve (CN V) to the trigeminal nucleus or the NST before reaching the VMP of the thalamus. From the thalamus, the somatosensory information is transmitted to the primary somatosensory cortex (SI) and the secondary somatosensory cortex (SII). The primary somatosensory cortex also relays information to the primary motor cortex, secondary somatosensory cortex, ACC and insula (Marciani et al., 2010).

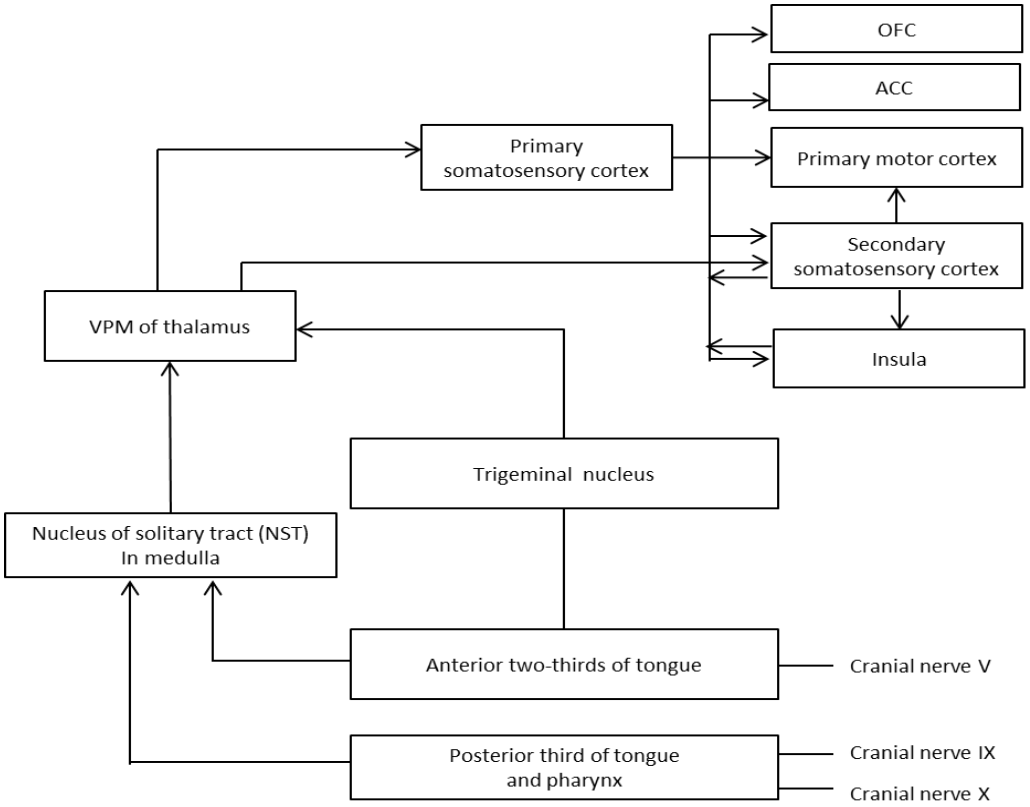

Figure 1-7 oral somatosensory central pathway (Marciani et al., 2010)

1.8.4 Multimodal flavour perception

Flavour perception is the combination of multisensory modalities including gustation, olfaction, and somatosensory sensations during food consumption (Auvray and Spence, 2008, Wallace, 2015). Taste, aroma and texture (somatosensory stimuli) do not only affect the perceived flavour as an individual modality, but their cross-modal interactions also shape our overall flavour perception. The interactions between oral sensory stimuli can happen at a physicochemical level within the food matrix, and at perceptual and neural levels involving cognitive or psychological integration (Wallace, 2015). Psychophysics, electrophysiology, and neuroimaging research have shown compelling evidence of the perceptual cross-modal interactions.

1.8.4.1 Taste-aroma interaction

1.8.4.1.1 Physiochemical interaction

Tastants such as sugar, salt, and acid can interact with aroma at a physicochemical level (Taylor et al., 2000). The physicochemical interaction between taste and aroma stimuli may affect the aroma release from the food matrix into the gaseous phase and then delivery to nasal receptors (Friel et al., 2000). In an aqueous food system, the release of aroma depends on the nature and concentration of both aroma and taste compounds. However, noticeable physicochemical interactions between aroma and taste compounds on aroma release seem to appear only at relatively high concentrations (Friel et al., 2000, Pfeiffer et al., 2006). The physicochemical impact of tastants on aroma delivery to the nose can be measured by *in vivo* atmospheric pressure chemical ionisation mass spectrometry (APCI-MS), via the evaluation of gas phase aroma concentration, in the nose, in real time (Taylor et al., 2000).

1.8.4.1.2 Cross-modal interaction

Without direct modification of the physical-chemical properties of a food, the interactions of aroma and taste stimuli may also affect flavour perception as results of cognitive or psychological effects (Noble, 1996). It has been debated whether the perceptual interactions between taste and aroma stimuli are a result of contextual effects, taste-smell confusion, or a cognitive/psychological integration in the central system. Dalton et al. (2000) found that the threshold of an aroma stimulus (benzaldehyde) delivered orthonasally was decreased with the simultaneous delivery of a congruent subthreshold tastant (saccharin) in the mouth (Dalton et al., 2000). This study provides evidence that taste and aroma integrate at the central nerve system. In addition, the aroma-taste interaction depends on the congruency of aroma and taste stimuli, and non-congruent aroma and taste did not show the same perceptual interaction (Dalton et al., 2000). Congruent stimuli are two stimuli that are appropriate for combination in a

food or drink (Schifferstein and Verlegh, 1996). Further psychophysics studies discover that the cross-modal interaction of congruent taste and aroma enhances the perceived flavour intensity more than the sum of taste or aroma stimuli presented independently (Hewson et al., 2009, Pfeiffer et al., 2006).

The cross-modal perception was supported by neuroimaging studies. Gustatory and olfactory systems are anatomically dissociated, but signals may integrate at the central nerve system. Rolls and Baylis (1994) firstly discovered the bimodal neurons in the OFC, which responds to individual taste or aroma stimuli, and also combined taste and aroma stimuli (Rolls and Baylis, 1994). A few overlapping areas in the brain that are activated by both aroma and taste stimuli have been identified, and these areas including the insula, OFC, amygdala and ACC, activated by both taste and odour (Eldeghaidy et al., 2011, Rolls, 2015). In some brain areas, i.e. the OFC, the simultaneous presence of taste and aroma stimuli activated signals higher than the sum of taste or aroma stimuli presented separated (de Araujo et al., 2003). In addition, an anterior part of the OFC was only activated by the combination of aroma and taste stimuli, but not by aroma or taste stimuli alone (de Araujo et al., 2003). These neuroimaging studies, together with psychophysics studies, provide compelling evidence that the perception of flavour is a result of combined perception of taste and aroma modalities rather than a result of the simple addition of independent taste modality and aroma modality.

1.8.4.2 Taste-texture interaction

Increasing the thickness (viscosity) of a food or drink has been consistently shown to decrease taste intensity (Cook et al., 2002, Lethuaut et al., 2003, Tournier et al., 2009). Conversely, increasing the concentration of sucrose (sweet taste) increased the perceived firmness of custards (Lethuaut et al., 2003). However, the concentration of sucrose does not always affect thickness perception (Tournier et al., 2009).

Changing the type of thickeners without changing the viscosity of a food was found to influence the diffusion of sucrose in the food (Brossard et al., 2006). This suggests that texture-taste interaction is at least partly due to the physicochemical interaction between a thickener and the taste substance.

Increasing thickness was found to reduce sweetness perception without affecting the diffusion of a sweet tastant (sucrose) in saliva (Tournier et al., 2009). It has been proposed that the texture-taste-aroma may interact at the psychological or cognitive level. The same cranial nerves CN IX and CN X convey both gustatory and somatosensory information, and these two cranial nerves unite with the trigeminal (CN V, somatosensory) and CN VII (gustatory) nerve at the NST (Marciani et al., 2010). The gustatory and somatosensory information can potentially integrate at the cranial nerves, NST, thalamus or cortex. Indeed, several common areas in the brain, including the insula, the rolandic, frontal and temporal operculum, are activated by both taste stimuli and somatosensory stimuli (Cerf-Ducastel et al., 2001).

1.8.4.3 Texture-aroma interaction

An increased viscosity of foods has been shown to reduce perceived aroma or flavour intensity (Boland et al., 2006, Ferry et al., 2006, Tournier et al., 2009). Saint-Eva et al. (Saint-Eve et al., 2006) reported that a reduction in viscosity led to an increase in aroma perception due to an increased aroma release. This suggests a physicochemical level interaction.

However, in other studies, higher thickness led to a reduction in perceived aroma (or flavour) intensity without changing the release of aroma into the nasal cavity (Cook et al., 2003, Hollowood et al., 2002). Bult et al. (2007) observed that increasing viscosity reduced creamy aroma perception when the aroma was delivered directly to nasal cavity by an olfactometer. These results together suggest that texture-aroma interaction can happen

at a physiological or cognitive level. In addition, a reverse effect of aroma-texture interaction was observed. A coconut and butter aroma increased perceived thickness greater than an apple aroma (Saint-Eve et al., 2004). This was also proposed to be a result of a texture-aroma physiological interaction.

Electrophysiological research proposes that somatosensory stimuli may have an inhibitive impact on olfactory information transmitted to the brain (Kobal and Hummel, 1988). Further neural and brain research is needed to investigate the neural and psychological basis of the texture-aroma interaction.

1.9 The influence of food flavour on appetite and eating behaviour

1.9.1 Flavour-nutrient associations

A large part of our dietary eating behaviour is learned from a lifetime of eating experiences (Brunstrom, 2007). Humans may have learnt to associate the flavour properties of a food with its post-digestive consequences (Brunstrom, 2007, Yeomans, 2012). This association may be one of the foundations for the flavour to impact on appetite and eating behaviour.

One associative learning outcome is ‘acquired liking’; that we learn to like or dislike the flavour of a food as a consequence of its positive or negative ingestion and metabolic consequences (Yeomans, 2012). For example, adults learn to like the bitterness of caffeinated products possibly because of their metabolic benefits, while infants dislike them (Yeomans et al., 2005). Liking (palatability) may affect our food choice and energy intake (Yeomans, 2012). Increased liking is associated with increased food intake. Based on the acquired liking, more palatable flavours may promote appetite and increase food intake (Yeomans, 2012).

Another outcome of flavour-nutrient association is ‘learned satiety or satiation’, that we associate the flavour properties of a food to how filling

the food is (satiation) and to what extent the food suppresses subsequent hunger (satiety) (Yeomans, 2012). The learned satiation and satiety may be related to the energy content and nutrient value of the food, and the post-digestive and post-absorptive effects of the food (Yeomans, 2012). Greater learned satiation or satiety may suppress appetite and reduce food intake (Yeomans, 2012).

Yeomans (2012) suggests that 'acquired liking' and 'learned satiety or satiation' may be confounding associations to influence the effect of flavour on appetite and feeding behaviour (Yeomans, 2012). However, the quantification of the effect of each association ('acquired liking' or 'learned satiety/satiation'), how independently they work, and to what extent they interact remains unresolved and still under debate (Yeomans, 2012). It is likely that effects of the two flavour-nutrient associations on appetite and eating behaviour depend on the quality of the flavour.

1.9.2 The role of taste in appetite and eating behaviour

Information of the taste stimuli can reach many areas of the brain including the OFC (pleasure, hedonic and sensory-specific satiety), amygdala (emotion and memory), hypothalamus (energy homeostasis) and ACC (cognitive control and attention) that potentially affect appetite and feeding behaviour (Marciani et al., 2010). Signals of satiation or satiety from taste stimuli may have complex mechanisms, including sensory, cognitive, digestive and hormonal.

We may associate the taste of a food with its metabolic consequences through instinct or learned association, and such association may partly affect our appetite and feeding behaviours (Yeomans, 2012). For instance, we may associate sweet taste with the intake of carbohydrates or high-energy foods, and umami with the intake of high-value protein (Hogenkamp et al., 2011, Chandrashekar et al., 2006). Taste stimuli therefore could help to orient our eating behaviour towards meeting our internal nutrients need. There are five basic tastes, including sweet, sour,

salty, bitter and umami, of which the effect sweet taste on appetite and food intake has been mostly studied.

1.9.2.1 Effect of sweet taste

Sweet stimuli, sugars, and artificial sweeteners, are perceived by the taste receptors T1R2 and T1R3 in the mouth, and are perceived as sweet (Fernstrom et al., 2012). Although taste receptors T1R2 and T1R3 were first discovered in the mouth and function in taste perception, it is now observed that both T1R2 and T1R3 are expressed in the gastrointestinal tract (Fernstrom et al., 2012). The T1R2 and T1R3 in the GI tract, in response to sugars and artificial sweeteners, are potentially involved in the release of satiety hormones glucagon-like peptide-1, and the maintenance of glucose homeostasis (Fernstrom et al., 2012).

Both sugars and artificial sweeteners can activate the sweet receptors, but they may bind to different sites on the receptors (Anderson and Woodend, 2003). For example, sucrose binds to the venus-flytrap (VFT) domains of T1R2 and T1R3, while aspartame can only bind to the VFT domain of T1R2 (Anderson and Woodend, 2003). The difference in binding in sweetness receptors determines the different sweetness perception profiles (Pfeiffer et al., 2000). In addition, aspartame and stevia also bind and activate the bitter receptors. Therefore, sugars and high intense sweeteners may have a different impact on appetite.

Sugars have been shown to promote rapid satiation, stimulate satiety and reduce subsequent food intake in the short-term (Lavin et al., 2002b, Vickers et al., 2001, Anderson and Woodend, 2003). Sugars are monosaccharides or disaccharides including glucose, fructose, sucrose, lactose, and maltose (Anderson and Woodend, 2003). Sucrose (135g) presented in a beverage increased the feeling of fullness, compared with a water placebo (Lavin et al., 2002a). Sugars have a 4 kcal/g energy loading and the intake of sugars increase the blood glucose level within 30 minutes, which sends satiation signals to the hypothalamus of the brain.

Mayer and colleagues first proposed the glucose-static theory of satiety (Mayer, 1953). They suggested that eating was initiated in response to low glucose uptake by body organs, and inhibited by high glucose uptake. However, the mechanisms by which sugars induce satiation cannot be attributed solely to their effect on blood glucose (Anderson and Woodend, 2003). Sugars have been shown to promote satiation and satiety via their sweet taste (Bellisle et al., 2012). Prolonged stimulation of sucrose over 10 minutes suppressed subsequent food intake greater compared to an iso-energetic drink consumed in only 2 minutes (Lavin et al., 2002b).

High intensity artificial sweeteners provide the sweetness but offer no or low energy. This has raised a concern that low caloric sweeteners (LCS) may confuse the body's internal appetite control systems (Bellisle et al., 2012). Some argue that the artificial sweeteners might stimulate hunger and increase intake of sugars. However, little evidence supports this argument. LCS in beverages do not increase hunger and do not lead to overconsumption, compared with non-sweet beverages (Mattes and Popkin, 2009). In many exciting studies, LCS have been shown to reduce the intake of sugar-sweetened foods and facilitate weight loss (Anderson and Woodend, 2003).

Sweetness influences, to a large extent, the perceived food flavour in many foods and drinks, and it is one of the most important sensory attributes that drives consumer liking towards foods and drinks (Pfeiffer et al., 2006). The high palatability of sweet-tasting food is believed to trigger overeating, as palatability has been shown to be positively corrected with the *ad libitum* intake of foods (Bellisle et al., 2012). On the other hand, enhanced oral sensory stimulation from sweet food has been shown to suppress food intake (Lavin and Read, 2000). This makes the role of sweet stimuli in satiation and satiety highly complex.

1.9.2.1.1 Sweetness and *ad libitum* intake (satiation)

The intensity of food flavour may play an important role in determining the energy intake of a meal (Sorensen et al., 2003). Increasing the duration of oral sensory exposure per bite of food has been found to induce stronger satiation and reduce *ad libitum* food intake (Bolhuis et al., 2011). It is likely that increasing the intensity of food flavour increases the intensity of oral sensory exposure per bite, and therefore may result in a faster satiation and decreased *ad libitum* food intake (Bolhuis et al., 2010).

Sweetness has been suggested to contribute to the reduced feeling of hunger and increased the feeling of fullness (Lavin and Read, 2000, Anderson and Woodend, 2003). This may be due to the increased oral sensory exposure by increased sweetness, which leads to enhanced satiation and reduced food intake. On the other hand, sweetness evidently increases the palatability of many foods and drinks. Most studies have suggested that food intake increased as palatability increased (Yeomans, 1998, Sorensen et al., 2003). Therefore, the effect of sweetness intensity on *ad libitum* intake is complex as increased sweetness (likely reduce intake) may be confounded by increased palatability (increase intake) in affecting intake. In Chapter 3, the effect of sweetness intensity of a drink on the *ad libitum* intake of the drink was investigated, while the palatability of the drink was controlled.

1.9.2.1.2 Sweetness and sensory-specific satiety

Apart from its effect on general satiation, the sweetness of a food may also affect the subjects' sensory-specific satiety or satiation to general sweet foods (Rolls et al., 1981, Rolls, 1986). One of the important issues that remain unclear is whether the intensity of a flavour modality (i.e. aroma, taste, texture) affects the extent and duration of the sensory-specific satiety or satiety (Hetherington and Havermans, 2013), as conflicting results were found in the literature. Following monotonous intake of sweet juices for several days, the pleasantness ratings of the sweetest juice decreased greater than the less sweet juices (Essed et al., 2006). This suggests that sensory-specific satiety is enhanced with

increased intensity of a flavour modality (i.e. sweetness). In contrast, Chung and Vickers (2007) observed that the sensory-specific satiety for a sweet tea was strong when the sweetness of tea was lower than when the sweetness of the tea was higher. This suggested that more intense sweetness induced weaker sensory-specific satiety compared with less intense sweetness. Havermans *et al*. (2009) reported that the pleasantness rating of a lemonade drink was not affected by its intensity of strawberry flavour (overall flavour), suggesting that sensory-specific satiety or satiety is unaffected by the intensity of flavour perception. Therefore, one of the objectives of this thesis is to investigate the effect of the sweetness intensity (as a flavour modality) on the extent of sensory-specific satiety (Chapter 3).

1.9.3 The role of aroma in satiation and satiety

The primary olfactory cortex receives information of the aroma perception, then connects and interacts with many areas of the brain including the OFC (pleasure, hedonic and sensory-specific satiety), hypothalamus (energy homeostasis), amygdala and hippocampus (emotion and memory) (Marciani et al., 2010). Therefore, aroma cues influence not only odour discrimination and perception but also cognitive, motivational and emotional responses that are related to feeding behaviours (Bear et al., 2001). It may be less likely for aroma volatiles to contribute to the post-digestive and post-absorptive signals in the satiety cascade, but they may be involved in satiation and short-term satiety via sensory, cognitive, hedonic and other brain mechanisms, and possibly the gastric and vagal nerve mechanisms (Rolls, 2015).

The orthonasal and retronasal aroma stimuli might influence appetite and feeding behaviours differently, possible due to the differences in their delivery efficiency, perceptual experiences and difference in associative learning (Small et al., 2005).

1.9.3.1 Effect of orthonasal aroma

Aroma volatiles delivered orthonasally are perceived as arising from the external environment. Orthonasal aroma delivery is associated with the anticipation of a potent food reward; hence, orthonasal aroma may signal that a food may be perceived as appetising (Ruijschop et al., 2009a).

A few studies have found that a food odour from the environment can stimulate the release of saliva and gastric acid and promote appetite (Yeomans, 2006). Ferriday and Brunstrom (2011) reported that the sight and smell of freshly baked pizza increased the salivation of overweight subjects. It suggested that the aroma cues together with vision cues might help prepare the body for nutrient ingestion.

Ramaekers et al. (2014) reported an increase of appetite for an aroma-specific food during 20 minutes exposure to the aroma present in a room (Ramaekers et al., 2014). For example, a specific appetite for tomato soup increased when subjects were exposed to a tomato soup aroma, compared to no aroma or another aroma (banana) (Ramaekers et al., 2014). This aroma-specific appetite was observed for all test food aromas including tomato soup, meat soup, bread, chocolate, brownie, and banana (Ramaekers et al., 2014). It indicates that a specific aroma cue may prepare the body for ingestion of the aroma-cued food (Ramaekers et al., 2014). Furthermore, food aroma also increased the general appetite for foods. However, the increase in the general appetite (5%) was relatively smaller when compared to the increase in the aroma-specific appetite (5-20% increase depending on the type of the aroma) (Ramaekers et al., 2014). Potentially, the exposure to any food aroma signals the availability of food in the environment and promotes the start of an eating event (Ramaekers et al., 2014).

However, other studies have shown contradicting evidence, that orthonasal aroma could suppress appetite. Massolt et al. (2010) reported that subjective appetite was suppressed by smelling dark chocolate for only 5 minutes. It was supported by physiological evidence that the suppressed appetite was correlated with a decreased ghrelin level.

Decreased ghrelin indicated the suppression on hunger (Cummings, 2006). In addition, Rolls (1997) have shown that orthonasal aroma can induce sensory-specific satiety.

In the three studies from Massolt (2010), Jansen (2003) and Rolls (1997), subjects were instructed to smell the food closely so that the aroma delivered to the nasal cavity might be intense. By contrast, in the study done Ramaekers et al. (2014), food aroma was distributed in a room and the intensity may be modest. One of the characteristic differences between retronasal aroma and orthonasal aroma delivery is the concentration of aroma reaching the olfactory epithelium (receptors) (Rozin, 1982). The aroma concentration reaching olfactory epithelium is usually much smaller during orthonasal delivery than retronasal delivery because the orthonasal delivery lacks the oral food processing including salivation, warming and mastication (Burdach et al., 1984, Ruijschop et al., 2009a). It is thought that by asking subjects to smell the food closely allowed a higher concentration of aroma reaching olfactory epithelium, inducing satiation similar to a retronasal aroma profile (Massolt, 2010). However, another possible explanation for the observed contrasting effect of orthonasal aroma in those studies may be due to the difference in the quality of the aroma.

It suggests that orthonasal aroma plays a role in appetite and satiation. However, whether it initiates appetite and prepares the body for ingestion, or promote satiation may depend on the type and intensity of the orthonasal aroma, the individual subject' characteristics, and the experimental settings.

1.9.3.2 Effect of retronasal aroma

Retronasal aroma delivery is associated with food intake in the mouth, and possibly a receipt of a food intake (Ruijschop et al., 2009a). Retronasal aroma delivery is typically associated with flavour perception during eating,

therefore its manipulation has the potential to enhance the satiating power of a food or drink (Small et al., 2005).

Consuming solid foods needs more intense oral processing due to its complex texture, which generates a long and more intense retronasal aroma stimulation. In contrast, the consumption of a liquid drink produces a short and spiked retronasal aroma profile (Brauss et al., 1999, Ruijschop et al., 2008b, Weel et al., 2003). The difference in aroma release profile may contribute to some extent to the higher satiation of solid foods when compared to liquid foods.

Ruijschop et al. (2008) compared the satiation effect of a sweetened milk drink of a liquid-like aroma profile, with the same drink of a solid-like aroma profile (Ruijschop et al., 2008a). The strawberry aroma was delivered retronasally but separated from the gustatory system, by olfactometer with a silicon tube placed into the subjects' nasal cavity (Ramaekers et al., 2014). Subjects felt significantly more satiated (10%, 10mm higher on 100 mm scale) when consuming the drink with a solid-like aroma profile, compared with the drink with a liquid-like profile during 10 minutes administration of aroma (Ramaekers et al., 2014). However, 5 minutes after the drink preload, the difference in appetite sensation between two drinks was not significant, and the subsequent intake of a strawberry-flavoured drink was not different (Ramaekers et al., 2014). This study suggests that retronasal aroma could induce satiation but not satiety. One limitation of the study was that the retronasal aroma delivery method may be invasive and uncomfortable to subjects, and it did not represent the real-life retronasal aroma delivery.

In addition to aroma intensity, the complexity of retronasal aroma has also been found to influence satiation. In another study by Ruijschop et al. (2010), subjects felt more satiated (5-10%) when consuming a yogurt with a multi-component strawberry aroma, compared to the same yogurt with a single-component strawberry aroma (Ruijschop et al., 2010). The muli-component aroma was perceived as more complex than the single-

component aroma, but both were perceived as similar in aroma intensity and pleasantness (Ruijschop et al., 2010). Potentially, the multi-component aroma increased satiation by activating more aroma receptors in the brain, which contributed to an enhanced overall flavour perception (Ruijschop et al., 2010).

Despite clear evidence of inducing satiation, the extent of retronasal aroma did not appear to alter the food intake. It is likely that the observed magnitude of the increase on satiation by retronasal aroma was too small (<10%) to have a significant effect on intake (Benelam, 2009).

There is limited knowledge about the complex mechanisms behind the observed effect of retronasal aroma on satiation. One possible explanation is that increasing the extent of retronasal aroma perception contributes to the increased oral sensory exposure, which leads to increased satiation (Bolhuis et al., 2011, Ruijschop et al., 2009a). Another explanation is that humans learn to associate enhanced retronasal aroma with increased intake of energy and nutrient through flavour-nutrient associative learning, and such associate enhanced perceived satiation (Yeomans, 2012).

Although it seems promising to induce satiation via the manipulation of retronasal aroma release in a food product, the current findings in the research field are still preliminary and limited studies have been done (Ruijschop et al., 2009a). The observed effect on satiation was small, and it will be challenging to obtain an actual effect on food intake (Ruijschop et al., 2009a). More evidence is needed to confirm this observation on a variety of food-related aroma cues, and to explore the mechanisms.

1.9.4 Aroma-taste interaction on satiation and satiety

So far, studies have focused on the independent effect of taste or aroma perception on appetite and eating behaviour. As far as the author is aware, the interactive effect of aroma-taste on appetite and eating behaviour has not been studied in comparison to their independent effects.

The enhancing effect on flavour perception from the interaction of congruent taste and aroma has been well studied and confirmed in both the psychophysical and neural studies (Wallace, 2015). On the one hand, congruent taste and aroma presented together increased overall perceived flavour intensity, which may lead to an increased extent of oral sensory exposure. A greater extent of oral sensory exposure has been associated with enhanced satiation (Bolhuis et al., 2011). On the other hand, congruent taste and aroma were found to increase activation in the hedonic areas of OFC and ACC, which were correlated with increasing pleasantness ratings (Rolls, 2012). Certain combinations of taste and aroma could promote food intake and increase appetite by producing highly pleasant food (Rolls, 2012, Rolls, 2009b). Therefore, whether aroma and taste interact to promote satiation or increase appetite remain unclear. It may also depend on the type of the flavour combination.

1.9.5 The role of texture in satiation and satiety

Food texture has been shown to influence satiation, satiety and food intake, which may be explained by both its apparent influence on the oral sensory processing of foods, and its effect on post-digestive physiological feedback.

A thicker beverage (extra addition of 0.1g / 325 ml microcrystalline cellulose compared to the thinner one) reduced the feeling of hunger greater and longer than a thinner beverage for over 4 hours postprandial (Mattes and Rothacker, 2001). The two beverages were matched for appearance, energy density, macronutrient content, palatability, and only differed in viscosity. This study suggests that texture alone, independent of energy and nutrients, could influence satiety.

Zijlstra et al. (2008) investigated the effect of viscosity on *ad libitum* intake and explored the possible mechanism of viscosity-induced satiation. Three chocolate-flavoured milk drinks were tested, and their ingredients differed only in the modified starches used, in order to achieve liquid, semi-liquid or

semi-solid textures. *Ad libitum* consumption of the milk drinks decreased with increasing thickness. Meanwhile, consumption rate was positively correlated with *ad libitum* intake and negatively correlated with thickness. They suggested that viscosity reduced the drink intake partly through slowing the consumption rate, which leads to a higher and longer oral sensory exposure (Zijlstra et al., 2008).

de Wijk et al. (2008) reported that food viscosity reduced intake via reducing bite size and increasing bite effort. In a first study, subjects consumed 47% more of a chocolate-flavoured milk drink than a semi-solid equivalent. The two test drinks were only different in the type of starch used. The liquid was consumed in larger bite sizes and with less bite effort than the semi-solid (de Wijk et al., 2008). In a second study, the difference in bite effort was removed by using a peristaltic pump while subjects could adjust the bite size. The consumption of two drinks was not significantly different, but the bite size was positively correlated with hunger, desire to eat and reduced fullness. Increased bite effort was associated with reduced bite size (de Wijk et al., 2008). The authors suggested that increasing food viscosity promotes satiation by reducing bite size and increasing bite effort.

Zhu et al. (2013) investigated the effect of viscosity on *ad libitum* intake of a food and explored possible mechanism of viscosity-induced satiation. Subjects consumed the same portion of a high viscosity (HV) and standard viscosity (SV) semi-solid meals, which were different only for the guar gum addition. Following consumption of the HV meal, rated feeling of hunger were significantly reduced for over 3 hours postprandial, compared to the feeling of hunger following the consumption of the SV meal. In addition, the plasma glucose concentration was significantly higher following HV meal; the HV meal also delayed gastric emptying (Zhu et al., 2013).

1.9.6 Food flavour and expected satiation and expected satiety

Apart from its role in satiation and satiety, food flavour may play an important role in shaping our cognitive expectation of the satiating capacity of the food through flavour-nutrient association (Yeomans, 2012).

Hogenkamp et al. (2011) compared expected satiation of a number of commercial dairy products, including milk, yogurts, and custards. Expected satiation was positively correlated with thickness, sweetness and creaminess intensities, but it was negatively correlated with sourness and freshness intensity across different products. This indicates a role of flavour and texture in affecting expected satiation. However, the independent effect of each attribute could not be concluded because those commercial products were distinctly different in many other uncontrolled attributes. Hogenkamp et al. (2011) then designed a custard product as a food model to study the independent effect of thickness intensity and type of flavourings. Varying thickness intensities of the custard was achieved by adding different modified corn-starch; and two flavourings (i.e. lemon or meringue) were added to achieve different flavour. The thickness of custards was positively correlated with expected satiation while the two flavourings did not affect expected satiation. In contrast to its effect on commercial products, sweetness intensity showed a slightly negative correlation with expected satiation of custards. The suppressed sweetness intensity was likely due to a reflection of increased thickness, as thickness has been found to suppress the sweetness perception (Hollowood et al., 2002). Therefore, the independent role of sweetness on expected satiation remains inconclusive.

Hogenkamp et al. (2012) investigated expected satiation of vegetable soups with different energy density and sensory attributes. The high energy dense soup was expected to be more satiating than the low energy dense soup (Hogenkamp et al., 2012). Moreover, expected satiation of the soups was positively correlated with the thickness and intensity of overall taste (Hogenkamp et al., 2012). However, the role of a specific taste, e.g. sweet and salty taste, in expected satiation was unclear.

McCrickerd et al. (2012) assessed expected satiation and expected satiety of a fruit yogurt drink with high or low thickness and creaminess. The thickness of the yogurt was manipulated by adding a different amount of tara gum while the creaminess was changed by adding different amount of aroma (vanilla and milk caramel) (McCrickerd et al., 2012a). The observed effect of thickness and creaminess on expectation was independent of the effect of energy content. Although increasing both the thickness and creaminess intensity increased subjects' expected fullness, only thickness enhanced the expected satiety of the yogurt drink (McCrickerd et al., 2012a). Possibly, the difference in the creaminess was too subtle (5-16% on VAS) to have a significant effect on expected satiety, compared with the large difference in thickness (20-35% on VAS). However, flavour may have a potential impact on expected satiety or satiation if the difference in flavour is large enough. In addition, 'creaminess' is not only a flavouring attribute, but also a combination of flavour and texture attributes (McCrickerd et al., 2012a, Kirkmeyer and Tepper, 2005). The observed effect of creamy flavour on expected fullness may be an indirect reflection of increased thickness, which also enhanced expected 'fillingness'. Therefore, further investigation is required on the role of flavour on expected satiation and expected satiety. However, higher expected satiation has been found to be associated with higher self-selected portion size. Subjects selected a larger portion of a thicker soup and consumed more of it than a thinner soup (McCrickerd et al., 2014).

1.9.6.1 Summary of literature gaps

In summary, there is clear evidence of thickness enhancing both the expected satiation and expected satiety of foods. However, the relative importance of a specific taste (e.g. sweetness) and aroma, on the expected satiation and expected satiety of foods need further investigation, which will be studied and discussed in Chapter 5. The relative relationship between expected satiation and expected satiety is not established, which will be explored in Chapter 5.

1.10 Aim and objectives of the thesis

In view of global overweight and obesity, the main aim of this thesis was to investigate the role of flavour perception of foods in appetite and eating behaviour; and therefore to explore how managing food flavour as a novel approach might help weight management.

Important flavour modalities studied include aroma, taste, and texture (thickness). This thesis focused on the influence of food flavour on subjective appetite sensation, food intake, sensory-specific satiety, and expected satiation and expected satiety. Specifically, three behaviour studies were reported in this thesis to answer unresolved questions in the field (**Table 1-1**):

- Study 1 (Chapter 3): Whether the sweetness intensity of a beverage affects *ad libitum* energy intake of the beverage, independent of the effect of palatability of the beverage; and whether the sweetness intensity of the beverage affected sensory-specific satiety;
- Study 2 (Chapter 4): How retronasal aroma, taste and aroma-taste interaction affect appetite sensation and subsequent food intake;
- Study 3 (Chapter 5): How aroma, taste, and texture (thickness) affect the expected satiation and expected satiety of a food.

Table 1-1 Summary of the objectives of the thesis

Flavour modalities	Outcome measurements			
	Subjective appetite sensations	**Food intake**	**SSS**	**Expected satiation & Expected satiety**
Taste	Chapter 4	Chapter 3, 4	Chapter 3	Chapter 5
Aroma (retronasal)	Chapter 4	Chapter 4		
Taste-aroma interaction	Chapter 4	Chapter 4		
Texture				

1.11 Layout of the thesis

Following the general introduction (Chapter 1), a review of current literature methods and general methods used in this thesis are described in Chapter 2.

Three independent behaviour studies are reported in Chapters 3, 4 and 5, to address the defined objectives of the thesis. Each chapter has an individual introduction section with a literature review relevant to that study. Each chapter has an individual discussion section.

Lastly, the general discussion chapter in Chapter 6 provides a summary of the key findings and their implication, strength and weakness of the studies, and opportunities for future research.

Chapter 2. General Methods

2.1 Introduction

This chapter will first review the common methodologies used in assessing appetite and eating behaviour in the current literature (section 2.2). Section 2.3 will outline the general methodologies used in the thesis. Methods and materials used in a particular study will be described in the method and material sections of chapters 3, 4 and 5.

2.2 Literature review of the methods

2.2.1 Measuring satiation and satiety

Satiation and satiety by definition are the elements and processes involved in influencing eating behaviour. Amongst most behaviour studies, both of satiation and satiety are commonly measured via subjective assessment of appetite sensation and objective evaluation of food intake. However, the experimental designs and procedures for measuring satiation and satiety can be very different, and should be tailored depending on the objective of the study. (Chapelot, 2013)

2.2.1.1 Measuring satiation

Satiation is the processes, which leads to the termination of a meal. The method of ‘concurrent evaluation’ is usually used in measuring satiation, where the study attributes are present within the measured food (Chapelot, 2013). Satiation is most commonly measured via meal intake or subjective appetite ratings at several time intervals within the meal.

2.2.1.1.1 *Ad libitum* energy intake

The satiation of a food can be measured as the *ad libitum* (at one’s pleasure) amount of the food eaten until reaching satiation, in comparison to a control food in a laboratory setting (Benelam, 2009). However, food intake in humans can be affected by many factors apart from internal

satiation or satiety signals, induced by an intervention treatment that is studied (Benelam, 2009). These factors may include the palatability of the food, the eating environment, the participant's emotional status, physiological, social and culture influences (Mattes et al., 2005). In order to minimise the influence of such factors, studies on food intake are usually conducted in a laboratory with a careful design and controlled environment (Benelam, 2009).

Ad libitum intake measurements conducted in a laboratory, enable the study of the determinants of food intake under tightly controlled conditions. Participants are given a standard portion of a single or a multi-item meal, and they are asked to eat until they feel comfortably full. The foods should be palatable or at least acceptable to participants, otherwise, they may not eat them (Benelam, 2009). Portion size should be reasonable large to allow *ad libitum* intake but also close to the normal presentation size. A very large portion size may increase intake and lead to overconsumption (Rolls et al., 2004a). However, this issue can be usually overcome by using refillable/self-refilling bowls (Wansink et al., 2005).

2.2.1.1.2 Appetite sensations during the meal

Measuring *ad libitum* intake is straightforward, but it does not provide the dynamic change in satiation within the eating event (Booth, 2009, Chapelot, 2013). Subjective appetite sensation, such as hunger and fullness, can be rated by participants at several time intervals within the meal, hence describing the changes of satiation over time. It also provides a cognitive dimension to the satiation measurement.

2.2.1.2 Measuring satiety

Satiety is the persistent suppression of appetite after a meal and before the next meal. Measures for satiety include satiating intensity, the duration between two meals and subsequent food intake. The duration of satiety is usually neglected because humans have fixed mealtimes, however,

humans may have 24/7 snacking (Chapelot, 2013). Generally, satiety is assessed by measuring the satiating intensities using subjective appetite scales at several time intervals following the meal (preload) and by measuring the subsequent meal energy intake, using a 'preloading paradigm'. In a 'preloading paradigm' study, the study attribute is present in the preload prior to record *ad libitum* intake of a subsequent meal (Kissileff, 1985). A control (blank) preload is conducted, in addition to the test preloads under the same experimental conditions. The differences between the satiety characteristic of interest, following the control preload and the test preloads are compared to evaluate whether the study variables in the test preload has a valid effect on satiety.

2.2.1.2.1 Subsequent *ad libitum* energy intake

Measuring subsequent meal intake provides a direct and concrete evaluation of changes in eating behaviour caused by a preload (Blundell et al., 2010). Meal intake can be affected by factors other than internal appetite signals; therefore, caution about the design and presentation of the test meal must be taken (section 2.2.1.1.1). Where possible, macronutrients in the test meal should be balanced, rather than disproportional high in one macronutrient (Chapelot, 2013). High fat content has been shown to increase meal size more than high carbohydrate content (Green et al., 2000). In addition, experimental control over the time interval between a preload and a subsequent meal is crucial; thus, participants should stay in the controlled laboratory setting when possible (Benelam, 2009).

2.2.1.2.2 Appetite sensations after the meal

To measure the satiety of a preload, subjective appetite sensations can be recorded frequently covering the entire time interval between the preload and the subsequent test meal (Blundell et al., 2010). Since satiety is time-dependent and different factors are involved in different phases of the satiety cascade, the time interval between a preload and a test meal

should be carefully designed to meet the study objective. A time interval of 2 to 6 hours is usually applied when studying the post-absorptive factors influencing satiety (Chapelot, 2013). In order to study the effects of sensory, cognitive and gastric factors on satiety, the time interval is approximately 40 minutes or less (Benelam, 2009). If the time interval is too long, the effect of the preload on subsequent food intake may not be detected (Blundell et al., 2010). In addition, time intervals between two ratings vary from 15 to 60 minutes in most studies, to study short-term factors such as sensory factors, ratings should frequently be recorded.

2.2.1.3 Subjective appetite sensations by VAS

Subjective appetite sensations are commonly measured by multiple scales including feelings of hunger, fullness, and satiation, desire to eat and prospective consumption (Blundell et al., 2010). Additional scales including the desire to eat a specific food can be applied when studying the effect of sensory properties on appetite, such as, desire to eat something sweet or savoury (de Ggraaf et al., 1992). Among all scale types, the visual analogue scales (VAS) are the most commonly used (Blundell et al., 2010). A VAS scale consists of a 100 or 150mm continuous line, with an appetite sensation or motivation question above the line, and the terms describing the minimum and maximum intensity at two ends of the scale, respectively. Participants draw a vertical mark on the line to indicate the intensity of the appetite sensation.

2.2.1.3.1 Repeatability, power and validity

Overall, VAS scales have good repeatability for the group means and comparisons of different foods or stimuli, with an appropriate experimental design and under a well-controlled laboratory condition (Flint et al., 2000, Blundell et al., 2010). Among all measures, hunger scale has been shown to have the best reproducibility (Merrill et al., 2002). However, there were large variations between individual participant’s ratings (Blundell et al., 2010). The large variation may reflect the true biological variation and

differences in participants' ways of using the scales. For example, some participants prefer to rate among the middle range of the scales, while others prefer to rate towards the end. The repeatability between different individual participants can also vary. To reduce between-participant variation, a 'within-subject design' should be applied where all participants complete both the control and the test conditions (Blundell et al., 2010).

The VAS scales generally have acceptable validity in the short term (Flint et al., 2000). The validity of VAS scales is difficult to determine since it is a subjective self-reported measure. One way to investigate the validity of VAS is to check the correlation of VAS ratings with energy intake. Flint (2000) reported that the mean postprandial values of VAS ratings over the time interval were correlated with subsequent food intake. However, the correlation between VAS ratings and energy intake is generally modest in appetite studies (Blundell et al., 2010). A lack of correlation between VAS and food intake does not invalidate the measure as a reflection of the eating motivational state of the participants, as humans often eat regardless of the presence of hunger (Blundell et al., 2010). Ideally, food intake and VAS appetite ratings should be measured.

2.2.2 Measuring sensory-specific satiety (SSS)

SSS can be characterised by changes in the subjective ratings of pleasantness or desire to eat before and after intake, using VAS scales; and by comparing the subsequent intake of foods with a similar and a different sensory characteristic (Hetherington and Havermans, 2013).

2.2.3 Measuring expected satiation or satiety

2.2.3.1 Subjective assessment

De Graaf et al. (1992) were the very first to quantify human participant's expectation about the satiating effect of foods. Expected satiety of sandwiches was measured by the estimation of the time that the sandwich was expected to stave off hunger, and by the expected satiating intensity

on a 20-point category scale. This method was able to distinguish the expected satiating effect of sandwiches with different macronutrients. However, it has been questioned whether humans can reliably assess the time length of satiety (Brunstrom et al., 2008b).

Green and Blundell (1996) assessed the 'expected filling' of snacks after consuming a mouthful of the snack using a 100 mm VAS scale (Green and Blundell, 1996). Participants were able to rate and discriminate their expected 'fillingness' among several snacks. This method was quick and easily conducted, but it may be inefficient in assessing products of varying categories (Poulton, 1979). Despite that, VAS scales are still used in some studies assessing the expected satiation/satiety as a quick and easily implemented method (McCrickerd et al., 2015, McCrickerd et al., 2014). VAS scale was used to assess the expected satiation of several beverage samples in Chapter 4.

2.2.3.2 Method of constant stimuli

Brunstrom et al. (2008) developed a relatively bias-free methodology to measuring satiety expectations using a classical psychophysical technique called the 'method of constant stimuli' (Brunstrom et al., 2008b). It is a highly sensitive method and can compare differences in expectations across a large variety of foods on a calorie-to-calorie basis (Brunstrom et al., 2008b). A 'standard' food with fixed and known energy content was displayed on a computer screen, next to which, was a picture of a 'comparison' food. The energy content of the comparison food changed over 56 trials, and on each trial, the participants were to indicate which of the standard food and the comparison food was expected to stave off hunger for longer. A plot was then made to show the probability that the standard food will be selected over the comparison food for a range of energy values (40 pictures=40 energy values). The energy content of the comparison food that is selected for a probability of 50% times was the amount of energy content it was expected to be equal as satiating as the standard food. Results show that this method is robust, reliable and highly

sensitive in comparing expected satiety across a large number of common foods. This method is highly reproducible, with a very low coefficient of variation (CV) of less than 5% across experiments (Brunstrom et al., 2008b). However, one disadvantage of the method of constant stimuli was that participants needed to give many responses, which was burdensome and time-consuming (Brunstrom, 2011).

2.2.3.3 Method of adjustment

Brunstrom and Rogers (2009) then developed a methodology called 'method of adjustment' in assessing expected satiation and satiety, which is a quicker version of the 'method of constant stimuli' (Brunstrom and Rogers, 2009). In this method, participants were presented with a fixed amount of a standard food, and a comparison food in 40 to 70 pictures with varying energy content. Participants could adjust the size of comparison food and select a picture with the energy content that was expected to stave off hunger for the same time length as the standard food. The energy content of the selected picture was the energy content of the comparison food that was equally satiating as the standard food. Similar to the 'method of constant stimuli', the method of adjustment had been shown to be highly sensitive and valid in assessing 17 different foods (Brunstrom and Rogers, 2009). It was then used in a number of studies for comparing the expected satiety of foods with different properties (Hogenkamp et al., 2011, Forde et al., 2013, McCrickerd et al., 2015, de Graaf, 1995). The coefficient of variation in assessing the expected satiation or satiety of a food varied from 10% to 40% across studies.

Expected satiation can be measured similarly to expected satiety by asking slightly different questions (Brunstrom et al., 2008b). For example, participants are asked to select the picture of a comparison food that is as equally satiating as the standard food immediately after consumption. However, the precise relationship between the expected satiation and expected satiety remains unclear. In this thesis (Chapter 5), the relationship between the expected satiation and expected satiety of a

product (custard) was investigated, using a modified method based on the ‘method of adjustment.’

2.3 General methods used in this thesis

2.3.1 Introduction

This section outlines the general methodologies used throughout the thesis. Methods used in a particular study will be described in the method sections of chapter 3, 4 and 5.

2.3.2 Study location

All studies were carried out in the Sensory Science Centre at the University of Nottingham. Sessions were conducted in an air-conditioned room at 18°C, under Northern Hemisphere daylight, in individual booths designed to meet ISO: 8589:2007(ISO, 2011). Studies in the following chapters were approved by the School of Biosciences Research Ethics Committee or the Medical Ethical Committee of the University of Nottingham prior to conduction.

2.3.3 Participants

2.3.3.1 Recruitment

Prospective participants were approached via email or poster. They were asked to participate voluntarily in the study. All who expressed interest in participating were given the participant’s information sheet before attending a screening session. General participants were recruited from the staff and students of the University of Nottingham. The trained panellists, required in Chapter 5, were recruited from the Sensory Science Centre panel, all having more than 10-year experience as a trained sensory panellist.

2.3.3.2 General participant criteria

General inclusion criteria are listed below. Participants were required to meet all the appropriate criteria before taking part. The study with different inclusion criteria will be mentioned in the chapter method section.

General inclusion criteria for consumer participants:

- Female (except Chapter 5: male and female)
- Aged 18-45 years
- BMI with 18.9~24.9 kg/m^2
- Healthy-no history of disease (self-report)
- Non-smokers
- Normal gustatory and olfactory senses (self-report)
- Not taking any medications except oral contraceptives
- Not clinically depressed, defined as a score ≤ 9 on the Beck Depression Inventory
- Not pregnant or breastfeeding (female participants)
- No allergy or intolerant to food ingredients used in each study (self-report)
- No weight loss or gain of more than 4 kg in the past 6 months (required only for studies involving *ad libitum* food intake: Chapters 3 &4)
- Non-restrained eaters (a score of < 7 for restraint factor on the Three Factor Eating Questionnaire, TFEQ)

2.3.4 Screening

All participants attended a screening session before taking part in the study sessions. During the screening session, a comprehensive verbal explanation of the study was given to the participants, and they were given the chance to ask any questions. Informed written consent was obtained once participants had indicated they were willing to participate. Their

height and weight were measured by the experimenter, and screening questionnaires regarding the study criteria were completed.

2.3.4.1 Weight, height and BMI

Body weight was measured using a digital scale with a capacity of 150kg (Seca, Germany), and height was measuring using a 0-220 cm wall-mounted roll-up tape (Seca, Germany). Participants were wearing light clothing and with their shoes removed during the measurements. The Body Mass Index (BMI) was then calculated according to the following Equation 2-1:

Equation 2-1: $BMI\ (kg/m^2) = Body\ mass\ (kg) / Height^2\ (m^2)$

2.3.4.2 Screening Questionnaires

The following questionnaires were completed by participants.

- General Information (Appendix I)
- Three Factor Eating Questionnaire (Appendix II)
- Beck Depression Inventory (Appendix III)

2.3.4.2.1 Three Factor Eating Questionnaire (TFEQ)

Dietary restraint refers to the cognitive self-control to restrict food consumption, to achieve certain goals like weight loss (Herman and Mack, 1975). Restrained eaters may hold an eating style that is under cognitive control, which may override the internal physiological control and satiation (or satiety) cues from the food itself (Johnson et al., 2012). At times, restrained eaters may successfully restrict food intake through cognitive self-control. However, they may develop reduced sensitivity to internal satiety signals over time, which often leads to disinhibition and overeating when cognitive control is undermined (Polivy and Herman, 1985). Restrained eaters behave very differently in food consumption, compared to non-restrained eaters in measuring appetite and eating behaviour. Therefore, restrained eaters were excluded from taking part in the studies,

in order to avoid bias from dietary restraint and to reduce between-subject variation.

The TFEQ was chosen in this thesis, as it has shown good participant consistency as one of the tools to measure the level of dietary restraint (Stunkard and Messick, 1985). The TFEQ contains 51 questions covering three measurements of eating behaviour:

- Cognitive dietary restraint (Factor I), 21 questions;
- Disinhibition (Factor II), 16 questions;
- Hunger (Factor III), 14 questions.

Each question scored by a participant either 1 or 0. The sum of scores for the dietary restrained questions was used to indicate the level of restraint, and higher scores associated with higher level of restraint in everyday life. In this thesis, participants who scored 7 or above, on the restraint factor of the TFEQ questionnaire in the screening session, were excluded from participating in the studies. Previous studies have used a restrained score above 10 for high restrained eaters (Bellisle et al., 2009), and a score of 7 is often used as a cut-off for high restrained eaters to exclude borderline scores (Astbury et al., 2011).

2.3.4.2.2 Beck Depression Inventory

The symptoms of clinical depression include a significant increase or decrease in appetite for food, and weight loss or gain (Paykel, 1977, Hall and McMahon, 2007). In addition, some patients with depression symptoms displayed changes in their perception of taste and odour stimuli, including changes in detection threshold, perceived intensity, odour identification, and a decreased pleasure towards the stimuli (Amsterdam et al., 1987). Therefore, participants who were clinically depressed were excluded from the study.

The Beck Depression Inventory (1961) was chosen because it is a commonly used self-report multiple-choice questionnaire to evaluate the

severity of the symptoms of clinical depression in individuals aged over 13. Participants completed 21 questions covering symptoms of hopelessness, irritability, cognitive belief of guilt, as well as physical symptoms such as fatigue, weight change, and loss of interest in sex, perceived by the participant in the last week. A score of 0 to 3 was assigned to different response choices for each question, and a higher score indicating a more severe symptom of depression. The sum score was therefore calculated to determine the overall severity of the depression symptoms, according to Beck et al. (1988):

- A score of 0-9: minimal depression
- A score of 10-18: mild depression
- A score of 19-29: moderate depression
- A score of 30-63: severe depression

Participants who demonstrated symptoms of clinical depression, with a score ≥10 on the Beck Depression Inventory, were excluded from taking part in the studies in this thesis.

2.3.5 Measuring subjective appetite sensations using VAS

The appetite sensations were assessed by using a 100 mm Visual Analogue Scale in this thesis. The statements of questions and anchors of VAS scale were based on a recommendation from Blundell (2010), **Figure 2-1**.

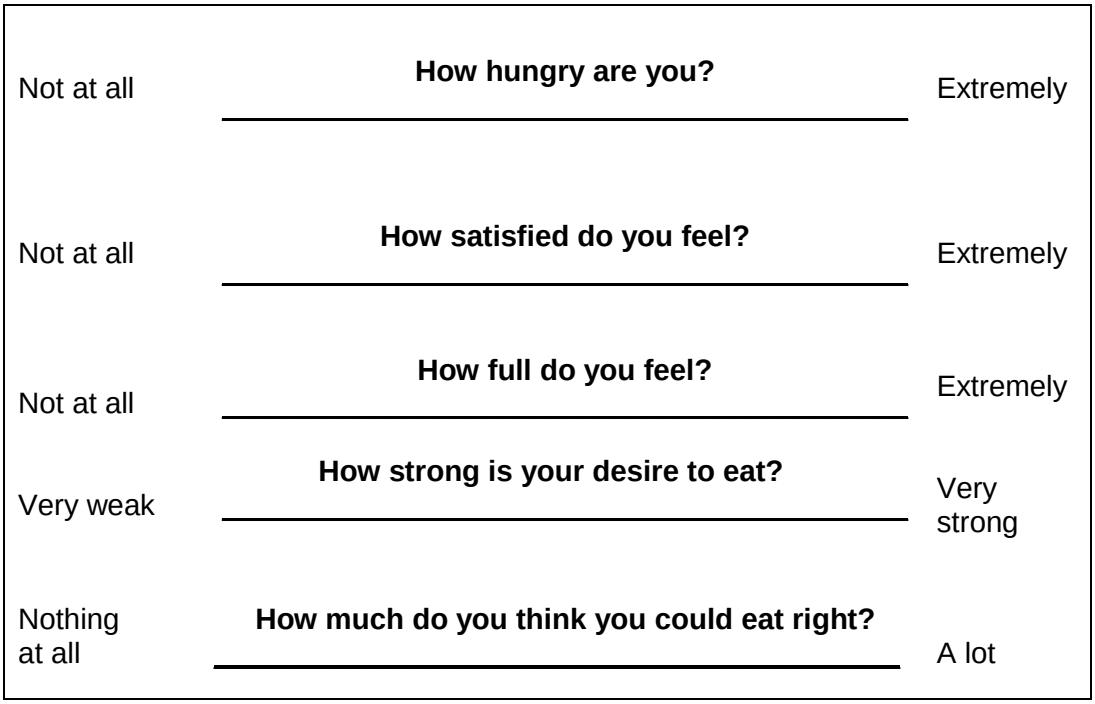

Figure 2-1 Subjective appetite sensations VAS questionnaire (Blundell et al., 2010).

2.3.5.1 Electronic VAS

Subjective appetite ratings were rated on computerised VAS using FIZZ software (Biosystems, Couternon, France), in Chapters 3 and 4. The length of the VAS on screen was 100 mm. Participants moved a vertical bar on the VAS line to the desired position using the computer mouse. Data was collected automatically by Fizz as 0 to 100. At each time point, a separate VAS questionnaire was presented on the screen.

2.3.5.2 Paper based VAS

Paper-based VAS scales were used in Chapter 5. The questionnaire for appetite sensations (Figure 2-1) were presented on A4 paper sheets. Each sheet was presented to the participant at the measuring time point, and the participants were instructed not to look at their ratings on previous sheets. Participants were asked to insert a vertical line at the point best describe the intensity of the perceived appetite sensation on the VAS

scale. Ratings were scored from 1 to 100 and measured using a steel ruler (precision: 1 mm).

Both the paper and electronic VAS are equally reliable but they were not used interchangeably (Benelam, 2009). In each study of the thesis, either paper-based or electronic based VAS was used. Participants were trained on the use of the VAS scales before commencing each study.

2.3.6 Measuring food energy intake

Energy intake of the test drink (Chapter 3, milkshake) and food (Chapter 4, pasta) were measured in *ad libitum* settings, at the Sensory Science Centre. In separate individual booths, each participant was provided with a standard serving portion of the test food or drink, and was instructed to 'consume the food or drink until whenever they felt comfortable full. Participants were encouraged to ask for another portion, or terminate consumption even if the portion was not finished, according to their feeling of fullness. The amount of food or drink consumed was recorded, and the total energy intake was calculated by multiplying the weight (g) consumed by the energy density (kcal/g) of the food or drink.

In a preliminary study, male participants showed significantly higher *ad libitum* intake compared to female participants. Therefore, male participants were excluded from this study to reduce any variation caused by gender differences in perception and food intake (Sudo et al., 2004, Olofsson and Nordin, 2004).

Chapter 3. *Ad libitum* intake and sensory-specific satiety

3.1 Introduction

3.1.1 Effect of sweetness intensity on *ad libitum* intake

Increasing the extent of oral sensory exposure to food has been reported to induce greater satiation and reduce the *ad libitum* intake of the food (Chapter 1, section 1.7.3.2) (Bolhuis et al., 2011). The intensity of the oral sensory perception of food (including aroma, taste or texture modality) may play be important in determining the total amount of the food consumed, through its influence on the overall intensity of oral sensory exposure (Sorensen et al., 2003). Increasing the perceived saltiness intensity of a soup was found to result in a faster satiation and decreased *ad libitum* intake of the soup (Bolhuis et al., 2011).

It is important to understand the impact of sweetness intensity of a drink on satiation, satiety and energy intake of the drink, since over-consumption of highly palatable sugar-sweetened drinks has been associated with the rise in overweight and obesity (Anderson and Woodend, 2003, Ludwig et al., 2001). On the one hand, the sweet taste of sugars and non-caloric sweeteners has been shown to suppress the feeling of hunger and increase the feeling of fullness (Lavin and Read, 2000, Anderson and Woodend, 2003). This may be due to an increased oral sensory exposure, resulting from increased sweetness intensity and leading to enhanced satiation. On the other hand, sweetness is a key contributor to the palatability of foods and drinks (Sorensen et al., 2003). Many studies have suggested that the increased palatability of foods is linked to increased food intake. (Yeomans, 1998, Sorensen et al., 2003). Therefore, the effect of sweetness intensity on *ad libitum* food intake is complex, because the anticipated reduction in food intake resulting from increased sweetness intensity could be confounded by increased palatability.

Several studies have investigated the role of sweetness intensity on the *ad libitum* intake of food. Contradicting results were found, which were potentially due to the contrasting effect of palatability and sweetness intensity. Vickers et al. (1998) observed that participants consumed a high sweetness yogurt more than a low sweetness yogurt, and the high sweetness yogurt also had higher palatability than the low sweetness yogurt. In another study by Vickers et al. (2001), they observed an opposite result: the low sweetness yogurt was consumed more than the high sweetness yogurt, and the low sweet yogurt also had a higher palatability than the high sweet yogurt. In addition, the ideal sweetness yogurt with the highest liking score was consumed the most. The palatability seemed to be positively correlated with the *ad libitum* intake of the yogurts throughout these studies. Therefore, it could be either, or both, the palatability or the sweetness intensity that affected the consumption of yogurts. However, the effect of sweetness intensity independent of palatability on *ad libitum* intake of yogurt could not be confirmed. In addition, in those studies, as the sweetness increased with the increasing sugar concentrations, the sourness of the yogurts was suppressed. It might not be the sweetness alone, but in combination with reduced sourness, which affects the overall liking and *ad libitum* intake of the yogurts.

Therefore, to study the independent effect of sweetness intensity on *ad libitum* intake, the initial palatability (before consumption) of foods should be kept as similar as possible. Meanwhile, a model food system with a sourness component should be avoided, in order to eliminate the suppressing interactive effects between sweetness and sourness perception (Savant and McDaniel, 2004).

3.1.2 Effect of sweetness intensity on sensory-specific satiety

It has been reported that the consumption of sweet food results in the development of sensory-specific satiety to foods belong to the general sweet category. A detailed description of SSS was summarized in chapter

1, section 1.5. SSS can be characterised as a reduction in liking (palatability/pleasantness) and wanting (motivation/desire to eat) of the eaten food. As sweet foods are consumed, the pleasantness of sweet foods reduces faster than the pleasantness of savoury (non-sweet) foods (de Graaf et al., 1993, Griffioen-Roose et al., 2009). Meanwhile, a sweet food consumed in a preload suppresses the subsequent intake of other sweet foods more than the subsequent intake of savoury foods (de Graaf et al., 1993). Conversely, pre-consumption of a savoury food suppresses the pleasantness of savoury foods, and reduces the subsequent intake of savoury foods more than sweet foods (Hetherington and Boyland, 2007, Bolhuis et al., 2011, Bolhuis et al., 2012, de Graaf et al., 1993).

It is likely that different sweetness intensities of foods may influence the extent of sensory-specific satiety to sweetness. However, little is known about such an influence and contradicting results were observed. In a study carried out by Essed et al. (2006), participants repeatedly consumed three orange juices of different sweetness, each for a continuous 12-day period. At the end of the study, the sweetest juice showed the largest reduction in the pleasantness ratings; only the *ad libitum* consumption of the sweetest juice reduced while the consumption of the other two juices remained unchanged (Essed et al., 2006). This study suggested that more intense sweetness induced stronger sensory-specific satiety (SSS) to sweet foods. However, one limitation of this study was that the three orange juices, which were commercially purchased, were not the same in other sensory properties (e.g. viscosity, sourness, freshness). Therefore, observations from this study may not be due to the simple effect of sweetness intensity. In contrast, Chung and Vickers (2007) observed that the sensory-specific satiety for a sweet tea, developed after drinking the tea, was stronger when the sweetness of the tea was lower than when the sweetness of the tea was higher. This suggested that more intense sweetness induced weaker sensory-specific satiety. In addition, Havermans *et al*. (2009) reported that the pleasantness rating of a lemonade was not affected by its intensity of strawberry flavour (overall

flavour: including taste and aroma), suggesting that sensory-specific satiety or satiety is unaffected by the intensity of flavour perception. Therefore, whether and how the intensity of sweetness (as a flavour modality) of a food affects the extent of sensory-specific satiety remains unresolved.

3.1.3 Study objectives

A diagram describing the objectives and measurements in this study is shown in **Figure 3-1**. The first objective of the study was to investigate the effect of the sweetness intensity of milkshakes on the *ad libitum* intake of the milkshakes while two of the milkshakes had similar initial palatability (before consumption). Measuring the *ad libitum* intake of the milkshake was a direct method of measuring the satiation of the milkshakes (Blundell et al., 2010).

The second objective was to study the effect of sweetness intensity of the milkshakes on sensory-specific satiety (SSS). SSS developed following milkshake consumption was characterised by 1) the changes (Δ) in the pleasantness of the milkshake; and 2) the changes (Δ) in the desire to drink the milkshake. In addition, it was hypothesised that a wider effect of SSS towards general sweet foods may develop following the consumption of sweet milkshakes, and the extent of SSS towards sweet foods may be affected by the sweetness intensity of the pre-consumed milkshake. The SSS towards general sweet foods was characterised by 1) the changes (Δ) in the general desire for something sweet; and 2) the difference in the subsequent consumption amount of savoury and sweet snacks.

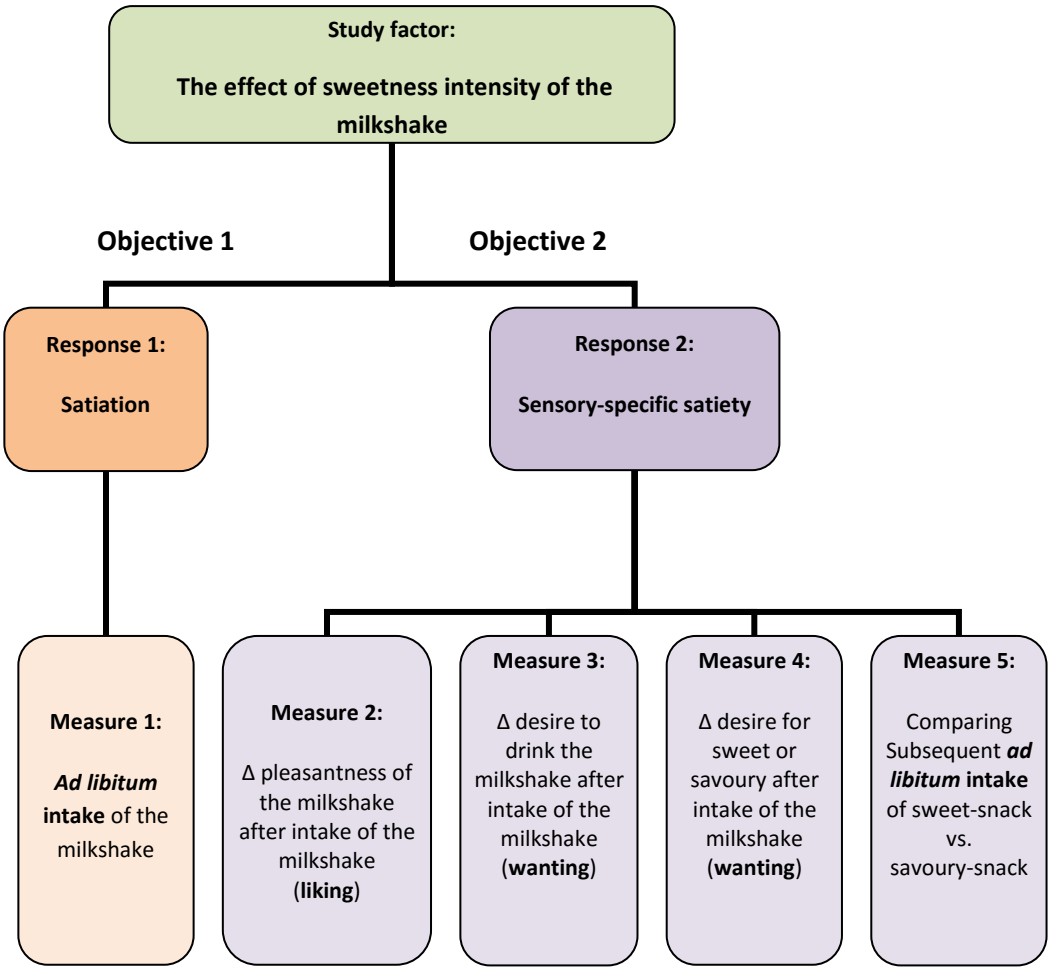

Figure 3-1 Objectives of the study, and the corresponding measurements

3.2 Methods and material

3.2.1 Study design and protocol

3.2.1.1 First phase experiment

Twenty-four participants completed this study in two separate study phases. The purposes of the first phase experiment were to select three sweetener concentrations for high sweetness (HS), ideal sweetness (IS) and low sweetness (LS) milkshakes, individually. For duplicate assessments, participants visited the Sensory Science Centre (SSC) for two identical sessions on two separate days. During each session, they rated the sweetness intensity, banana flavour intensity, pleasantness and

relative-to-ideal sweetness intensity of these 10 milkshake samples. The HS, IS and LS milkshake samples were determined for each individual, and the procedure was described in section 3.2.5. The IS milkshake was selected as the closest to the just-about-right point on the relative-to-ideal sweetness scale, and it was the most pleasant one. HS milkshake had sweetener concentration above the IS milkshake, and LS milkshake had lower sweetener concentration than IS milkshake. In addition, selected HS and LS milkshakes shared similar pleasantness ratings, with a distance of less than 10 mm on a 100mm long VAS scale.

3.2.1.2 Second phase experiment

The protocol of a session in the second phase of the study is shown in **Figure 3-2**. The same participants completed 3 sessions on 3 separate days, according to a cross-over experimental design. At each session, each participant consumed one of the three selected LS, IS or HS milkshakes (3 different experimental treatments), in a randomised and balanced order, on three separate visits. They were asked to come to each study session only during their luteal phase of menstrual cycle (day 18 to day 26), in order to minimise the differences in their gastric emptying, appetite and food intake across the menstrual cycle (Hirschberg, 2012, Li et al., 1999).

Participants were requested to refrain, for 24 hours prior to each visit, from intensive exercise, alcohol consumption and taking any medication. They were also asked to consume the same self-chosen dinner with similar energy content, the evening before each session between 20.00 to 21.00 hours, and then fast (except water) until arriving in the laboratory next morning at 9.00 hours.

Baseline subjective appetite ratings of hunger, fullness and satiation were taken immediately before breakfast at 9.00 hours using the VAS scales (Chapter 2, section 2.2.1.3). The appetite sensations include hunger, fullness, satiation, desire to eat something sweet, and desire to eat

something savoury. Participants consumed 50g porridge (So Simple Original Porridge Pot, Quaker Oat, UK) dissolved in 100ml hot water as a standard breakfast between 9.00 and 9.20 hours. Then participants fasted from any foods and drinks from 9.20 to 10.25 hours.

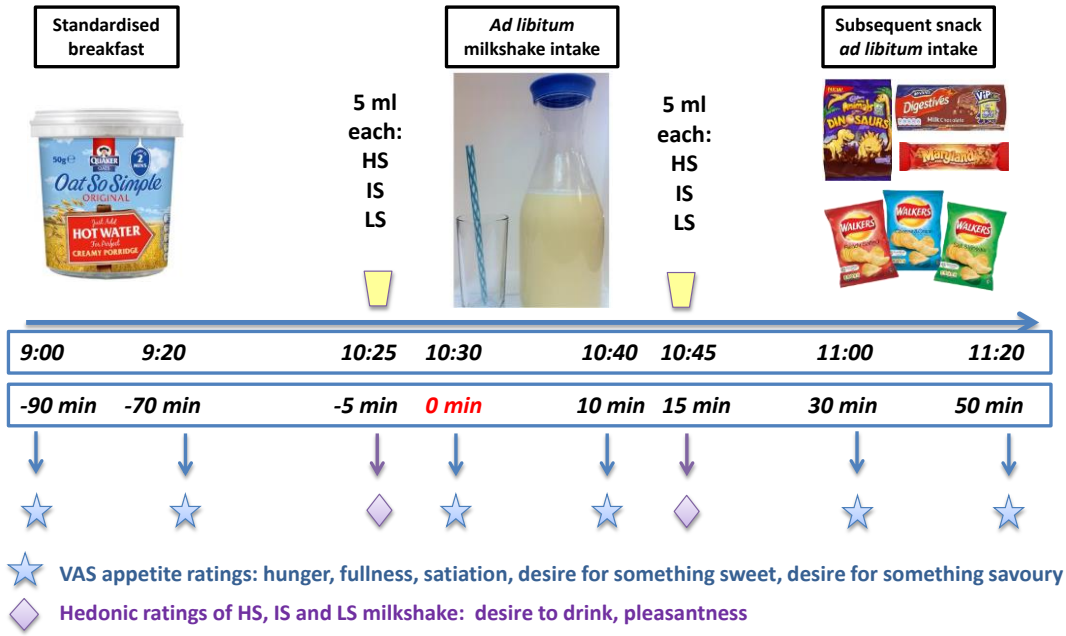

Figure 3-2 Study day protocol of the second phase of the study. Each participant completed three experiment treatments on three separate days: 1) *ad libitum* intake of HS milkshake, 2) *ad libitum* intake of LS milkshake, 3) *ad libitum* intake of IS milkshake

At each session, each participant was presented with the same self-selected savoury and sweet snacks, which had been chosen by him or her in the first session. They were asked to eat one, or both of the sweet and savoury snacks, as much as they wanted. During the snack consumption, participants had limited access to water (100ml). The volume of the *ad libitum* milkshake and the weight of each snack consumed were recorded. Subjective appetite ratings were completed immediately before and after consumptions of breakfast, the *ad libitum* intake of milkshake, and the *ad libitum* intake of snacks.

In addition, before (at -5 min) and after (at 15 min) the *ad libitum* intake of the milkshake, participants tasted and rated 5ml of each of the HS, IS and

LS milkshakes (presented monadically in a 30ml cup), in a random order, on the desire to drink and pleasantness of the milkshakes.

3.2.2 Participants

Potential female participants attended a screening session, and they were selected according to the general participant criteria (chapter 2, section 2.3.3). The recruitment procedure and screening session were conducted according to the standard protocol in chapter 2, sections 2.3.3.1 and 2.3.4. Twenty-four (24) selected participants completed the study. Participants were told that the objective of the study was to taste and drink different milkshake products, and were provided with the ingredients list, nutritional and energy information. They were not informed of the study objectives. The Medical Ethical Committee of the University of Nottingham approved this study with the ethics reference number R14032013 SBS Food, 15/03/2013.

3.2.3 Sample size

The primary outcome measurement is the amount of *ad libitum* intake milkshakes. Vickers *et al.* (2001) detected a significant (α=0.05) mean difference of 52g between the consumption of a high sweetness yogurt and a low sweetness yogurt, using 19 participants. Therefore, the sample size of 24 participants in the current study is considered sufficient to detect a difference (> 52g) in the consumption amounts of milkshakes with different sweetness intensities.

3.2.4 Milkshake sample preparation

The 10 different milkshakes, in every 100ml, consisted of 50ml mineral water (Evian, Danone Group, France), 50ml of a commercial milkshake drink (Yazoo banana, Friesland Campina, Belgium), and varying concentrations of a low-caloric sweetener (Canderel Spoonful artificial sweetener, Merisant, UK) (**Table 3-1**). The milkshake samples were prepared by mixing the ingredients in a container using a tube-roller mixer

(Denley Spiramix, Thermo Electron, UK). They were made one day before each session day and stored at in a 5 °C fridge before serving.

Canderel is an aspartame and acesulfame-K based sweetener. The granular Canderel sweetener had an energy of 3.8 kcal/g, slightly lower than sugar, but its sweetness was approximately 10 times as the sweetness of sugar, when compared on a weight-to-weight basis (claimed by the producer). Therefore, in this study, the addition of 0.1g Canderel sweetener was estimated to give the equal sweetness to the addition of 1g sugar. The milkshake sample S1, without the addition of Canderel sweetener, had a baseline sugar concentration of 4.8 g, which was comparable to the addition of 480 mg Canderel sweetener. The total calculated Canderel sweetener concentration in milkshake sample S2 equals to the sweetener concentration of S1 multiplying by a ratio of 1.2, which was 480*1.2 = 576 mg (**Table 3-2**). The total calculated sweetener concentration in S3 equals to the concentration in S2 (576 mg) multiplying by the same ratio of 1.2, which is 691 mg. Thus, the total calculated Canderel sweetener concentrations in 10 the milkshake samples increase by a ratio of 1.2. Every 100 ml milkshake sample contained 0.6 g fat, 1.5 g protein, and 4.8 to 6.8 g carbohydrates (depended on the addition of sweetener).

Table 3-1 Addition of ingredients in 100 ml milkshake

Samples	Yazoo Milkshake (ml)	Evian water (ml)	Canderel sweetener (mg)	Energy content (kcal)
S1	50	50	0	30
S2	50	50	96	30.4
S3	50	50	211	30.8
S4	50	50	349	31.3
S5	50	50	515	32
S6	50	50	714	32.7
S7	50	50	953	33.6
S8	50	50	1240	34.7
S9	50	50	1584	36.2
S10	50	50	1997	37.6

Table 3-2 Total calculated Canderel sweetener concentration in 100ml milkshake

Weight	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Canderel Sweetener(mg)	0	96	211	349	515	714	953	1240	1584	1997
Sugar (g)	4.8 g (= 480 mg Canderel sweetener)									
Total calculated sweetener (mg)	480	576	691	829	995	1194	1433	1720	2064	2477

3.2.5 Selection of HS, IS and LS milkshakes

To select the HS, IS and LS milkshake samples on an individual basis, each participant tasted 10 g of each of the 10 samples, and rated their perceived intensities of sweetness, relative-to-ideal sweetness, banana flavour and pleasantness, and desire to drink the milkshake on 100 mm VAS scales, **Figure 3-3**.

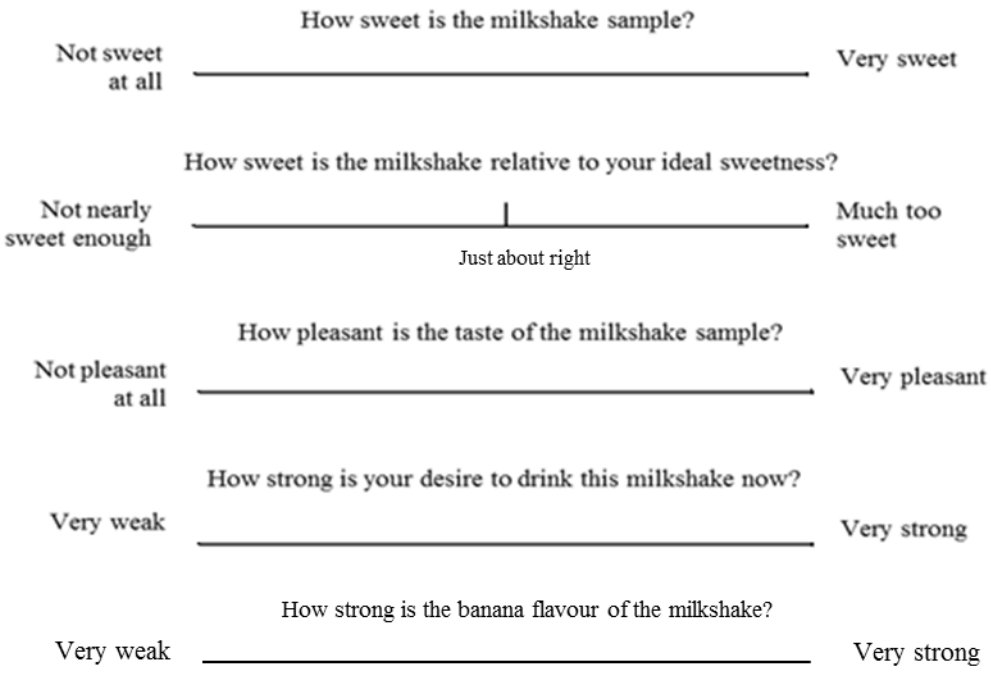

Figure 3-3 Sensory characterization scales for each milkshake sample. Each scale was 100mm in actual length.

The presentation order of milkshake samples was based on an interactive procedure, developed from the method by Booth (1983). This procedure was conducted as a fast method to find the samples with individual's ideal sweetness. Milkshake sample 5 (S5: 995 mg/ml sweetener addition ≈ 10 g/100ml sugar addition) was presented first to all participants. Because S5 was estimated to give the ideal sweetness for most consumers, as its sweetness was expected to be the closest to a commercial milkshake product (9.6 g/100ml sugar). Depending on the rating of S5, the next sample was chosen with the sweetener concentration expected to be rated on the other side of the just-about-right point. For example, if the S5 was rated on the left side of the just-right point, the next sample should have the sweetener concentration that was expected be rated on the right side of the just-right point. The procedure was continued until all samples were assessed. Participants were asked to take a 15-minute break after assessing five samples. Water and crackers were provided for palate cleansing before tasting each sample.

Each participant completed two sessions following the above identical procedure, on two separate days. For each individual, the mean of duplicate measurements was plotted against the total calculated geometric sweetener concentration. The IS milkshake sample was selected as the sample that was rated closest to the 'just-about-right' point (0 mm). The HS and LS samples were chosen on each side of the IS sample but with a similar pleasantness (difference in pleasantness ratings < 10 mm). The geometric distance of the sweetener concentrations between each pair of HS and LS samples for a participant was similar, which was 3-4 fold of the common ratio 1.2 (section 3.2.4).

3.2.6 Measuring *ad libitum* intake of the milkshake

Participants were presented with a large serving portion (800 ml) of milkshake sample (HS, IS or LS) in a 1-litre transparent plastic bottle, a 200 ml glass cup and a plastic straw (diameter: 0.3mm) (Figure 3-2). They were asked to pour the milkshake from the 1-litre bottle into the glass cup

and consume the milkshake using the straw until they felt comfortably full. They were encouraged to ask for more milkshake if they wanted. The volume of the *ad libitum* milkshake consumed was calculated by subtracting the volume of milkshake left after consumption from the total volume of milkshake provided. The total energy intake of each milkshake sample was calculated by multiplying the volume (ml) consumed by its energy density (kcal/ml).

3.2.7 Measuring *ad libitum* intake of subsequent snack

Participants were asked to select one favourite sweet snack and one favourite savoury snack from six popular commercial snacks in the UK (**Table 3-3**). The six snacks were similar in their macronutrient composition and energy density (Hetherington and Boyland, 2007). The energy content of the portions was similar between the sweet snack (284, 292, or 297 kcal) and the savoury snack (285, 286, or 289 kcal). During all the three separate sessions in the second phase of the study, each participant was given the same pre-selected sweet snack and savoury snack, and they were asked to consume as much as they wanted. The sweet snack was served as a 60g portion, and the savoury snack was served a 55g portion. Both were served in a 500 ml transparent bowl. Participants were encouraged to ask for more portions, and to stop eating whenever they wanted. They were also informed that they could eat both of the sweet and savoury snacks, only the sweet snack, or only the savoury snack, as required. The total weight of the consumed sweet or savoury snack was calculated by subtracting the weight of leftover snacks from the weight of total snacks provided. The total energy intake was calculated as the weight (g) consumed multiplied by the energy density (kcal/g) of the snack.

Table 3-3 Name, description, producer and nutritional information of the 6 snacks

Snack product Name	Values per 100g					
	Calorie (kcal)	Protein (g)	Fat (g)	Carbohydrate/ sugar (g)	Salt (g)	Fibre (g)
Savoury snack						
1.Cheese & Onion Crisps (Walkers)	518	5.9	30.5	52.9/0.1	1.5	4.3
2.Salt & Vinegar Crips (Walkers)	519	5.9	30.8	52.6/0.1	1.6	4.2
3.Ready Salted Crisps (Walkers)	526	6.1	31.9	51.5/0.1	1.4	4.3
Sweet snack						
4.Animals Biscuits (Cadbury)	474	7.4	19.6	66.8/27.7	0.6	2.3
5.Maryland Cookies (Burton's)	487	5.4	22.6	63.8/36.8	0.5	3.5
6.Chocolate Digestives (McVitie's)	495	6.7	23.6	62.2/29.5	1	3

3.2.8 Measuring subjective appetite ratings

Subjective appetite ratings, including feelings of hunger, fullness, and satiation, desire for sweet food and desire for savoury food, were rated on 100 mm long visual analogue scales. The questionnaire statements for hunger, fullness and satiation referred to Figure 2-1, (Chapter 2). The statements for 'desire for something savoury or sweet' are listed in **Figure 3-4.**

How strong is your desire to eat something sweet?

Very weak _____ Very strong

How strong is your desire to eat something savoury?

Very weak _____ Very strong

Figure 3-4 Questionnaires for measuring subjective appetite sensation of desire for something sweet or savoury (de Ggraaf et al., 1992).

3.2.9 Statistical analysis

Values were presented with mean ± standard deviation (SD). A p-value <0.05 was considered as statistically significant for all tests. All data

analysis was performed using IBM SPSS Statistics (version 21.0; IBM Corporation, USA).

3.2.9.1 The first phase data analysis

In the first phase experiment, the effect of sweetener concentration on sweetness intensity, banana flavour intensity, relative-to-ideal sweetness intensity, and pleasantness of 10 milkshake samples were analysed using one-way repeated measures ANOVA (one factor: sweetener concentration). The rated intensities of pleasantness, desire to drink, sweetness, relative-to-ideal sweetness, and banana flavour were compared between the individually selected HS, IS and LD milkshakes, using one-way repeated measures ANOVA (one factor: milkshake samples -- HS, IS and LS). P*ost hoc* comparison tests with Bonferroni adjustment were used to determine which milkshake samples were significantly different in these attributes.

3.2.9.2 The second phase data analysis

3.2.9.2.1 Energy intake

In the second phase, the effect of sweetness intensity of milkshakes (HS, IS and LS) on the *ad libitum* intake (volume or energy) of milkshakes were assessed using one-way repeated measures ANOVA (one factor: 3 experiment treatments). Similarly, the *ad libitum* intake of the subsequent snacks was compared between the three experiments treatments, using one-way repeated measures ANOVA. The consumption amount of sweet snacks and savoury snacks were compared using two-way repeated measures ANOVA (two factors: 3 experiment treatments × 2 snacks). P*ost hoc* comparison tests with Bonferroni adjustment were used to determine where the differences were.

3.2.9.2.2 Desir to drink and pleasantness of milkshakes

Before *ad libitum* intake of the milkshake (at time = -5 minutes), the initial ratings of ‘desire to drink’ and ‘pleasantness’ were analysed using two-way

repeated measures ANOVA (two factors: 3 sweetness intensities × 3 treatments).

The initial ratings (-5 minutes) of the pleasantness of the milkshake (HS, IS or LS) were compared with the ratings of pleasantness of the milkshake collected after *ad libitum* milkshake consumption (15 minutes), using paired sample t-tests. Similarly, the initial ratings (-5 minutes) of the desire to drink the milkshake were compared with the ratings of desire to drink the milkshake collected after *ad libitum* milkshake consumption (15 minutes), using paired sample t-tests.

Changes in the pleasantness (Δ pleasant) of the milkshakes were calculated by subtracting the pleasantness ratings collected at - 5 minutes from the pleasantness ratings collected at 15 minutes. Similarly, changes in the desire to drink (Δ desire to drink) the milkshakes were calculated by subtracting the rating of desire to drink the milkshake collected at - 5 minutes from the ratings of desire to drink the milkshake collected at 15 minutes. The ratings of Δ pleasant and Δ desire to drink were compared between three experiment treatments using one-way repeated measures ANOVA (one factor: experiment treatments). Where significant differences were identified in repeated measures AVONA, *post hoc* comparisons with Bonferroni adjustment were used to determine where the differences were.

3.2.9.2.3 VAS appetite ratings

VAS appetite ratings (hunger, fullness, satiation, desire for something sweet, and desire for something savoury) from the three treatments over the time course of the study were compared using two-way repeated measures ANOVA (two factors: 3 experiment treatments × 8 time points). The VAS appetite ratings immediately before the consumption of milkshakes (at time = 0 minutes) were not significantly different between the three experiment treatments ($p>0.05$). Therefore, Δ VAS appetite ratings were calculated by subtracting the appetite ratings collected immediately before *ad libitum* intake of the milkshake (time = 0 minutes),

from the ratings collected after the milkshake consumption (time = 10 minutes and 30 minutes). Δ VAS appetite ratings following the *ad libitum* intake of the milkshake were then compared between the three experiment treatments, using two-way repeated measures ANOVA (two factors: 3 experiment treatments × 3 time points at 0, 10 and 30 minutes). Where significant differences were identified in the main effect obtained by repeated measures AVONA, *post hoc* comparisons with Bonferroni adjustment were used to determine where the differences were.

3.3 Results

3.3.1 Participant characteristics

All participants met the study criteria and completed the study. They had a mean score of 5 ± 2 on the restrained factor of the TFEQ, a mean score of 2 ± 2 on Beck Depression Inventory. They were aged from 19 to 27 years (mean = 22 ± 2 years), had a mean BMI of 20.2 ± 1.6 kg/m^2 (ranged from 19 to 23 kg/m^2).

3.3.2 Phase 1 experiment results

3.3.2.1 Sensory characteristics of 10 milkshake samples

Overall, sweetness intensity ratings increased significantly with increasing geometric sweetener concentrations, $F(2.828, 65.039) = 169.673$, $p<0.001$ (**Figure 3-5)**. *Post hoc* test revealed that the mean values of sweetness intensities were significantly different among samples S1, S2, S3, S4, S5, S6 and S7 ($p<0.01$). S8 (1720mg), S9 (2064mg) and S10 (2477mg) were significantly different from the rest of the seven samples in sweetness intensity ($p<0.05$), but S8, S9 and S10 were not statistically different from each other ($p>0.05$).

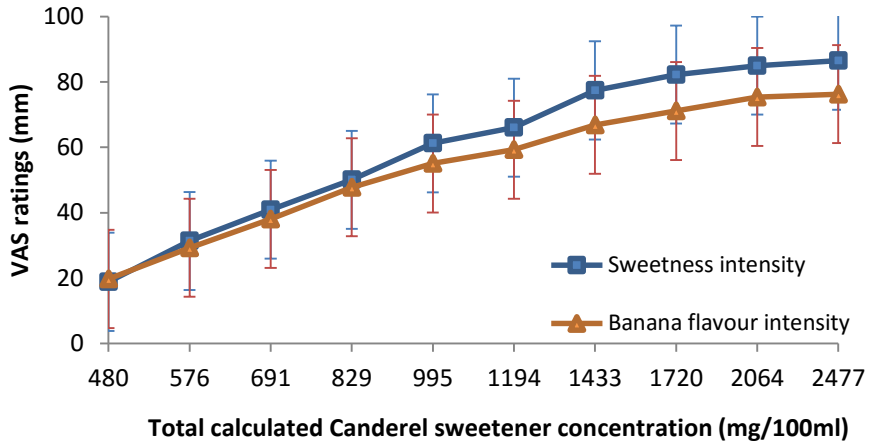

Figure 3-5 Mean intensities (n=24) of sweetness and banana flavour with increasing total calculated sweetener concentrations. Error bars represent the standard deviations.

Relative-to-ideal sweetness intensity rated on the just-about-right scale also increased significantly with increasing sweetener concentrations, $F(2.914, 67.021) = 102.910$, $p<0.001$. The sweetener concentration for ideal sweetness was where the relative-to-ideal sweetness curve crosses the x-axis (sweetener concentration) (**Figure 3-6**) (Bolhuis et al., 2010). As we can observe from **Figure 3-7**, there were clearly individual differences in the ideal sweetener concentration and their sweetness perception of milkshake samples.

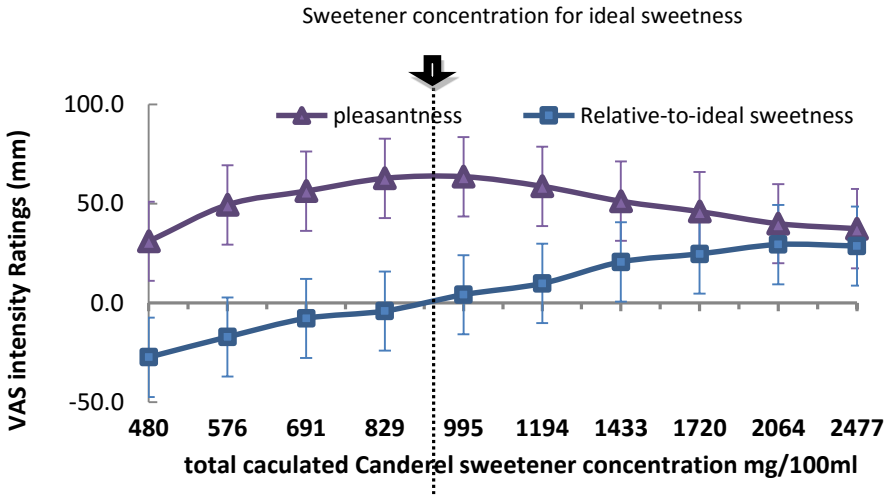

Figure 3-6 Mean ratings (n=24) for relative-to-ideal sweetness (■) and for pleasantness (▲) of 10 milkshake samples with increasing Canderel sweetener concentrations. Error bars represent the standard deviations.

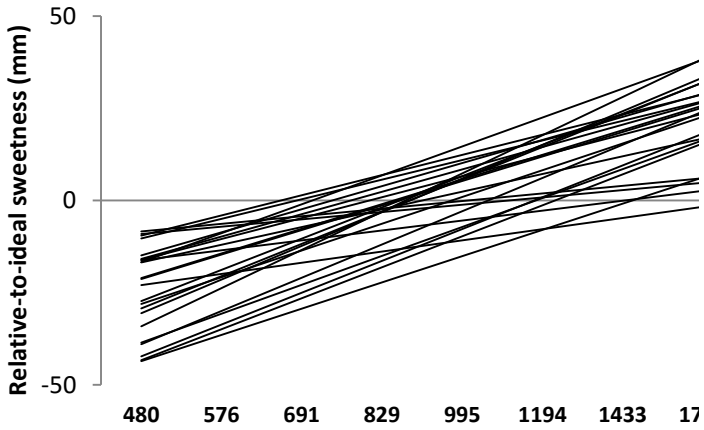

Figure 3-7 Individual (24) relative-to-ideal sweetness trend lines as a function of total calculated sweetener concentrations

The pleasantness ratings of the milkshakes were influenced by the sweetener concentration significantly, $F(2.567, 59.042) = 11.406$, $p<0.001$. The pleasantness ratings showed an inverted U-shape against the sweetener concentrations (**Figure 3-6**). The pleasantness ratings for the milkshake initially increased with increasing sweetener concentration until it reached its maximum at the ideal sweetener concentration, then it decreased with further increases in sweetener concentration. As clearly demonstrated by Figure 3-6, the sweetener concentration for the highest point of the pleasantness curve coincides with the concentration for the ideal sweetness.

3.3.2.2 Characteristics of selected HS, IS and LS milkshakes

The relative-to-ideal sweetness and pleasantness curves for each individual were plotted for selecting personalised HS, IS and LS milkshake samples. Milkshake samples selected for each individual participant were listed in **Table 3-4**.

Table 3-4 LS, IS and HS milkshake samples selected individually for 24 participants

Participant	LS	IS	HS	Participant	LS	IS	HS
1	S2	S4	S6	**13**	S4	S5	S7
2	S3	S5	S7	**14**	S3	S4	S6
3	S3	S6	S7	**15**	S4	S6	S7
4	S4	S6	S7	**16**	S3	S5	S7
5	S4	S5	S7	**17**	S3	S5	S7
6	S2	S3	S5	**18**	S5	S7	S8
7	S2	S4	S6	**19**	S2	S3	S6
8	S1	S4	S5	**20**	S4	S6	S7
9	S3	S5	S7	**21**	S2	S4	S6
10	S1	S3	S5	**22**	S5	S6	S8
11	S1	S3	S5	**23**	S2	S4	S5
12	S3	S4	S5	**24**	S1	S3	S4

The ideal sweetness milkshake was the one closest to the just-about-right point (0 mm) while low sweetness and high sweetness samples were at each side of the IS milkshake with similar pleasantness (difference < 10 mm). As we can see from the Table 3-4, S9 and S10, the two extremely sweet samples, were not selected as HS samples by any participant. The mean calculated total sweetener concentration selected for LS, IS, and HS milkshakes were 681 ± 153 mg/100ml, 943 ± 206 mg/100ml, and 1272 ± 242 mg/100ml, respectively.

Mean sweetness intensity ratings were significantly different among LS (33±16 mm), IS (55±16 mm) and HS milkshakes (73±16 mm), $p<0.001$ (**Figure 3-8**). Similarly, mean relative-to-ideal sweetness ratings were significantly different among LS (13±13 mm), IS (0.36±7 mm) and HS (-15±13 mm) milkshake samples, $p<0.001$. As for the pleasantness ratings, IS milkshakes (68±19 mm) were perceived more pleasant than LS (51±21 mm) and HS (55±19 mm) milkshakes, $p<0.001$; while LS was not different from HS in pleasantness ratings, $p=0.6$.

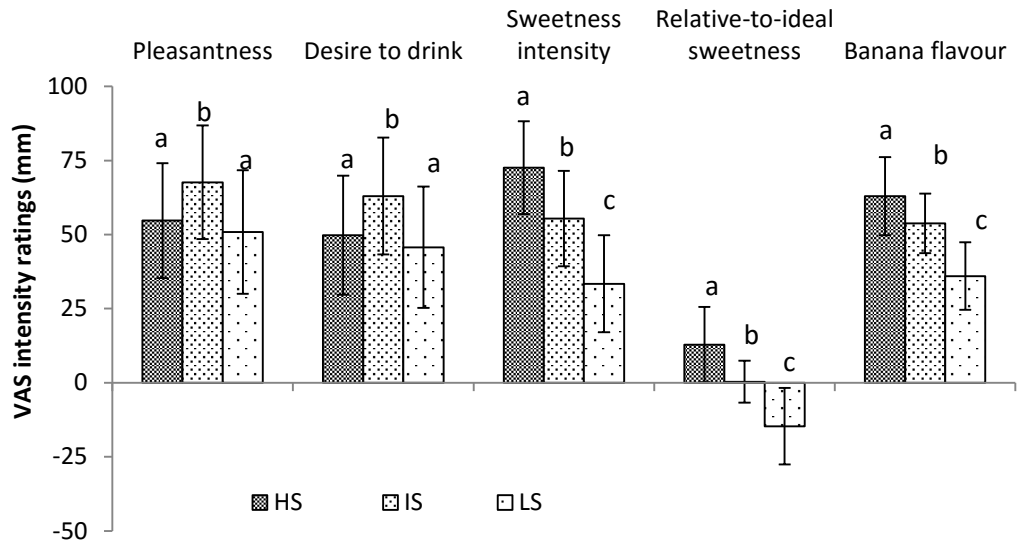

Figure 3-8 Mean (n=24) of the initial pleasantness, desire to drink, sweetness, relative-to-ideal sweetness and banana flavour for HS, IS and LS milkshakes. For each measurement, the samples without a same small letter were significantly different. Error bars represent the standard deviations

3.3.3 Phase 2 experiment results

3.3.3.1 *Ad libitum* intake of milkshake

None of the participants consumed all 800 ml milkshake in a serving portion. All participants finished *ad libitum* drinking of each milkshake within 10 minutes. There was no significant difference between the mean volume intake of HS milkshake (292 ± 87 ml), IS milkshake (325 ± 114 ml) and LS (297 ± 110 ml) milkshakes, F (2, 46) =2.22, p=0.12 (**Figure 3-9A**). Meanwhile, there was no significant difference between the energy intake of HS (98 ± 30 kcal), IS (100 ± 35 kcal), and LS (93 ± 35 kcal) milkshakes, F (2, 46) = 0.83, p = 0.44 (**Figure 3-9B**). This indicates that the consumption of milkshakes was not affected by the sweetness intensity of the milkshake.

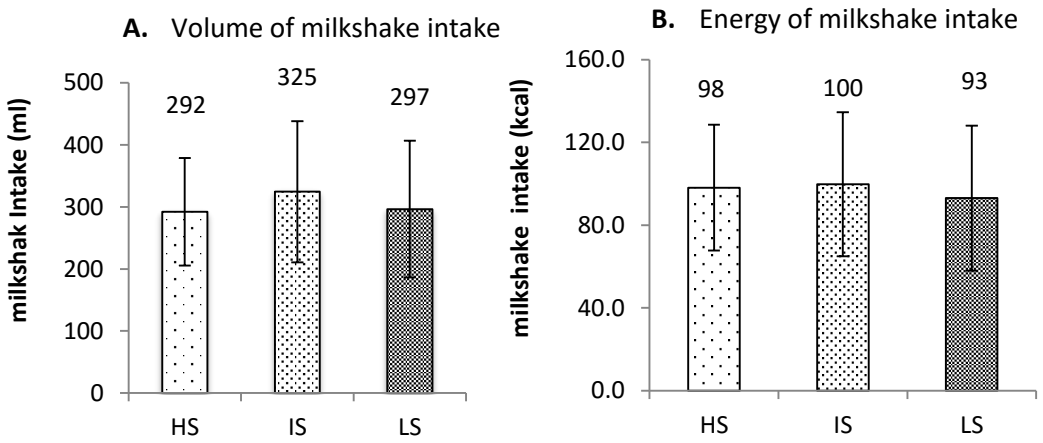

Figure 3-9 Mean values of *ad libitum* intake of HS, IS and LS milkshake samples measured as total volume (A) and energy (B) consumed. Error bars represent the standard deviations.

3.3.3.2 Ratings of 'desire to drink' and 'pleasantness'

3.3.3.2.1 Initial ratings of 'desire to drink' and 'pleasantness'

There was a significant main effect of the sweetness of the milkshake on the ratings of initial desire to drink the milkshake, $F(2, 46) = 13.552$, $p<0.0001$. Post hoc test review that the initial ratings of the 'desire to drink' the IS milkshake, collected at time= - 5 minutes, was significantly higher than the initial ratings for the LS ($p < 0.0001$) and HS ($p = 0.001$) milkshake (**Table 3-5**). The initial desire to drink the HS milkshake was similar to the initial desire to drink the LS milkshake ($p = 1.0$). There was no significant difference in initial ratings of desire to drink the HS, IS or LS milkshake between the three treatment sessions, $F(2, 46) = 0.093$, $p = 0.911$.

Table 3-5 initial ratings (mean ± SD) of desire to drink the HS, IS and LS milkshakes before *ad libitum* intake of HS, IS or LS milkshake in three sessions of the second stage of the study

Experimental treatment	**Initial desire to drink the milkshakes (T= -5 minutes)**		
	desire for HS	desire for IS	desire for LS
Before ad libitum HS intake	48±22[a]	65±21[b]	43±22[a]
Before ad libitum IS intake	49±13[a]	65±20[b]	45±17[a]
Before ad libitum LS intake	49±21[a]	65±17[b]	46±22[a]
Mean ratings of 3 sessions	49±19[a]	65±19[b]	45±20[a]

Variables in a column or a row without a same small letter are significantly different, $p<0.05$

Similar to the ratings of 'desire to drink', the initial ratings of the 'pleasantness' of HS and LS milkshake were similar ($p>0.05$), while the initial pleasantness of the IS milkshake was significantly higher than HS ($p = 0.007$) and LS ($p < 0.0001$) milkshake ($p<0.05$) (**Table 3-6**). There was no significant difference in initial ratings of pleasantness of the HS, IS or LS milkshake between the three treatment sessions, $F (2, 46) = 0.0126$, $p = 0.88$.

Table 3-6 Initial pleasantness ratings (mean ± SD) of the milkshakes

Condition	**Initial pleasantness of the milkshakes (T= -5 minutes)**		
	HS	IS	LS
Before ad libitum HS intake	56±19[a]	67±18[b]	53±21[a]
Before ad libitum IS intake	55±18[a]	68±20[b]	50±19[a]
Before ad libitum LS intake	54±22[a]	69±21[b]	49±23[a]
Mean ratings of 3 sessions	55±19[a]	68±19[b]	51±21[a]

Values in each row without a same small letter are significantly different, $p<0.05$

3.3.3.2.2 Changes in ratings of 'desire to drink' and 'pleasantness'

Following *ad libitum* intake of HS, IS or LS milkshake, the desire to drink the HS milkshake reduced significantly ($p < 0.05$), while the desire to drink IS and LS milkshake appeared to show a non-significant decrease ($p> 0.05$) (**Figure 3-10**). Meanwhile, there was no significant mean effect of

the experiment treatments on the Δ ratings of desire to drink the HS (p = 0.732), IS (p = 0.342) or LS (p = 0.726) milkshake.

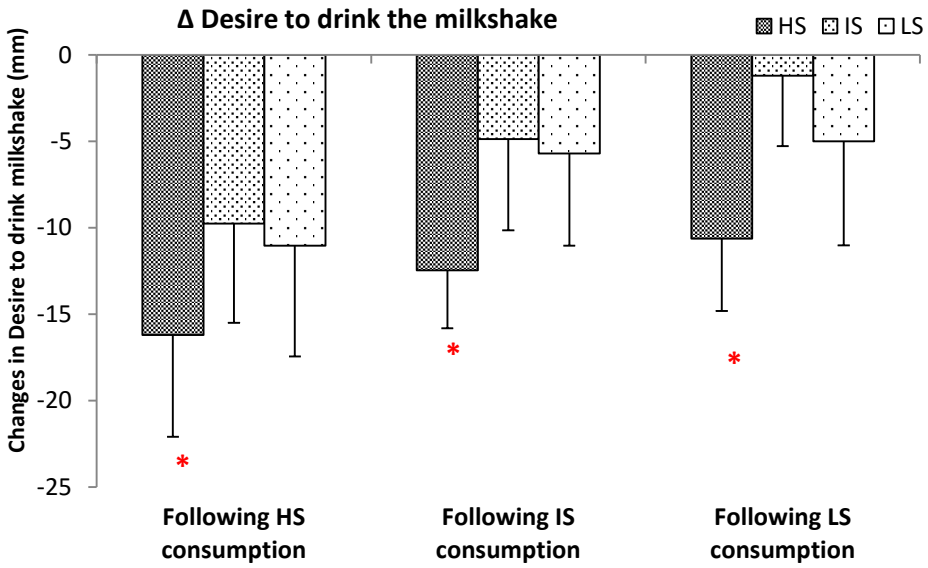

Figure 3-10 Change in the desire to drink the HS, IS and LS milkshakes following *ad libitum* intake of HS, IS or LS milkshake. Δ desire to drink ratings were calculated by subtracting the ratings before *ad libitum* intake of the milkshake (time = -5 minutes) from the ratings collected after *ad libitum* intake of the milkshake (time = 15 minutes). Error bars represent the standard errors. A sample bar marked with '*' represent a significant reduction in the ratings collected at 15 minutes, compared with the ratings collected at -5 minutes, p< 0.05.

Similarly, the pleasantness of HS milkshake decreased significantly (p<0.05), while the pleasantness of LS and IS stayed unchanged (p>0.05) (**Figure 3-11**). There was no significant mean effect of the experimental treatments on the Δ pleasantness of HS (p = 0.961), IS (p = 0.828) or LS milkshake (p = 0.932).

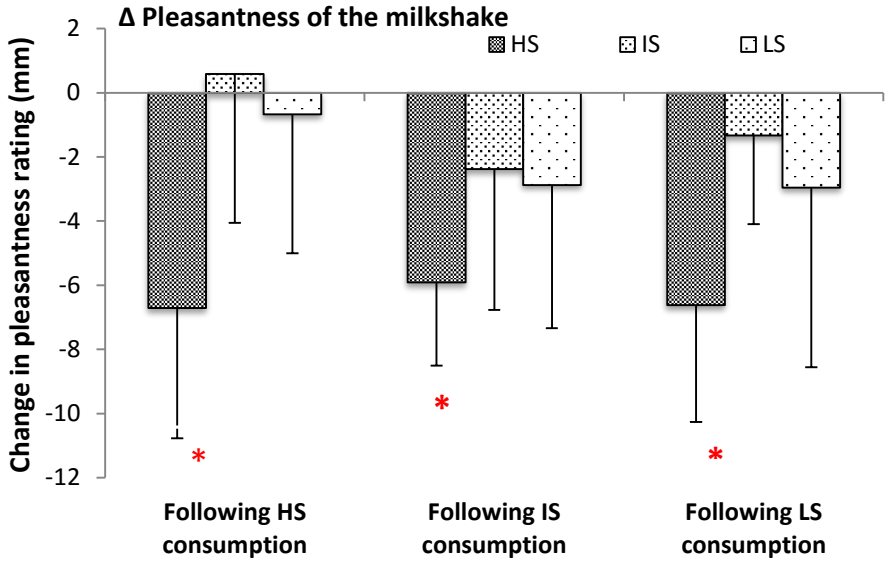

Figure 3-11 Change in the pleasantness of HS, IS and LS milkshakes following *ad libitum* intake of HS, IS or LS milkshake. Δ pleasantness ratings were calculated by subtracting the ratings before *ad libitum* intake of the milkshake (time = -5 minutes) from the ratings after *ad libitum* intake of the milkshake (time = 15 minutes). Error bars represent the standard errors. A sample bar marked with '*' represents a significant reduction in the ratings collected at 15 minutes, compared with the ratings collected at -5 minutes, $p < 0.05$.

3.3.3.3 Subjective appetite ratings

3.3.3.3.1 Hunger, fullness and satiation

The originally rated hunger, fullness, and satiation at the time point before breakfast were not significantly different ($p>0.05$) across the three sessions, indicating that participants arrived each time with similar baseline appetite sensation. There was a significant main effect of time on VAS appetite ratings ($p<0.001$), suggesting rated hunger, fullness and satiation were changing over the time course of the study. However, there was no significant main effect of treatments (*ad libitum* intake of HS, IS or LS milkshake in each session) on the ratings of hunger, fullness and satiation ($p>0.05$). **Figure 3-12 A1, B1** and **C1** show the original mean ratings of hunger, fullness and satiation over the time course of the study.

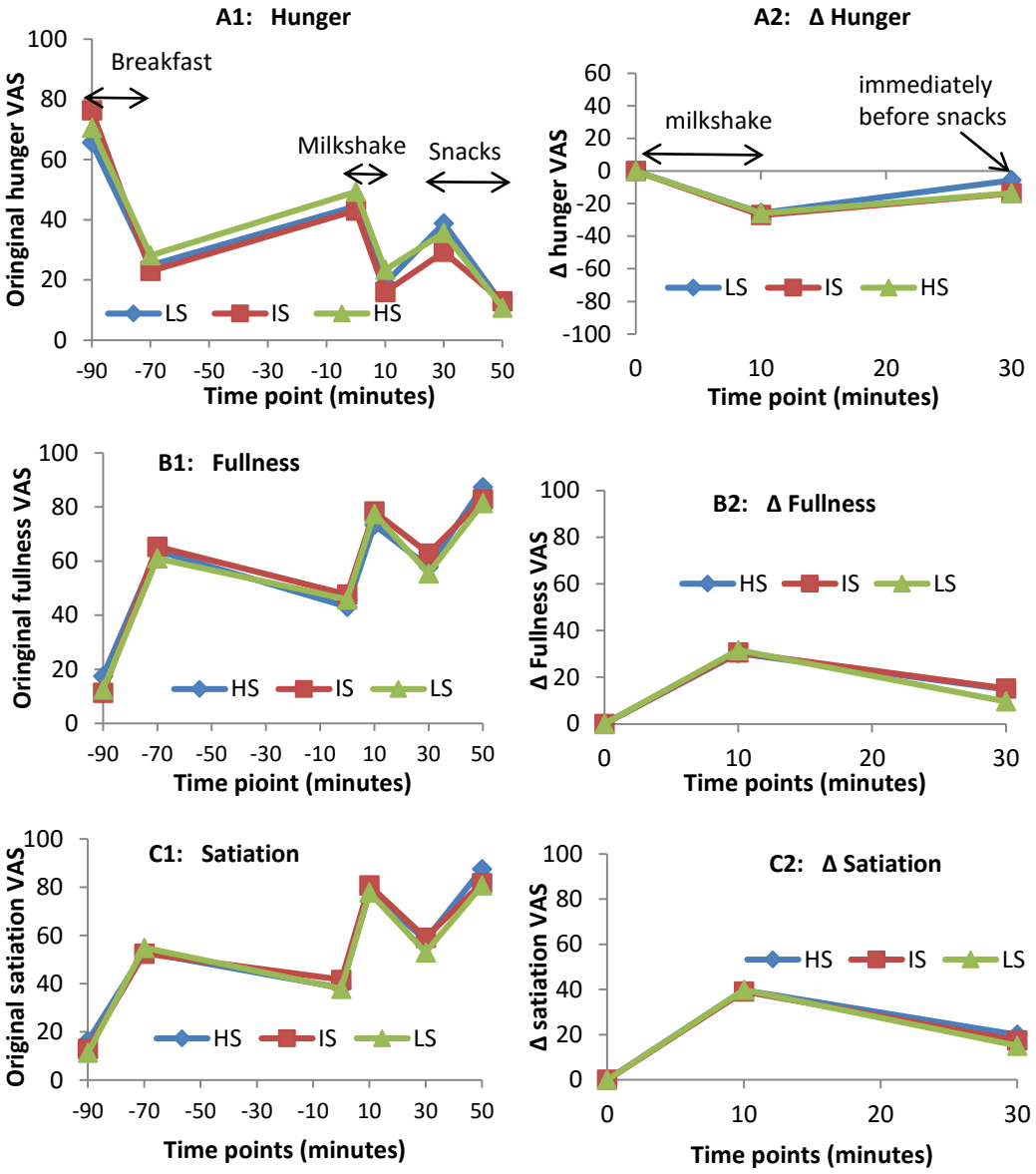

Figure 3-12 Original hunger, fullness and satiation ratings (A1, B1 and C1) and Δ hunger, Δ fullness and Δ satiation ratings (A2, B2 and C2); Δ VAS were determined by subtracting original hunger ratings at T=0 minutes from the hunger ratings T = 10 minutes, and T= 30min. Data points were mean values of 24 participants

Ratings of hunger, fullness and satiation immediately before the *ad libitum* intake of the milkshake were not significantly different ($p>0.05$) between the three treatment sessions. Therefore, the Δ hunger, Δ fullness and Δ satiation were determined by subtracting the ratings at time T=0 point (immediately before milkshake consumption) from the two subsequent time points before subsequent intake of snacks (at T=10 and 30 minutes).

Figure 3-12 A2, B2 and **C2** show the changes in mean ratings of Δ hunger, Δ fullness and Δ satiation following the *ad libitum* intake of the milkshakes. There was no significant treatment-time interaction ($p>0.05$), indicating that Δ hunger, Δ fullness and Δ satiation followed similar trend between the three treatment sessions. There was a significant main effect of time on Δ ratings ($p< 0.05$). The Δ hunger ratings was significantly reduced after all milkshake intake at 10 min from the ratings at 0 min ($p<0.05$). There was no significant difference observed in the Δ hunger, Δ fullness or Δ satiation between the three treatment sessions when the *ad libitum* intake of HS, IS or LS milkshake was measured ($p>0.05$).

3.3.3.3.2 Desire for something sweet or savoury

There was a significant main effect of time on the ratings of desire for something sweet and the ratings of desire for something savoury ($p<0.05$) over the time of the study (-90 to 50 min) (**Figure 3-13**). The ratings of 'desire for something sweet', or the 'desire for something savoury' before the *ad libitum* intake of the milkshakes (-90 minutes, -70 minutes, and 0 minutes) were not significantly different between the three treatment sessions, $p>0.05$ (**Figure 3-13**). Therefore, Δ desire for something sweet and Δ desire for something savoury were calculated by subtracting the ratings collected immediately before the milkshake intake (time = 0 minutes) from the ratings collected after milkshake intake and before the snack intake (time = 10 minutes and 30 minutes).

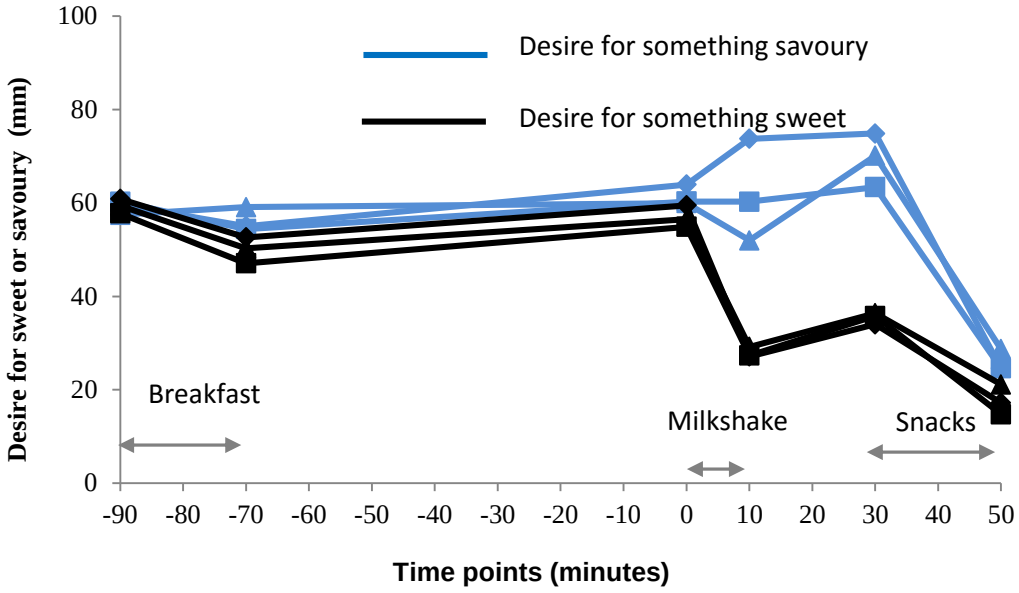

Figure 3-13 Mean ratings of desire for something sweet (black lines) or desire for something savoury (blue lines) under the three experiment treatments: *ad libitum* intake of LS (▲), IS (■) or HS (♦) milkshakes

3.3.3.3.2.1 Δ desire for something sweet

There was no significant main effect of time-treatment interaction on ratings of Δ desire for something sweet, F (2, 92) = 0.328 p = 0.859, suggesting that the changes in the Δ desire for something sweet followed a similar pattern over time (0, 10 and 30 min) between the three treatments. There was a significant main effect of time (0, 10 and 30 min) on ratings of the Δ desire for something sweet, F (2, 46) = 37.706, p<0.0001. Immediately after *ad libitum* intake of HS, IS or LS milkshake (at time = 10 minutes), the ratings of Δ desire for something sweet reduced significantly from the ratings collect at 0 min (p <0.0001) (**Figure 3-14**). Immediately after *ad libitum* intake of snacks (at time = 30 minutes), ratings of Δ desire for something sweet recovered a little from ratings at 10 min (p = 0.135), but were still significantly lower than the ratings collected before the *ad libitum* intake of milkshakes (at time = 0 minutes) (p < 0.0001) (**Figure 3-14** and **Table 3-7**). There was no significant main effect of treatment on the ratings of Δ desire for something sweet, F (2, 46) = 0.747, p= 0.48

(**Table 3-7**), indicating that the sweetness intensity of the *ad libitum* milkshake did not affect the ratings of Δ desire for something sweet after the milkshake intake.

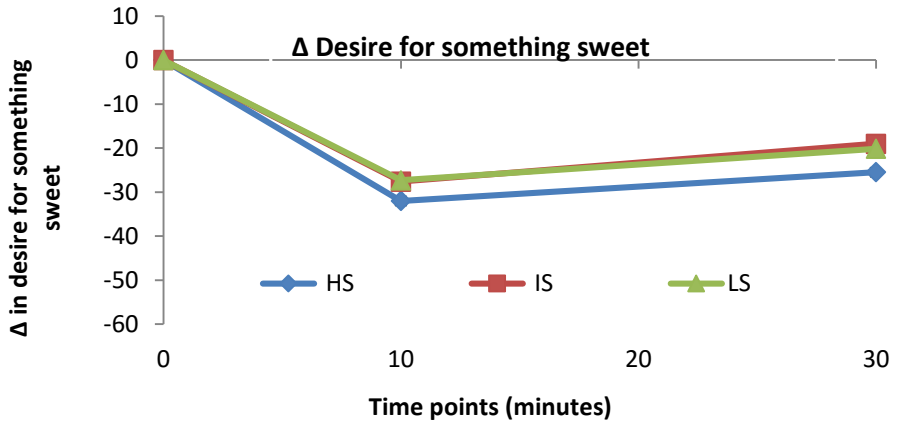

Figure 3-14 Mean Δ desire for something sweet after *intake* of HS, IS and LS milkshakes.

Table 3-7 Changes (**Δ**) in desire for something sweet after intake of HS, IS and LS milkshakes (mean ± SD)

Measurement	**Treatment (session)**	Before milkshake	After milkshake	Before snack
		T=0 min	T=10 min	T=30 min
Δ Desire for sweet	*Ad libitum* intake of LS	0 a	-27±29 b	-20±32 c
	Ad libitum intake of IS	0 a	-28±23 b	-19±24 c
	Ad libitum intake of HS	0 a	-32±20 b	-25±23 c

Within a column (treatment) or row (time), values without a same small letter are significantly different, $p<0.05$

3.3.3.3.2.2 Δ desire for something savoury

There was a significant main effect of time-treatment interaction on the ratings of Δ desire for something savoury, $F(2.235, 51.406) = 3.093$, $p=0.019$, suggesting that the changes in Δ desire for something savoury followed a different pattern over time between the three treatments (**Figure 3-15**). Therefore, the simple main effect of time on the ratings of Δ desire for something savoury was analysed using separate one-way repeated measures ANOVAs under each milkshake treatment. The simple main effect of treatment on the Δ rating of desire for something savoury

was analysed using separate one-way repeated measures ANOVAs at each time point (0, 10 and 30 min).

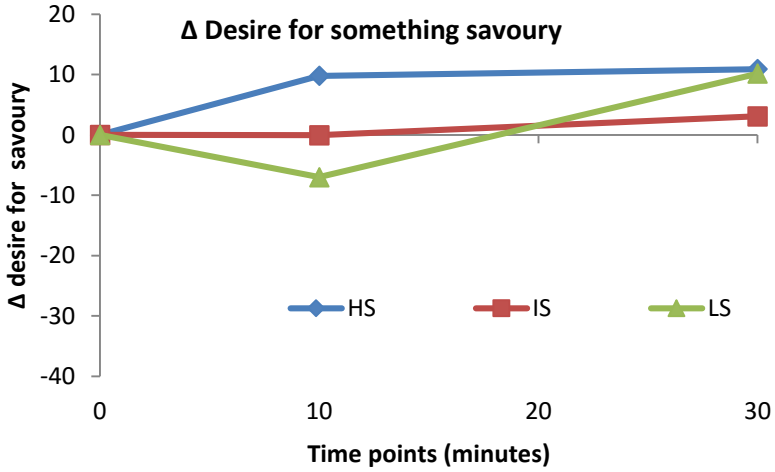

Figure 3-15 Mean of Δ desire for something savoury following *ad libitum* intake of HS, IS and LS milkshakes.

Table 3-8 Changes (Δ) (mean ± SD) in desire for something savoury after intake of HS, IS and LS milkshakes

Measurement	**Treatment (session)**	Before milkshake	After milkshake	Before snack
		T=0 min	T=10 min	T=30 min
Δ Desire for savoury	*Ad libitum* intake of LS	0 a	-8±28 a	10.2±27 a
	Ad libitum intake of IS	0 a	0±17 ab	3.1±19 a
	Ad libitum intake of HS	0 a	9.8±21 b	10.9±16 a *

Within a row (treatment), only the value marked with a '*'was significantly different from the ratings collected at 0 min. Within a column (time), values without a same small letter were significantly different, $p<0.05$

There was no significant main effect of time (0, 10, and 30 min) on the Δ desire for something savoury under the IS or LS treatments ($p>0.05$) (**Table 3-8**), indicating that the ratings of Δ desire for something savoury did not changed following the intake of IS and LS milkshake (0 – 30 min). In comparison, there was a significant main effect of time on the ratings of Δ desire for something savoury under the HS treatment, $F(2, 46) = 4.213$, $p = 0.021$. *Post hoc* comparison tests revealed that the ratings of Δ desire for something savoury collected at 30 min was significantly higher than the relevant ratings collected at 0 min, under the HS treatment ($p = 0.008$)

(Table 3-8). However, these ratings collected at 10 min was not significantly different from the ratings collected at 0 min (p = 0.091) or 30 min (p = 1.0), under the HS treatment.

There was no significant main effect of treatment on the ratings of Δ desire for something savoury at 0 min or 30 min (p>0.05) (**Table 3-8)**. However, there was a significant main effect of treatment on the ratings of Δ desire for something savoury at 10 min F (2, 46) = 5.056, p = 0.01. *Post hoc* comparison test revealed that the ratings of Δ desire for something savoury collected at 10 min under the HS milkshake treatment was significantly higher than the relevant ratings under the LS milkshake treatment (p = 0.04). This suggested that a high sweetness milkshake enhanced the ratings of Δ desire for something savoury greater than a low sweetness milkshake.

3.3.3.4 *Ad libitum* intake of sweet or savoury snacks

Following *ad libitum* intake of HS, IS and LS milkshakes, there was no significant difference in the *ad libitum* intake of sweet snacks in weight (p = 0.952) or in energy (p = 0.97) (**Figure 3-16**). There was also no significant difference in the *ad libitum* intake of savoury snack in weight (p = 0.315) or in energy (0.314). The total snack intake in weight (p = 0.599) or in energy did not differ following intake of HS, IS and LS (p = 0.511). However, the intake of savoury snack was significantly higher compared with the intake of sweet snack in weight (p<0.0001) or in energy (p<0.0001), under all three treatments.

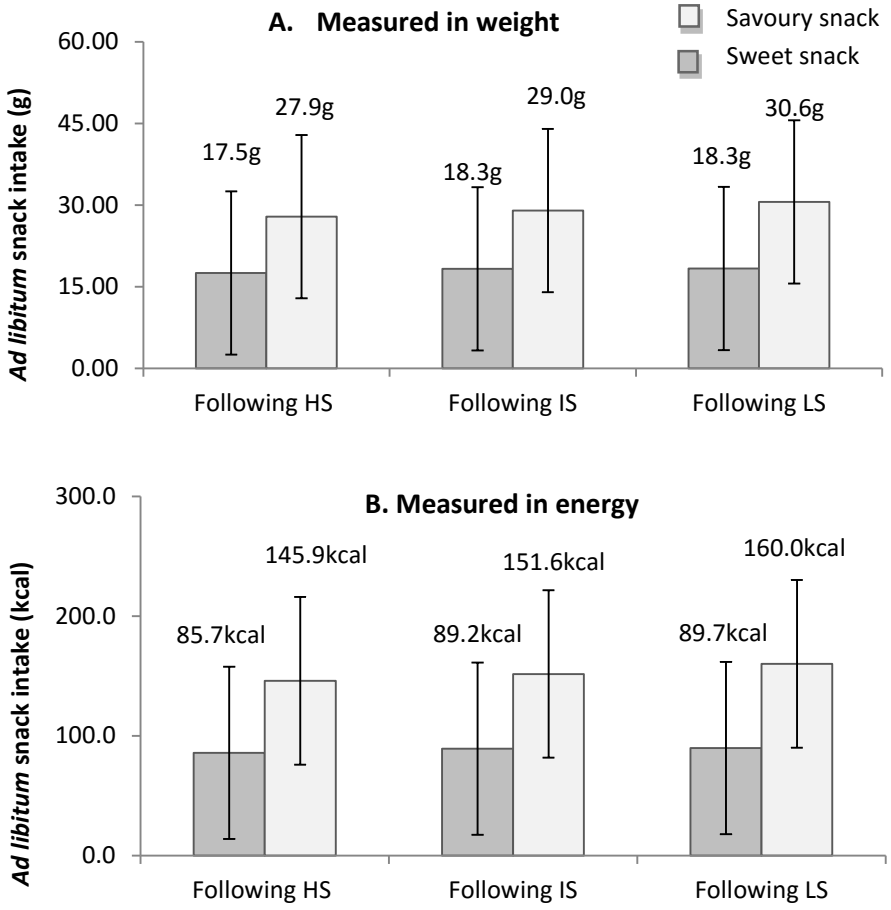

Figure 3-16 Mean values of *Ad libitum* intake of sweet snack and savoury snack and total snack intake following HS, IS and LS milkshake *ad libitum* intake: A) measured in gram; B) measured in kcal. Error bars represent the standard deviation of the total snack intake. Actual intake data for the sweet and the savoury snack were labelled within relative bars. Error bars represent standard deviation

3.3.3.5 Total *ad libitum* energy intake (milkshake + snack)

Total *ad libitum* energy intake was calculated by adding the energy intake of the milkshake with the energy intake of the total snacks consumed. There was no significant difference in the total *ad libitum* energy intake under HS (329.8 ± 90.8kcal), IS (340.6 ± 128.3kcal) and LS (342.7 ± 106.8kcal) milkshake treatments, $F(2, 46) = 0.285$, $p = 0.75$.

3.4 Discussion

3.4.1 Summary of the key findings

The first objective of the study was to investigate the effect of sweetness intensity of milkshakes on *ad libitum* intake of the milkshakes, while the palatability of the milkshakes was controlled. Results show that the amount of *ad libitum* intake of a high sweetness milkshake was similar to the intake of a low sweetness milkshake, when the two milkshakes shared a similar palatability. This suggests that satiation measured as the *ad libitum* intake of milkshake, developed following intake of the milkshake, was not influenced by the sweetness intensity of the consumed milkshake.

The second objective of the study was to investigate the effect of sweetness intensity of milkshake on sensory-specific satiety (SSS). The SSS for the milkshakes was characterised by changes in the pleasantness ratings of the milkshake, and changes in the ratings of desire to drink the milkshake, following *ad libitum* intake of the milkshake. Results show that ratings of the desire to drink (or the pleasantness of) the HS milkshake was reduced significantly, following the *ad libitum* intake of the HS, IS or LS milkshake. This suggests that SSS for the high sweet milkshake was developed following *ad libitum* intake of all sweet milkshakes. However, the reduction in the desire to drink (or the pleasantness of) the HS milkshake was not affected by the sweetness intensity of the milkshake. This indicated that the extent of SSS for the milkshake was not affected by the sweetness intensity of the milkshake that had been consumed.

In addition, the SSS for general sweet foods was characterised by the changes in ratings of ‘desire for something sweet’. Results show that the desire for something sweet was reduced significantly following the *ad libitum* intake of all milkshakes. In comparison, ratings of desire for something savoury remained relatively unchanged or even showed a slight increase after milkshake intake. This suggested that SSS for sweet foods was developed after intake of sweet milkshakes. However, the desire for something sweet did not differ among the treatment when HS,

IS or LS milkshake was consumed. Therefore, the sweetness intensity of the milkshake in a preload did not affect the extent of SSS for sweet foods.

The ratings of desire for something savoury increased significantly following the intake of HS milkshake, suggesting a sensory-specific appetite (SSA, opposite to SSS) for savoury foods was developed following the HS milkshake consumption. The ratings of desire for something savoury after intake of HS milkshake were significantly higher than the ratings after the intake of LS milkshake. It indicated that the sweetness intensity of the milkshake in a preload influenced the extent of SSA for savoury foods. It appears that a high milkshake induced a stronger SSA for savoury foods compared with a low sweet milkshake.

3.4.2 Evaluation of the study design

A considerable individual variation in participants' sweetness perception was observed in this study. Thus, certain sweetness intensities may be too sweet for one participant, but ideal or even not sweet enough for another participant. Meanwhile, the pleasantness ratings showed inverted U-shape to the sweetness intensity of the milkshake; thus, the ideal sweetness milkshakes were rated the most pleasant, while high and low sweetness samples were less pleasant. Therefore, if HS, IS or LS milkshake was selected at the same concentration of sweetener for all participants, it would give a large between-subject variation in their ratings of the pleasantness of HS, IS and LS milkshakes. The pleasantness of food is considered a strong prediction of consumption (Yeomans, 1998, Sorensen et al., 2003). If the pleasantness of milkshakes was uncontrolled, the effect of pleasantness might confound the independent effect of sweetness intensity on *ad libitum* intake of the milkshake.

In the current study, the individual variations in the pleasantness ratings of HS, IS and LS milkshakes were minimized by selecting tailored sweetener concentrations for each individual. For each individual participant, their selected ideal sweetness milkshake had the highest pleasantness ratings,

while the selected high sweetness and low sweetness milkshakes shared similar pleasantness ratings. In this way, the effect of sweetness intensity on *ad libitum* intake of the milkshake was investigated, independently of the effect of palatability.

A commercial low-energy sweetener was used to provide different sweetness intensities rather than sucrose in this study. The energy content of the sweetener was one tenth of the sucrose. Therefore, any observed differences in intake or SSS following the *ad libitum* intake of the milkshakes were likely to be a result of differences in the perceived sweetness intensity, rather than due to differences in energy values provided.

One limitation of this study design was that the sweet snack Chocolate Digestives has relatively a high salt content of 1g/100g as a sweet snack which may have a noticeable salt taste. However, the consumption of Chocolate Digestives showed similar trend as the other two sweet snacks (Animals Biscuits and Maryland Cookies), indicating the results of this study seems unaffected.

3.4.3 Effect of palatability on satiation (*ad libitum* intake)

Palatability of a food has repeatedly been reported to reduce the food intake and increase satiation (Yeomans, 1998). In this study, the milkshake with the ideal sweetness intensity and the highest palatability seemed to be consumed the most, in volume and energy, compared to the high or low sweetness milkshake. However, this observation was not significant ($p=0.12$).

The lack of a significant effect of palatability on the *ad libitum* intake may be due to the relatively small differences in the pleasantness ratings of the milkshakes. The mean pleasantness ratings of HS, IS and LS milkshakes were 55±19, 68±19, and 51±19 mm, obtained from the VAS scale. Therefore, the mean pleasantness rating of IS was only 13 mm, and 17 mm higher than ratings of HS and LS milkshakes, respectively. Vickers et

al. (1997) observed a difference in *ad libitum* intake of yogurts when the difference in pleasantness ratings was greater than 29 mm on the 100 mm VAS scale (Vickers et al., 1998).

3.4.4 Effect of sweetness intensity on ad libitum intake

Despite a non-significant effect of palatability on *ad libitum* intake of the milkshake, the conclusion of the study was not affected. The objective here was to investigate if two milkshakes, different in sweetness intensity but similar in palatability, were consumed in different amounts in an *ad libitum* setting. HS milkshake (sweetness rating: 73±16mm) was perceived to be significantly sweeter than LS milkshake (sweetness rating: 33±16 mm), and both shared very similar palatability ratings (55±19 mm vs. 51±19mm).

No difference in *ad libitum* intake of high sweetness (HS) and the low sweetness (LS) milkshake was observed, measured as the total volume or energy consumed. This suggested that sweetness intensity of the milkshake did not affect participants' *ad libitum* intake of the milkshake. Participants were given the standard instruction to consume as much as they wanted until they felt comfortably full. Therefore, they may reach a similar status of satiation sensation after the *ad libitum* intake of milkshakes. This explains why the ratings of hunger, fullness and satisfaction were not significantly different after intake of the milkshakes. It cannot rule out the possibility that, if the energy contents of milkshakes were fixed across the sessions, sweetness intensities of milkshake may have an impact on the subjective appetite sensation ratings following the intake of the milkshake

The sensory-specific satiation for the milkshake may have contributed, to some extent, to the termination of the intake of the milkshake. It is often difficult to separate the effect of sensory-specific satiation, from (sensory-induced) satiation (Ruijschop et al., 2008a). By definition, (sensory-induced) satiation is affected by all the physiological or psychological

consequences of the exposure to sensory cues that bring an eating event to the end. Sensory-specific satiation especially refers to the consequences of changes in the brain's hedonic and reward responses towards a specific sensory property. A reduction in the pleasantness (liking) and desire (wanting) towards the milkshakes, may contribute to the termination of the milkshake intake.

3.4.5 Effect of the sweetness intensity on sensory-specific satiety

Participants developed SSS for the high sweetness (HS) milkshake after consumption of all milkshakes. This was demonstrated in a reduction in both the hedonic response (pleasantness) and motivation to drink (desire to drink) the high sweetness milkshake.

Sensory-specific satiety can extend to uneaten foods with similar sensory qualities as the eaten food. Rolls (1986) observed that the liking of uneaten foods declined when their sensory qualities were broadly similar to the eaten foods. After consuming all three sweet milkshakes, participants developed sensory-specific satiety to sweet foods and showed a clear preference for savoury foods over sweet foods. This was demonstrated in a reduction in their desire for something sweet compared with the unchanged or even slight increased ratings of desire for something savoury. It appears that sensory-specific satiety for the sweet milkshake extended to general sweet foods.

SSS is most intense immediately after eating and stays relatively strong for 2 h after consumption (Weenen et al., 2005). The decrease in desire to eat a food can affect food choice and intake in subsequent meals (Hetherington, et al., 1989). Since the amount of milkshake consumed in a preload was not significantly different, therefore, it is valid to study the effect of sweetness intensity of the milkshake consumed on the intake of subsequent snacks. At time=30 minutes, following milkshake consumption, participants consumed savoury snacks significantly more than the sweet snacks, under all three-milkshake treatments. This result is consistent with

a previous study, where a sweet food consumed in a preload suppressed the subsequent intake of sweet foods more than the intake of savoury foods (de Graaf et al., 1993). Similar results were found in another study where participants consumed a higher amount of sweet snacks than savoury snacks following consumption of a savoury lunch (Hetherington and Boyland, 2007). Regarding the nature of the taste signals which may have an impact on food choice and intake, the sweet-savoury domain is probably the most important dimension (Blundell et al., 2010). The majority of energy that we ingest comes from food with either a dominant sweet taste or a dominant savoury taste. The results of this study suggested that SSS limits the intake of foods within a similar sensory characteristic (sweet foods), and promotes diet balance by encouraging intake of foods with different sensory characteristics (savoury foods).

However, the sensory-specific satiety to sweet foods was not affected by the sweetness intensity of the pre-consumed sweet milkshakes. The changes in ratings of pleasantness and desire to drink the milkshakes, the ratings of desire for something sweet, and the subsequent *ad libitum* intake of sweet or savoury snack were not significantly different among three milkshake treatments. This finding is in agreement with a study by Havermans et al. (2009), who reported SSS of a lemonade was unaffected by flavour intensity (aroma + taste). They did not obtain a significant difference in the changes of the pleasantness ratings of the strawberry lemonade, following *ad libitum* intake of the lemonade of high concentration strawberry syrup, compared with a low concentration of strawberry syrup. Thus, the manipulation of flavour intensity in lemonade did not affect the extent of SSS for lemonade drinks.

The lack of a difference in the changes of the pleasantness ratings of the milkshake following intake of milkshakes with different sweetness supports Rolls' ideas about SSS (2012). Rolls (2012) proposed that the intensity of taste stimuli is processed separately from the hedonic-reward process of the taste stimuli in the brain. The intensity and recognition of taste stimuli

are processed in the primary taste cortex (i.e. the anterior insula and post-central gyrus) (Rolls, 2012). The hedonic and reward evaluation of the taste or flavour stimuli is processed in the secondary taste cortex, i.e. the OFC (Rolls, 2012). When a food of certain sensory properties was eaten to SSS, the signals in the pleasantness centre of the OFC were reduced specifically to the eaten food, while the signals in the primary taste cortex remained relatively unchanged (Rolls and Grabenhorst, 2008, Rolls, 2012). In other words, SSS reflected in changes in the pleasantness of the stimuli but not the perceived intensity of the stimuli. Conversely, manipulation of the taste intensity (processed in the primary taste cortex) does not seem to interact with the changes in the hedonic signals (processed in the OFC) over continues or repeated consumption of taste stimuli (Havermans, 2009).

3.4.5.1 Sweetness intensity and the desire for savoury

Participants developed sensory-specific appetite (SSA) for savoury foods in comparison to sweet foods, following the intake of sweet milkshake. Similarly, Weenen, et al. (2005) observed an increase in the desire for sweet foods following consumption of a savoury food. This observed contrasting effect between sweet-taste foods and savoury-taste foods on the desire to eat something sweet or savoury may be a function to promote food variety and nutrient balance (Hyde and Witherly, 1993).

In addition, the sweetness intensity of the pre-consumed milkshake affected the extent of the SSA for savoury foods. After the *ad libitum* consumption of the high sweetness milkshake, the ratings of desire for something savoury were significantly higher than the ratings after the consumption of the low sweetness milkshake (at 10 min). However, this difference in the ratings of desire for something savoury did not result in a difference in the subsequent intake of sweet or savoury snacks. As far as the author knows, this has been the first study to demonstrate that consumption of a higher sweetness drink could induce a stronger sensory-specific appetite for savoury food, compared with a low sweetness drink.

The measurement of ‘desire for something savoury’ corresponds to ‘wanting for the savoury foods.’ Wanting is more complex than liking. Wanting is affected by both the sensory and cognitive inputs (Berridge, 1996). The developed SSA for savoury foods after the consumption of sweet milkshakes was unlikely through the sensory stimuli pathway, which transmits the savoury stimuli from the primary cortex to the OFC, because participants were not exposed to the actual stimulation of savoury stimuli. Therefore, the developed SSA for savoury foods was potentially a consequence of cognitive processes. After intake of high sweet milkshake, participants may believe that savoury foods became more rewarding (desirable) than the already eaten sweet foods.

3.5 Conclusions

Changing the sweetness intensities of milkshakes by adding a different amount of a low-caloric sweetener (Canderal) did not affect the *ad libitum* intake of the milkshakes, even when the milkshakes were similar in pleasantness. After consuming all sweet milkshakes, participants developed the sensory-specific satiety for the HS milkshake, and an extended SSS for general sweet foods. The sweetness intensity of the milkshakes did not affect the extent of sensory-specific satiety for the milkshake, or general sweet foods. However, the consumption of the high-sweetness milkshake induced a sensory-specific appetite for general savoury foods, while the sensory-specific appetite for savoury foods was unchanged following IS and LS milkshake consumption.

Chapter 4. Appetite sensation and subsequent food intake

4.1 Introduction

Our perception of food flavour is an integration of multiple concurrent sensory modalities, of which aroma and taste are primary drivers (Noble, 1996). Literature has been focusing on the independent effect of aroma or taste stimuli on satiation and satiety (Chapter 1, section 1.9). Retronasal aroma delivery is typically associated with food consumption, therefore, its manipulation has the potential to enhance the satiating power of a food or drink (Small et al., 2005). Indeed, a more intense (or more complex) retronasal aroma stimulation led to enhanced feelings of satiation compared with a less intense (or single-component) aroma stimuli (Ruijschop et al., 2010, Ruijschop et al., 2008a). As for taste stimuli, we may associate the taste of a food with its post-consumption consequences through instinct or learned association, and such association may affect our appetite and feeding behaviours (Yeomans, 2012). For example, the sweet taste of sugars or non-caloric sweeteners has been shown to increase the feeling of fullness (Lavin et al., 2002b, Vickers et al., 2001, Anderson and Woodend, 2003).

Flavour perception is synergistic of the combination of taste and aroma modalities (Chapter 1, section 1.8.4). Thus, the cross-modal interaction of congruent taste and aroma stimuli enhances the perceived flavour intensity more than the sum of taste and aroma stimuli presented independently (Hewson et al., 2009, Pfeiffer et al., 2006). In some brain areas, the simultaneous presence of taste and aroma stimuli activate stronger signals than the sum of taste or aroma stimuli presented separated (de Araujo et al., 2003). In addition, a lateral anterior part of the OFC was only activated by the combination of aroma and taste stimuli, but not by aroma or taste stimuli alone (de Araujo et al., 2003). These studies provide compelling evidence that the perception of flavour is a result of combined perception of taste and aroma modalities rather than the result of the simple addition of independent taste or aroma modality.

As far as the author is aware, the interactive effect of aroma-taste on appetite and food intake has not been studied specifically, in comparison to their independent effects. On the one hand, increased flavour intensity leads to increased oral sensory exposure, which has the potential to enhance satiation (Bolhuis et al., 2011). On the other hand, congruent taste and aroma were found to increase the activation in the hedonic areas of OFC and ACC, leading to increased pleasantness ratings (Rolls, 2012). Highly pleasant foods may promote overeating (Yeomans, 1998). Therefore, whether aroma and taste interact to promote or delay satiation remains unknown.

4.2 Study objectives and hypothesis

The main objectives of this study were to investigate the effects of taste modality, aroma modality and their cross-modal interaction on subjective appetite ratings and subsequent food intake. It is hypothesised that when congruent taste and aroma stimuli are presented concurrently, appetite and food intake may be suppressed and that this effect may be stronger than that is elicited by taste or aroma stimuli presented individually.

Therefore, a model water-based drink was of different combinations of strawberry aroma and taste substances (sucrose and citric acid). Strawberry aroma, sweetness (due to sucrose), and sourness (due to citric acid) are congruent modalities which have been found to enhance overall strawberry flavour intensity more than the sum of their parts (Pfeiffer et al., 2006).

In addition to perceptual interactions, taste substances such as sugar, salt and acid can also interact with aroma at a physicochemical level. The physicochemical interaction between taste and aroma stimuli may affect the aroma release from the food matrix into the gaseous phase and then delivery to the nasal receptors (Friel et al., 2000). In order to evaluate the physicochemical interaction between taste substances (sucrose + citric

acid) and strawberry aroma compounds, the aroma release to the nose was monitored by APCI-MS.

Flavour perception of food may also affect our psychological expectations about the satiating capacity of food. The expectation on satiety has been found to affect the subjective appetite sensations such as hunger and fullness (Brunstrom et al., 2011). Therefore, participants' expected satiation of the sample drinks were measured using a 100 mm VAS scale (McCrickerd et al., 2014). This was to investigate if the effect of flavour on expected satiation was potentially correlated with its potential effect on appetite sensations.

The Medical Ethical Committee of the University of Nottingham approved this study with the ethics reference number R14032013 SBS Food, 15/03/2013.

4.3 Materials and methods

Two experiment phases were conducted. In the first phase, the effect of different sample drinks on subjective appetite sensation and subsequent intake were evaluated. In the second session, the characteristics of the samples drinks were compared, including flavour and taste intensities, in vivo aroma release, palatability (liking), and expected satiation.

4.3.1 The first phase of the study

4.3.1.1 Study design

The study was a single-blind, randomised crossover design. A 'preloading paradigm' was used to investigate the effects of aroma alone, taste alone, and aroma and taste together in a preload drink on subjective appetite ratings and subsequent food intake. Water without any taste and aroma substances was used as a control preload in parallel to the three sample drinks with stimuli. All 26 selected female participants completed all four drink-preload treatments over 4 days, separated by at least 3 days.

4.3.1.2 Participants

Twenty-six participants completed this study. The standard procedures of recruitment and screening applied to this study; and participants were selected using the general participants' criteria (Chapter 2, 2.2.3). Participants were only informed of the fact that this study was about food and appetite, and the ingredients information of the test foods and drinks. No further information was given to prevent response bias.

4.3.1.3 Sample size

The primary outcome measure is the subjective appetite sensation. The number of participants (sample size) needed to detect a difference in subjective appetite sensations was decided based on the numbers of participants used in previous studies. A number of 20-25 participants is adequate to detect a mean difference of 10% on VAS appetite scale between two foods (Blundell et al., 2010, Flint et al., 2000). Ruijschop (2008b) detected a significant (α=0.05) mean difference of 12 mm (SD of 20 mm), in a 100 mm VAS satiation scale, between two drinks with different aroma profiles, using 27 participants. Therefore, 26 participants (in the current study) was sufficient to detect a mean difference of 10% in appetite sensations measured on VAS scales, at α=0.05 significance level, with a power of 0.82 (Statistical Solutions, 2016).

4.3.1.4 Protocol

A brief training session on rating appetite using Visual Analogue Scale (VAS) was given to participants prior to the test study. Participants attended four test sessions. They were asked to come to each study session only during their luteal phase of menstrual cycle (day 18 to day 26), in order to minimise the differences in their gastric emptying, appetite and food intake across the menstrual cycle (Hirschberg, 2012, Li et al., 1999).

At least 24 hours prior to each visit, participants were required to refrain from intensive exercise, smoking, alcohol consumption and any

medications (Astbury et al., 2011). They were also asked to consume the same self-chosen dinner the evening before each session between 20.00 and 21.00 hours, and then to fast from after the dinner until arriving in the laboratory next morning. Water consumption was permitted while fasting.

For the test session, participants reported to the laboratory at 08.45 hours. Participants were requested to remove heavy shoes or clothing, and their body weight and height were assessed. Baseline subjective appetite ratings were measured at 09.00 hours using visual analogues scales (VAS). Participants were then served a standardised breakfast to be consumed within 20 minutes. At 11.00 hours, participants were provided with one of the four samples to be consumed over 15 minutes. VAS were completed immediately before, and at 5, 10, 15, 25, 35, 45, 80 and 100 minutes after starting the sample stimulation. At 12.20 hours, participants were served a pasta lunch which they were asked to consume until they felt comfortably full. Unlimited water was served together with the lunch. No other food or drink consumption was permitted. Participants were required to stay quietly in a waiting room when not required to undertake activities by the experiment. The protocol of a test day is shown in **Figure 4-1**.

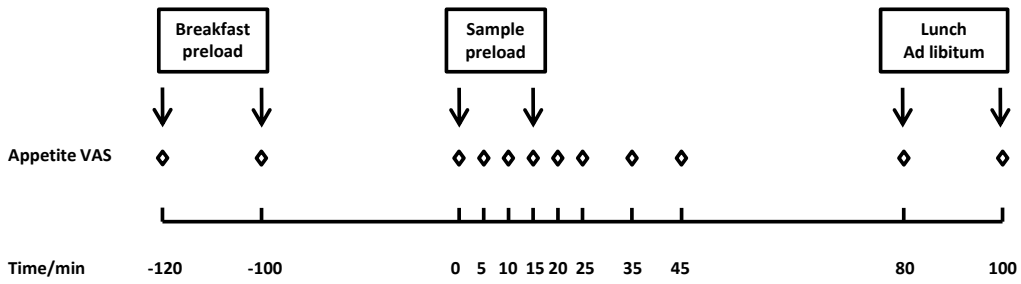

Figure 4-1 Protocol for each study day. The subjective feelings of hunger, satiation, fullness, desire to eat and prospective consumption were rated on 100 mm long VAS scale at time points indicated with open diamond markers. The arrows indicate start and end of breakfast, sample drink stimulation and lunch.

4.3.1.5 Sample drinks

The sample drinks consisted of Evian mineral water (Danone Group, France), Silver Spoon granulated sugar (British Sugar PLC, Peterborough,

UK), citric acid (Fisher Scientific, UK) and a multi-component strawberry aroma (Mane Co. Ltd., Derby, UK). The key strawberry aroma compounds were ethyl butyrate, ethyl 2-methyl butyrate, and ethyl hexanoate, which were diluted in propylene glycol. All samples were freshly made 2 hours before serving and were served at 18°C. Sample drink composition and energy content are shown in **Table 4-1**.

Table 4-1 Sample drink composition and energy content.

Drink sample	Strawberry aroma v/v	Sucrose w/v	Citric acid w/v	Energy kcal (150ml)
Sample 1 (S1)	0	0	0	0
Sample 2 (S2)	0.50%	0	0	0
Sample 3 (S3)	0	8%	0.10%	48 kcal
Sample 4 (S4)	0.50%	8%	0.10%	48 kcal

Each sample was served in 15 sealed cups coded with a three-digit random number, each containing 10 ml. Participants were asked to consume the drink slowly (10 ml/minutes) using a fine straw (diameter: 0.625 mm, Altec Ltd., UK). Participants fit the straw into a small hole on the lid of the sample cup before drinking. Therefore, the release of aroma to the external air (orthonasal aroma delivery) was minimised. Each participant, therefore, consumed 150 ml sample drink over 15 minutes per session. While drinking, participants were instructed to adherent to their normal breathing rate, swallow at a comfortable frequency, and focus on the perception of the sample.

4.3.1.6 Pre-visit Dinner

On the evening prior to the study participants were free to choose a dinner within their normal diet but requested to eat the same dinner with equal energy prior to each study day.

4.3.1.7 Breakfast

A breakfast of Rice Krispies (Kellogg's UK Limited, Manchester, UK) and semi-skimmed milk (Sainsbury's Supermarkets Ltd., London, UK). It

equivalent to 10% of the participant's estimated total daily energy requirement (TDER) and containing 14%, 14%, and 72% energy from fat, protein, and carbohydrate, respectively was provided (Astbury et al., 2011).

4.3.1.7.1 Calculating total daily energy requirement (TDER)

The energy requirement for a participant with stable weight is equal to his or her energy expenditure (Frayn, 2010). All participants in the study were required to have a weight change within ± 4 kg over the past 6 months, indicating that their weight was stable and they were in energy balance. Therefore, the TDER was equal to the participant's total daily energy expenditure (TDEE). Participants' TDEE can be estimated by multiplying their BMR with their activity factor, as stated in the following equation (Equation 4-1):

Equation 4-1: TDER = TDEE = BMR × Activity factor

4.3.1.7.1.1 Basal Metabolic Rate (BMR)

Basal Metabolic Rate is the minimum amount of energy (calories) that a person needs to keep the body functioning at rest. These processes include breathing, blood circulation, body temperature regulation, cell growth, brain and nerve function, and contraction of muscles. The BMR was calculated according to the equation (Equation 4-2) developed by Harris and Benedict (1918) below:

Equation 4-2: BMR (woman) = 655.1 + (9.563 x weight in kg) + (1.850 x height in cm) – (4.676 x age in years)

4.3.1.7.1.2 Activity factor

Estimated Physical Activity Level (PAL) for each individual can be assessed using the International Physical Activity Questionnaire (IPAQ) (Appendix IV) (Craig et al., 2003). In the IPAQ, participants completed questions assessing their physical activity pattern over the past one week. Based on participants' response to IPAQ questions, participants were characterised into three physical activity level groups (low, moderate or

high), each with a corresponding activity factor number of 1.375, 1.55 and 1.725, respectively (Harris and Benedict, 1919, Rosenblum et al., 2012). The activity factor, expressed as a number, is a way to indicate a person's daily physical activity (Weinstein, 2013). It is the ratio of the person's total daily energy expenditure (TDEE) to his or her basal metabolic rate (BMR).

4.3.1.8 Subjective appetite measurement

Participants were asked to rate their subjective appetite sensation of hunger, satiation, fullness, desire to eat and expected amount to eat using a 100 mm long visual analogue scale (VAS) (Flint et al., 2000) at defined time points across the session. Participants were asked to electronically score on the scales by placing a mark on the horizontal line using the computerised data acquisition system FIZZ 2.46 (Biosystems, France).

4.3.1.9 Pasta Lunch

The *ad libitum* test lunch consisted of penne pasta (Sainsbury's Supermarkets Ltd., London, UK), Dolmio Garden Vegetable pasta sauce (MARS Food, USA), olive oil (Sainsbury's Supermarkets Ltd., UK), and cheddar cheese (Sainsbury's Supermarkets Ltd., UK). It was cooked on the evening before each study day under standard food hygiene procedures (Appendix V). It contained a total energy content of 1.66 kcal/g; and 14%, 52%, and 34% energy provided by protein, carbohydrate, respectively (**Table 4-2**). The cooked pasta lunch was refrigerated in sealed containers overnight until being reheated in a microwave oven for 3 minutes and then stirred immediately before serving.

Table 4-2 Macronutrient composition of pasta per serving portion (530 g)

Composition	weight (g)	energy %
Protein	31.6	14%
Carbohydrate	113.5	52%
Fat	33.0	34%
Fibre	6	0

4.3.1.10 Measuring lunch energy intake

Food intake was assessed directly by measuring the subsequent pasta lunch energy intake (EI) following sample drink stimulation. Participants were given 530 g (878 kcal) portion of pasta and instructed to terminate the meal only when they felt comfortably full. They were instructed to ask for another portion once the previous one was finished. Lunch EI was calculated by multiplying the weight (g) of pasta consumed by the energy density of pasta lunch (1.66 Kcal/g).

4.3.2 The second experiment phase: sample characterisation

4.3.2.1 Participants

Sixty healthy female participants were recruited and completed three sensory tests (4.3.2.2, 4.3.2.3 and 4.3.2.4), for assessing the four sample drinks (Table 4-1), on separate days. In addition, five out of these participants also completed an extra session measuring their in-vivo aroma release by APCI-MS (4.2.3.5) on another day. Participants were selected to attend these sessions because they replied to an email advertise of the study and reported themselves as healthy, non-smoking, normal weight (BMI: 18.9-24.9 kg/m^2) females with no abnormal gustatory and olfactory senses and no allergy to the ingredients used. Prior to the session, their weight and height were measured in the lab, and any participants who had a BMI outside 18.9-24.9 kg/m^2 were excluded from the study. The 60 female participants who completed the study had a mean BMI of 21.4 ± 2.1 kg/m^2, and a mean age of 22±4 years.

4.3.2.2 Pairwise ranking test for flavour and sweetness

The four sample drinks were compared for perceived overall flavour intensity and sweetness intensity using two pairwise ranking tests on three separate sessions (Meilgaard et al., 2006a). One attribute was assessed during each session. For each attribute, each participant evaluated all six possible pairs formed from the four samples, one pair at a time. The question was “which sample is stronger in overall flavour intensity”, or

“which sample is stronger in sweetness intensity”. The order of sample presentation was fully randomised within pairs between pairs, and among participants. 10ml of each sample was present in a 30 ml sealed plastic cup coded with a 3-digit random number. Water and crackers were used to cleanse the palate between any two samples.

4.3.2.3 Hedonic liking ratings

The overall sensory acceptability of the four sample drinks was assessed using a 9-point hedonic scale (Peryam, 1952). The hedonic scale ranged from (**Figure 4-2**), 1 (dislike extremely) to 5 (neither like nor dislike), and to 9 (like extremely). All 60 participants assessed the four samples in a randomised and balanced order. 10ml of each sample was present in a 30 ml sealed plastic cup coded with a 3-digit random number. Water and crackers were used to cleanse the palate between any two samples.

Figure 4-2: 9-point vertical hedonic scale

4.3.2.4 Expected satiation

Participants' expected satiation (expected fullness and expected hunger) of the sample drinks was measured on a 100 mm long VAS scale (Hogenkamp et al., 2011, McCrickerd et al., 2012a) (**Figure 4-3**).

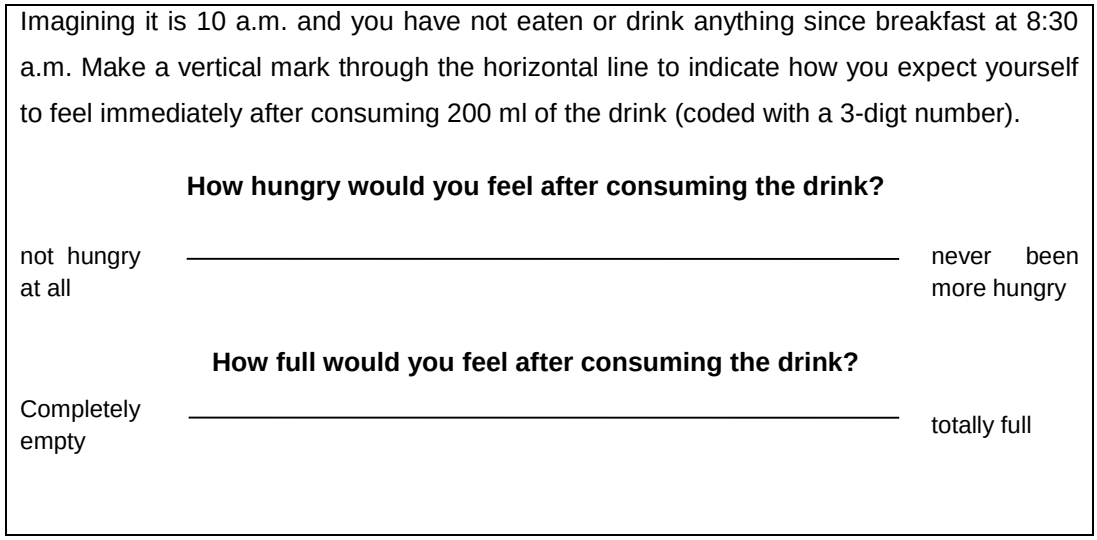

Figure 4-3 Sensory questionnaire for assessing expected satiation

4.3.2.5 APCI-MS analysis of in-vivo volatile release

Breath by breath APCI-MS analysis (Taylor and Linforth, 2003) was used to compare the in-nose aroma release during drinking between Sample 2 (only strawberry aroma) and Sample 4 (strawberry aroma + citric acid + sucrose). Five participants consumed both samples in triplicates. Participants were instructed to drink 30 ml of each sample in one go while positioning one nostril on the nasal sampling tube of APCI-MS while breathing and swallowing normally. Nose breath was sampled at a flow rate of 25 ml/minutes. The release of key strawberry aroma compounds, ethyl butyrate, ethyl 2-methyl butyrate, and ethyl hexanoate was determined by monitoring m/z 117, 131, and 145, which are the mass to charge ratios for each protonated molecule. The presentation order of the samples was randomised and balanced.

4.3.3 Data analysis

Data is presented as mean values ± standard deviation unless otherwise stated. A p-value < 0.05 was considered statistically significant for all tests. Subjective appetite ratings, subsequent lunch intake, hedonic liking ratings and APCI-MS data analysis were performed with IBM SPSS statistics (version 21.0; IBM Corporation, USA). Pairwise ranking test and triangle test were analysed in Microsoft Excel 2010 (Microsoft Corporation, USA).

4.3.3.1 VAS appetite ratings analysis

VAS appetite ratings were collected via Fizz software, and measured in millimetres from the left end to the point that the participants scored on the scale. Δ VAS was determined by subtracting the ratings collected immediately before sample drink stimulation (time=0 minutes) from the ratings after sample drink stimulation (time = 5, 10, 15, 20, 25, 35, 45, 80 minutes). One participant was identified as an outlier whose appetite ratings (hunger and fullness) had studentized residual values above ± 3 standard deviations. Therefore, data from 25 participants were left in the final appetite rating analysis. Δ hunger, Δ fullness, Δ satisfaction, Δ desire to eat, and Δ amount expected to eat were compared between the four sample drinks using two-way repeated measures ANOVA (two factors: 4 samples × 8 time points). Where a significant difference was identified in the main effect of the sample, *post hoc* testing with Bonferroni adjustment was used to determine which samples were significantly different. Paired-samples t-test was also used to determine at which time points two samples were significantly different.

4.3.3.2 Energy intake data analysis

Lunch energy intake and accumulated energy Intake (sample drink energy + lunch energy) were compared between four sample drink treatments using one-way repeated measures ANOVA (one factor: sample drink). *Post hoc* tests with Bonferroni adjustment were used to identify which samples were different.

4.3.3.3 Pairwise ranking and hedonic liking data

Pairwise ranking data for the perceived flavour and sweetness intensities of the four sample drinks was analysed using Friedman's test with *post hoc* Tukey's honestly significant difference (HSD) test according to a standard data analysis protocol (Meilgaard et al., 2006b). Hedonic liking ratings were analysed using Friedman statistic test with *post hoc* Wilcoxon signed-rank test in SPSS.

4.3.3.4 Expected satiation of sample drinks

VAS ratings of expected hunger and expected fullness were collected via Fizz software and measured in millimetres from the left end to the point that the participants scored on the scale. Data analysis was then analysed using one-way repeated measures ANOVA (one factor: sample) in SPSS. *Post hoc* tests with Bonferroni adjustment were used to identify which samples were significantly different.

4.3.3.5 APCI-MS data analysis

The peak height values from the chromatograms for each ion from S2 and S4 were obtained using MassLynx software (Micromass Ltd, UK). The mean peak values, n (15) = 5 participants × 3 replicates, of ions 117, 131 or 145 were compared between S2 and S4 using paired sample t-tests in SPSS. The objective was only to compare the intensities of aroma delivered from S2 and S4, therefore, aroma intensity measured as arbitrary units (relative intensity ratio) was sufficient to analysis for differences.

4.4 Results

4.4.1 The first phase results

4.4.1.1 Participants characteristics

All participants met the general subject's criteria and completed the first phase of the study. They had a mean BMI of 20.9 ± 1.9 kg/m^2, a mean age

of 24 ± 4 years (19-40 years), mean restraint score on TFEQ of 4 ± 2, and a mean score of 3 ± 2 on Beck Depression Inventory.

4.4.1.2 Subjective appetite ratings

All five subjective appetite ratings including hunger, fullness, satisfaction, desire to eat, and the amount expected to eat at the time point before breakfast were not significantly different between the four visits, indicating that participants arrived each time with a similar baseline appetite sensation. Subjective appetite ratings immediately before sample stimulation were not significantly different, Δ VAS appetite rating was determined by subtracting the ratings at time=0 point from all subsequent time points. There was a significant main effect of time on all Δ VAS appetite ratings, $p<0.001$, suggesting subjective appetite feelings were changing over the time course of the study. There were significant main effects of samples on the Δ ratings of hunger and fullness ($p<0.05$), but the Δ ratings of satiation, desire to eat and the amount expected to eat were not affected by the sample drinks in four sessions ($p>0.05$).

4.4.1.2.1 Δ Hunger ratings

Mean ± standard deviation of the original hunger ratings and Δ hunger ratings of the 4 sample drinks are listed in **Table 4-3**. **Figure 4-4** illustrates the changes of Δ hunger over time (from 0 to 80 minutes) under the four sample-drink treatments. The interaction between samples and time points on Δ hunger ratings were not statistically significant, $F (7.945, 190.692) = 1.698$, $p = 0.102$. This indicates that the four samples shared a similar pattern in Δ hunger ratings over time. There was a significant main effect of time (from 0 to 80 minutes) on Δ hunger ratings, $F (3.184, 75.564) = 57.22$, $p < 0.0001$, indicating that Δ hunger ratings changed significantly over the time course of the study. There was a significant main effect of sample drinks on Δ hunger ratings, $F (3, 72) = 6.025$, $p=0.001$. *Post hoc* test revealed that S4 (aroma + tastants) reduced Δ hunger ratings greater than S1 (water, $p=0.006$), S2 (aroma, $p=0.009$) and S3 (tastants, $p=0.041$)

(**Figure 4-5**). S1, S2 and S3 did not affect Δ hunger significantly different, p = 1.0. The time points where S4 reduced Δ hunger significantly greater than S1, S2 and S3 were shown in **Table 4-4** (paired sample t-tests). Specifically, S4 reduced Δ hunger significantly greater than S1 from 5 minutes to 45 minutes, and greater than S2 or S3 from 5 minutes to 35 minutes ($p<0.05$).

Table 4-3 Mean ± SD for original hunger and Δ hunger ratings, n=25 participants.

Time	Original hunger rating				Δ hunger rating			
(min)	S1	S2	S3	S4	S1	S2	S3	S4
0	55.0±17	55.5±19	53.8±19	57.8±18	0	0	0	0
5	53.4±15	53.7±19	53.3±20	49.8±17	-1.6±7	-1.8±8	-0.5±10	-8.0±11
10	52.8±16	54.3±19	51.1±21	42.0±16	-2.2±19	-1.2±16	-2.7±13	-15.8±16
15	49.8±20	51.7±20	47.7±22	38.0±19	-5.2±18	-3.8±15	-6.2±13	-19.8±18
20	55.6±18	53.0±18	49.2±20	41.4±17	0.6±16	-2.5±18	-4.6±12	-16.4±16
25	55.7±18	55.5±16	53.2±19	46.9±18	0.7±15	0.0±17	-0.6±15	-10.9±17
35	59.8±3.8	61.8±15	58.2 ±17	51.7±18	4.8±15	6.3±17	4.3±17	-6.1±15
45	66.2±16	66.3±17	62.8±18	58.2±16	11.2±15	10.8±18	9.0±18	0.4±14
80	80.2±11	78.9±16	74.5±14	74.4±14	25.2±18	23.4±17	20.7±21	16.6±20

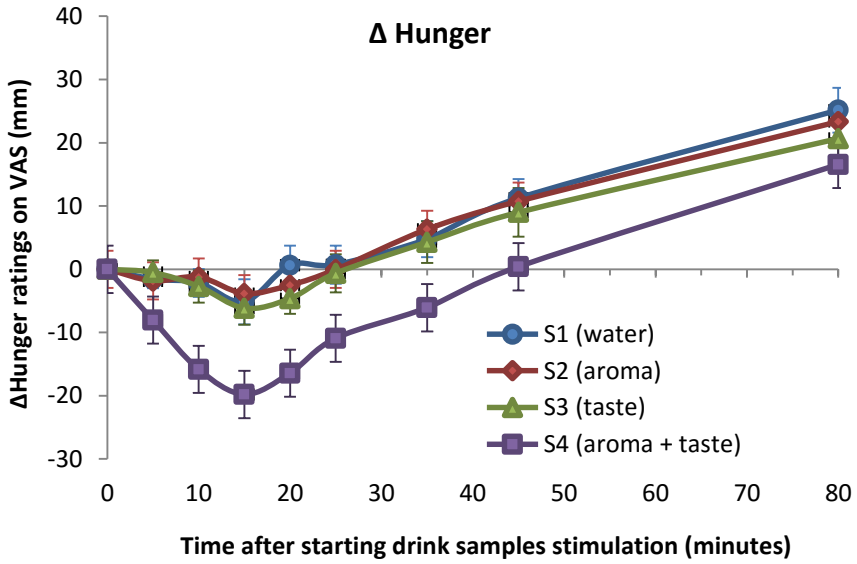

Figure 4-4 Mean Δ hunger ratings at time 5, 10, 15, 20, 25, 35, 45, and 80 minutes after starting the sample drink stimulation of S1, S2, S3 or S4, n=25 participants. Error bar represents standard error.

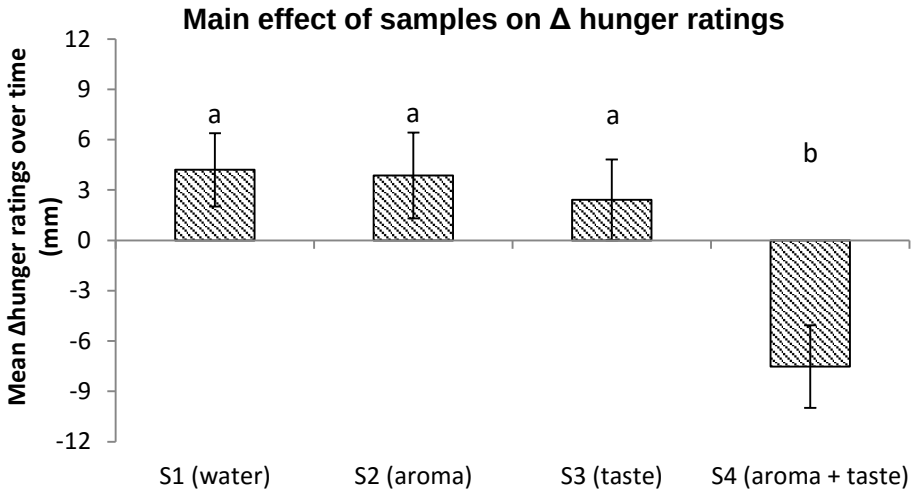

Figure 4-5 Mean Δ hunger ratings across the time course of the test session (average of 5, 10, 15, 20, 25, 35, 45, and 80 minutes) under S1, S2, S3 or S4 condition, n=25 participants. Sample bars without a same letter coding are significantly different ($p<0.05$). Error bar represents standard error.

Table 4-4 Mean ± SD of the differences in Δ Hunger between paired samples at time points following samples drink stimulation, n=25 participants.

Paired sample difference in Δ hunger ratings (paired sample t-test)			
Time (minutes)	S4 - S1	S4-S2	S4-S3
5	-6.5±14*	-6.2±14*	-7.5±13*
10	-13.7±18*	-14.6±16*	-13.1±20*
15	-14.6±27*	-16±26*	-13.6±21*
20	-17±21*	-14±13*	-12±18*
25	-11.6±22*	-11±17*	-10.3±20*
35	-10.8±17*	-12.4±21*	-10.4±20*
45	-10.8±17*	-10.4±25	-8.6±23
80	-8.6±25	-6.8±17	-4.1±28

Values were calculated by subtracting Δ hunger ratings under S1, S2 or S3 treatments from Δ ratings under S4 treatment. Values with '*' were statistically significant between the two samples, $p<0.5$

4.4.1.2.2 Δ Fullness ratings

The mean ± standard deviation of the original fullness ratings and Δ fullness ratings of the 4 sample drinks are listed in **Table 4-5**. **Figure 4-6** illustrates the changes of Δ fullness over time (from 0 to 80 minutes) under the four sample-drink treatments.

Table 4-5 Mean ± SD for original fullness and Δ fullness ratings, n=25.

Time	Original fullness rating				Δ fullness rating			
(min)	S1	S2	S3	S4	S1	S2	S3	S4
0	40.8±15	37.9±16	41.7±17	40.5±18	0	0	0	0
5	41.0±14	39.8±16	46.7±18	48.7±18	0.2±10	1.9±7	5.0±9	8.2±14
10	44.1±19	41.6±16	50.9±21	52.1±20	3.3±15	3.8±15	9.2±14	11.6±16
15	44.8±26	46.5±18	52.7±21	54.3±21	4.0±22	8.6±20	11.0±14	13.8±17
20	41.8±20	45.4±18	50.3±20	52.7±20	1.0±17	7.5±21	8.6±13	12.2±20
25	40.2±19	43.3±18	46.8±20	50.1±20	-0.6±14	5.4±21	5.1±14	9.6±18
35	35.2±17	38.0±16	41.6±15	44.9±18	-5.6±13	0.2±21	-0.1±15	4.4±15
45	29.7±16	32.8±15	37.0±17	40.2±17	-11.1±15	-5.1±20	-4.7±15	-0.2±17
80	16.4±15	19.4±11	24.8±13	23.0±17	-24.4±15	-18.5±15	-16.9±15	-17.4±20

The interaction between samples and time points on Δ fullness ratings were not statistically significant, $F(7.788, 186.915) = 0.937$, $p = 0.486$. This indicates that the four samples shared a similar pattern in Δ fullness over time. There was a significant main effect of time (from 0 to 80 minutes) on Δ fullness ratings, $F(2.639, 63.345) = 44.401$, $p < 0.0001$, indicating that Δ fullness ratings changed significantly over the time course of the study. There was a significant main effect of sample drinks on Δ fullness ratings, $F(3, 72) = 3.037$, $p = 0.033$. *Post hoc* test reviewed that S4 (aroma + tastants) reduced Δ fullness ratings significantly greater than S1 (water, p=0.023), but similar to S2 (p = 1.0) and S3 (p = 1.0) (**Figure 4-7**). S1, S2 and S3 did not affect Δ fullness significantly different, p = 1.0. The time points where S4 reduced Δ fullness significantly greater than S1, S2 and S3 were shown in **Table 4-6**. Specifically, S4 reduced Δ fullness significantly greater than S1 from 5 minutes to 45 minutes, and greater than S2 or S3 only at 5 minutes ($p<0.05$).

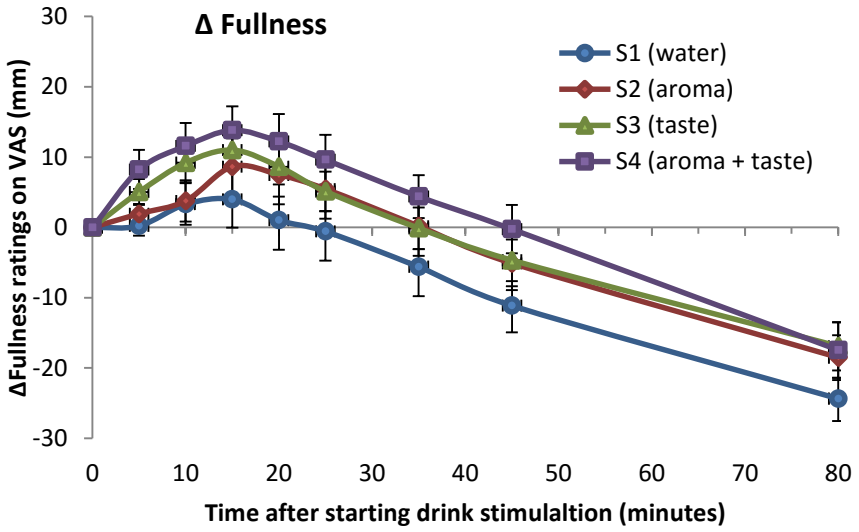

Figure 4-6 Mean Δ fullness ratings at time 5, 10, 15, 20, 25, 35, 45, and 80 minutes after starting the sample drink stimulation of S1, S2, S3 or S4, n=25 participants.

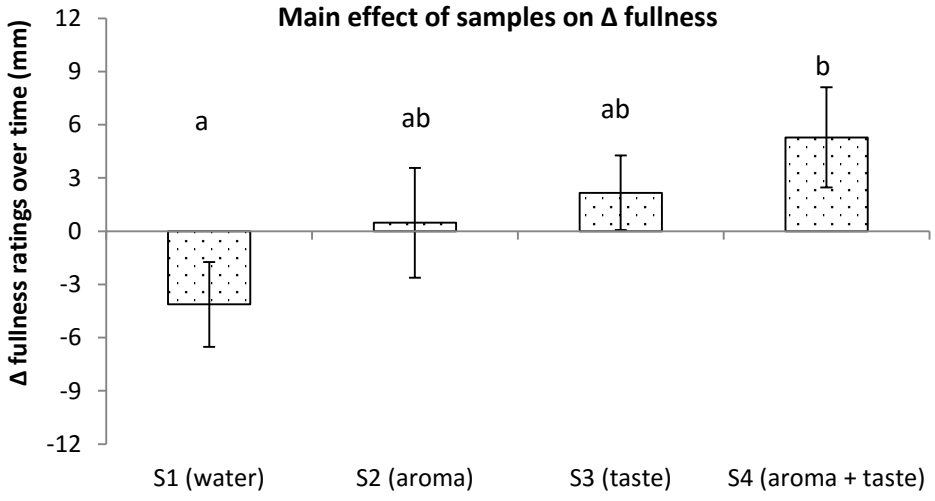

Figure 4-7 Mean Δ fullness across the time course of the test session (average of 5, 10, 15, 20, 25, 35, 45, and 80 minutes) under S1, S2, S3 or S4 condition, n=25 participants. Sample bars without the same letter coding above are significantly different ($p<0.05$).

Table 4-6 Mean ± SD of the differences in Δ fullness between paired samples at time points following samples drink stimulation, n=25 participants.

Paired sample difference in Δ fullness ratings (paired sample t-test)			
Time (minutes)	S4 - S1	S4-S2	S4-S3
5	8.0±14*	6.3±15*	3.2±13*
10	8.3±18*	7.8±19	2.4±16
15	9.8±20*	5.2±22	2.8±15
20	11.2±18*	4.8±23	3.6±18
25	10.2± 16*	4.2±22	4.5±18
35	10±17*	4.2±24	4.5±15
45	10.9±20*	4.9±27	4.4±18
80	6.9±26	1.1±25	-0.52±19

Values were calculated by subtracting Δ ratings of S1, S2 or S3 from Δ ratings of S4. Values with '*' were statistically significant between the two samples, p<0.5

4.4.1.3 Energy intake

There was no significant difference in the subsequent pasta lunch energy intake, F (2.126, 53.150) = 1.396, p=0.26. The accumulative energy intake (sample drink plus pasta lunch) was also not different, F (2.126, 53.150) = 2.686, p=0.074 (**Table 4-7** and **Figure 4-8**), although this is approaching significant level 0.05. By visual observation, the accumulative energy intake was slightly higher under S3 and S4 treatments than S1 and S2 treatments.

Table 4-7 Mean ± standard deviation (n=26) for energy values of lunch, preload (sample drinks) and accumulative intake (lunch + sample drink) under 4 preload drink treatments.

Treatments	Lunch (kcal)	Preload (kcal)	Accumulative energy (kcal)
S1 (water)	776 ± 96	0	776 ± 96
S2 (aroma)	781 ± 75	0	781 ± 75
S3 (taste)	759 ± 82	48	807 ± 82
S4 (aroma + taste)	757 ± 89	48	806 ± 89

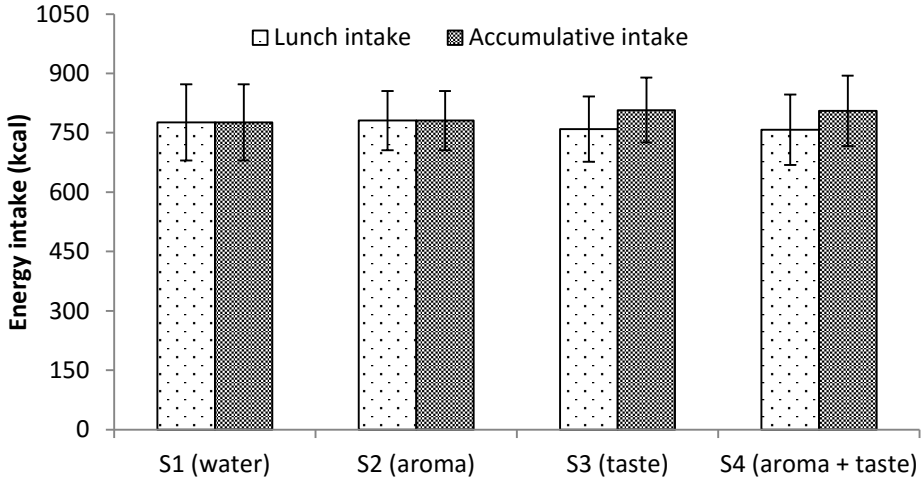

Figure 4-8 Mean (n=26 participants) pasta lunch energy intake and combined energy intake (sample + pasta) ± standard deviation following four sample drink treatments. Error bars represent standard deviation. No significant difference was observed (P>0.05) amongst four sample drink treatments.

4.4.2 The second phase results

4.5.3.1 Flavour and sweetness intensities

Overall flavour intensities of the four sample drinks were perceived to be different from each other ($p<0.05$), **Figure. 4-9A**. S4 containing both strawberry aroma and tastants (sucrose + citric acid) was perceived as the strongest in overall flavour intensity, followed by S3 (sucrose + citric acid), S2 (aroma), and S1 (water), $p<0.05$.

Strawberry aroma is actual tasteless without any sweetness stimuli. However, the fact participants perceived S2 (strawberry aroma) to be sweeter than S1 (water) (**Figure 4-9B**) may be that participants have learnt to associate strawberry aroma with a sweet taste. S4 and S3 containing sucrose were perceived sweeter than S1 and S2 ($p<0.05$). However, there was no significant difference between S4 and S3 ($p>0.05$), indicating that the addition of strawberry aroma did not interact with tastants (sucrose + citric acid) to affect the sweetness perception of drinks.

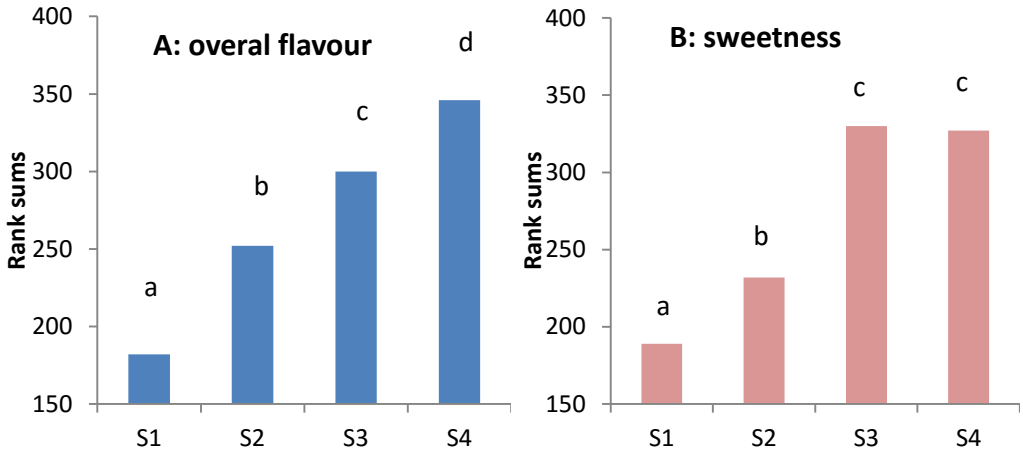

Figure 4-9 Rank sums of the 4 sample drinks in perceived flavour (7A), and sweetness (7B). Data were from the pairwise ranking tests, n=60. Higher rank sum indicated higher intensity. Samples without a same letter coding are different in the attribute ($p<0.05$).

4.5.3.2 Hedonic liking ratings

The percentage distribution of hedonic response for each sample drinks was demonstrated in **Figure 4-10**. Hedonic liking ratings for S4 (aroma + tastants) and S3 (tastants) were higher than S2 (aroma) and S1 (water), $p<0.05$ (**Table 4-8**). None of the sample drinks was disliked by participants ("1" to "5" = "dislike extremely"' to "neither dislike nor like").

Table 4-8 Median, Mode and Mean values for sample drinks from 9-point hedonic ratings (n=60).

Sample drinks	Median	Mode	Mean ± SD
Sample 1 (Water)	5	5	5.0±1.0
Sample 2 (aroma)	5	5	5.1±0.9
Sample 3 (sugar + citric acid)	6	6	6.4±1.4
Sample 4 (aroma + sugar + citric acid)	7	7	6.6±1.5

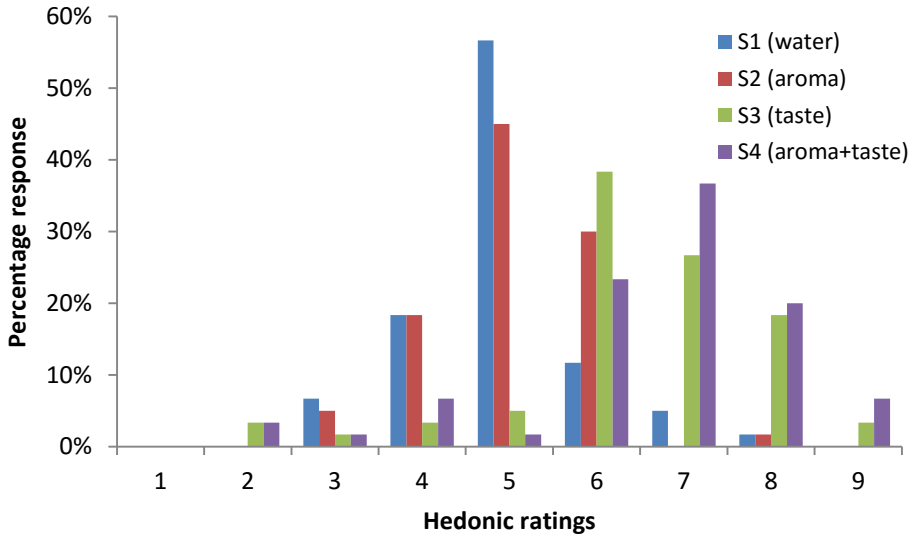

Figure 4-10: Histogram (with frequencies) of the overall liking scores for four sample drinks, n=60 participants.

4.5.3.3 Expected satiation

There were significant main effects of sample drink on the ratings of expected hunger and expected fullness immediately after consumption of 200 ml of the drinks($p<0.05$). Post hoc Bonferroni tests indicate that S1 (water) and S2 (aroma) were rated significantly lower in expected hunger and expected fullness, compared with S3 (taste) and S4 (taste + aroma), $p = 0.035 < 0.05$ (**Figure 4-11**). S3 and S4 were expected to give similar feelings of hunger and expected fullness. S1 and S2 were not different in ratings of expected hunger and expected fullness.

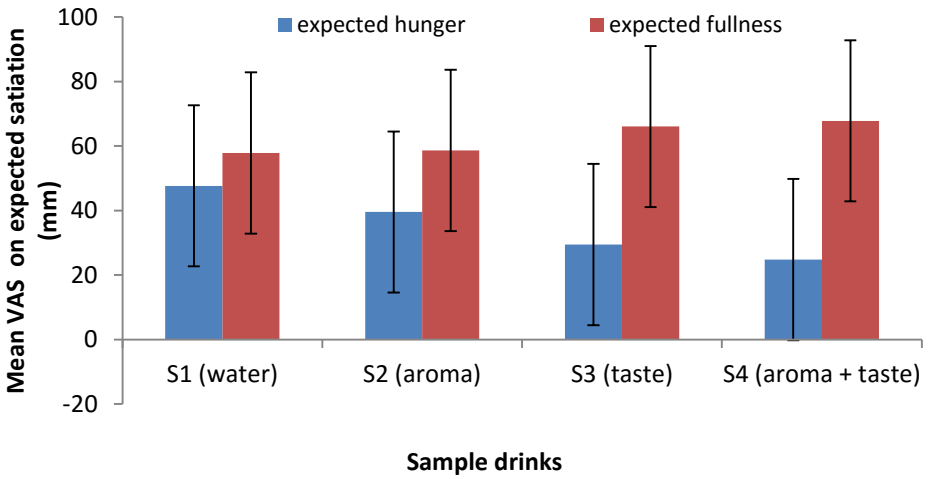

Figure 4-11 Mean expected hunger (blue bars) and expected fullness (red bars) of 60 participants. Error bar represents standard deviation.

4.5.3.4 In-vivo retronasal aroma release

There was no significant difference in the in-nose concentrations of ethyl butyrate (ion 117), ethyl 2-methyl butyrate (ion 131), and ethyl hexanoate (ion 145) between S2 and S4, $p>0.05$ (**Figure 4-12**). This indicates that the addition of sucrose and citric acid did not alter the retronasal delivery of key strawberry aroma compounds. An example chromatogram from one participant (**Figure 4-13**) illustrated that the APCI-MS analysis was reproducible (triplicate measurements) in the intensity and shape of strawberry aroma released retronasally from samples.

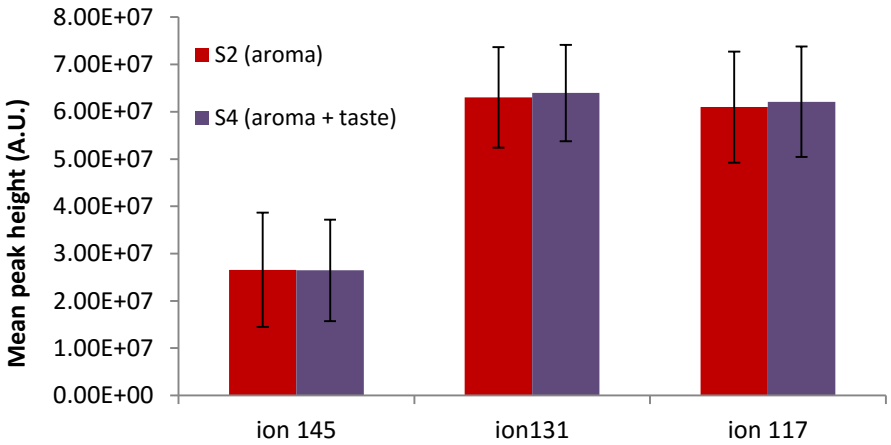

Figure 4-12 Mean peak height values (n= 5 participants × 3 replicates) of ion 145, 131 or 117 released retronasally from Sample 2 and Sample 4 as measured by APCI-MS. Error bars represents standard deviation.

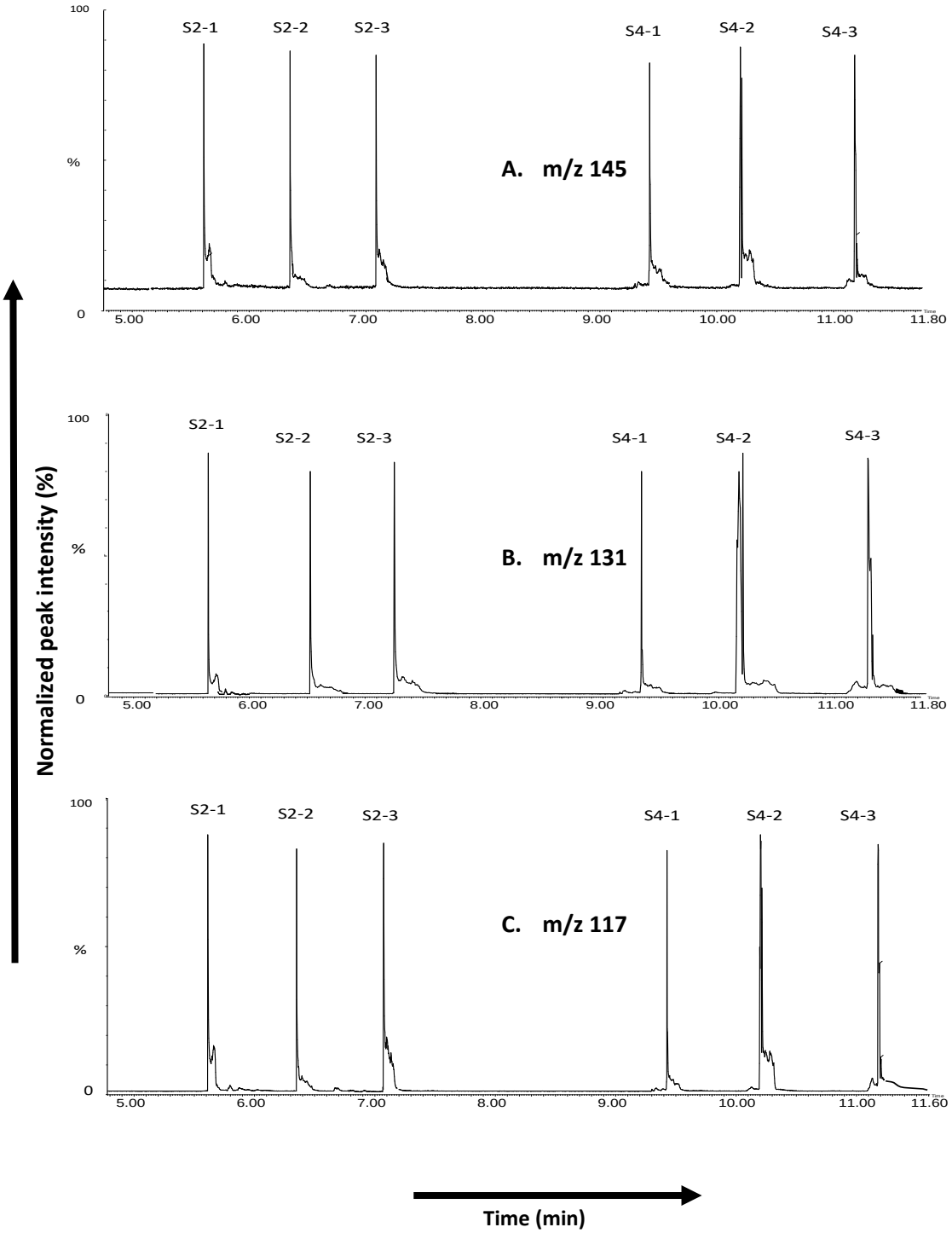

Figure 4-13 One participant's chromatogram of headspace aroma quantification by APCI-MS for **A**) ethyl hexanoate (m/z=145), **B**) ethyl 2-methyl butyrate (m/z=131), **C**) ethyl butyrate (m/z=117) in S2 and S4. Each sample was measured 3 times. S2-3 represents sample2- replicate 3.

4.5 Discussion

4.5.4 Summary of key findings

The objective of the study was to investigate the effects of taste, aroma and their cross-modal interaction on subjective appetite sensation and subsequent food intake. 26 healthy normal weight female participants visited the Sensory Science Centre on 4 occasions and consumed 4 sample drinks, in which aroma and taste stimuli were manipulated, after a light breakfast.

There was no significant difference in rated appetite sensation between taste drink preload (S3), aroma drink preload (S2) and the water control (S1). When taste and aroma stimuli were presented together in a drink (S4), rated hunger ratings were reduced significantly, compared with the water control and the drink with only aroma or taste. Meanwhile, taste and aroma presented together increased rated fullness significantly compared to the water control. Despite the small difference in the energy content of the drinks (0 kcal or 48 kcal) and the perceived flavour intensities of the drinks, the subsequent *ad libitum* intake of pasta lunch and the accumulative energy intake (pasta lunch + drink) were not significantly different under four sample drink treatments.

4.5.5 Evaluation of study design

Flavour-induced satiation or satiety is being likely developed and increased after a certain period, intensity and quality of oral sensory exposure. It has been proposed that a solid food is perceived to be more satiating than a liquid food because of the longer and stronger sensory exposure due to extensive chewing and swallowing (Bolhuis et al., 2011). This is supported by a study where a drink consumed with an aroma profile comparable to that of a solid food was perceived as more satiating than the same drink with a liquid-like aroma profile (Ruijschop et al., 2008a). In the current study, participants were requested to drink the

sample slowly over 15 minutes using a fine straw, allowing sufficient exposure to develop satiation from taste and aroma stimuli. Meanwhile, participants were instructed to focus their attention on the flavour of the drinks during consumption. Distraction, during an eating process, has been shown to increase *ad libitum* intake (Brunstrom and Mitchell, 2006). It is, therefore, likely that reducing distraction by asking participants to focus consciously, may maximise the differences in satiation generated from differently flavoured drinks.

4.5.6 The role of aroma

The strawberry aroma was diluted in water; participants perceived the aroma retronasally via the mouth to nasopharynx during drinking. This method of aroma delivery was closer to the reality, compared with Ruijschop's method where aroma was delivered using an invasive silicone tube to the nasal cavity (Ruijschop et al., 2008a). The concentration of strawberry aroma added in the drink (0.5% aroma in water) was slightly higher than a more common addition of 0.1% (claimed by the producer). This was to maximise the magnitude of the effect of aroma on inducing satiation or satiety. Despite that, participants still perceived all drinks as acceptable (average hedonic ratings ≥5 out of 9).

Aroma stimuli on its own in water (S2) did not affect the subjective appetite sensation and subsequent energy intake compared with the water control (S1). Retronasal strawberry aroma delivery has been shown to increase the subjective feeling of satisfaction, but this effect was observed with the concurrent delivery of sweet taste from a milk drink (Ruijschop et al., 2008a). The retronasal aroma in S2 was delivered in water without the presence of noticeable taste stimuli. Therefore, it appears that retronasal aroma might induce satiation only with the simultaneous presence of taste.

4.5.7 The role of taste

The taste preload (S3: sucrose + citric acid) did not differ from the water preload in rated appetite sensation and subsequent energy intake, indicating that the combination of sweet taste (sucrose) and sour taste (citric acid) have no noticeable effect on subjective appetite sensation and subsequent intake. It has been suggested that sweetness reduces hunger and increases fullness (Lavin and Read, 2000, Anderson and Woodend, 2003), but little is known about the effects of citric acid and its sourness on appetite sensations and intake. Potentially, sour taste might have an appetising effect, which could neutralise the effect of sweet taste on satiation. However, further investigations are needed to understand the individual effect of citric acid and the interaction of sucrose and citric acid on appetite sensations and food intake.

In addition, participants did not compensate for the small energy difference (12g, 48 kcal) between the drink containing sucrose from the water control at the subsequent lunch. This is also in line with previous literature showing that adult participants do not compensate for the energy in a sucrose preload by eating less at the subsequent meal, especially when the energy of the sucrose preload was small (Birch and Deysher, 1986, Anderson, 1995). Many studies show that a preload needs to have more than 50 g sucrose in order to have a significant suppression of the subsequent food intake (Anderson and Woodend, 2003). In comparison, 12 g sucrose in a preload, in the current study, was not large enough to have a significant effect on food intake.

4.5.8 Aroma-taste interaction

When aroma and taste presented together (S4), a significant reduction of rated hunger was observed compared to the water control (S1), and the drink with only aroma (S2) or only taste (S3). Since S4 and S3 shared the equal energy content (48 kcal), and were similarly liked, the fact that S4 reduced rated hunger more than S3 is potentially due to the significant

difference in their perception of flavour intensity. S4 containing both taste and aroma stimuli was perceived as the most intense in perceived flavour intensity, and it also demonstrated the greatest effect on suppressing hunger and increasing fullness amongst all drinks. Furthermore, the suppressing effect of S4 on subjective appetite ratings (reduced hunger and increased fullness) happened during the consumption of S4 drink and lasted continuously for up to 30 minutes after the consumption. This suggests that the enhanced flavour perception intensity, by aroma-taste interaction, can induce both satiation and short-term satiety. As far as the author is aware, this might be the first time that such results have been demonstrated.

In addition, APCI-MS analysis confirmed that there were no significant differences in the release of aroma volatiles to the nose regardless of the presence of tastants (sucrose + citric acid). Therefore, in this study, the interaction of taste and aroma on enhancing flavour perception was believed to be a result of cross-modal perceptual interaction rather than a physiochemical interaction. This would support the study hypothesis that a sample activating multiple sensory modalities (congruent taste and aroma) is more satiating than a sample activating a single sensory modality (taste or aroma).

Although S4 suppressed rated hunger, it did not affect the subsequent lunch energy intake. The difference in hunger ratings from different drink preloads disappeared just before the pasta lunch (t= 80 minutes). If the lunch was served earlier, when the difference in hunger ratings was still significantly among preloads, it might result in a reduction in the subsequent food intake. However, it may still be challenging to obtain an actual reduction in energy intake, as the effect of flavour on suppressing appetite is relatively small. Other studies did not obtain a reduction in food intake from low-energy flavour drinks, even when satiation was increased (Ruijschop et al., 2009b, Bellisle et al., 2012).

The mechanisms behind the effect of aroma-taste cross-modal interaction on suppressing hunger and increasing fullness are complex, which are potentially due to the interactive effects of physiological and psychological factors. Considering the noticeable differences in the flavour characteristics between sample drinks, participants might have been consciously aware of this difference. Therefore, it is potentially that participants might have some cognitive belief or expectation about the sample drinks. The cognitive expectation of the satiating capacities of foods can affect the appetite sensations of hunger and fullness (Brunstrom et al., 2011). However, such cognitive expectation does not seem to explain why S4 suppressed hunger more than S3. S3 (taste) and S4 (taste + aroma) shared similar cognitive expectation on satiation (expected immediately fullness and hunger), while both were expected to be more satiating than S1 (water) and S2 (aroma). It appears that participants did not make their expectation on the satiation of the drink based on the flavour intensity, but rather according to its sweetness intensity. S3 and S4 contained energetic sugars, which tasted sweeter than S1 and S2. Participants may have gradually learnt to associate sweetness with intake of sugars; thus, a sweeter food may be richer in nutritional and energy values and therefore, more satiating (Gibson and Brunstrom, 2007, Chandrashekar et al., 2006).

Currently identified brain areas that integrate taste and aroma include insula (flavour recognition and intensity), OFC (pleasure, reward and affection), amygdala (pleasure and reward) and ACC (cognitive control, e.g. reward and anticipation) (Marciani et al., 2010). Although not identified, taste and aroma may potentially integrate at the lateral hypothalamic area (LPA: the satiation and satiety centre), based on their neural pathways; thus, both projects into the hypothalamus (Chapter 1, Figure 1-4 &1-7) (Marciani et al., 2010). It has been found that the activation of LHA suppressed appetite and decrease intake (Anand and Brobeck, 1951). S4 (Taste + aroma) was equally liked to S3 (taste), and both shared similar cognitive expected satiation. Therefore, the observed suppression of the

hunger sensation from S4 is believed to be the possible results of the aroma-taste integration at LHA or other cortex, in addition to the integration in the pleasure and reward centre. Thus, when aroma and taste were presented together, the signals in the activation of LHA may be stronger than the sum effect of aroma and taste, resulting in significant suppression on the hunger feeling, compared with the presence of a single aroma or taste stimuli, and the water control.

Hypothalamus is believed to be the energy homeostatic centre which mediates hormone signals and gastrointestinal signals (Harrold et al., 2012). Further investigation is required to explain the complex multi-dimensional factors influencing flavour-regulated satiation and satiety, and to investigate whether the effects that have been noted are sustained at a similar level over repeated exposure.

4.6 Conclusion

To conclude, taste or aroma stimuli presented alone in a drink preload neither suppressed appetite nor reduced subsequent energy intake, compared to the water control. However, a beverage activating multiple flavour modalities (congruent taste and aroma) is more satiating than a beverage activating a single flavour modality (taste or aroma) and the water control. It appears to be a result of enhanced flavour perception caused by aroma-taste cross-modal interaction at central brain area, which may include the satiation centre of LHA. It is believed that these findings could facilitate the development of food products that could help suppress hunger and enhancing fullness sensations by solely manipulating the complexity and intensity of food flavour.

Chapter 5. Expected satiation and expected satiety

5.1 Introduction

Aroma, taste and texture properties of foods can enhance satiation and satiety signals from foods, and may affect *ad libitum* energy intake (Ruijschop et al., 2008a, Zijlstra et al., 2008). However, real-world energy intake is not solely affected by feeling of satiation and satiety induced after food intake, but it is also determined largely by decisions on portion size (Brunstrom, 2011, Fay et al., 2011). Such decision can be made even before a meal. Plate cleaning happens remarkably at 91% of meals, and it is highly correlated with pre-meal decisions on portion size (Fay et al., 2011). Humans have a strong cognitive ability to evaluate their expectation of the satiating capacities of foods, and such expectation rather as well as palatability of a food is known to determine our meal-size election (Brunstrom and Rogers, 2009, Brunstrom et al., 2008a). Selected portion size, hence energy consumption, was reduced when a food was expected to be more satiating (Brunstrom, 2011, McCrickerd et al., 2014). A detailed introduction of expected satiation and expected satiety has been described in Chapter 1, section 1.6.

Expected satiation of a food is defined as the relative satiation (feeling of fullness immediately after consumption) that a person expects from consumption of the food; expected satiety is the extent to which a food is expected by a person to stave off hunger before the next meal (Brunstrom and Rogers, 2009). Both the expected satiation and expected satiety of a food can be made by humans prior to the food consumption.

Expected satiation and expected satiety of foods correlate with apparent energy density, perceived volume and the familiarity of the food (Brunstrom and Rogers, 2009, Brunstrom et al., 2010b, Piqueras-Fiszman and Spence, 2012). Expected satiation or expected satiety is proposed to be a result of learned association between sensory properties of a food with its post-ingestion consequences (Brunstrom, 2011, Yeomans, 2012).

The role of flavour and texture on expected satiation and satiety has been explored in few studies (section 1.9.6). Those studies consistently reported that higher thickness (or viscosity) of a food was positively correlated with higher expected satiation or expected satiety of the food (Hogenkamp et al., 2011, Hogenkamp et al., 2012, McCrickerd et al., 2012b, Tarrega et al., 2014).

In comparison to the effect of texture, flavour properties of foods seem to have little or no impact on expected satiation or expected satiety. Changing the type of the aroma from lemon to meringue in a food did not change the expected satiation of the food (Hogenkamp et al., 2011). A subtle increase in perceived creamy flavour achieved by increasing the addition of vanilla extract showed no impact on expected satiety of the drink, but it increased its expected satiation (McCrickerd et al., 2012a). The quality of an aroma or flavour may play an important role in determining whether such aroma or flavour may have a potential influence on the expected satiation and expected satiety. Since only a couple of aroma (flavour) has been studies, it cannot exclude the possible influence of other aroma or flavour on expected satiation or expected satiety.

It appeared that the intensity of overall taste perception of a soup was positively correlated with the expected satiation of the soup (Hogenkamp et al., 2012). However, the role of a specific taste, such as sweetness, in expected satiation or satiety is still under-investigated. Conflicting results in the role of sweetness on expected satiation were reported; thus, some reported the sweetness intensity was positively correlated with expected satiation, while others reported that sweetness intensity was negatively correlated with expected satiation (Hogenkamp et al., 2011, McCrickerd et al., 2015). This may be due to a lack of control of other irrelevant factors in the food studies, i.e. using uncontrolled commercial food products rather than a controlled model food system. In these studies, the effect of sweetness intensity on expected satiation may be confounded by the

effects of other factors, such as energy density, micronutrients, the familiarity of the products, or other sensory attributes.

In this chapter, it is hypothesised that an intense caramel flavour (caramel aroma is congruent to sweet taste) may signal a high intake of sugars or carbohydrates, and therefore may result in high expected satiation or expected satiety. The sweet taste of a non-caloric sweetener may also signal an intake of sugars and carbohydrates. However, the role of non-caloric sweetener on expected satiation or satiety has not been previously studied. It is hypothesised that increasing sweetness intensity by increasing the concentration of a non-caloric sweetener may increase expected satiation or expected satiety. In addition, the potential interaction effect of between thickness, sweetness and caramel flavour on expected satiation and expected satiety will also be investigated. A controlled model food (custard) was therefore designed especially for this study.

5.1.1 Study objective

The main objective of this study was to investigate the effects of the perception of sweetness, caramel flavour, thickness and their interaction effect on human participants' expected satiation and expected satiety of custards. A set of 17 different custards were prepared with the same energy density, macronutrient composition and familiarity to participants. However, custards were designed to vary in their sensory attributes of sweetness, caramel flavour and thickness, according to an experimental design. The distinct sensory characteristics of custards were achieved by manipulating the concentrations of Truvia® sweetener, caramel aroma and carboxymethyl cellulose (CMC).

The main objective was achieved via two phases. In Phase 1, a precise quantification of the perceived intensities of sweetness, caramel flavour and thickness of custards was conducted by a trained panel. Statistical models were built to determine the impact of varying concentration of Truvia® sweetener, caramel aroma and CMC on the perception of

sweetness (Model 1), caramel flavour (Model 2) and thickness (Model 3). Ultimately, Models 1, 2 and 3 were to generate predictive equations to predict the perception of sweetness, caramel flavour and thickness for any concentrations of Truvia® sweetener, caramel aroma and CMC within the custard model system.

In Phase 2, the expected satiation and expected satiety of custards were assessed by consumer participants using the modified 'method of adjustment' (Brunstrom and Rogers, 2009). Statistical models were built to evaluate the impact of varying concentrations of Truvia sweetener, caramel aroma and CMC on expected satiation (Model-4) and expected satiety (Model-6). Additional statistical models were built to assess the impact of sweetness, caramel flavour and thickness intensities (predictive values from Phase 1: Models 1, 2 and 3) on expected satiation (Model-5) and expected satiety (Model-7), which was the main objective of the study.

Previous studies used the 'method of adjustment' (Brunstrom and Rogers, 2009) to measure the expected satiation or expected satiety of foods. Subjects were asked to match the expected satiation or expected satiety of foods (in a standard serving size) with a comparison food, on a calorie-to-calorie basis. In those studies, a comparison food was presented in a number of pictures (on computer screen), and each picture represents a different energy content; one picture was shown on one screen at a time. The procedure of the current study was similar to previous studies, but using paper-based pictures instead of computer-based pictures. Thus, comparison foods were presented on an A3 paper where 20 pictures (20 energy contents) of the comparison food were presented together. The reproducibility of this paper scale will be assessed. This study was approved by the School of Biosciences Research Ethics Committee of the University of Nottingham (approval code: SBREC140127A).

5.2 Materials and Methods

5.2.1 Custard sample design and preparation

A modified response surface model was used to generate 17 different custards with varying Truvia® sweetener, caramel aroma and CMC, in the Design Expert 7.0 software (Stat-ease, Minneapolis, MN). The 17 custards were chosen to generate a balanced design space (**Figure 5-1, Table 5-1**). Each composition factor (i.e. sweetener, caramel aroma or CMC) had five levels (**Table 5-2)**. The amounts of the remaining ingredients used in preparing custards were listed in **Table 5-3**. All custard samples had the same energy density of 71.4 kcal/100g and similar macronutrient composition. Every 100 g custard contained 2.8% fat, 3.2% protein, 8.4% sugars **Table 5-4**. Custards were prepared using a Thermo Mix-35 (Thermomix® Vorwerk, UK), in the Food Hall of Sutton Bonington Campus, University of Nottingham. Ingredients of custards were added slowly while being stirred in the Thermomix, and then were heated at 80°C while being stirred at speed 2 in the Thermo Mix-35 for 13min. The preparation of custards was conducted according to a standard procedure (Appendix VI). Cooked custards were stored in sealed plastic containers (30ml) in a 5°C fridge 24 hours prior to sensory tasting. Each plastic container contained 20ml custard when presenting to participants.

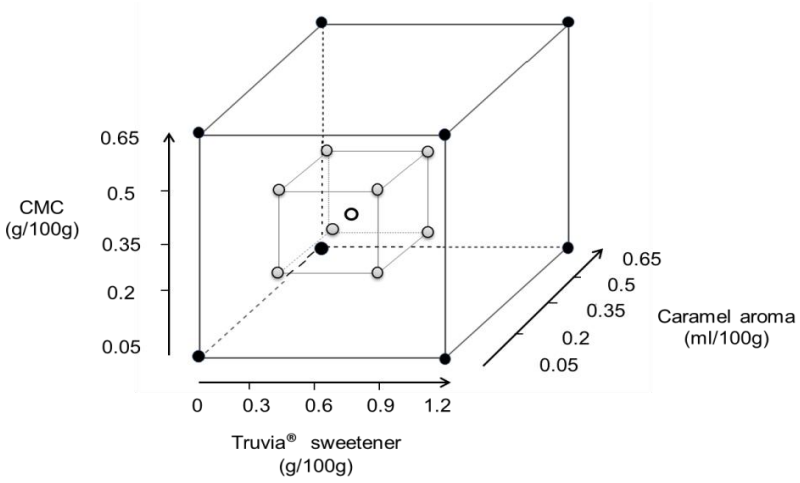

Figure 5-1 Diagrammatic representation of the experimental design space

Table 5-1 Ingredient factor levels in 17 custard samples

Custard Sample	Composition as factors %		
	Truvia®	**CMC**	**Aroma**
1	0.9	0.2	0.5
2	0.9	0.5	0.2
3	0.3	0.5	0.2
4	0.9	0.5	0.5
5	0	0.65	0.65
6	0.3	0.2	0.5
7	0.9	0.2	0.2
8	1.2	0.65	0.65
9	0	0.05	0.05
10	1.2	0.65	0.05
11	0	0.65	0.05
12	0	0.05	0.65
13	0.3	0.2	0.2
14	0.6	0.35	0.35
15	1.2	0.05	0.05
16	0.3	0.5	0.5
17	1.2	0.05	0.65

Table 5-2 Composition factors and the five levels present in 100g custard.

Factor	Additional level				
	1	**2**	**3**	**4**	**5**
Truvia® sweetener(g)	0.00	0.3	0.6	0.9	1.2
CMC(g)	0.05	0.20	0.35	0.50	0.65
Caramel aroma(ml)	0.05	0.20	0.35	0.50	0.65

Remaining ingredients expect water were kept constant across custard samples.

Table 5-3 Non-factor ingredients used in 100g custard samples

Ingredient	Addition amount
Semi-skimmed milk (Sainsbury's, UK)	90g
mineral water (Evian, Danone Group, France)	0.13-2.33g
double cream (Sainsbury's, UK)	2.5g
corn flour (Sainsbury's, UK)	2g
caster sugar (Sainsbury's, UK)	2.5g
carrageenan (Sigma-Aldrich, UK)	40mg
Yellow colouring (Sainsbury's, UK)	0.1ml

Table 5-4 Key macronutrients composition of custards

Key composition of custards	Weight (g) per 100 g
Fat	2.8
Protein	3.2
Carbohydrate	8.4

5.2.2 Phase 1 experiment: Measuring Sensory Perception

5.2.2.1 Sensory panel

A trained panel of 12 (11 females and 1 male) from the external sensory panel of the Sensory Science Centre (SSC) of University of Nottingham (UoN) completed the Phase 1 experiment. They performed a modified quantitative descriptive analysis (QDA) to assess the 17 custards based on a standard method (Stone et al., 1980). All panellists had 10-15 years' experiences as sensory panellists in the SSC. All had participated previously in evaluating the flavour and textural attributes of a broad range of food systems, from simple solutions to complex matrices.

5.2.2.2 Panel training

The panel completed 4 training sessions. They were trained in using VASs scale to rate the intensities of sweetness, caramel flavour and thickness of custards. The training aims were to improve group consistency and reproducibility and to promote the use of the ends of scales.

5.2.2.2.1 Define attributes and protocol

Each panellist firstly tasted a set of 4 distinct custards chosen from the design space (S11, S17, S15, and S8). They identified and agreed on the attributes perceived to be different between the 4 custards, including sweetness, caramel flavour and thickness. After a group discussion, they agreed on a standard assessment protocol (**Table 5-5**). Panellists were asked to taste a single spoonful of the custard (5g) and rate the intensities of the three attributes according to the assessment protocol. Custards

were to be swallowed within 5 seconds to minimise the dilution of custards by saliva.

Table 5-5 Sensory lexicon for custard samples

Attributes	Definition	Assessment protocol
Sweetness	the sweet taste from sugar	Taste the sample on tongue
Thickness	the resistance to flow in the mouth	Compress the sample 3 times using tongue
Caramel flavour	the flavour of toffee with subtle vanilla notes	Taste the sample in the mouth

5.2.2.2.2 Selection of reference samples

Before training on intensities, reference samples were chosen to represent the extreme low (0 mm) or high intensities (100 mm) on the scales of each attribute (sweetness, caramel flavour or thickness). For example, to select the low sweetness custard sample, the panel ranked S5, S9, S11 and S12 (Truvia® sweetener = 0%) in sweetness intensity according to a standard ranking test protocol (ISO, 2010). S11 was ranked the least sweet among the 4 samples and it was selected as the reference to illustrate the lowest sweetness intensity. Reference samples of the highest or the lowest intensity for each attribute were selected using ranking tests (**Table 5-6**).

Table 5-6 Reference samples for each attribute

Attribute	Lowest intensity (0 mm)	Highest intensity (100 mm)
Sweetness	S11	S17
Caramel flavour	S11	S17
Thickness	S15	S8

5.2.2.2.3 Training on rating perception intensities

The panel was trained on rating the intensity of reference samples (0 mm or 100 mm) using a 100 mm long visual analogue scale (VAS). On the sweetness and caramel flavour scales, the intensity of reference S11 was

illustrated to panellists as the lowest intensity point (0 mm), while the intensity of reference S17 was illustrated to panellists as the highest intensity point (100 mm). On the thickness scale, the intensity of reference S15 was illustrated to panellists as the lowest intensity point (0 mm), while the intensity of S8 represents the highest intensity point (100 mm). Panellists were asked to taste and memorise repeatedly the intensity of each reference sample and to rate such intensity at the end of a scale.

5.2.2.3 Sensory attributes evaluation (Final assessment)

Each panellist completed 3 assessment sessions on 3 separate days. At each session, they assessed 18 samples in total, including the 17 different custards plus a replicate of the sample at the centre of the design (S14). The sample presentation order was randomised and balanced. Custard samples were presented at 5 °C. Panellists were asked to rate each the sweetness, caramel flavour and thickness intensities of custards, on 100 mm long VAS scales, using the standard assessment protocol agreed on training sessions (Table 5-5). Panellists used water and crackers to cleanse their palate between samples. They were required to take a 10 minutes break after every 6 samples. Data were collected using Fizz (Biosystems, France).

5.2.2.4 Statistical data analysis

5.2.2.4.1 Panel performance monitoring

For each panellist, the coefficient of variance (CV) and probability FPROD value, obtained from Fizz (one-way ANOVA analysis), were used to evaluate the panellist's repeatability and discriminative ability, respectively. Data from two panellists were eliminated from further analysis due to poor performance (section 5.3.1.1). The means of the remaining 10 panellists were analysed using two-way ANOVA with interactions (sample × panellist) in FIZZ, where a significant main effect of the custards identified, Tukey's HSD multiple comparison tests were applied to determine which samples were significantly different.

5.2.2.4.2 Predictive model generation

The data for the remaining 10 panellists were analysed using ANOVA (with blocking) for a modified response surface methodology (RSM) in Design Expert 7.0 software (Stat-Ease Inc., Minneapolis). Each data input in Design Expert was the mean for the triplicate assessments from each panellist. A total of 540 experimental runs was split into 10 blocks for the 10 panellists. Each block contains all custard samples (17+1) rated by one panellist. The blocking design was chosen to minimise the variation due to individual differences. Then, each independent response (i.e. sweetness, caramel flavour or thickness) was submitted to multivariate regression analysis. The models to evaluate the effect of composition factors on the perception of sweetness, thickness and caramel aroma of custards were listed in **Table 5-7**.

Table 5-7 Composition factors and perception responses in models 1, 2 and 3

Response	Factor	Model
Perceived sweetness	A: Truvia® concentration	**Model-1**
Perceived caramel flavour	B: CMC concentration	**Model-2**
Perceived thickness	C: Caramel aroma concentration	**Model-3**

Predictive models were generated to illustrate the variations of perceived sweetness, caramel flavour and thickness as a function of Truvia® sweetener, caramel aroma and CMC concentrations. The initial models contained linear, quadratic, cubic or interaction terms. Terms with a p-value ≤ 0.05 were considered statistically significant to the models, and they were left in the final predictive equations.

Replicate assessments of S14, the centre point in the design space, were included in the models to provide the evaluation of Lack of Fit. The 'Lack of Fit' test compares the mean squares of the residual (actual value minus predicted value) to the pure error from replicate data. A model shows a lack of fit ($p<0.05$) would indicate that the predictive model does not fit the data well and more terms/variables may be needed.

Original R^2 values (coefficient of determination) (**Equation 5-1**) increase with the increase in non-significant terms from the model. An adjusted R^2 was calculated by the **Equation 5-2**, which stayed unchanged with the addition of non-significant terms. Therefore, the adjusted R^2 was used to evaluate the ability of a statistical model in explaining the observed variation in the data. An adjusted R^2 value of 1.0 indicates the ideal situation when 100 % of the variation in the data can be explained by the generated statistical model. The predicted R^2 (**Equation 5-3**) evaluated the amount of variation in predicted data by the model, and it indicated how well the final model predicted a response. The closer to 1.0 a predicted R^2, the better a model predicts a response. (Design Expert 7.0 software, DX help)

Adequate precision calculated by the Design Expert software describes the signal to noise ratio. An 'adequate precision ratio' greater than 4 suggests adequate model discrimination.

Equation 5-1: $R^2 = 1 - SS_{RESIDUAL} / (SS_{MODEL} + SS_{RESIDUAL})$

Equation 5-2: Adjusted $R^2 = 1 - \dfrac{(SS_{RESIDUAL}/DF_{RESIDUAL})}{(SS_{MODEL} + SS_{RESIDUAL}) / (DF_{MODEL} + DF_{RESIDUAL})}$

Equation 5-3: Predicted $R^2 = 1 - (PRESS/SS_{TOTAL})$

Where: $SS_{RESIDUAL}$ is the sum of squares of residual variation
SS_{MODEL} is the sum of squares of model variation
$DF_{RESIDUAL}$ is the degrees of freedom for the residual variation
DF_{MODEL} is the degrees of freedom for the model variation
PRESS is the predicted residual error sum of squares

5.2.3 Phase 2 experiment: Measuring Expectation

5.2.3.1 Consumer participants

Ninety healthy participants (65 females and 25 males), aged 18-45 years, completed the Phase 2 experiment. Participants were selected according to the general participant criteria (Chapter 2, section 2.3.3.2).

5.2.3.2 Sample size

Using 29 participants, Hogenkamp (2011) detected a significant (α=0.05) mean difference of 87 kcal (SD=120 kcal) in expected satiation of dairy products, providing a statistical power of 0.8. Therefore, 90 participants, in the current study, was expected to be sufficient for detecting a difference of 50 kcal (if SD=120 Kcal) in expected satiation (or satiety), at the 0.05 significant level, with a statistical power of 0.8 (Statistical Solutions, 2016).

5.2.3.3 Experimental design and protocol

The 17 different custard samples from the Phase 1 experiment were evaluated for their expected satiation and expected satiety by the 90 consumer participants, resulting in 90 experimental blocks (one participant = one block). Each block contained 17 different custards and a replicate of S14. Each consumer assessed the samples in two separate sessions (9 samples per session). Each session was scheduled between 10.00– 10.40 am, 11.00 -11.40 am, or 3.00 - 3.40 pm. Each participant completed his or her two sessions at the same time of day.

Participants were asked to avoid eating or drinking anything except water 2 hours prior to a session. At each session, subjects tasted 9 custard samples, one at a time, and rated its expected satiation and expected satiety. The custard samples were presented in a randomised and balanced order to each participant. Participants were requested to take a 5-minutes break after assessing every three samples. A limited amount of

water (≤ 100ml in a session) and plain cracker were given for plate cleansing samples.

5.2.3.4 Measuring expected satiation and expected satiety

The method of measuring expected satiation and expected satiety was modified from the methodology developed by Brunstrom and Roger (2009). Participants were presented with the custard sample in a 30 ml plastic cup and asked to taste a spoonful (5 g) of the sample and then to rate the expected satiation and expected satiety of a custard in comparison to three 'comparison foods' on three picture scales.

5.2.3.4.1 Picture Scales

The comparison foods were ripened banana (Sainsbury's Loose Fairtrade Bananas; 105kcal : 102g per unit), KitKat (milk chocolate wafer bar, Nestlé; one finger per unit), and cooked pork sausage (Frankfurters 4'S Hot Dogs, Herta; one sausage per unit (**Table 5-8**). The scales of each comparison food consisted of 20 pictures, numbered from 1 to 20, with increasing energy, **Figure 5-2, 5-3, and 5-4**. The energy contents of the foods on pictures varied from 26.3 kcal to 525.3 kcal for banana, 29.1 kcal to 582.5 kcal for KitKat, and 33.3 kcal to 665.6 kcal for sausage (**Table 5-9**). The difference in energy content between two continuous pictures was 26.3 kcal for banana, 29.1 kcal for KitKat and 33.3 kcal for sausage. An actual banana, a finger KitKat and a sausage were presented to with the picture scales to minimise individual variation in how they perceived the size of the comparison foods.

Table 5-8 Key nutritional compositions in each comparison food.

comparison food	Energy content, kcal	Fat, g	Carbohydrate, g	Protein, g
Banana	103	0.5	23	1.2
Sausage	285	25	2	12.5
KitKat	518	26	65	7

Values given per 100 g

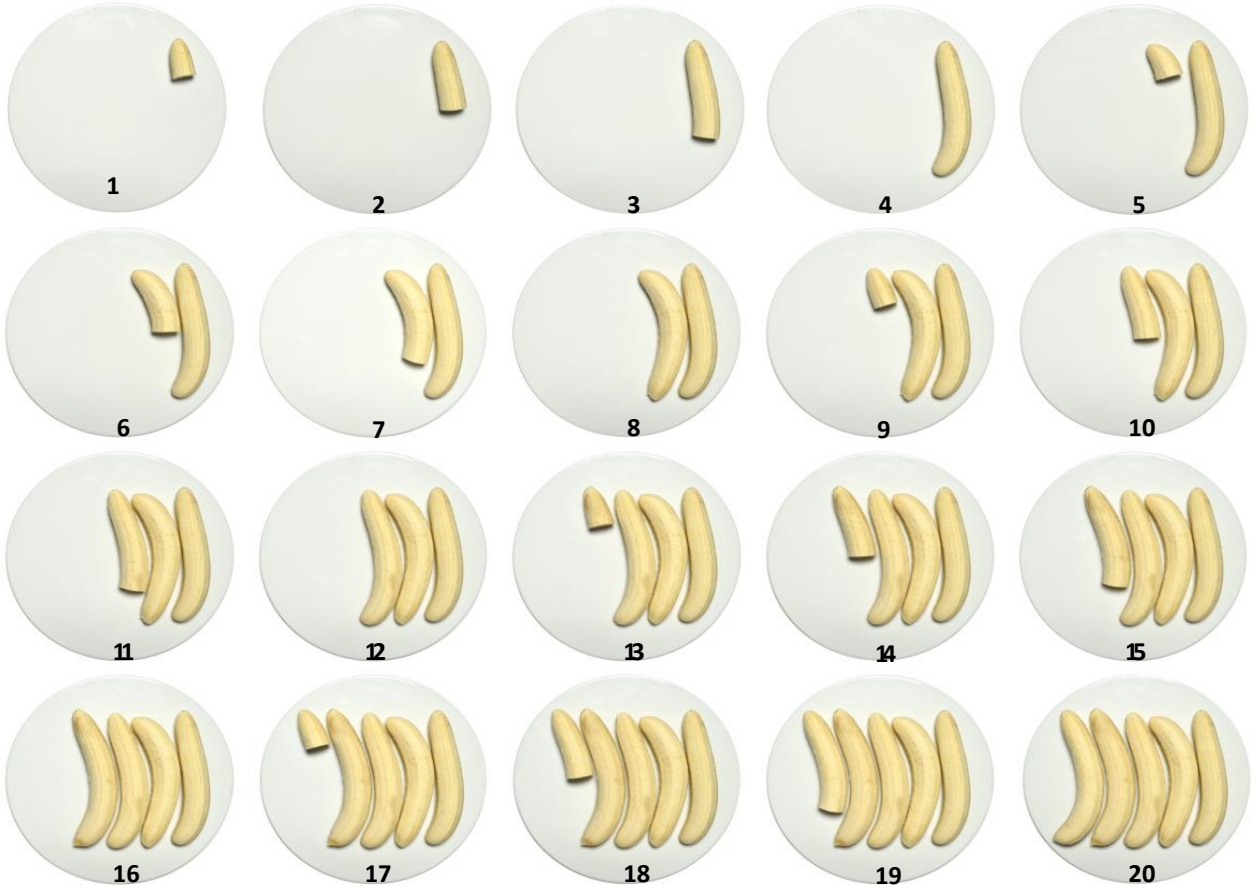

Figure 5-2 Picture scale of banana as a comparison food used in measuring satiety/satiation expectation

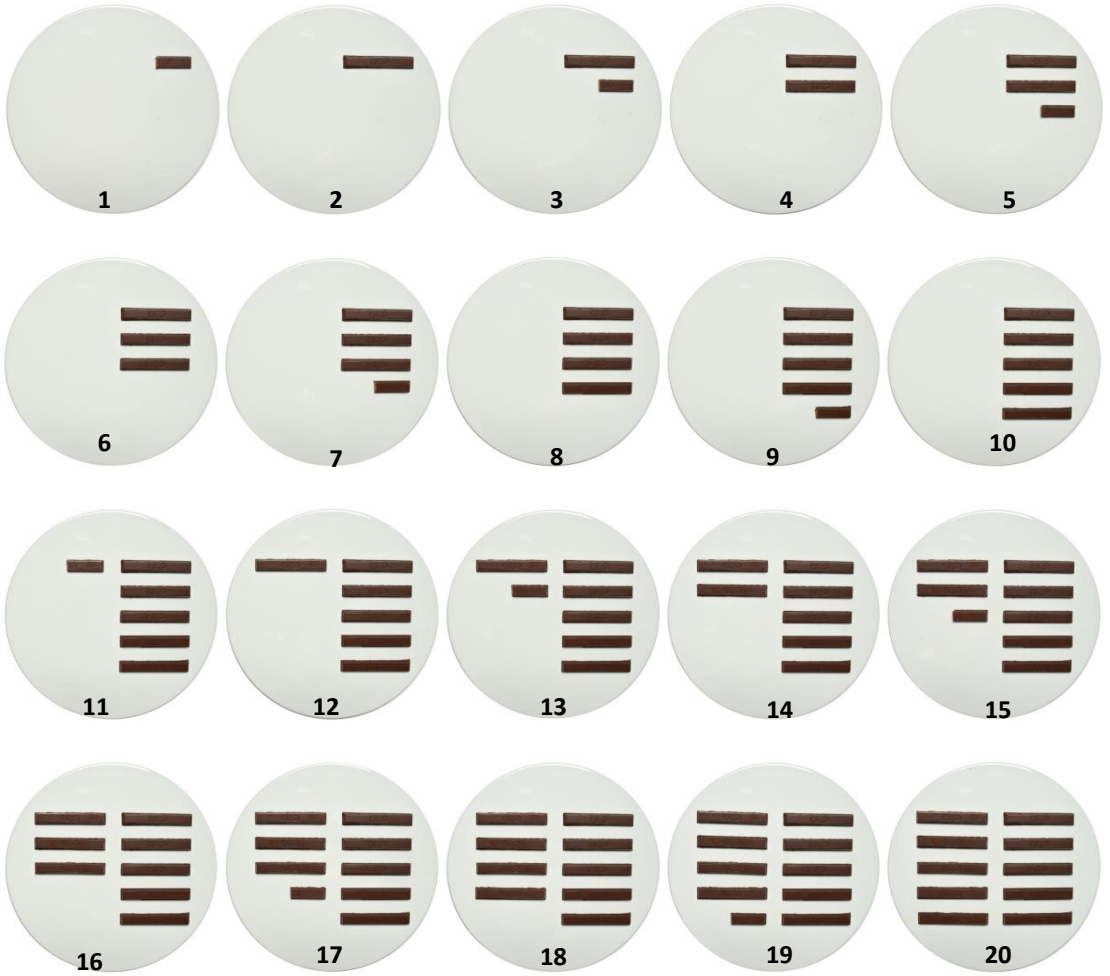

Figure 5-3 Picture scale of KitKat as a comparison food used in measuring satiety/satiation expectation

Figure 5-4 Picture scale of pork sausage as a comparison food used in measuring satiety/satiation expectation

Table 5-9 Energy contents of each picture on three paper scales

	Energy (kcal)		
Picture	Banana	KitKat	Sausage
1	26.3	29.1	33.3
2	52.5	58.3	66.5
3	78.8	87.4	99.8
4	105.1	116.5	132.7
5	131.3	145.6	166.6
6	157.6	174.8	199.6
7	183.9	203.9	232.5
8	210.1	233.0	266.4
9	236.4	262.1	299.4
10	262.7	291.3	332.3
11	288.9	320.4	366.2
12	315.2	349.5	399.2
13	341.4	378.6	432.1
14	367.7	407.8	466.0
15	394.0	436.9	499.0
16	420.2	466.0	531.9
17	446.5	495.1	565.8
18	472.8	524.3	598.7
19	499.0	553.4	631.7
20	525.3	582.5	665.6

5.2.3.4.2 Procedure

A standard serving portion of 350g (250kcal) custard was presented in a 455 ml transparent plastic bottle. Participants did not eat directly from this standard portion, since this portion was just to indicate the consumption amount for them to imagine. Instead, participants taste each sample from a 30 ml plastic sample cup.

To rate the expected satiation of a custard, participants tasted the 1st spoonful of the custard and answered the Question 1. Question 1 was ‘Imagine you would consume this custard at 13.00 pm before lunch, assuming you had your breakfast at 8:30 am, please select the amount of a food (banana, KitKat or sausage) on each picture scale that would give

you a similar feeling of fullness as the custard (in a standard serving portion) immediately after consumption.'

To rate the expected satiety of a custard, participants tasted the 2nd spoonful of the custard sample and answered the Question 2. Question 2 was 'Imagine you will consume this custard at 13.00pm before lunch, assuming you had your breakfast at 8:30 am, please select the amount of a food (banana, KitKat or sausage) on each picture scale that would give you a similar feeling of hunger as the custard (in a standard serving portion), 3 hours after consumption.'

The statement questions for expected satiation and expected satiety were decided based on previous studies (Hogenkamp et al., 2011, McCrickerd et al., 2012b, Tarrega et al., 2014).

5.2.3.5 Statistical data analysis

The expected satiation or expected satiety of custards was calculated as the arithmetic mean of the selected energy content (kcal) of three comparison foods from picture scales. Data were entered in the Design Export 7.0 for statistical analysis. Polynomial models were generated in Design Export 7.0 to explain variations in expected satiation or expected satiety as a function of composition factors, or as a function of perception factors (**Table 5-10**). Predictive polynomial models were generated using a similar approach as described in phase 1, section 5.2.2.4.2.

In addition, the mean expected satiation values (or mean expected satiety values) of 17 different custards were compared using one-way ANOVA (factor: sample custard) with Tukey's HSD *post hoc* test in SPSS (version 21.0; IBM Corporation, USA). A Pearson's correlation test was performed in IBM SPSS Statistics to see if there is any correlation between expected satiation and expected satiety.

Table 5-10 Factors and responses used in Model 4 to Model 9

Response	Factor		Model
Expected satiation	Composition	Truvia®	**Model-4**
		CMC	
		caramel aroma	
	Perception	sweetness	**Model-5**
		thickness	
		caramel flavour	
Expected satiety	Composition	Truvia®	**Model-6**
		CMC	
		caramel aroma	
	Perception	sweetness	**Model-7**
		thickness	
		caramel flavour	

5.3 Results

5.3.1 Phase 1 experiment results

5.3.1.1 Assessment of panel performance

5.3.1.1.1 Repeatability and discriminative ability

Figure 5-5, generated by Fizz Calculation software, demonstrated the discriminative ability and repeatability of each panellist in assessing sweetness, thickness and caramel flavour intensities of custards. In Figure 5-5, different colour circles represent data points for different panellists. Panellists appearing in the bottom right-hand relating to high FPROD (good discrimination) and low coefficient of variation (CV) (good repeatability) are the best performers. In contrast, panellists in the top left-hand relating to low FPROD value (poor discrimination) and high CV value (poor repeatability) performed the worst. In this study, a CV below 0.6 was a cut-off for acceptable repeatability; a FPROD p-value below 0.05 suggested the panellist could discriminate between custards.

Visual inspection of **Figure 5-5a** suggests that the majority of panellists could discriminate between the custard samples in sweetness intensity, except Panellist-1 whose probability FPROD p value (x-axis) was higher

than 0.5. Panellist-1 also showed poor repeatability (CV > 0.6) in rating sweetness compared with the rest of the panel (CV between 0.2-0.6).

The CV values of the majority of panellists in assessing caramel flavour were between 0.3 and 0.6 except Panellist-1 (CV: 1.2) and Panelist-12 (CV: 1.6) (**Figure 5-5b**). Panellist-1 and Panelist-12 showed very poor repeatability in assessing caramel flavour intensity compared with the remaining panellists. Panellist-12 also could not discriminate between the samples in caramel flavour (probability FPROD value > 0.2), while the remaining panellists showed good discriminative ability in rating the caramel flavour (probability FPROD value < 0.05).

All panellists could identify the difference in perceived thickness of the custards (probability FPROD value <0.05) (**Figure 5-5c**). The CV values of panellists for assessing thickness intensity were within 0.2 to 0.6, indicating acceptable group repeatability.

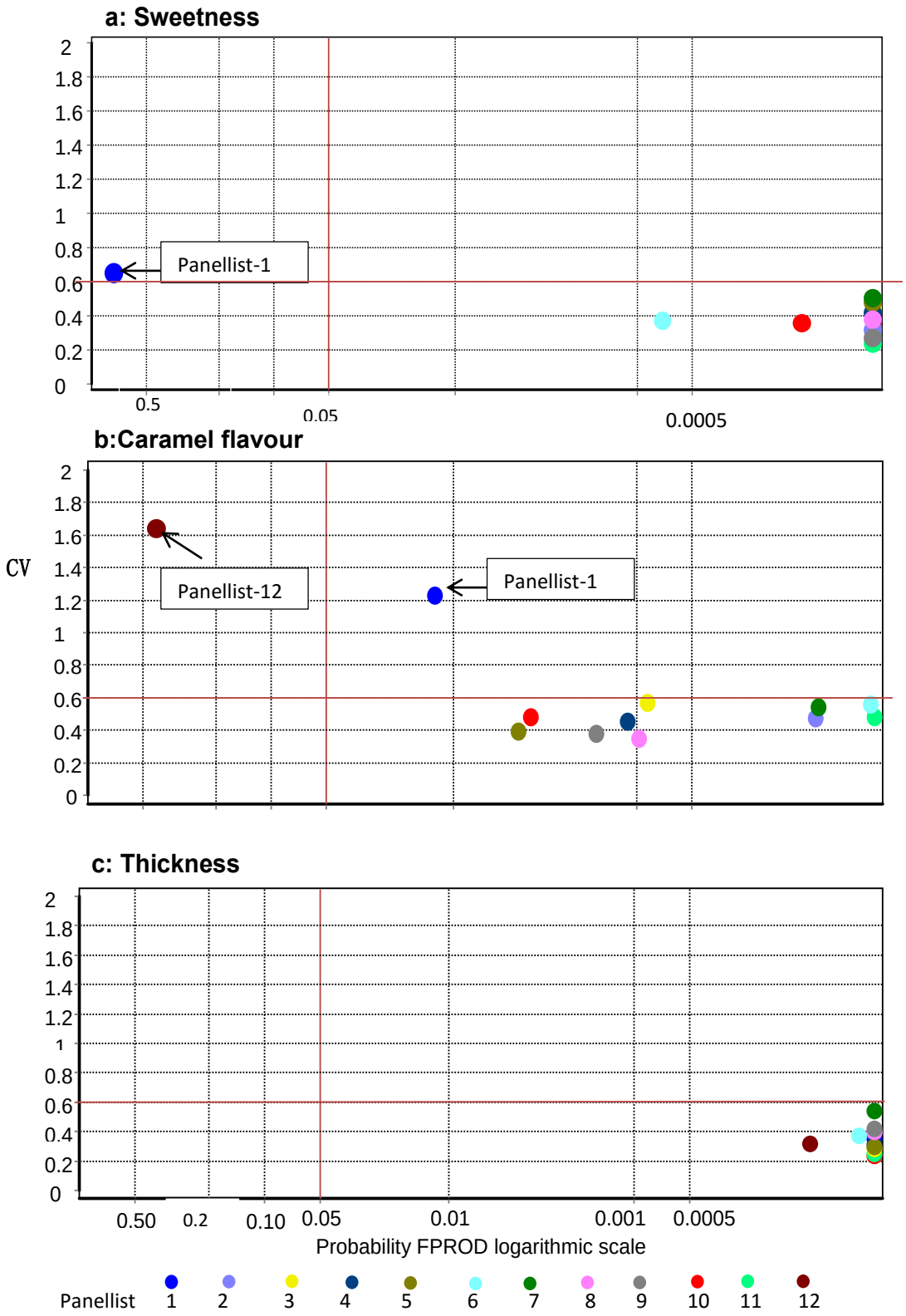

Figure 5-5: Assessment of each panellist's performance: repeatability (y-axis CV) and discrimination ability (x-axis FPROD).

5.3.1.1.2 Accuracy

Mean perception values of the reference samples (S11, S17, S8 and S9), rated by all panellists from three replicate assessments, are presented in **Figure 5-6**. Assessing the references allowed the evaluation of panellist accuracy. With 100% accuracy, a panellist should rate S11 at 0 mm and S17 at 100 mm for both sweetness and caramel flavour intensities; rate S15 at 0 mm and S8 at 100 mm for thickness intensity. A shift from ideal ratings, if within 20mm, is considered accurate (Kemp et al., 2009).

For perceived sweetness intensity (**Figure 5-6a**), the panel means for both the high-sweet reference (S17: 88.9mm) and the low-sweet reference (S11: 8.3 mm) were within 13mm from ideal ratings (100mm and 0 mm, respectively), indicating a good group accuracy. The majority of panellists showed good accuracy: their means are within 20 mm from the ideal scores. However, the means of the high-sweet reference (S17), obtained from panellist-1 and panellist-12, was more than 25 mm higher than the ideal rating, indicating a lack of accuracy.

For caramel flavour (**Figure 5-6b**), the panel mean of the low-caramel reference (S11: 7.9 mm) was within 10 mm higher the ideal (0mm), while the panel mean of the high-caramel reference (S17: 74.6 mm) was 25.5 mm lower the ideal rating (100mm). Individually, panellist-1 and panellist-12 showed a particularly poor accuracy in rating S17, and their means that are more than 30 mm lower the ideal caramel flavour intensity.

The panel showed good group accuracy in rating thickness (**Figure 5-6c**). The panel means of both the high-thickness and low-thickness references were within 10mm away from the ideal ratings. However, panellists-1 showed poor accuracy in rating S8 (ideally 100 mm), with a mean about 40 mm away from the ideal (100 mm). In addition, panellist-1 and panellist-12 showed poor accuracy in rating S15 (ideally 0 mm), with mean ratings more than 35 mm higher than the ideal rating.

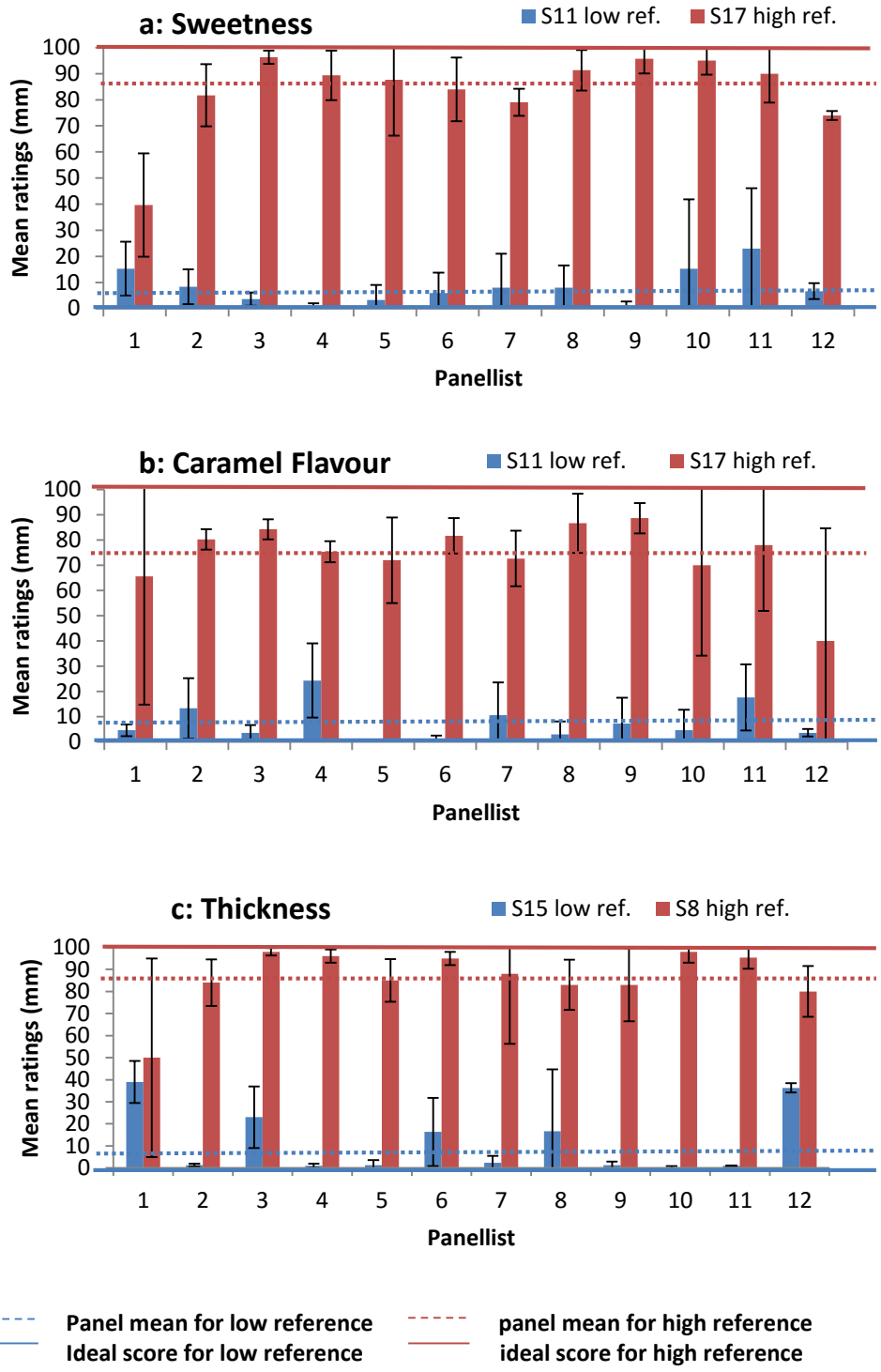

Figure 5-6 Panellists' mean ratings of reference samples for evaluating accuracy. Error bars represent standard deviations

Panellist-1 and panelist-12 were eliminated from further analysis due to their particular poor performance compared with the rest of panellists. Specifically, panellist-1 could not discriminate between custards in sweetness intensity ($p>0.05$); had poor repeatability (high CV) in rating

caramel flavour of custards compared with the repeatability of the group; and had poor accuracy in rating the reference samples (S17, S15 and S8). Panellist-12 could not discriminate between custards in caramel flavour intensity ($p>0.05$); had poor repeatability (high CV) in rating caramel flavour of custards compared with the repeatability of the rest of the group, and had poor accuracy in rating the reference samples (S17 and S15).

5.3.1.1.3 Two-way ANOVA with interaction

Two-way ANOVA with interaction (sample × panellist) was performed in Fizz software with the remaining 10 panellists' data, and results are shown in **Table 5-11**.

Table 5-11 P-values for the two-factor ANOVA with interaction

Source of variation	P value		
	Sweetness	**Caramel Flavour**	**Thickness**
Sample	<0.0001*	<0.0001*	<0.0001*
Panellist	<0.0001*	<0.0001*	<0.0001*
Sample*Panellist interaction	0.5158	0.0004*	0.1944

P value ≤0.05 is considered statistically significant, which is indicated by '*'. N (sample) =18 (17+1), n (panellist) =10.

There was a significant main effect of 'sample' on perceived intensities of sweetness, flavour and thickness ($p<0.0001$), indicating that the panel could discriminate the samples in all three attributes.

There was a significant main effect of 'panellist', but no effect of sample-panellist interaction on ratings of sweetness and thickness. This suggests that panellists might have used the scale differently but their rank orders of samples in sweetness and thickness intensities were similar.

There was a significant main effect of 'panellist' ($p<0.0001$) and a significant 'sample-panellist' interaction in caramel flavour intensity ($p<0.05$), suggesting that panellists ranked the order of samples in caramel flavour intensities differently. Flavour is a relatively complex term compared with sweetness and thickness. The cross-over interactions between panellist and sample in flavour assessment were not uncommon

even for the trained panels (Hewson et al., 2009). The custard sample was a complex food system where sweetness, caramel aroma and thickness intensities changed together, making ratings of the overall caramel flavour more complicated than a simple liquid solution. A large number of samples were assessed which may also have contributed to the significant interaction (Hewson, 2007). However, the graph of individual ratings showed a similar trend, indicating that the results were acceptable.

5.3.1.1.4 Multiple comparison tests

The means of 17 custards obtained from the remaining 10 panellists were compared using one-way ANOVA with *post hoc* Tukey's HSD multiple comparison tests. Mean and standard deviation of each sample for each attribute were listed in **Table 5-12**, where samples without the same capital letter coding were significantly different ($p<0.05$). Custards were rated across a broad range on the VAS scales for sweetness (7.8-88.9mm), flavour (8.1-78.8 mm) and thickness (6.5-90.5 mm).

Table 5-12 Composition and perception values of the 17 custards, n=10 panellists

Sample	Composition %			Sweetness			Flavour			Thickness		
	Truvia®	**CMC**	**Aroma**	Mean	Std. Dev.		Mean	Std. Dev.		Mean	Std. Dev.	
1	0.9	0.2	0.5	82.2	15.8	**EF**	58.3	17.9	**BC**	29.7	19.0	**B**
2	0.9	0.5	0.2	76.6	16.9	**EF**	43.5	17.5	**BC**	83.4	12.7	**C**
3	0.3	0.5	0.2	30.9	10.2	**B**	29.9	21.9	**AB**	81.6	14.2	**C**
4	0.9	0.5	0.5	73.6	12.0	**DEF**	65.3	18.7	**C**	79.8	12.8	**C**
5	0	0.65	0.65	14.6	12.3	**AB**	16.9	15.0	**AB**	86.8	10.6	**C**
6	0.3	0.2	0.5	50.5	20.1	**C**	39.6	25.4	**B**	21.6	13.8	**AB**
7	0.9	0.2	0.2	79.9	12.2	**EF**	48.8	22.2	**BC**	16.9	13.9	**AB**
8	1.2	0.65	0.65	84.7	13.9	**EF**	65.2	20.8	**C**	90.5	8.9	**C**
9	0	0.05	0.05	14.5	13.7	**AB**	8.1	11.0	**A**	23.4	11.8	**AB**
10	1.2	0.65	0.05	80.0	8.3	**EF**	38.3	22.9	**B**	90.3	9.5	**C**
11	0	0.65	0.05	7.8	6.8	**A**	8.6	7.9	**A**	86.9	11.3	**C**
12	0	0.05	0.65	24.8	18.5	**B**	37.6	22.9	**B**	19.9	10.6	**AB**
13	0.3	0.2	0.2	38.8	21.5	**B**	28.4	14.1	**AB**	28.8	21.3	**B**
14	0.6	0.35	0.35	67.8	16.9	**DE**	51.7	22.7	**BC**	43.9	21.5	**B**
15	1.2	0.05	0.05	84.1	13.1	**EF**	25.1	23.2	**AB**	6.5	8.6	**A**
16	0.3	0.5	0.5	33.1	11.2	**B**	44.0	15.7	**BC**	78.7	18.6	**C**
17	1.2	0.05	0.65	88.9	10.8	**F**	78.8	16.6	**C**	23.7	17.2	**AB**

Samples in a column without a same capital letter are significantly different ($p<0.05$)

5.3.1.2 Perception models

5.3.1.2.2 Model-1: perceived sweetness

Truvia® sweetener (A), a quadratic term for Truvia® sweetener (A^2), CMC (B) and caramel aroma (C) were the significant factors influencing perceived sweetness intensity (**Table 5-13**). Overall, the addition of Truvia® sweetener enhanced the perceived sweetness ($p<0.0001$), to a maximum rise of about 4 fold (**Figure 5-7a**). Compared to Truvia® sweetener, CMC and caramel aroma had relatively minor but significant effects on the perceived sweetness. Increasing caramel aroma concentration increased perceived sweetness ($p=0.0063$) (**Figure 5-7c**), while CMC showed a slight suppression of the sweetness intensity ($p=0.0004$) (**Figure 5-7b**).

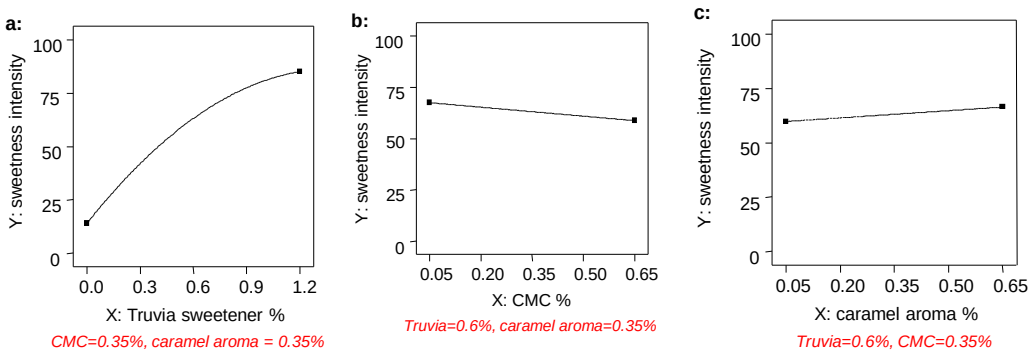

Figure 5-7 One-factor plots describing the compositional effects on sweetness intensity

Table 5-13 Statistical models for sensory perception intensity as a function of composition factors, n=10

Model	Responses	Factor terms	P value	Model equation	Lack of fit (p)	CV %	R^2	adjusted R^2	predicted R^2	Adequate precision
Model-1	Sweetness	A: sweetener %	<0.0001	15.2+104.6*A -14.6*B+11.1*C -37.8*A^2	0.60	22	0.85	0.85	0.82	33
		B: CMC %	0.0004							
		C: caramel aroma %	0.0063							
		A^2	<0.0001							
Model-2	Caramel Flavour	A: sweetener %	<0.0001	1.1+67.4*A+14.6*B +51.8*C+28.9*A*C -57.8*B*C-40.5*A^2	0.74	38	0.61	0.59	0.53	24
		B: CMC %	0.29							
		C: caramel aroma %	<0.0001							
		AC	0.0027							
		BC	0.0027							
		A^2	<0.0001							
Model-3	Thickness	A: sweetener %	0.59	29.2-7.0*A-210.9*B -5.67*C+16.9*A*C +1162*B^2-1061*B^3	0.91	23	0.88	0.87	0.85	32
		B: CMC %	<0.0001							
		C: caramel aroma %	0.26							
		AC	0.02							
		B^2	0.05							
		B^3	<0.0001							

Evaluation of Model-1: perceived sweetness intensity

Sweetness intensity ratings were normally distributed (**Figure 5-8a**). There was no significant outlier since the data were all within ±3 standard deviation (**Figure 5-8b**). The adequate precision measures the signal to noise ratio. The ratio of 33 (Table 5-13) was greater than 4 indicating adequate model discrimination.

There was no significant lack of fit ($p>0.05$) and the residuals were randomly scattered around zero. This indicates that the Model-1 fits the expected satiation data (Figure 5-8b). Actual values for sweetness intensity correlated well with the predicted values (**Figure 5-8c**), which was also indicated by a high adjusted R^2 of 0.85 (Table 5-13). It suggests that the model fits the data well. A predicted R^2 of 0.82 suggested that the model was robust for prediction (Table 5-13).

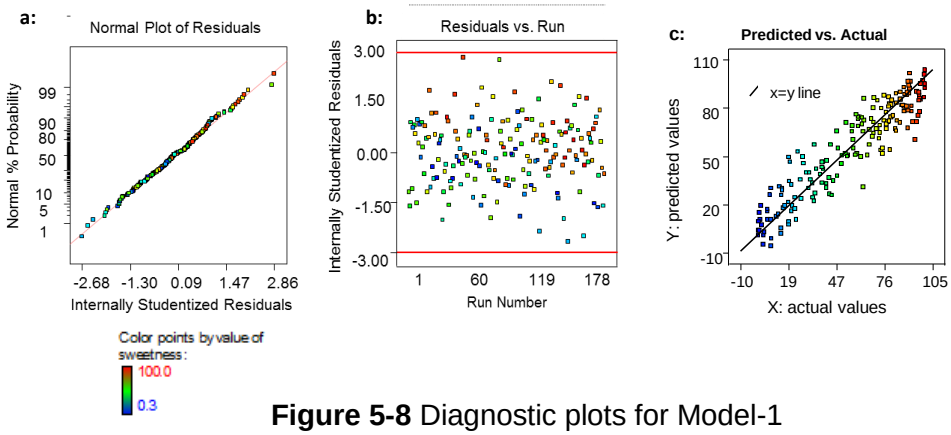

Figure 5-8 Diagnostic plots for Model-1

5.3.1.2.3 Model-2: perceived caramel flavour

The Model-2 included a linear term for caramel aroma (C, $p<0.0001$), a linear and a quadratic term for the Truvia® sweetener (A and A^2, $p<0.0001$), an interaction term between Truvia® sweetener and caramel aroma (AC, $p=0.0027$), and an interaction term between CMC and caramel aroma (BC, $p=0.0027$) (Table 5-13)**.** CMC (B) alone was not

significant (p=0.29) but it was kept in the model to retain the hierarchy for the inclusion of the significant BC interaction.

Increasing the Truvia® sweetener from 0 % to 0.9% increased the perceived caramel flavour intensity (**Figure 5-9a**). The increase in caramel flavour flattened out as the Truvia® concentration reached above 0.9%.

Perceived caramel flavour increased consistently with increasing concentration of caramel aroma (**Figure 5-9b**). A greater enhancement (sharper gradient) of perceived caramel flavour by increasing caramel aroma concentration was observed when custards contained a higher concentration of Truvia® sweetener (**Figure 5-9b**). This suggested caramel aroma and Truvia® sweetener (sweetness) acted synergistically to enhance perceived caramel flavour.

CMC alone does not have a significant influence on perceived caramel flavour intensity (p=0.29), but it interacted with caramel aroma to affect perceived caramel flavour intensity (p=0.0027<0.05). This interaction reflects the change in magnitude of the effect of caramel aroma on caramel flavour perception, in the presence of CMC. When CMC concentration was low, increasing caramel aroma concentration increased perceived caramel flavour intensity greater than when the CMC concentration was high (Figure 5-9c).

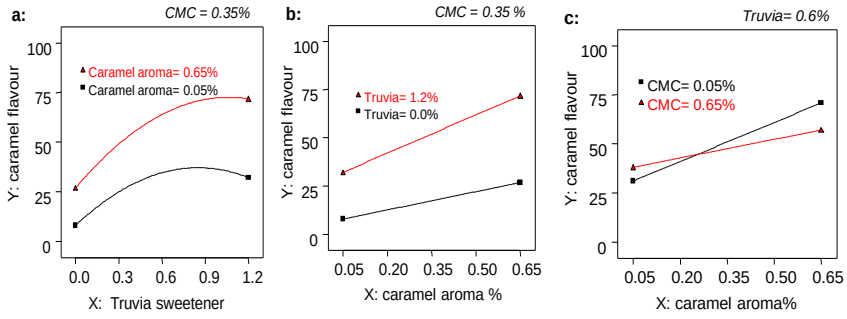

Figure 5-9 Interaction plots describing the effects of composition factors on caramel flavour intensity

Evaluation of Model-2: perceived caramel flavour

Observed data for perceived caramel flavour were normal disturbed. There were no significant outliers (**Figure 5-10b**). The adequate precision ratio of 24 for model-2 (Table 5-13) was greater than 4, indicating the model had adequate discrimination. There was no significant lack of fit observed (p=0.74>0.05). The residuals were randomly and symmetrically scattered around zero (**Figure 5-10b**), which indicates that the model-2 fits the data.

The actual values for caramel flavour intensity correlated with the predicted values (**Figure 5-10c**), which was also indicated by a modest adjusted R^2 of 0.59 (Table 5-13). The predicted R^2 for the model-2 predicting caramel aroma was 0.53, which was relatively low compared to that for the model-1 (sweetness, 0.82) and model-3 (thickness, 0.85).

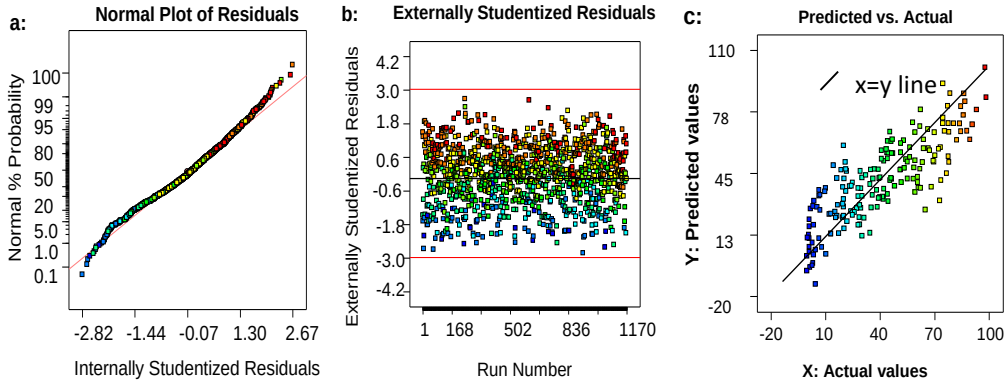

Figure 5-10 Diagnostic plots for Model-2

5.3.1.2.4 Model-3: perceived thickness

Significant terms affecting perceived thickness in model-3 included CMC (B), B^2, B^3 and AC interaction of (Table 5-13). The term Truvia® sweetener (A) or caramel aroma (C) alone was not significant (p>0.2) but both were kept in the equation to retain the model hierarchy for the inclusion of the AC interaction.

At low concentration, the increase of CMC from 0.05% to 0.2% resulted in little change in the thickness perception. Above 0.2% of CMC, the thickness intensity increased rapidly with the increasing concentration of CMC, and plateaued at a CMC concentration above approximately 0.6% (**Figure 5-11a)**.

The interaction of Truvia® sweetener and caramel aroma affected the thickness perception significantly. At a low concentration of caramel aroma (0.05%), the addition of Truvia® sweetener suppressed thickness intensity. At a high concentration of caramel aroma (0.65%), the addition of Truvia® enhanced the thickness intensity slightly (**Figure 5-11b**).

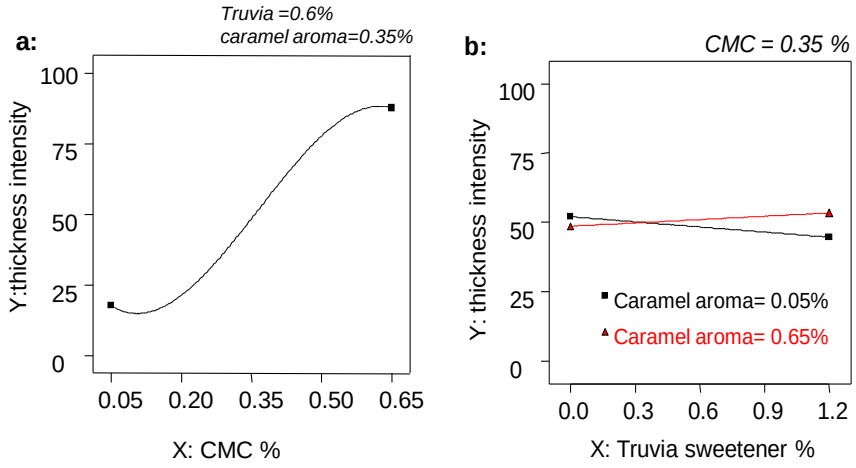

Figure 5-11 Plots describing the perceived thickness intensity

Evaluation of Model-3: perceived thickness

Observed data for thickness intensity were normally distributed (**Figure 5-12a**). There was one outlier, which fell outside the ±3 standard deviations (**Figure 5-12b**). The outlier was kept in the final analysis because that the results were not affected by the outlier. The adequate precision ratio of 32 (Table 5-13) indicates adequate model discrimination.

The model-3 fits the thickness data very well, as noted by both the lack of fit test ($p=0.91>0.05$) and the residual plots (Figure 5-12b). The actual values for thickness intensity were highly correlated with the predicted

values (**Figure 5-12c**, R^2=0.88). A high predicted R^2 of 0.85 suggested that the model-3 is robust to predict the thickness perception within the system.

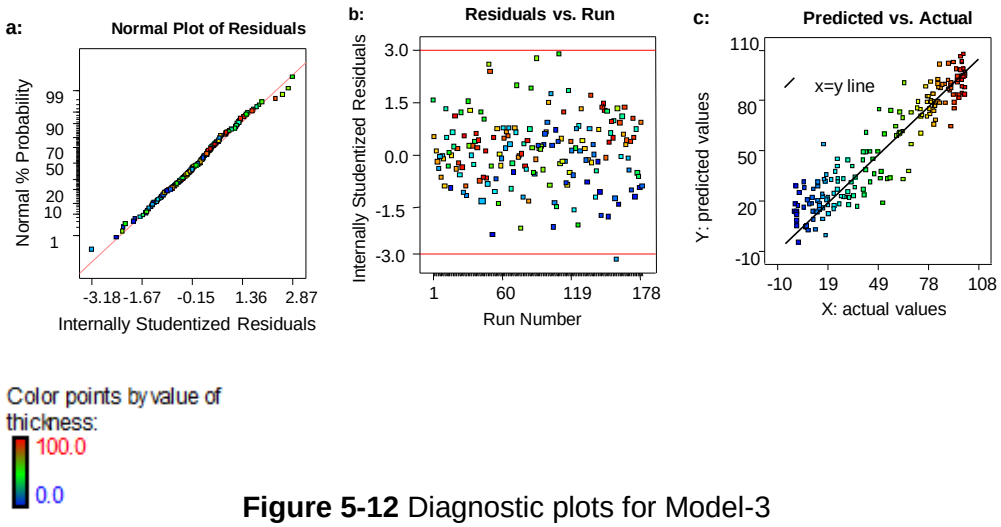

Figure 5-12 Diagnostic plots for Model-3

5.3.2 Phase 2 experiment results

5.3.2.1 Selected energy content of each comparison food

The three picture scales showed a similar pattern in comparing the effected satiation and expected satiety of the custards (**Table 5-14**). Therefore, the expected satiation or expected satiety of custards (kcal) was calculated as the arithmetic mean of the selected energy contents of three comparison foods from picture scales. Arithmetic mean data were entered in the Design Export 7.0 for statistical analysis.

Table 5-14 Energy content of each comparison food expected to have equal satiation and satiety of custards (Mean ± SD)

Custards	Energy content (kcal)	Energy content (kcal)					
		Equal expected satiation			Equal expected satiety		
		banana	KitKat	Sausage	banana	KitKat	Sausage
S1	250	270±90	319±110	347±117	279±86	331±109	360±95
S2		312±101	362±108	401±116	312±91	365±109	403±99
S3		300±99	355±116	386±125	303±97	364±113	398±103
S4		317±102	357±112	396±123	308±102	358±117	385±105
S5		317±105	359±127	403±134	323±106	372±130	412±118
S6		268±89	315±97	347±109	280±90	328±100	366±92
S7		282±95	318±100	351±110	281±88	327±97	355±86
S8		333±98	384±114	422±120	330±96	386±117	422±104
S9		267±79	308±98	329±96	276±99	322±113	347±110
S10		333±106	379±120	412±132	339±102	385±120	425±107
S11		307±107	354±119	389±129	316±101	368±1119	410±107
S12		268±94	316±106	339±121	285±106	332±123	355±111
S13		271±82	316±101	339±107	287±88	340±105	367±94
S14		288±86	330±94	361±102	301±95	351±110	380±96
S15		279±96	325±104	349±106	279±97	332±111	355±101
S16		293±101	347±116	372±122	307±93	370±109	401±99
S17		275±90	321±112	346±112	275±94	322±119	352±103
Mean		293±95	239±117	370±112	299±96	350±122	382±113

5.3.2.2 Expected satiation

The means and standard deviations of the expected satiation of the custard samples are listed in **Table 5-15**. Visual observation of **Figure 5-13** indicated that the means of custards fell in a narrow range (300 to 380 kcal) across the middle part of expectation scales (26 – 525 kcal for banana, 29-583 kcal for KitKat, and 33- 666 kcal for sausage).

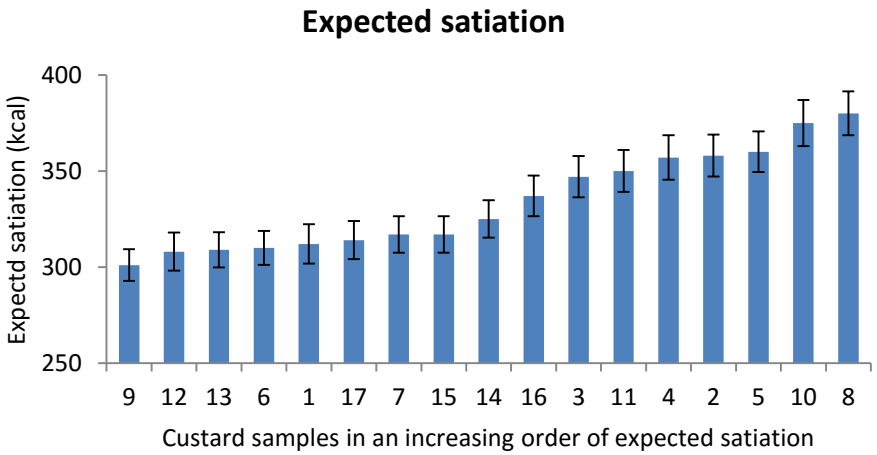

Figure 5-13 Means and standard errors (error bars) of the expected satiation of custards, n=90 consumers

Table 5-15 Means and standard deviations of the expected satiation and satiety of 17 custards, n=90 consumers

Sample	Composition as factors %			Predicted Perception as factors			Response					
							Expected satiation kcal			*Expected satiety kcal*		
	Truvia®	*CMC*	*Aroma*	*sweetness*	*Caramel flavour*	*thickness*	Mean	Std. Dev.		Mean	Std. Dev.	
1	0.9	0.2	0.5	81.4	65.0	23.5	312	97	**ABCD**	323	95	**AB**
2	0.9	0.5	0.2	73.7	46.0	77.3	358	100	**CDEF**	360	99	**ABC**
3	0.3	0.5	0.2	38.1	31.3	79.4	347	103	**ABCDEF**	355	103	**ABC**
4	0.9	0.5	0.5	77.0	60.7	80.1	357	103	**BCDEF**	351	105	**ABC**
5	0	0.65	0.65	12.9	19.9	88.0	360	114	**DEF**	369	118	**BC**
6	0.3	0.2	0.5	45.8	45.1	22.6	310	84	**ABCD**	324	92	**AB**
7	0.9	0.2	0.2	78.0	45.1	20.7	317	90	**ABCD**	321	86	**AB**
8	1.2	0.65	0.65	84.0	64.9	92.8	380	100	**F**	379	104	**C**
9	0	0.05	0.05	15.0	4.3	21.2	301	78	**A**	315	100	**A**
10	1.2	0.65	0.05	77.4	35.6	84.0	375	108	**EF**	383	107	**C**
11	0	0.65	0.05	6.3	11.3	91.4	350	110	**ABCDEF**	365	107	**ABC**
12	0	0.05	0.65	21.7	33.7	17.8	308	94	**AB**	324	110	**AB**
13	0.3	0.2	0.2	42.5	30.4	22.8	309	87	**ABC**	331	94	**ABC**
14	0.6	0.35	0.35	63.1	49.2	49.6	325	100	**ABCDE**	343	107	**ABC**
15	1.2	0.05	0.05	86.1	28.5	13.8	317	92	**ABCD**	321	101	**AB**
16	0.3	0.5	0.5	41.4	40.8	79.2	337	102	**ABCDEF**	359	99	**ABC**
17	1.2	0.05	0.65	92.8	78.7	22.6	314	94	**ABCD**	316	103	A

Samples in a column without a same capital letter are significantly different in the expectation values ($p<0.05$).

5.3.2.2.2 Model-4: composition and expected satiation

Significant composition factors affecting the expected satiation in model-4 contained only CMC (B) and Truvia® sweetener (A), **Table 5-16**. The concentration of caramel aroma in custards did not affect consumers' expected satiation of the custards. Consumers expected the custards to make them feel fuller immediate after consumption (higher expected satiation) when the custards contained a higher concentration of Truvia® sweetener or CMC (**Figure 5-14**). CMC showed a greater enhancement on expected satiation than Truvia® sweetener, as there were more contour lines (larger difference in expected satiation) across changes in the CMC concentration (X1).

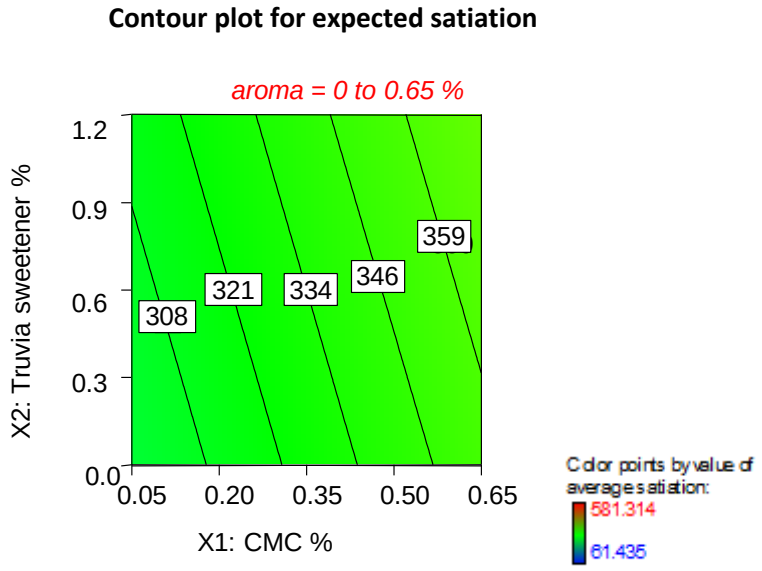

Figure 5-14 Contour plot describing the expected satiation as a function of composition factors

Table 5-16 Models for expected satiation and expected satiety

Model	Response	Factor		F value	p-value	Model equation	Lack of fit (p)	R^2	Adjusted R^2	Predicted R^2	Adequate precision
Model-4	Expected satiation	Composition	A: Truvia® %	16.2	<0.0001	289.9+14.6*A +99.8*B	1.0	0.12	0.12	0.01	25.7
			B: CMC %	190.3	<0.0001						
Model-5		Perception	A: sweetness	14.0	0.0002	281+0.22*A +0.77*B	1.0	0.12	0.12	0.01	25.3
			B:thickness	203.1	<0.0001						
Model-6	Expected satiety	Composition	B: CMC %	201.6	<0.0001	310.5+94.4*B	1.0	0.12	0.12	0.008	27.6
Model-7		Perception	B: thickness	194.3	<0.0001	307.8+0.68*B	1.0	0.12	0.12	0.004	27.4

5.3.2.2.3 Model-5: perception and expected satiation

Model-5 describes the expected satiation as a function of perceptual factors, it included a linear term for perceived sweetness ($p<0.0001$) and a linear term for perceived thickness ($p<0.0001$), as seen in Table 5-15. The expected satiation increased with the increasing thickness perception and with the increasing sweetness perception (**Figure 5-15**). Thickness showed a greater enhancement on expected satiation than sweetness, as there were more contour lines (larger difference in expected satiation) across changes in the thickness (X2) than across changes in the sweetness (X1). Caramel flavour perception did not affect the expected satiation of custards ($p>0.05$).

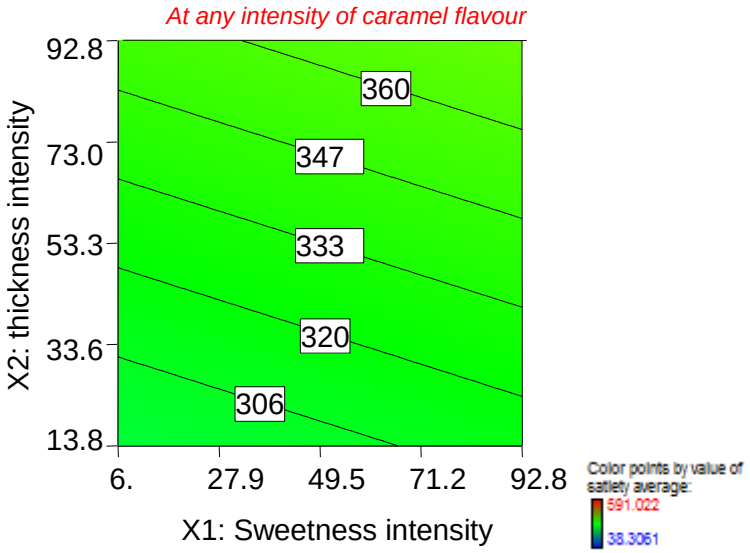

Figure 5-15 Contour plot describing the expected satiation as a function of sensory perception factors

Evaluation of Model-4 and Model-5 for the expected satiation

Expected satiation data was normally distributed (**Figure 5-16a**). There were five outliers (**Figure 5-16b**), but they were kept in the final analysis since it did not affect the models. The adequate precision ratio was 25.7 (Table 5-16), indicating adequate model discrimination. It should be noted

that the adjusted R^2 value in model-4 and model-5 was relatively low (0.12), compared to the values in models 1 (0.85), 2 (0.59) and 3 (0.87) (Table 5-13). Despite a low R^2, both the model-4 and model-5 fitted the data since there was no significant lack of fit (Lack of fit test, p=1.0>0.05). The actual values correlated linearly with the predicted values (**Figure 5-16c** and **5-16d**).

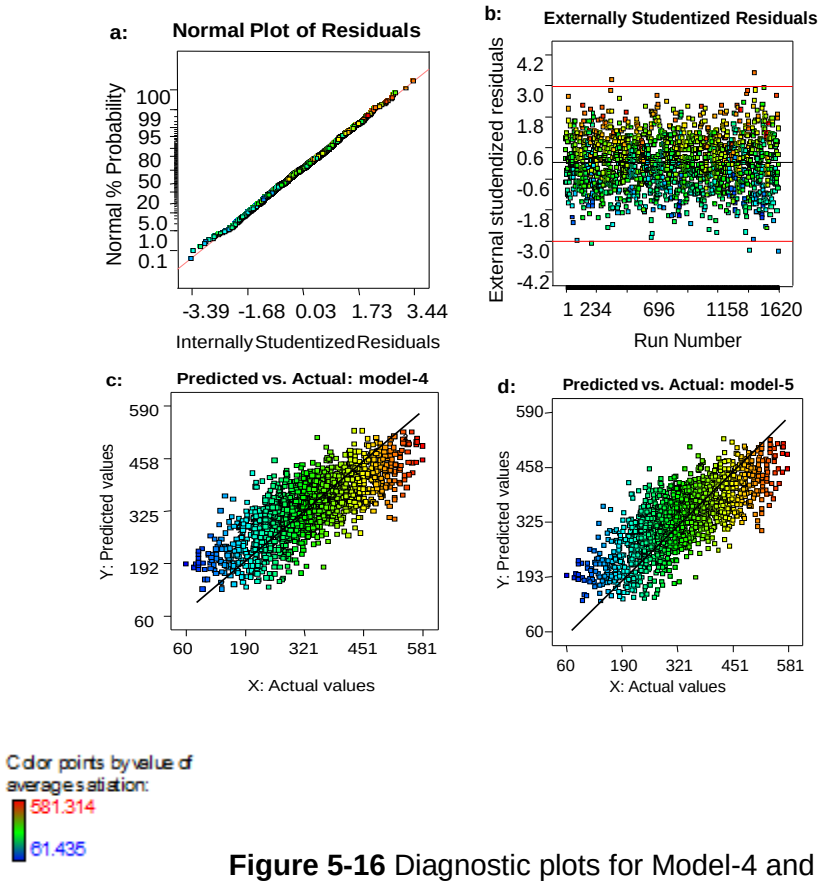

Figure 5-16 Diagnostic plots for Model-4 and Model-5

5.3.2.3 Expected satiety

The means of the expected satiety of custards are presented in Table 5-15 and **Figure 5-17**. The means of expected satiety of custards varied from 315 to 383 kcal.

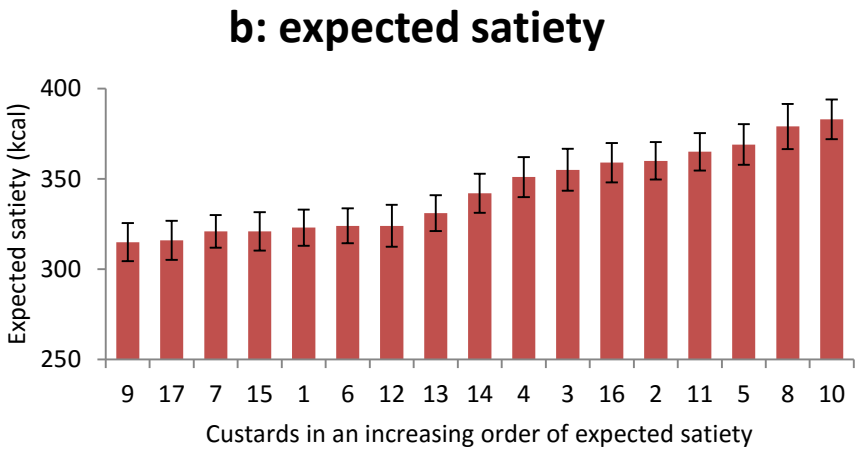

Figure 5-17 Means and standard errors (error bars) of the expected satiation of custards, n=90 consume

5.3.2.3.2 Model-6: composition and expected satiety

Only CMC influenced the model-6 in predicting expected satiety ($p<0.0001$) (Table 5-16). The concentration of Truvia® sweetener or caramel aroma did not influence participants' expected satiety of the custards ($p>0.05$). Expected satiety increased with increasing concentration of CMC, but was unaffected by varying addition of Truvia® or caramel aroma (**Figure 5-18**).

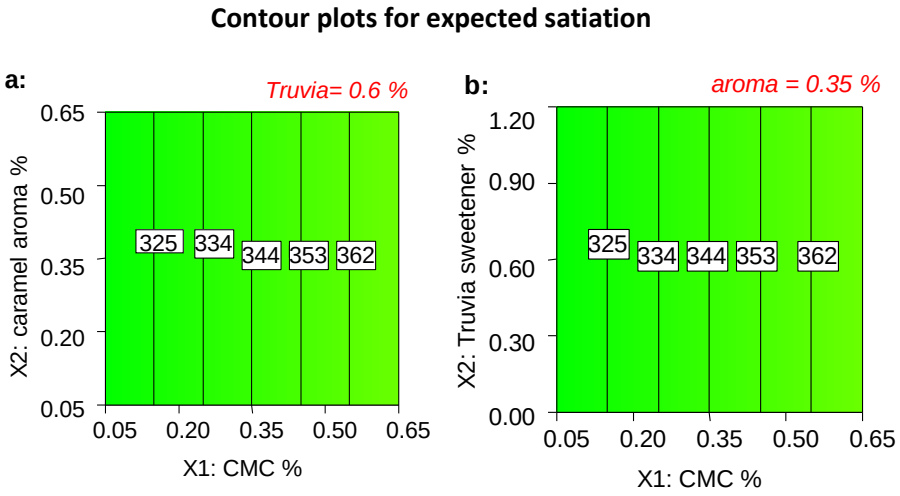

Figure 5-18 Contour plots describing the expected satiety as a function of composition factors. Each contour line represents a value of expected satiation at specific concentrations of sweetener, caramel aroma, CMC

5.3.2.3.3 Model-7: perception and expected satiety

The model-7 describes the expected satiety of custards as a function of perception factors, and it contained only a linear term of perceived thickness (Table 5-16). Increased perceived thickness intensity enhanced expected satiety ($p<0.0001$). Neither sweetness nor caramel flavour intensity affected on the expected satiety of custards (**Figure 5-19**).

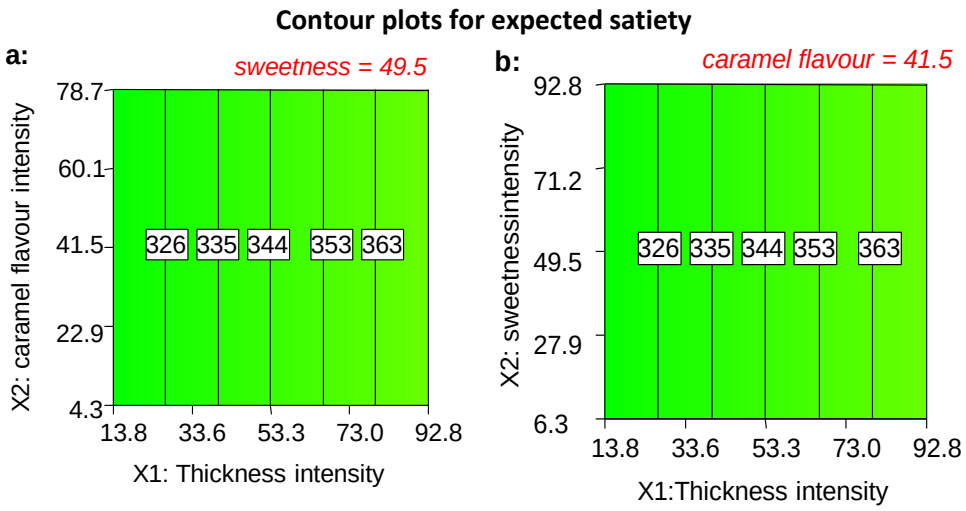

Figure 5-19 Contour plots for expected satiety as factors of perception of sweetness, caramel flavour and thickness. Each contour line represents a value of expected satiety at specific concentrations of sweetener, caramel aroma, CMC

Evaluation of model-6 and model-7 for the expected satiety

Data for expected satiety were normally distributed (**Figure 5-20a**). There were four outliers (**Figure 5-20b**). However, the outlier was kept in the final analysis because they did not affect the model. The adequate precision ratio was 25.3 (Table 5-16), indicating adequate model discrimination.

The adjusted R^2 value in model-6 and model-7 was 0.12 (Table 5-16). Despite a low R^2, both the model-6 and model-7 fitted the data since there was no significant lack of fit (Lack of fit test, p=1.0>0.05) and the actual values correlated linearly with the predicted values (**Figure 5-20c** and **5-20d**).

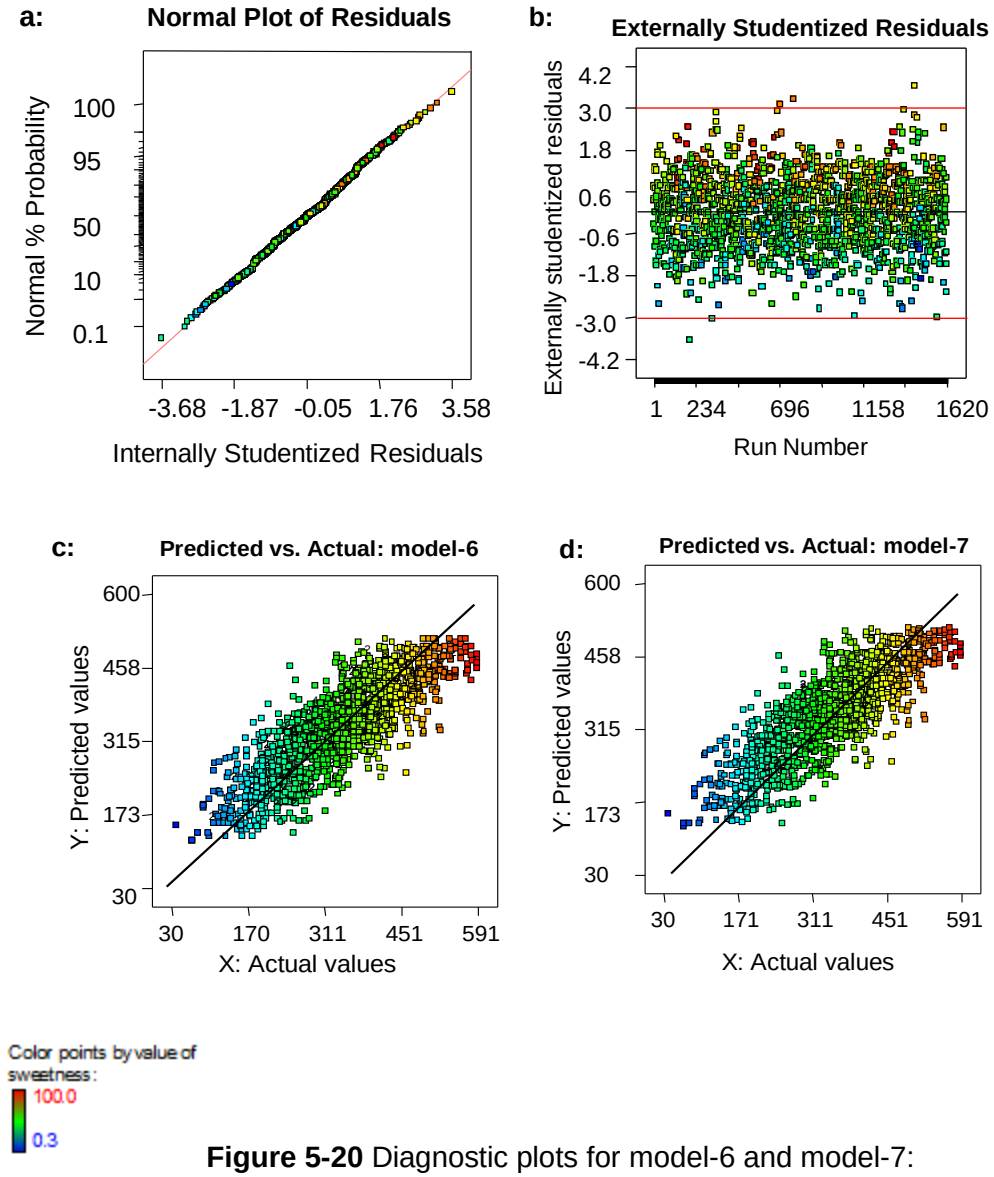

Figure 5-20 Diagnostic plots for model-6 and model-7:

5.3.2.4 Expected satiation vs. expected satiety

A Pearson's correlation test indicated that the expected satiation of custards had a strong positive correlation with the expected satiety of custards ($r=0.95$, $p<0.00001$). The mean values of custards for expected satiety were plotted against the mean values for expected satiety of custards (**Figure 5-21**).

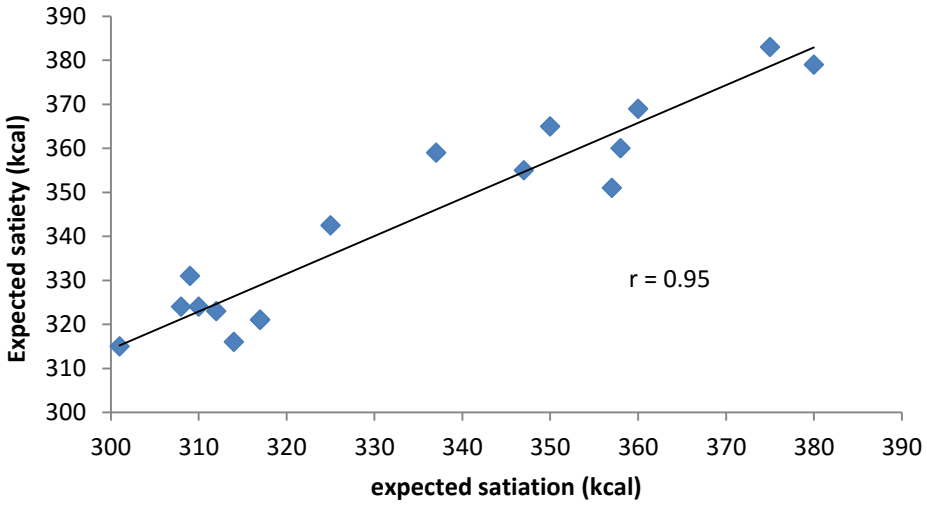

Figure 5-21 Correlation between expected satiety and expected satiation of the 17 different custard samples. 'r' represents Pearson's correlation coefficient.

5.4 Discussion

5.4.1 Summary of key findings

The objective of the phase 1 experiment was to quantify the perceived intensities of sweetness, caramel flavour and thickness of custards with varying concentrations of sweetener, caramel aroma and CMC. The sweetness intensity of custards was increased with the increasing concentration of Truvia® sweetener or caramel aroma, and it was decreased with increasing concentration of CMC. Perceived caramel flavour intensity increased with increasing concentration of caramel aroma or Truvia® sweetener, and it was unaffected by the overall increase in CMC concentration. However, when the CMC concentration was lower, the enhancement by caramel aroma on the perceived caramel flavour intensity was greater (significant CMC and caramel aroma interaction). The perceived thickness of custards was increased with increasing CMC concentration, and it was not influenced by the overall changes in the concentration of caramel aroma or Truvia® sweetener.

The main objective of this study (Phase 2 experiment) was to investigate the effects of perceived sweetness, caramel flavour and thickness of the custards on the expected satiation and expected satiety of the custards. Increasing both sweetness and thickness intensities increased participants' expected satiation of the custards; caramel flavour perception did not affect the expected satiation of the custards. The expected satiety of custards was only increased with the increasing thickness intensity; it was not affected by the perceived sweetness or caramel flavour intensities.

5.4.2 Evaluation of experimental design

In the phase 1 experiment, the perceived intensities of sweetness, caramel flavour and thickness of custards were quantified by a trained panel. Sweetness, caramel flavour and thickness intensities were predicted as a function of Truvia sweetener, caramel aroma and CMC concentrations by statistical models 1, 2 and 3, respectively. High adjusted R^2 values for statistical model-1 (sweetness) and model-3 (thickness) (>0.8) suggest that the two models fitted very well with the data. In comparison, a relatively moderate adjusted R^2 (=0.59) was obtained from the model-2 (caramel flavour), which was potentially due to that the term 'caramel flavour' was more complex. Caramel flavour perception was a result of multimodal interaction between caramel aroma, sweet taste, and texture. The perception of caramel flavour was expected to be more complicated than sweetness or thickness perception.

In the phase 2 experiment, the statistical models 5 and 7 were built to evaluate the expected satiation and expected satiation of the custards as a function of sweetness, caramel flavour and thickness intensities. Models 5 (expected satiation) and 7 (expected satiety) had relatively low adjusted R^2 (0.12), indicating that the final models explained only 12% of the observed variability of the expectation data. This was not uncommon because humans' psychological expectation response is usually harder to predict. In psychology studies, a model attempted to predict human behaviour typically has a R^2 lower than 50% (Minitab, 2016). Besides,

there were a large number of custard samples, which made rating more difficult. There is no clear-cut of an acceptable R^2 value since the objective of the study was not to make a prediction (Design Expert 7.0 software, DX help content). Regardless of low R^2 values, all models fitted the data and there were highly statistically significant terms (e.g. thickness enhanced expected satiation or expected satiety significantly). Low R^2 did not compromise the conclusion of this study, since the objective was to investigate if there were any significant influence of sweetness, caramel flavour and thickness perception on the expected satiation and the expected satiety of the custards, rather than to establish statistical models to predict the expectation values within the model custard system.

5.4.3 Multimodal flavour perception

5.4.3.1 Aroma-taste interaction

Caramel aroma enhanced sweetness perception of the custards. A reverse aroma-taste interaction was also observed: Truvia® sweetener enhanced perceived caramel flavour. This is consistent with a study by Tournier et al. (2009). They reported that the sweetness intensity of custards was increased by increasing pineapple or almond aroma; and the pineapple or almond aroma perception was increased by increasing sugar concentration.

The caramel aroma itself was tasteless but it enhanced sweetness perception when being added to a sweet solution. Sweet-congruent aroma naturally appears to be co-exposed with a sweet taste (Auvray and Spence, 2008). The enhancement of sweetness perception by sweet-congruent aroma is not simply a result of chemical interactions between aroma and taste substances; because the enhancement of an aroma on sweetness perception disappeared when participants were wearing a nose clip (Tournier et al., 2009). It is now widely accepted that a sweet-congruent aroma, such as caramel aroma, can increase sweetness perception by the cross-modal interaction between gustatory and olfactory signals at neural and psychological levels (Wallace, 2015).

Sweetness increases perceived caramel flavour intensity. The aroma-taste interaction on flavour perception is mainly due to psychological interaction rather than physicochemical interaction (Tournier et al., 2009). For example, the perceived strawberry flavour increased when the strawberry aroma was co-exposed with taste substances (sucrose and citric acid), but the strawberry aroma release by APCI analysis was unaffected by the presence of taste substances (Chapter 4) (Pfeiffer et al., 2006).

5.4.3.2 Texture-flavour interaction

The sweetness of custards was suppressed by the increased concentration of CMC. CMC increased the perceived thickness intensity. The suppressing impact of thickness on sweetness perception was in agreement with results from other studies (Hollowood et al., 2002, Tournier et al., 2009).

Thickness (viscosity) affects taste perception may partly be due to a physicochemical interaction. For example, thickness (or thickener, here CMC) might affect the release of sucrose into saliva, through its impact on the diffusion of sucrose in foods (Boland et al., 2006, Hollowood et al., 2002). However, increase thickness can increase sweetness perception without affecting the in-mouth sucrose release, suggesting that the interaction of taste and viscosity is likely at neural and psychological level (Tournier et al., 2009). The observed suppressing effect of thickness (CMC) on sweetness perception is potentially results of both physiochemical and physiological interactions.

CMC alone did not affect the perceived caramel flavour intensity. However, it interacted with caramel aroma to affect the perceived caramel flavour. When CMC concentration was reduced, increasing caramel aroma concentration increased the perceived caramel flavour intensity greater. Potentially, CMC may interact with caramel aroma at a physicochemical level, which might reduce the release of caramel aroma into the nasal cavity. Reduced the release of caramel aroma may result in a reduction in

the overall caramel flavour perception. Alternatively, CMC might suppress the perceived caramel flavour by suppressing the perceived sweetness.

In addition, the perceived thickness of the custards was increased with the increasing CMC concentration, and it was not affected by the overall changes in the concentration caramel aroma or Truvia® sweetener. This is in agreement with previous literature that perceived thickness was not affected by the concentration of aroma or taste substances (Hollowood et al., 2002, Tournier et al., 2009). However, there was a significant interactive effect between caramel aroma and Truvia sweetener on the perceived thickness. At a low concentration of caramel aroma, the increase in Truvia® sweetener suppressed the perceived thickness intensity. At a high concentration of caramel aroma, the increase in Truvia® sweetener enhanced the thickness intensity. This may be due to a physicochemical interaction between Truvia® sweetener, caramel aroma and CMC.

5.4.3.3 Effect of thickness perception on expectation

The role of thickness intensity on the expectations of the satiating capacity of food, observed in this study, is consistent with the literature (Hogenkamp et al., 2011, McCrickerd et al., 2012b). Results of this study confirmed that increasing the perceived thickness intensity increased consumers' expectations about satiation (immediately after consumption) and satiety (3 hours after consumption) of the custards. In other words, participants expected a thicker custard to make them feel fuller immediately, and to have stronger suppression on their feeling of hunger for at least 3 hours post-consumption, compared with a thinner custard.

Humans may have learned to anticipate a thicker food with high energy and nutrients, through their previous eating experience of foods with varying thickness (McCrickerd et al., 2012a). Thicker foods may be expected to be richer in energy and have stronger satiation or satiety effect than thinner foods. In fact, the higher thickness of foods have been

found to enhance satiation and satiety of foods. In a study by Mattes and Rothacker (2001), a beverage of a thicker texture reduced the feeling of hunger more than the iso-energy beverage of a thinner texture for up to 4 hours post-consumption (Mattes and Rothacker, 2001). The consumption of thicker foods have been shown to delay gastric emptying more than the consumption of thinner foods (Zhu et al., 2013). Therefore, this suggests that participants may have learned to associate thicker foods with higher satiating effect (e.g. reduce feeling of hunger and delay gastric emptying), resulting in high expectations for satiation and satiety.

In addition, increasing thickness of a food has been found to reduce eating rate, thereby increase the duration of orosensory exposure (Zhu et al., 2013). Longer orosensory exposure has been shown to increase satiation effect of a food and decreased *ad libitum* intake of the food (Bolhuis et al., 2011). Therefore, the longer oral exposure of a thicker food may contribute to higher expectations on satiation and satiety, through a learned association between the oral sensory processing characteristic of a food and its digestive consequences.

5.4.4 Effect of sweetness perception on expectation

Increasing the sweetness of a custard increased expected satiation but did not affect expected satiety of the custard. Participants were not aware that while the sweetness intensity was changed, the energy content of the custards did not change. Informal verbal feedback from some of the participants following the study confirmed this. In typical foods and beverages, the sweet taste reflects the intake of sugars, which have the energy of 4 kcal/g. Participants may have assumed sweeter custards have higher sugar content, and hence produce more energy. Independent of energy content, the sweet taste alone from sugars and some non-caloric sweeteners has been shown to reduce actual feeling hunger and increase the feeling of fullness (Lavin et al., 2002b, Canty and Chan, 1991, Anderson and Woodend, 2003). Therefore, participants may have

associated sweeter custards with stronger satiating effect (e.g. reduce hunger, increase fullness), resulting in increased expected satiation.

It has previously been shown that the suppression of hunger from sugar (sucrose) preloads, especially with a low concentration, is a short-term effect, less than 2 hours (Anderson and Woodend, 2003). For example, sucrose showed no effect on satiety below 25g at a single dose, and only above 50g had sucrose been shown to reduce subsequent food intake 20-60 minutes later (Anderson, 1995). This may explain the absence of a significant impact of sweetness intensity on expected satiety (defined as the expected hunger 3h after consumption in the current study). Participants expected the sweetness of custards did not suppress hunger 3 hours after consumption.

5.4.5 Effect of caramel flavour on expectation

The intensity of perceived caramel flavour did not affect the expected satiation and expected satiety of the custards. This is consistent with the literature where flavour played no significant role in influencing expectations of the satiating capacities of a food (Hogenkamp et al., 2011, McCrickerd et al., 2012a). At the same time, the concentration of caramel aroma did not influence the expected satiation or expected satiety of the custards. Retro-nasal aroma release has been shown to induce satiation but not affect the subsequent food intake (Ruijschop et al., 2008a). Therefore, participants did not expect a higher intensity of perceived caramel flavour (or aroma) with a higher energy intake or a higher satiating effect.

5.4.6 Comparison with other factors with respect to expectation

Expected satiety has been found to vary markedly by up to 6-fold (600%) between different foods with differences in energy density, fat content and physical and sensory characteristics (Brunstrom et al., 2008b). In comparison, this thesis suggests that the model custards, same in

macronutrient composition and energy density but different in sweetness, caramel flavour and thickness perception, had a smaller but significant difference in expected satiation or expected satiety. Specifically, increasing the perceived thickness of the custards could increase the expected satiation by up to 21%, and increase expected satiety by up to 17%. Increasing the sweetness of the custards showed a smaller but also significant increase on expected satiation, by up to 6%. It might be the first time an explicit comparison of the effects of thickness and sweetness on expected satiating power of a food has been determined.

Expected satiation and expected satiety have been found to vary remarkably among foods; with some foods are expected to be 5-6 fold more satiating than other foods (Brunstrom et al., 2008a). The observed differences between different food categories were large, whereas differences between foods with similar characteristics were relatively subtle (Brunstrom, 2011).

5.4.7 Relationship between expected satiation and expected satiety

The expected satiation was highly correlated with the expected satiety of the custards. The expected immediate fullness of a food has been reported to correlate with the expected hunger 1 hour after the consumption of the food (McCrickerd et al., 2015). The confirmed correlation between expected satiation and expected satiety by the current study is not surprising. Because, by definition, expected satiation and expected satiety differed mainly in the anticipated time (immediately vs. 3 hours post consumption).

5.4.8 Evaluation of the paper scale

Banana, KitKat and sausage were chosen as the comparison foods, because they are widely consumed in the UK and were expected to be familiar to the UK consumers. In addition, the different amount of each selected food can be easily visualised and discriminated on pictures. Each

comparison food (banana, KitKat or sausage) was presented on an A3 paper scale. The size of a comparison food in each picture was smaller than its real size. Participants were presented with an actual food (e.g. a real banana), which would visually help them to imagine the amount of the comparison food that a picture represented. Participants informally reported that it was easy for them to compare their expectation of custards with the comparison foods on the paper scales. The between-subject coefficient of variation (CV) was 24-32% for the expected satiation (or satiety) of a custard. This was similar to the between-subject CV (21-35%) reported by Hogencamp (2011), who used a computer-based scale. In addition, custard sample S14 was rated twice by each participant. This allowed the assessment of the reproducibility of assessing the expectation using the paper scales. The expected satiation of S14 was reported as 315 ± 98 kcal (replicate 1) and 335 ±102 kcal (replicate 2), with a difference of 20 kcal. Its expected satiety was reported as 353 ± 110 kcal and 321 ± 102, with a difference of 22 kcal. This was comparable to another study, where the difference between two replicates was reported approximately 16 kcal (Brunstrom et al., 2008b). Therefore, the paper scales used in this study was considered reproducible and reliable in assessing expected satiation and expected satiety.

5.4.9 Significance of the findings

This study demonstrates that manipulating perceived thickness and sweetness without changing the energy content of custards can modify expectations about the satiating capacities of the custards. Expected satiation or expected satiety of a food determines the self-selected portion size of the food (Brunstrom and Rogers, 2009; Fay et al., 2011). A high-expected satiation was associated with a smaller selected portion size. In addition to its impact on self-selected portion size, a higher expected satiety of a food appears to make people feel less hungry and much fuller for up to three hours after the consumption of the food (Brunstrom et al., 2011). Expected satiety and expected satiation of a food are believed to

have been learned by humans from long-time eating experience, and they are not easily altered over repeated consumption (Hogenkamp et al., 2012). Therefore, increasing both the thickness and sweetness of foods may contribute to a smaller self-selected portion size, reduce energy intake, and might be helpful for weight control in a long term.

5.5 Conclusion

Decisions on the expected satiation and expected satiety of the custards depended on its perceived thickness and sweetness intensities, not the perceived caramel flavour intensity. The thickness of the custards played the most important role, which enhanced both expected satiation and expected satiety of custards. The increased sweetness intensity provided by a non-caloric sweetener also increased the expected satiation but not the expected satiety of the custards. This indicates a novel way to control self-selected portion size and regulate appetite sensations through the manipulation of perceived thickness and sweetness of a food without changing its actual energy content. However, participants were unaware that the differences in perceived sweetness intensity of custards were due to the varying addition of a non-caloric sweetener. Future research can be done to investigate if the sweetness from non-caloric sweeteners still affects the expected satiation of a food when participants are informed of the presence of non-caloric sweeteners. A next step could be to measure the actual portion size and the consumption of the custards, and to investigate to what extent the difference in expectation, caused by the difference in sweetness and thickness perception, affects the real-world meal size and energy intake.

Chapter 6. General Discussion

6.1 Aim of the thesis

The overall aim of the thesis was to investigate the role of food flavour (aroma, taste texture, or their cross-modal interaction) in human appetite control and eating behaviour; and therefore to explore how managing food flavour, as a novel approach, might help appetite and weight management.

6.2 Objectives and connection of individual studies

The overall aim of the thesis was achieved through three connected objectives which were explored in three independent studies (Chapters 3, 4 and 5). A summary of the key findings in each chapter is presented in **Table 6-1**.

Table 6-1 Summary of findings (grey areas: no measurements were conducted)

Flavour modalities	**Outcome measurements**						
	SSS Chapter 3	**Food intake**		**Subjective appetite sensations**		**Expected satiation** Chapter 5	**Expected satiety** Chapter 5
		Satiation Chapter 3	**Satiety** Chapter 4	**Satiation** Chapter 3	**Satiety** Chapters 4		
Taste	Intake of a sweet drink induced SSS for sweet foods; Sweetness intensity did not affect the extent of SSS; High sweetness intensity induced greater SSA for savoury foods.	No impact	No impact	No impact	No impact	Enhance	No impact
Aroma		No impact	No impact	No impact	No impact	No impact	No impact
Taste-aroma interaction		No impact	No impact	Reduce hunger during stimulation	Suppress hunger until 30 min after stimulation	No impact	No impact
Texture						Enhance	Enhance

In Chapter 3, the independent effect of taste (sweetness) on satiation and sensory-specific satiety was studied. The consumption of sweet milkshakes induced sensory-specific satiety for sweet foods in general; but satiation as measured by *ad libitum* intake of the milkshakes was not affected by sweetness intensity of the milkshakes. Moving beyond the independent effect of a single flavour modality (i.e. taste), the synesthetic effect of taste and aroma cross-modal interaction on satiation and satiety was further investigated in Chapter 4. The 'concurrent evaluation' of satiation (Chapter 2, section 2.2.1.1) through directly measuring the *ad libitum* milkshake intake (Chapter 3) did not provide the dynamic change in satiation within the event of milkshake consumption (Booth, 2009, Chapelot, 2013). Therefore, satiation was measured through a 'preloading paradigm' design (Chapter 2, section 2.2.1.2) in the study described by Chapter 4, which provided addition information of subjective appetite ratings during milkshake consumption (satiation) and after consumption (satiety).

Aroma and taste stimuli, when presented together, suppressed the feeling of hunger stronger, compared to the effect of individual aroma or taste stimulation (Chapter 4). The potential psychological mechanism behind this finding was further explored in Chapter 5. It was hypothesised that stronger flavour perception as a result of aroma-taste interaction may be associated by participants as more satiating than less intense flavour perception from single aroma or taste modality, through learned association (Brunstrom 2007, Yeomans, 2012). One of this association is expected satiation or expected satiety, which was evaluated in Chapter 5. Real-world energy control is not only influenced by feeling of hunger, but it is also determined, to a large extent, by expected satiation or expected satiety of the food decided even before the food consumption (Brunstrom, 2001, Fay et at., 2011). Understanding the effect of flavour on expected satiation or satiety provided additional understanding of the psychological aspect of flavour affecting eating behaviour.

It is worth mention that different sweeteners were used in different studies. Commercial low-caloric sweeteners (LCS) were used in the first study (Chapter 3: Canderal- mainly aspartame) and the third study (Chapter 5: Truvia- mainly stevia) in order to minimise or remove the difference in the energy content of samples. Sucrose was used in the second study (Chapter 4) due to that the samples were water-based beverage which may be difficult to mask any aftertaste of the artificial sweeteners (such as bitterness) in the samples. Bitterness is not congruent with the strawberry aroma which may affect the validity of the hypothesis and results of the study. However, it would be important to investigate the different influence of sugars and LCS sweeteners on appetite and eating behaviours in future research.

In addition, strawberry aroma was used in the second study (Chapter 4) while caramel aroma was used in the third study (Chapter 5). Strawberry liquid drink is commonly seen in the market and it is pleasant, but the strawberry aroma is usually matched with both sweet and sour tastes for flavour enhancement. In order to study the independent effect of sweetness on expected satiation, caramel aroma, which is usually associated with sugars and candies, was selected. Future research can explore the effect of a variety of aromas or flavours on appetite and eating behaviours.

6.3 Implications of findings and opportunity for future research

6.3.1 The role of taste in satiation and satiety

6.3.1.1 Summary of findings and implications

Consumption of all sweet milkshakes induced sensory-specific satiety (SSS) for sweet foods and the subsequent intake of savoury snacks were significantly higher than the intake of sweet snacks (Chapter 3). However, both the extent of SSS for sweet foods and subsequent snack intake was not affected by the sweetness intensity of the milkshakes. This supports the finding of another study, which shows that SSS is not affected by the

flavour intensity of a drink (Hetherington and Havermans, 2013, Bolhuis et al., 2010). Interestingly, a sensory-specific appetite (SSA) for savoury foods was induced after the consumption of a high-sweetness milkshake, but the SSA did not change after the consumption of low or ideal sweetness milkshakes. This suggests that SSA is affected by sweetness intensity of the pre-consumed milkshake. It might be the first time that an increased SSA for savoury foods has been demonstrated following the consumption of a sweeter drink compared with a less sweet drink. Observed SSA for savoury foods is unlikely to be driven by homoeostasis hunger, but rather it might be driven by either a hedonic hunger, resulting in an increased preference for the intake of uneaten savoury foods compared with the decreased preference for the eaten sweet foods (Hetherington and Havermans, 2013).

The sweetness-induced SSS or SSA limits the intake of eaten sweet foods and promotes intake of uneaten savoury food, which is biological adaptive to encourage the variety and balance of food intake (Hetherington and Havermans, 2013). This finding can potentially be applied to manipulate the intake of foods with sweet or savoury characteristics. For example, presenting consumers with a less sweet appetiser may lower their sensory-specific appetite for a subsequent savoury main meal, compared to a highly sweet appetiser that may promote stronger SSA for savoury foods and potentially result in a higher intake of subsequent savoury meal.

In Chapter 3, the effect of sweetness intensity of milkshakes on the *ad libitum* intake of the milkshakes was evaluated. Increasing the sweetness intensity of the milkshake did not affect the *ad libitum* intake of the milkshake. The novelty of this study, compared with the existing literature, was that the palatability of the milkshake was successfully controlled. In comparison, Zata et al. (1997) demonstrated a reduction in the *ad libitum* intake of a low-sweetness yogurt, compared with the same yogurt of high sweetness. However, the low-sweetness yogurt also had a significantly lower liking rating than the high-sweetness yogurt, indicating that the

observed difference in yogurt intake might have been a result of the difference in palatability of the yogurts. In comparison, in the current study (Chapter 3), having a similar palatability rating (difference < 10 % on VAS scale), a low-sweetness milkshake was consumed in a similar amount as a high-sweetness milkshake.

A small amount of sucrose (12g, 48 kcal), presented together with citric acid in the preload, did not change participants' subjective appetite sensation or subsequent food intake, when compared with a water preload (Chapter 4). In addition, participants did not compensate for the energy from sucrose by eating less at a subsequent meal. Some suggest that a sugar preload needs to be more than 50g in order to have any significant suppression on the subsequent energy intake (Anderson and Woodend, 2003). However, the lack of impact on subsequent energy intake by sugar preload (Chapter 4) may be a genius reflecting of the lack of compensation in energy intake following the intake of liquid sugar solution (Libuda and Kersting, 2009). Results from Chapter 4 supports the assumption that overconsumption of sugar-sweetened drinks might contribute, to some extent, to the excessive total energy intake which eventually results in overweight (Olsen and Heitmann, 2009). This implies that reducing sugar-sweetened beverage may be used to prevent overweight or obesity. In addition, high intense low caloric artificial sweeteners, when used in a beverage, may be helpful for weight control simply through reducing the total energy intake by replacing sugars.

In humans, food intake is not only controlled by our feelings of hunger and satiation within an eating event, rather, we learn to control our meal size through cognitive activities such as decisions about meal planning made even before eating (Brunstrom, 2011). The role of sweetness intensity on expected satiation was unresolved in the literature, where contrasting results were found (Hogenkamp et al., 2011). This may due to other uncontrolled attributes which may override or confound the independent effect of sweetness intensity on expected satiation. In the study described

in Chapter 5, the independent effects of sweetness in custards were studied as well as texture and flavour attributes through a robust experimental design approach where other factors, such as energy density, nutritional composition and familiarity were kept similar between custard samples. This thesis has confirmed that increasing the perceived sweetness intensity increased participant' expected satiation in a controlled food model. Although liking was uncontrolled across custards, it was unlikely to affect the results since expected satiation has been found to be unaffected by liking (Hogenkamp et al., 2011).

The fact that increasing the sweetness of custards increases the expected satiation but not expected satiety (3h after consumption), may be due to a learned association. Participants may learn to associate the sweet taste with the metabolic consequences of sugars. Sugars have been shown to induce satiation but its effect on satiety lasts often than 2 hours (Anderson and Woodend, 2003). It seems that participants showed a good ability to express their cognitive expectation with the capability to differentiate between satiation and satiety effects. As far as the author knows, this is the first study show that increasing sweetness intensity affected expected satiation, but not expected satiety. Expected satiation determines the meal size, to a greater extent than liking, especially in a self-selected setting (Brunstrom and Rogers, 2009). Therefore, it suggests that the participants might select a smaller portion if the food is perceived to be much sweeter. In addition, the sweetness in custards was manipulated by the concentration of non-energetic artificial sweetness. This implies that artificial sweeteners, used as sugar substances, have a potentially positive role in limiting self-selected portion size and energy consumption, in addition to reducing the energy content of the product by replacing sugars.

6.3.1.2 Opportunities for future research

A sensory-specific appetite (SSA) for savoury foods was observed after the consumption of a high-sweetness milkshake (Chapter 3). Similarly, an increasing in SSA for sweet foods has been inducted after the

consumption of savoury foods (Weenen, et al., 2005). Sweet and savoury are contrasting tastes that divide foods into general sweet and savoury categories. Future research may be interesting to explore whether SSA for an aroma could be induced by the stimulation of its contrasting aromas, such as beef aroma (savoury) vs chocolate aroma (sweet). Understanding the effect of contrasting taste or aroma on SSA may direct our diet to a healthy balance.

Sucrose, when presented with citric acid and strawberry aroma in a liquid preload, reduced subjective feeling of hunger (Chapter 4). Artificial sweeteners may have different sweetness perception profile from sugars. For example, the time and intensity profile for sweetness intensity has been shown to be different between sucrose and aspartame (Pfeiffer et al., 2000). Sucrose reached its maximum sweetness intensity higher and quicker than aspartame; while the sweetness of aspartame lasted longer than the sweetness of sucrose (Pfeiffer et al., 2000). Future research could investigate if high intense low-caloric sweeteners (LSC) such as stevia or aspartame could have a similar effect on suppression hunger as sucrose as demonstrated in Chapter 4.

Increasing the sweetness intensity of custards by increasing the concentration of a commercial low caloric artificial sweetener increased expected satiation of custards (Chapter 5). However, participants were not aware of the addition of the non-caloric sweetener. Future research can be done to investigate if the sweetness from non-caloric sweeteners still affects the expected satiation of a food when participants are informed of the presence of non-caloric sweeteners. A next step would be to investigate if actual portion size and energy intake corresponds to the expected satiation of both sugar and LCS sweetened foods.

Sweetness has been shown to induce satiation and short-term satiety (Anderson and Woodend, 2003). Potentially, the sour taste in the beverage might have an appetising effect, which neutralises the effect of sweet taste on satiation (Chapter 4). However, little is known about how

sour taste and citric acid affects appetite and intake. Further investigation should be undertaken to investigate the effect of other taste, such as sour and bitter, on appetite and eating behaviour.

6.3.2 The role of aroma in satiation and satiety

6.3.2.1 Summary of findings and implications

Strawberry aroma alone, delivered retronasally via drinking, did not affect subjective appetite sensation and subsequent food intake, when compared with water control (Chapter 4). Increasing the intensity of the retronasal strawberry aroma, delivered in a milk drink, has previously been shown to increase the sensation of satiation, but this effect was observed with the concurrent delivery of sweet taste in the context of a milk drink (Ruijschop et al., 2008a). In this thesis, when the strawberry aroma was delivered together with sweet and sour tastes in a drink, the sensation of hunger reduced significantly, more than when water control was delivered. Therefore, it suggests that retronasal aroma can promote satiation, but only when the aroma is delivered simultaneously with congruent taste stimuli. Thus, retronasal aroma needs to be associated with congruent taste sensation in order to have a noticeable impact on appetite sensation. This may be due to that the enhancement of flavour perception because of aroma-taste cross-modal interaction suppresses hunger feelings more than the effect of a single flavour modality (aroma or taste).

Sugar-sweetened beverages have been implicated in the increasing obesity worldwide, because of the low satiation and satiety capacities of the highly palatable sugars (Malik et al., 2006). This thesis suggests a positive role of sweet-congruent aroma in increasing satiation effect of sugar-sweetened beverages. The amount of aroma present in a food or drink is typically very low when compared to other ingredients and it is energy free. Adding a sweet-congruent aroma into a sugar-sweetened drink may increase the feeling of fullness and reduce the feeling of hunger following consumption without increasing the total energy intake.

This thesis investigated the effect of aroma on consumer's cognitive expected satiation and satiety of foods (Chapter 5). The self-selected portion size, hence energy consumption, is reduced when food is expected to be more satiating (Brunstrom, 2011). Increasing the concentration of caramel aroma in the custard samples did not change consumer's expectation about the satiation capacities of the custards (chapter 5). This finding is consistent with the current literature where increasing the addition of aroma did not affect the expected satiation or expected satiety (Hogenkamp et al., 2011, McCrickerd et al., 2012a). In other words, people do not make a decision about the size of their meal that would make them feel satiated, based on the aroma profile of the meal. Potentially, participants did not associate the increase in aroma perception with increased energy intake through learned association. However, the addition of caramel aroma did increase the perception of sweetness intensity slightly and higher sweetness intensity increased expected satiation. If an aroma increases the perception of sweetness, such aroma might indirectly increase expected satiation via its enhancement on sweetness perception.

6.3.2.2 Opportunities for future research

It is suggested that retronasal aroma needs to be associated with a congruent taste sensation in order to have a noticeable impact on suppressing the feeling of hunger (Chapter 4). This theory can be further tested by presenting subjects with incongruent taste and retronasal aroma in following-up research. For example, it would be interesting to investigate whether subjective feeling of hunger would be suppressed if strawberry aroma was presented with salty taste (incongruent).

Caramel aroma did not have a significant impact on expected satiation but it enhanced the sweetness perception (Chapter 5). A future study could investigate whether a highly sweet-congruent aroma which enhances sweetness intensity to a greater extent could enhance expected satiation.

6.3.3 The role of texture in satiation and satiety

6.3.3.1 Summary of findings and implication

The effect of texture on satiation and satiety has received much more attention than aroma and taste. This thesis focused on a less studied question about texture: how it may affect consumer's expectation about the satiating capacities of foods. Participants expected thicker custard to make them feel fuller than thin custard, immediately after consumption and 3 hours after consumption (Chapter 5). These findings supported the results of studies by Hogenkamp et al. (2011). They demonstrated a positive correlation between expected satiation and thickness. In addition, McCrickerd et al. (2012) found a positive correlation between expected satiety and thickness. The increase in expectation by increasing thickness may be due to a longer oral sensory exposure time (Zijlstra et al., 2009), and or due to a learned association of the thickness with actual satiation and satiety.

Texture has been expected to have a stronger effect on satiation and satiety than taste (Rolls, 2012, Hogenkamp et al., 2011). This study is the first to show a clear difference in the magnitude and duration of such expectation between texture and taste. Increasing the thickness of custards increased the expected satiation and expected satiety 3 hours after consumption, while increasing sweetness only increased the expected satiation but not expected satiety. In addition, the increase in expected satiation resulting from the increased thickness was greater than by the increased sweetness. This implies manipulation of texture may make a more effective contribution to portion-size control than manipulating flavour.

6.3.3.2 Opportunities for future research

Increasing the concentration of CMC enhanced the expected satiation and expected satiety by increasing the thickness of custards (Chapter 5). Tarrega A. et al. (2014) have shown that the thickened capacity (viscosity)

rather than the elasticity of 4 different hydrocolloids (hydroxypropyl methylcellulose HPMC, carrageenan, alginate and xanthan gum) enhanced expected satiety of milk products. Future work can be done to explore the effect of other texture properties, such as mouthfeel, hardness, chewiness or smoothness on expected satiation and expected satiety. For example, harder or more chewiness foods may be associated with a longer oral sensory processing time, greater effort for consumption, or a delayed gastric emptying, resulting in increased expectation on the satiating capacity of such foods (Forde et al., 2013). Further investigation of the role texture properties of foods in cognitive expectation is promising for effective portion-size control and helpful for the understanding of cognitive mechanism of eating our behaviour.

6.3.4 The role of multi-modal perception in satiation and satiety

6.3.4.1 Summary of findings and implication

The interactive effect of different flavour modalities, i.e. tastes, aroma and texture have not been particularly studied in comparison to the independent effect of each modality on appetite. However, flavour perception is a synergistic effect of different modalities rather than simply the sum of them. This thesis studied the interactive effect of aroma and taste on appetite and intake. When congruent taste and aroma were presented together in drinks, the overall flavour perception increased and the subjective feeling of hunger reduced, compared to a single taste or aroma modality, or the water control (Chapter 4). Strawberry aroma worked together with sweet-sour taste, to reduce the feeling of hunger, which is possibly a result of their cross-modal enhancement of flavour perception. Strawberry aroma and sweet-sour taste are congruent flavour modalities, which enhance the overall flavour perception more than the sum of the contribution of each modality (Pfeiffer et al., 2006). Enhanced flavour perception leads to a greater oral sensory exposure, which might be associated with increased satiation (Bolhuis et al., 2011). The

mechanism behinds this observation is unknown, but it is potentially due to the combined effects of physiological and psychological processes.

Currently, the areas of the brain identified as integrating taste and aroma include insula (flavour recognition and intensity), OFC (pleasure, reward and affection), amygdala (pleasure and reward) and ACC (cognitive control, e.g. reward and anticipation) (Marciani et al., 2010). Although not identified, taste and aroma may integrate at the lateral hypothalamic area (LHA: the satiation and satiety centre), based on their neural pathways; thus, both project into the hypothalamus (Marciani et al., 2010). It has been found that the activation of LHA suppressed appetite and decrease intake (Anand and Brobeck, 1951). The observed suppression of the hunger sensation from the aroma-taste preload is potentially the result of aroma-taste integration at LHA, in addition to the integration in the pleasure and reward centre of OFC. For example, when aroma and taste were presented together, the activation signals of LHA may be stronger than the sum effect of aroma and taste, resulting in significant suppression of the hunger feeling, compared with the presence of a single aroma or taste stimuli, and the water control.

It is suspected that higher flavour intensity is associated, consciously or unconsciously, with higher nutrition values, and hence it is perceived to be more satiating in foods (Yeomans, 2012). Until recently, little was known about the flavour-nutrient association. One of the conscious association is expected satiation and expected satiety (Yeomans, 2012, Brunstrom, 2007). Expected satiation and expected satiety is the cognitive expectation of how full the food is going to make a person feel, prior to consumption. Cognitive expectation has been shown to influence the actual subjective feelings of hunger and fullness (Brunstrom et al., 2011). However, in this thesis, the drink with both aroma and taste was not expected to be more filling compared to the drink with only taste; but the former reduced the feeling of hunger more significantly than the later (Chapter 4). Results in Chapter 5 further confirmed that the expected satiation and expected

satiety of custards was not affected by the perceived caramel flavour intensities. Therefore, cognitive psychological process, at least with respect to the expectation in the satiating capacities of foods, might not explain why increased flavour intensity through cross-modal interaction suppressed the feeling of hunger.

The hunger suppressing effect of an intense flavour perception from aroma-taste interaction may have a physiological mechanism. Hunger was suppressed by only smelling a dark chocolate, and it correlated with a decrease in the ghrelin (the hunger hormone) (Massolt et al., 2010). Potentially, when aroma and taste were presented together, the release of hunger hormone ghrelin may be suppressed and the release of satiety hormones such as CCK, GLP-1 or even PYY may be increased accordingly.

In addition, no change in subsequent energy intake by manipulation of flavour preloads was observed (Chapter 4). The subsequent pasta lunch was served to participants 65 minutes after they consumed the flavour drink preload. The difference in hunger ratings from the different preloads was no longer observed by lunchtime. If the lunch were served earlier, when the difference in hunger ratings was still significant across preloads, potentially, it might result in an actual reduction in food intake. However, it may still be challenging to obtain an actual reduction in energy intake, since other studies did not obtain a reduction in food intake from low-caloric flavour drinks (Ruijschop et al., 2009b, Bellisle et al., 2012).

Despite a lack of an observed reduction in the actual food intake, the fact that the manipulation of flavour intensity reduced the feeling of hunger is promising for weight management. For example, the flavour may simply be used to reduce the dysphoria of hunger feelings when the energy intake has already been controlled by consumption of low-energy diets, reduced-energy foods or meal replacements (Blundell et al., 2010).

6.3.4.2 Opportunities for future research

Further study could be done to investigate if the observed hunger suppressing effect from congruent taste and aroma can be repeated in other flavour combination, such as savoury taste and beef flavour. Subsequent energy intake can be measured more closely following the flavour preload, in order to investigate if the observed hunger suppressing effect can result in a significant reduction in food energy intake.

Clinical trials would further be carried out to investigate the physiological response behind observed behaviour response of flavour induced satiation and satiety. This may include the evaluation of changes in hunger hormone Ghrelin, satiation hormone CCK and GLP-1, and satiety hormone PYY (Harrold et al., 2012). Gut hormonal responses to the flavour perception can be determined by analysing the blood samples at different time points (Blom et al., 2004). Corresponding neurology response at the LHA of hypothalamus can also be monitored by fMRI technique (Rolls, 2012).

Furthermore, other modalities could be studied to understand the cross-modal flavour induced satiation or satiety. For example, sensory modalities including colours, sounds and temperature have been shown to influence flavour perception at a cross-modal level (Auvray and Spence, 2008). It would be important to investigate whether colours, sounds or temperature can interact with taste and aroma to influence satiation and satiety in future research.

The studies on the effect of flavour perception of foods on subjective appetite sensations, food intake and sensory-specific satiety were conducted only on normal subjects (BMI within18.9 - 24.9 kg/m^2). Findings show positive implications for food intake control and weight management. Obese and overweight individuals have demonstrated different appetite behaviour responses towards food consumptions compared with the normal weight group (Ekuni et al., 2013). Future studies should be done to investigate if the same findings observed in this thesis could be applied to obese and overweight individuals for intake and weight reduction.

6.4 Strength, limitation and improvement for future work

One of the strengths of this thesis is that participants' baseline appetite sensation was well controlled when studying the effect of food flavour on subjective appetite sensation and food intake (Chapters 3 & 4). Before the exposure of food flavour stimuli, participants were served with a standard breakfast, and then fast while sitting in the laboratory, in order to standardise their baseline appetite sensations. The baseline appetite status of participants might influence appetite response towards a food cue (Bolhuis et al., 2012). Therefore, in this thesis, the careful experimental control of participants' initial appetite status might have increased the likelihood of finding a significant effect of a flavour cue on appetite sensation and food intake.

Participants voluntarily consumed a test meal in a standard serving size until they felt comfortably full. One limitation of this method was that participants were able to monitor visually the amount of foods or drinks that had consumed from the containers. Eating behaviours are highly habitual, and plate cleaning habitually happens at most of our meal events (Khare and Inman, 2006, Wansink et al., 2005, Fay et al., 2011). Individuals may habitually consume a fixed amount of the meal according to their visual monitoring of the portion size, instead of responding to their internal satiation or satiety cues. In this thesis, the effect of habitual eating (e.g. plate cleaning) on food intake was minimised by giving participants clear instructions: to terminate eating only according to their feeling of fullness. Once reaching their satiation, participants were encouraged to stop eating even if the food or drink in the serving container was not complete, and to ask for another portion once the current portion was finished. A future study could use a bottomless bowl (e.g. self-refilling bowl) so that it might be difficult for participants to monitor the amount of foods that have been consumed (Bolhuis et al., 2011, Wansink et al., 2005).

In addition, the number of participants completed the study in Chapter 3 was 24. According to a pre-study power calculation, 24 participants might

be able to detect a significant difference in milkshake intake, if the mean difference was more than 52g. However, the maximum mean difference in intake between two milkshakes with different sweetness was 35g. Therefore, the sample size in the study might be not large enough to detect such a small difference in energy intake. Based on the results from Chapter 3 study, in order to detect a 35g difference in *ad libitum* intake of milkshakes, with a standard deviation of 100g, a minimum of 65 participants is needed at a statistical power of 80%. The number of participants completed the study in Chapter 4 was 26, which was sufficient to detect a difference in appetite sensations according to the power calculation. However, a sample size of 26 might not be sufficient for the detection of a small difference in food intake. Based on results from Chapter 4 study, in order to detect a 22kcal difference in the intake subsequent drinks, with a standard deviation of 80kcal, a minimum of 104 participants is needed at a statistical power of 80%. Therefore, future study should use a large number of participants, e.g. above 150 participants, in order to obtain a significant impact of flavour on food energy intake.

6.5 General conclusion

Changing the sweetness intensities of milkshakes by adding a different amount of a low-caloric sweetener (Canderal) did not affect the *ad libitum* intake of the milkshakes, even when the milkshakes were similar in pleasantness. This might be the first time when the effect of sweetness intensity of a food on its *ad libitum* intake has been studied independently of the effect of palatability of the food. After consuming the sweet milkshakes of all sweetness intensities, participants developed the sensory-specific satiety for the sweetest milkshake and extended sensory-specific satiety for general sweet foods, which is consistent with the current literature. However, the sweetness intensity of the milkshakes did not affect the extent of sensory-specific satiety for the milkshake or general sweet foods. Interestingly, pre-consumption of the sweetest milkshake induced a sensory-specific appetite for general savoury foods, while the sensory-specific appetite for savoury foods was unchanged following the consumption of less sweet milkshakes. This might be the first study that demonstrates the development of stronger sensory-specific satiety for savoury foods following the consumption of a sweeter food.

Taste or aroma stimuli presented alone in a drink preload neither suppressed appetite sensation or reduced the subsequent energy intake. However, a beverage activating multiple flavour modalities (congruent taste and aroma) is more satiating than a beverage activating a single flavour modality (taste or aroma) and the water control. This suggests that enhanced flavour perception caused by aroma-taste cross-modal interaction can suppress hunger and increase fullness, which might add new knowledge to the current literature.

Decisions on the expected satiation and expected satiety of a model food (custard) depended on its thickness and taste properties, rather than the aroma cues. Thickness enhanced both expected satiation and expected satiety. The increased sweetness intensity provided by a non-caloric sweetener also increased expected satiation but not expected satiety. This

indicates a way to control self-selected portion size through the manipulation of the thickness and sweetness of a food without changing its actual energy content.

In conclusion, manipulating food flavour, as a novel approach, is a promising area to explore with the respect to suppressing hunger and increasing fullness sensations; affecting sensory-specific satiety and sensory-specific appetite; and its contribution to a cognitive control of portion size. Therefore, it might be potentially helpful in appetite control, but it seems difficult to obtain an actual reduction in energy intake by only manipulating food flavour.

Reference

ABBOTT, C. R., MONTEIRO, M., SMALL, C. J., SAJEDI, A., SMITH, K. L., PARKINSON, J. R. C., GHATEI, M. A. & BLOOM, S. R. 2005. The inhibitory effects of peripheral administration of peptide YY3-36 and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Research,* 1044**,** 127-131.

ADRIAN, T. E., FERRI, G. L., BACARESEHAMILTON, A. J., FUESSL, H. S., POLAK, J. M. & BLOOM, S. R. 1985. Human Distribution and Release of a Putative New Gut Hormone, Peptide-Yy. *Gastroenterology,* 89**,** 1070-1077.

AHIMA, R. S. & ANTWI, D. A. 2008. Brain Regulation of Appetite and Satiety. *Endocrinology and Metabolism Clinics of North America,* 37**,** 811-+.

AKIEDA-ASAI, S., POLENI, P. E. & DATE, Y. 2014. Coinjection of CCK and leptin reduces food intake via increased CART/TRH and reduced AMPK phosphorylation in the hypothalamus. *American Journal of Physiology-Endocrinology and Metabolism,* 306**,** E1284-E1291.

AMSTERDAM, J. D., SETTLE, R. G., DOTY, R. L., ABELMAN, E. & WINOKUR, A. 1987. Taste and Smell Perception in Depression. *Biological Psychiatry,* 22**,** 1481-1485.

ANAND, B. K. & BROBECK, J. R. 1951. Hypothalamic Control of Food Intake in Rats and Cats. *Yale Journal of Biology and Medicine,* 24**,** 123-140.

ANDERSON, G. H. 1995. Sugars, Sweetness, and Food-Intake. *American Journal of Clinical Nutrition,* 62**,** 195s-202s.

ANDERSON, G. H. & WOODEND, D. 2003. Consumption of sugars and the regulation of short-term satiety and food intake. *American Journal of Clinical Nutrition,* 78**,** 843s-849s.

ASTBURY, N. M., TAYLOR, M. A. & MACDONALD, I. A. 2011. Breakfast Consumption Affects Appetite, Energy Intake, and the Metabolic and Endocrine Responses to Foods Consumed Later in the Day in Male Habitual Breakfast Eaters. *Journal of Nutrition,* 141**,** 1381-1389.

AUVRAY, M. & SPENCE, C. 2008. The multisensory perception of flavor. *Consciousness and Cognition,* 17**,** 1016-1031.

BACHMANOV, A. A. & BEAUCHAMP, G. K. 2007. Taste receptor genes. *Annual Review of Nutrition,* 27**,** 389-414.

BATTERHAM, R. L., COHEN, M. A., ELLIS, S. M., LE ROUX, C. W., WITHERS, D. J., FROST, G. S., GHATEI, M. A. & BLOOM, S. R. 2003. Inhibition of food intake in obese subjects by peptide YY3-36. *New England Journal of Medicine,* 349**,** 941-948.

BATTERHAM, R. L., COWLEY, M. A., SMALL, C. J., HERZOG, H., COHEN, M. A., DAKIN, C. L., WREN, A. M., BRYNES, A. E., LOW, M. J., GHATEI, M. A., CONE, R. D. & BLOOM, S. R. 2002. Gut hormone PYY3-36 physiologically inhibits food intake. *Nature,* 418**,** 650-654.

BATTERHAM, R. L., HEFFRON, H., KAPOOR, S., CHIVERS, J. E., CHANDARANA, K., HERZOG, H., LE ROUX, C. W., THOMAS, E. L., BELL, J. D. & WITHERS, D. J. 2006. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metabolism,* 4**,** 223-233.

BAVER, S. B., HOPE, K., GUYOT, S., BJORBAEK, C., KACZOROWSKI, C. & O'CONNELL, K. M. S. 2014. Leptin Modulates the Intrinsic Excitability of AgRP/NPY Neurons in the Arcuate Nucleus of the Hypothalamus. *Journal of Neuroscience,* 34**,** 5486-5496.

BEAR, M. K., CONNORS, B. W. & PARADISO, M. A. 2001. *Neuroscience: exploring the brain,* USA, Lippincott Williams & Wilkins

BECK, A. T., STEER, R. A. & GARBIN, M. G. 1988. Psychometric Properties of the Beck Depression Inventory - 25 Years of Evaluation. *Clinical Psychology Review,* 8**,** 77-100.

BECK, A. T., WARD, C. H., MENDELSON, M., MOCK, J. & ERBAUGH, J. 1961. An inventory for measuring depression. *Archives of General Psychiatry,* 4**,** 561-571.

BEGLINGER, C., DEGEN, L., MATZINGER, D., D'AMATO, M. & DREWE, J. 2001. Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* 280**,** R1149-R1154.

BELL, E. A., CASTELLANOS, V. H., PELKMAN, C. L., THORWART, M. L. & ROLLS, B. J. 1998. Energy density of foods affects energy intake in normal-weight women. *American Journal of Clinical Nutrition,* 67**,** 412-420.

BELLISLE, F. & BLUNDELL, J. E. 2013. Satiation, satiety: concepts and organisation of behaviour. *Satiation, Satiety and the Control of Food Intake: Theory and Practice***,** 3-11.

BELLISLE, F., DALIX, A. M., AIRINEI, G., HERCBERG, S. & PENEAU, S. 2009. Influence of dietary restraint and environmental factors on meal size in normal-weight women. A laboratory study. *Appetite,* 53**,** 309-313.

BELLISLE, F., DREWNOWSKI, A., ANDERSON, G. H., WESTERTERP-PLANTENGA, M. & MARTINS, C. K. 2012. Sweetness, Satiation, and Satiety. *Journal of Nutrition,* 142**,** 1149s-1154s.

BEN SLAMA, F., JRIDI, N., BEN RAYANA, M. C., TRIMECHE, A., HSAIRI, M. & BELHADJ, O. 2015. Plasma levels of leptin and ghrelin and their correlation with BMI, and circulating lipids and glucose in obese Tunisian women. *Asian Biomedicine,* 9**,** 161-168.

BENELAM, B. 2009. Satiation, satiety and their effects on eating behaviour. *Nutrition Bulletin,* 34**,** 126-173.

BERRIDGE, K. C. 1996. Food reward: Brain substrates of wanting and liking. *Neuroscience and Biobehavioral Reviews,* 20**,** 1-25.

BERTHOUD, H. R. 2007. Interactions between the "cognitive" and "metabolic" brain in the control of food intake. *Physiology & Behavior,* 91**,** 486-498.

BERTHOUD, H. R. & MORRISON, C. 2008. The brain, appetite, and obesity. *Annual Review of Psychology,* 59**,** 55-92.

BIRCH, L. L. & DEYSHER, M. 1986. Caloric Compensation and Sensory Specific Satiety - Evidence for Self Regulation of Food-Intake by Young-Children. *Appetite,* 7**,** 323-331.

BLOM, W., DE GRAAF, C., SMEETS, P., STAFLEU, A. & HENDRIKS, H. 2004. Biomarkers of satiation and satiety: A review. *International Journal of Obesity,* 28**,** S214-S214.

BLUNDELL, J., DE GRAAF, C., HULSHOF, T., JEBB, S., LIVINGSTONE, B., LLUCH, A., MELA, D., SALAH, S., SCHURING, E., VAN DER KNAAP, H. & WESTERTERP, M. 2010. Appetite control: methodological aspects of the evaluation of foods. *Obesity Reviews,* 11**,** 251-270.

BLUNDELL, J. E. & BELLISLE, F. 2013. Satiation, satiety: concept and organisation of behaviour. *Satiation, Satiety and the Control of Food Intake: Theory and Practice.* Cambridge: Woodhead Publishing.

BLUNDELL, J. E. & BURLEY, V. 1987. Satiation, Satiety and the Action of Fiber on Food-Intake. *International Journal of Obesity,* 11**,** 29-29.

BLUNDELL, J. E. & FINLAYSON, G. 2004. Is susceptibility to weight gain characterized by homeostatic or hedonic risk factors for overconsumption? *Physiology & Behavior,* 82**,** 21-25.

BLUNDELL, J. E., LAWTON, C. L., COTTON, J. R. & MACDIARMID, J. I. 1996. Control of human appetite: implications for the intake of dietary fat. *Annu Rev Nutr,* 16**,** 285-319.

BLUNDELL, J. E., ROGERS, P. J. & HILL, A. J. 1987. Evaluating the satiating power of foods: implications for acceptance and consumption *Food acceptance and nutrition***,** 205-220.

BOLAND, A. B., DELAHUNTY, C. M. & VAN RUTH, S. M. 2006. Influence of the texture of gelatin gels and pectin gels on strawberry flavour release and perception. *Food Chemistry,* 96**,** 452-460.

BOLHUIS, D. P., LAKEMOND, C. M. M., DE WIJK, R. A., LUNING, P. A. & DE GRAAF, C. 2010. Effect of Salt Intensity on Ad Libitum Intake of Tomato Soup Similar in Palatability and on Salt Preference after Consumption. *Chemical Senses,* 35**,** 789-799.

BOLHUIS, D. P., LAKEMOND, C. M. M., DE WIJK, R. A., LUNING, P. A. & DE GRAAF, C. 2011. Both Longer Oral Sensory Exposure to and Higher Intensity of Saltiness Decrease Ad Libitum Food Intake in Healthy Normal-Weight Men. *Journal of Nutrition,* 141**,** 2242-2248.

BOLHUIS, D. P., LAKEMOND, C. M. M., DE WIJK, R. A., LUNING, P. A. & DE GRAAF, C. 2012. Effect of salt intensity in soup on ad libitum intake and on subsequent food choice. *Appetite,* 58**,** 48-55.

BOMER, I., SAURE, C., CAMINITI, C., RAMOS, J. G., ZUCCARO, G., BREA, M., BRAVO, M. & MAZA, C. 2015. Comparison of energy expenditure, body composition, metabolic disorders, and energy intake between obese children with a history of craniopharyngioma and children with multifactorial obesity. *Journal of Pediatric Endocrinology & Metabolism,* 28**,** 1305-1312.

BOOTH D.A., T. A., SHAHEDIAN B. 1983. A robust brief measure of an individual's most preferred level of salt in an ordinary foodstuff *Appetite,* 4**,** 301-302.

BOOTH, D. A. 2009. Learnt reduction in the size of a meal. Measurement of the sensory-gastric inhibition from conditioned satiety. *Appetite,* 52**,** 745-749.

BORZOEI, S., NEOVIUS, M., BARKELING, B., TEIXEIRA-PINTO, A. & ROSSNER, S. 2006. A comparison of effects of fish and beef protein on satiety in normal weight men. *European Journal of Clinical Nutrition,* 60**,** 897-902.

BRAND, G. 2006. Olfactory/trigeminal interactions in nasal chemoreception. *Neuroscience and Biobehavioral Reviews,* 30**,** 908-917.

BRAUSS, M. S., BALDERS, B., LINFORTH, R. S. T., AVISON, S. & TAYLOR, A. J. 1999. Fat content, baking time, hydration and temperature affect flavour release from biscuits in model-mouth and real systems. *Flavour and Fragrance Journal,* 14**,** 351-357.

BROSSARD, C. D., LETHUAUT, L., BOELRIJK, A. E. M., MARIETTE, F. & GENOT, C. 2006. Sweetness and aroma perceptions in model dairy desserts: an overview. *Flavour and Fragrance Journal,* 21**,** 48-52.

BRUNSTROM, J. M. 2007. Associative learning and the control of human dietary behavior. *Appetite,* 49**,** 268-271.

BRUNSTROM, J. M. 2011. The control of meal size in human subjects: a role for expected satiety, expected satiation and premeal planning. *Proceedings of the Nutrition Society,* 70**,** 155-161.

BRUNSTROM, J. M., BROWN, S., HINTON, E. C., ROGERS, P. J. & FAY, S. H. 2011. 'Expected satiety' changes hunger and fullness in the inter-meal interval. *Appetite,* 56**,** 310-315.

BRUNSTROM, J. M., COLLINGWOOD, J. & ROGERS, P. J. 2010a. Perceived volume, expected satiation, and the energy content of self-selected meals. *Appetite,* 55**,** 25-29.

BRUNSTROM, J. M. & MITCHELL, G. L. 2006. Effects of distraction on the development of satiety. *British Journal of Nutrition,* 96**,** 761-769.

BRUNSTROM, J. M. & ROGERS, P. J. 2009. How Many Calories Are on Our Plate? Expected Fullness, Not Liking, Determines Meal-size Selection. *Obesity,* 17**,** 1884-1890.

BRUNSTROM, J. M., ROGERS, P. J., POTHOS, E. M., CALITRI, R. & TAPPER, K. 2008a. Estimating everyday portion size using a 'method of constant stimuli': In a student sample, portion size is predicted by gender, dietary behaviour, and hunger, but not BMI. *Appetite,* 51**,** 296-301.

BRUNSTROM, J. M., SHAKESHAFT, N. G. & ALEXANDER, E. 2010b. Familiarity changes expectations about fullness. *Appetite,* 54**,** 587-590.

BRUNSTROM, J. M., SHAKESHAFT, N. G. & SCOTT-SAMUEL, N. E. 2008b. Measuring 'expected satiety' in a range of common foods using a method of constant stimuli. *Appetite,* 51**,** 604-614.

BRUNSTROM, J. M. & SHAKESHAFT, N. S. G. 2009. Measuring affective (liking) and non-affective (expected satiety) determinants of portion size and food reward. *Appetite,* 52**,** 108-114.

BRYANT, B. & MEZINE, I. 2002. Pungency and tingling: Sensations and mechanisms of trigeminal chemical sensitivity. *Chemistry of Taste: Mechanisms, Behaviors, and Mimics,* 825**,** 202-212.

BUCK, L. B. 2004. Olfactory receptors and odor coding in mammals. *Nutrition Reviews,* 62**,** S184-S188.

BULT, J. H. F., DE WIJK, R. A. & HUMMEL, T. 2007. Investigations on multimodal sensory integration: Texture, taste, and ortho- and retronasal olfactory stimuli in concert. *Neuroscience Letters,* 411**,** 6-10.

BURDACH, K. J., KROEZE, J. H. A. & KOSTER, E. P. 1984. Nasal, Retronasal, and Gustatory Perception - an Experimental Comparison. *Perception & Psychophysics,* 36**,** 205-208.

CALLAHAN, H., CUMMINGS, D., MATTHYS, C., BREEN, P. & WEIGLE, D. 2003. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load in humans. *Diabetes,* 52**,** A347-A347.

CANTY, D. J. & CHAN, M. M. 1991. Effects of Consumption of Caloric Vs Noncaloric Sweet Drinks on Indexes of Hunger and Food-Consumption in Normal Adults. *American Journal of Clinical Nutrition,* 53**,** 1159-1164.

CARPENTER, R. H. S. 2003. *Neurophysiology,* London, Oxford University Press.

CARSTENS, E., SIMONS, C. T., DESSIRIER, J. M., CARSTENS, M. I. & JINKS, S. L. 2000. Role of neuronal nicotinic-acetylcholine receptors in the activation of neurons in trigeminal subnucleus caudalis by nicotine delivered to the oral mucosa. *Experimental Brain Research,* 132**,** 375-383.

CEGLA, J., TAN, T. M. & BLOOM, S. R. 2010. Gut-brain cross-talk in appetite regulation. *Current Opinion in Clinical Nutrtion & Metabolic Care,* 13**,** 588-93.

CERF-DUCASTEL, B., VEN DE MOORTELE, P. F., MACLEOD, P., LE BIHAN, D. & FAURION, A. 2001. Interaction of gustatory and lingual somatosensory perceptions at the cortical level in the human: a functional magnetic resonance imaging study. *Chemical Senses,* 26**,** 371-383.

CHANDRASHEKAR, J., HOON, M. A., RYBA, N. J. P. & ZUKER, C. S. 2006. The receptors and cells for mammalian taste. *Nature,* 444**,** 288-294.

CHANDRASHEKAR, J., KUHN, C., OKA, Y., YARMOLINSKY, D. A., HUMMLER, E., RYBA, N. J. P. & ZUKER, C. S. 2010. The cells and peripheral representation of sodium taste in mice. *Nature,* 464**,** 297-U182.

CHANDRASHEKAR, J., MUELLER, K. L., HOON, M. A., ADLER, E., FENG, L. X., GUO, W., ZUKER, C. S. & RYBA, N. J. P. 2000. T2Rs function as bitter taste receptors. *Cell,* 100**,** 703-711.

CHAPELOT, D. 2013. Quantifying satiation and satiety. *Satiation, Satiety and the Control of Food Intake: Theory and Practice***,** 12-39.

CHENG, K. C., LI, Y. X., ASAKAWA, A. & INUI, A. 2010. The role of ghrelin in energy homeostasis and its potential clinical relevance (Review). *International Journal of Molecular Medicine,* 26**,** 771-778.

CHUNG, S. J. & VICKERS, Z. 2007. Influence of sweetness on the sensory-specific satiety and long-term acceptability of tea. *Food Quality and Preference,* 18**,** 256-264.

COOK, D. J., HOLLOWOOD, T. A., LINFORTH, R. S. T. & TAYLOR, A. J. 2002. Perception of taste intensity in solutions of random-coil polysaccharides above and below c. *Food Quality and Preference,* 13**,** 473-480.

COOK, D. J., HOLLOWOOD, T. A., LINFORTH, R. S. T. & TAYLOR, A. J. 2003. Oral shear stress predicts flavour perception in viscous solutions. *Chemical Senses,* 28**,** 11-23.

CRAIG, C. L., MARSHALL, A. L., SJOSTROM, M., BAUMAN, A. E., BOOTH, M. L., AINSWORTH, B. E., PRATT, M., EKELUND, U., YNGVE, A., SALLIS, J. F. & OJA, P. 2003. International physical activity questionnaire: 12-country reliability and validity. *Medicine and Science in Sports and Exercise,* 35**,** 1381-1395.

CUMMINGS, D. E. 2006. Ghrelin and the short- and long-term regulation of appetite and body weight. *Physiology & Behavior,* 89**,** 71-84.

CUMMINGS, D. E., FRAYO, R. S., MARMONIER, C., AUBERT, R. & CHAPELOT, D. 2004. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *American Journal of Physiology-Endocrinology and Metabolism,* 287**,** E297-E304.

CUMMINGS, D. E. & OVERDUIN, J. 2007. Gastrointestinal regulation of food intake. *Journal of Clinical Investigation,* 117**,** 13-23.

CUMMINGS, D. E. & SHANNON, M. H. 2003. Roles for ghrelin in the regulation of appetite and body weight. *Archives of Surgery,* 138**,** 389-396.

DALTON, M. & FINLAYSON, G. 2013. Hedonics, satiation and satiety. *In:* BLUNDELL, J. E. & BELLISLE, F. (eds.) *Satiation, satiety and the control of food intake.* Woodhead Publishing.

DALTON, P., DOOLITTLE, N., NAGATA, H. & BRESLIN, P. A. S. 2000. The merging of the senses: integration of subthreshold taste and smell. *Nature Neuroscience,* 3**,** 431-432.

DE ARAUJO, I. E. T., ROLLS, E. T., KRINGELBACH, M. L., MCGLONE, F. & PHILLIPS, N. 2003. Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain. *European Journal of Neuroscience,* 18**,** 2059-2068.

DE GGRAAF, C., HULSHOF, T., WESTSTRATE, J. A. & JAS, P. 1992. Short-Term Effects of Different Amounts of Protein, Fats, and Carbohydrates on Satiety. *American Journal of Clinical Nutrition,* 55**,** 33-38.

DE GRAAF, C. 1995. Expected Satiety of Food Items. *Appetite,* 24**,** 300-300.

DE GRAAF, C., BLOM, W. A. M., SMEETS, P. A. M., STAFLEU, A. & HENDRIKS, H. F. J. 2004. Biomarkers of satiation and satiety. *American Journal of Clinical Nutrition,* 79**,** 946-961.

DE GRAAF, C., DE JONG, L. S. & LAMBERS, A. C. 1999. Palatability affects satiation but not satiety. *Physiology & Behavior,* 66**,** 681-688.
DE GRAAF, C., SCHREURS, A. & BLAUW, Y. H. 1993. Short-Term Effects of Different Amounts of Sweet and Nonsweet Carbohydrates on Satiety and Energy-Intake. *Physiology & Behavior,* 54**,** 833-843.
DE GRAAF, C., STAFLEU, A., STAAL, P. & WIJNE, M. 1992. Beliefs About the Satiating Effect of Bread with Spread Varying in Macronutrient Content. *Appetite,* 18**,** 121-128.
DE WIJK, R. A., POLET, I. A. & BULT, J. H. F. 2009. Bitesize is Affected by Food Aroma Presented at Sub- or Peri Threshold Concentrations. *Chemical Senses,* 34**,** A38-A38.
DE WIJK, R. A., ZIJLSTRA, N., MARS, M., DE GRAAF, C. & PRINZ, J. F. 2008. The effects of food viscosity on bite size, bite effort and food intake. *Physiology & Behavior,* 95**,** 527-532.
DEL PARIGI, A., GAUTIER, J. F., CHEN, K. W., SALBE, A. D., RAVUSSIN, E., REIMAN, E. & TATARANNI, P. A. 2002. Neuroimaging and obesity - Mapping the brain responses to hunger and satiation in humans using positron emission tomography. *Lipids and Insulin Resistance: The Role of Fatty Acid Metabolism and Fuel Partitioning,* 967**,** 389-397.
DELWICHE, J. 2004. The impact of perceptual interactions on perceived flavor. *Food Quality and Preference,* 15**,** 137-146.
DESSIRIER, J. M., SIMONS, C. T., CARSTENS, M. I., O'MAHONY, M. & CARSTENS, E. 2000. Psychophysical and neurobiological evidence that the oral sensation elicited by carbonated water is of chemogenic origin. *Chemical Senses,* 25**,** 277-284.
DEURENBERG, P., WESTSTRATE, J. A. & SEIDELL, J. C. 1991. Body-Mass Index as a Measure of Body Fatness - Age-Specific and Sex-Specific Prediction Formulas. *British Journal of Nutrition,* 65**,** 105-114.
DICTIONARIES, O. 2016. *fullness* [Online]. Available: http://www.oxforddictionaries.com/definition/english/fullness [Accessed 22 Feb 2016].
DICTIONARY, A. H. 2007. satiation. *In:* DICTIONARY, A. H. (ed.) *The American Heritage Medical Dictionary.* Houghton Mifflin Company.
DICTIONARY, F. P. M. 2012. *'appetite'* [Online]. Available: http://medical-dictionary.thefreedictionary.com/appetite.
DICTIONARY, T. A. H. S. 2016. *'hunger'* [Online]. Available: http://dictionary.reference.com/browse/from--hunger [Accessed 22 Feb 2016].
DOCKRAY, G. J. & BURDYGA, G. 2011. Plasticity in vagal afferent neurones during feeding and fasting: mechanisms and significance. *Acta Physiol (Oxf),* 201**,** 313-21.
DOTSON, C. D., VIGUES, S., STEINLE, N. I. & MUNGER, S. D. 2010. T1R and T2R receptors: The modulation of incretin hormones and potential targets for the treatment of type 2 diabetes mellitus. *Current Opinion in Investigational Drugs,* 11**,** 447-454.
DREWNOWSKI, A. 1998. Energy density, palatability, and satiety: Implications for weight control. *Nutrition Reviews,* 56**,** 347-353.
DRUCKER, D. J. 2006. The biology of incretin hormones. *Cell Metab,* 3**,** 153-65.
EKUNI, D., FURUTA, M., TOMOFUJI, T., IRIE, K., AZUMA, T., IWASAKI, Y. & MORITA, M. 2013. Effects of Eating Behaviors on Being Overweight in Japanese University Students: A Cross-sectional Survey at the Okayama University. *Asia-Pacific Journal of Public Health,* 25**,** 326-334.
ELDEGHAIDY, S., MARCIANI, L., PFEIFFER, J. C., HORT, J., HEAD, K., TAYLOR, A. J., SPILLER, R. C., GOWLAND, P. A. & FRANCIS, S. 2011. Use of an Immediate Swallow

Protocol to Assess Taste and Aroma Integration in fMRI Studies. *Chemosensory Perception,* 4**,** 163-174.
ESSED, N. H., VAN STAVEREN, W. A., KOK, F. J., ORMEL, W., ZEINSTRA, G. & DE GRAAF, C. 2006. The effect of repeated exposure to fruit drinks on intake, pleasantness and boredom in young and elderly adults. *Physiology & Behavior,* 89**,** 335-341.
FAY, S. H., FERRIDAY, D., HINTON, E. C., SHAKESHAFT, N. G., ROGERS, P. J. & BRUNSTROM, J. M. 2011. What determines real-world meal size? Evidence for pre-meal planning. *Appetite,* 56**,** 284-289.
FERNSTROM, J. D., MUNGER, S. D., SCLAFANI, A., DE ARAUJO, I. E., ROBERTS, A. & MOLINARY, S. 2012. Mechanisms for Sweetness. *Journal of Nutrition,* 142**,** 1134s-1141s.
FERRY, A. L., HORT, J., MITCHELL, J. R., COOK, D. J., LAGARRIGUE, S. & PAMIES, B. V. 2006. Viscosity and flavour perception: Why is starch different from hydrocolloids? *Food Hydrocolloids,* 20**,** 855-862.
FINLAYSON, G., KING, N. & BLUNDELL, J. E. 2007. Liking vs. wanting food: Importance for human appetite control and weight regulation. *Neuroscience and Biobehavioral Reviews,* 31**,** 987-1002.
FISHER, J., LIU, Y., BIRCH, L. L. & ROLLS, B. J. 2007. Effects of portion size and energy density on young children's intake at a meal. *American Journal of Clinical Nutrition,* 86**,** 174-179.
FLINT, A., RABEN, A., BLUNDELL, J. E. & ASTRUP, A. 2000. Reproducibility, power and validity of visual analogue scares in assessment of appetite sensations in single test meal studies. *International Journal of Obesity,* 24**,** 38-48.
FORBES, G. B. & REINA, J. C. 1970. Adult Lean Body Mass Declines with Age - Some Longitudinal Observations. *Metabolism,* 19**,** 653-&.
FORDE, C. G., VAN KUIJK, N., THALER, T., DE GRAAF, C. & MARTIN, N. 2013. Oral processing characteristics of solid savoury meal components, and relationship with food composition, sensory attributes and expected satiation. *Appetite,* 60**,** 208-219.
FRAYN, K. N. 2010. *Metabolic regulation: a human perspective,* Chichester, U.K. ; Malden, MA, Wiley-Blackwell Pub.
FRIEDMAN, J. 2015. Leptin and the Regulation of Food Intake and Body Weight. *Journal of Nutritional Science and Vitaminology,* 61**,** S202-S202.
FRIEL, E. N., LINFORTH, R. S. T. & TAYLOR, A. J. 2000. An empirical model to predict the headspace concentration of volatile compounds above solutions containing sucrose. *Food Chemistry,* 71**,** 309-317.
GANDY, J. & GARROW, J. S. 2007. The impact of the Journal of Human Nutrition and Dietetics. *Journal of Human Nutrition and Dietetics,* 20**,** 145-147.
GEHLERT, D. R. 1999. Role of hypothalamic neuropeptide Y in feeding and obesity. *Neuropeptides,* 33**,** 329-338.
GIBSON, E. L. & BRUNSTROM, J. M. 2007. Learned influences on appetite, food choice and intake: evidence in human beings. *In:* COOPER, C. M. & KIRKHAM, T. C. (eds.) *Progress in Brain Research: Appetite and Body Weight.* London: Elsevier.
GOLDSTEIN, D. J. 1992. Beneficial Health-Effects of Modest Weight-Loss. *International Journal of Obesity,* 16**,** 397-415.
GREEN, S. M. & BLUNDELL, J. E. 1996. Subjective and objective indices of the satiating effect of foods. Can people predict how filling a food will be? *European Journal of Clinical Nutrition,* 50**,** 798-806.

GREEN, S. M., WALES, J. K., LAWTON, C. L. & BLUNDELL, J. E. 2000. Comparison of high-fat and high-carbohydrate foods in a meal or snack on short-term fat and energy intakes in obese women. *British Journal of Nutrition,* 84**,** 521-530.

GRIFFIOEN-ROOSE, S., MARS, M., FINLAYSON, G., BLUNDELL, J. E. & DE GRAAF, C. 2009. Satiation Due to Equally Palatable Sweet and Savory Meals Does Not Differ in Normal Weight Young Adults. *Journal of Nutrition,* 139**,** 2093-2098.

GUINARD, J. X. & BRUN, P. 1998. Sensory-specific satiety: Comparison of taste and texture effects. *Appetite,* 31**,** 141-157.

HALL, G. C. & MCMAHON, A. 2007. The association of weight change with depression. *Pharmacoepidemiology and Drug Safety,* 16**,** S220-S220.

HALTON, T. L. & HU, F. B. 2004. The effects of high protein diets on thermogenesis, satiety and weight loss: A critical review. *Journal of the American College of Nutrition,* 23**,** 373-385.

HARRIS, J. A. & BENEDICT, F. G. 1918. A biometric study of human basal metabolism. *Proceedings of National Acadamic Sciences of United States of America,* 4**,** 370-373.

HARRIS, J. A. & BENEDICT, F. G. 1919. A biometric study of basal metabolism in man. Washington (DC): Carnegie Institute of Washington.

HARROLD, J. A., DOYEY, T. M., BLUNDELL, J. E. & HALFORD, J. C. G. 2012. CNS regulation of appetite. *Neuropharmacology,* 63**,** 3-17.

HAVERMANS, R. C., GESCHWIND, N., FILLA, S., NEDERKOORN, C. & JANSEN, A. 2009. Sensory-specific satiety is unaffected by manipulations of flavour intensity. *Physiology & Behavior,* 97**,** 327-333.

HERMAN, C. P. & MACK, D. 1975. Restrained and Unrestrained Eating. *Journal of Personality,* 43**,** 647-660.

HERRMANN, S. D., WILLIS, E. A., HONAS, J. J., LEE, J., WASHBURN, R. A. & DONNELLY, J. E. 2015. Energy intake, nonexercise physical activity, and weight loss in responders and nonresponders: The Midwest Exercise Trial 2. *Obesity,* 23**,** 1539-1549.

HETHERINGTON, M. & HAVERMANS, R. C. 2013. Sensory-specific satiation and satiety. *Satiation, Satiety and the Control of Food Intake: Theory and Practice***,** 253-269.

HETHERINGTON, M., ROLLS, B. J. & BURLEY, V. J. 1989. The Time Course of Sensory-Specific Satiety. *Appetite,* 12**,** 57-68.

HETHERINGTON, M. M. & BOYLAND, E. 2007. Short-term effects of chewing gum on snack intake and appetite. *Appetite,* 48**,** 397-401.

HEWSON, L. 2007. *Multimodal interactions in a carbonated beverage system.* PhD, University of Nottingham.

HEWSON, L., HOLLOWOOD, T., CHANDRA, S. & HORT, J. 2009. Gustatory, Olfactory and Trigeminal Interactions in a Model Carbonated Beverage. *Chemosensory Perception,* 2**,** 94-107.

HILL, J. O., WYATT, H. R. & PETERS, J. C. 2012. Energy Balance and Obesity. *Circulation,* 126**,** 126-132.

HIRSCHBERG, A. L. 2012. Sex hormones, appetite and eating behaviour in women. *Maturitas,* 71**,** 248-256.

HOGENKAMP, P. S., BRUNSTROM, J. M., STAFLEU, A., MARS, M. & DE GRAAF, C. 2012. Expected satiation after repeated consumption of low- or high-energy-dense soup. *British Journal of Nutrition,* 108**,** 182-190.

HOGENKAMP, P. S., STAFLEU, A., MARS, M., BRUNSTROM, J. M. & DE GRAAF, C. 2011. Texture, not flavor, determines expected satiation of dairy products. *Appetite,* 57**,** 635-641.

HOLLOWOOD, T. A., LINFORTH, R. S. T. & TAYLOR, A. J. 2002. The effect of viscosity on the perception of flavour. *Chemical Senses,* 27**,** 583-591.

HOUDE, A. A., LEGARE, C., BIRON, S., LESCELLEUR, O., BIERTHO, L., MARCEAU, S., TCHERNOF, A., VOHL, M. C., HIVERT, M. F. & BOUCHARD, L. 2015. Leptin and adiponectin DNA methylation levels in adipose tissues and blood cells are associated with BMI, waist girth and LDL-cholesterol levels in severely obese men and women. *Bmc Medical Genetics,* 16.

HYDE, R. J. & WITHERLY, S. A. 1993. Dynamic Contrast - a Sensory Contribution to Palatability. *Appetite,* 21**,** 1-16.

IKEDA, H., WEST, D. B., PUSTEK, J. J., FIGLEWICZ, D. P., GREENWOOD, M. R. C., PORTE, D. & WOODS, S. C. 1986. Intraventricular Insulin Reduces Food-Intake and Body-Weight of Lean but Not Obese Zucker Rats. *Appetite,* 7**,** 381-386.

IMERYUZ, N., YEGEN, B. C., BOZKURT, A., COSKUN, T., VILLANUEVA-PENACARRILLO, M. L. & ULUSOY, N. B. 1997. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *American Journal of Physiology,* 273**,** G920-7.

ISO 2010. ISO 8587:2006 Sensory analysis -- Methodology -- Ranking.

ISO 2011. ISO 8589:2007 Sensory analysis -- General guidance for the design of test rooms.

JANG, H. J., KOKRASHVILI, Z., THEODORAKIS, M. J., CARLSON, O. D., KIM, B. J., ZHOU, J., KIM, H. H., XU, X., CHAN, S. L., JUHASZOVA, M., BERNIER, M., MOSINGER, B., MARGOLSKEE, R. F. & EGAN, J. M. 2007. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proceedings of National Acadamic Sciences of United States of America,* 104**,** 15069-15074.

JOHNSON, F., PRATT, M. & WARDLE, J. 2012. Dietary restraint and self-regulation in eating behavior. *International Journal of Obesity,* 36**,** 665-674.

JOHNSON, F. & WARDLE, J. 2014. Variety, Palatability, and Obesity. *Advances in Nutrition,* 5**,** 851-859.

KAIRUPAN, T. S., AMITANI, H., CHENG, K. C., RUNTUWENE, J., ASAKAWA, A. & INUI, A. 2016. Role of gastrointestinal hormones in feeding behavior and obesity treatment. *Journal of Gastroenterol,* 51**,** 93-103.

KAIYA, H., KANGAWA, K. & MIYAZATO, M. 2014. Ghrelin/ghrelin receptors. *Journal of Molecular Endocrinology,* 52**,** T87-T100.

KATSUURA, Y., HECKMANN, J. A. & TAHA, S. A. 2011. mu-Opioid receptor stimulation in the nucleus accumbens elevates fatty tastant intake by increasing palatability and suppressing satiety signals. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* 301**,** R244-R254.

KEMP, S. E., HOLLOWOOD, T. A. & HORT, J. 2009. *Sensory Evaluation*, Willey Blackwell.

KHARE, A. & INMAN, J. J. 2006. Habitual behavior in American eating patterns: The role of meal occasions. *Journal of Consumer Research,* 32**,** 567-575.

KIRKMEYER, S. V. & TEPPER, B. J. 2005. Consumer reactions to creaminess and genetic sensitivity to 6-n-propylthiouracil: A multidimensional study. *Food Quality and Preference,* 16**,** 545-556.

KISSILEFF, H. R. 1985. Effects of Physical State (Liquid-Solid) of Foods on Food-Intake - Procedural and Substantive Contributions. *American Journal of Clinical Nutrition,* 42**,** 956-965.

KOBAL, G. & HUMMEL, C. 1988. Cerebral Chemosensory Evoked-Potentials Elicited by Chemical-Stimulation of the Human Olfactory and Respiratory Nasal-Mucosa. *Electroencephalography and Clinical Neurophysiology,* 71**,** 241-250.

KRISTENSEN, P. 1999. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Progress in Obesity Research: 8*, 351-355.
KUYUMCU, S., GOETZE, O., FOX, M. R., REHFELD, J. F., HOLST, J. J., MENNE, D., BOESIGER, P., MILOS, G., FRIED, M., SCHWIZER, W. & STEINGOETTER, A. 2013. Gastric Emptying Rate and CCK and GLP-1 Release After a Solid Test Meal Are Dependent on Body Mass Index. *Gastroenterology,* 144, S742-S743.
LAVIN, J. & READ, N. 2000. An investigation of the role of oro-sensory stimulation in sugar satiety. *Proceedings of the Nutrition Society,* 59, 122a-122a.
LAVIN, J. H., FRENCH, S. J. & READ, N. W. 2002a. Comparison of oral and gastric administration of sucrose and maltose on gastric emptying rate and appetite. *International Journal of Obesity,* 26, 80-86.
LAVIN, J. H., FRENCH, S. J., RUXTON, C. H. S. & READ, N. W. 2002b. An investigation of the role of oro-sensory stimulation in sugar satiety. *International Journal of Obesity,* 26, 384-388.
LE PAGE, M. 2016. Food additives are obesity suspects. *New Scientist,* 229, 12-12.
LETHUAUT, L., BROSSARD, C., ROUSSEAU, F., BOUSSEAU, B. & GENOT, C. 2003. Sweetness-texture interactions in model dairy desserts: effect of sucrose concentration and the carrageenan type. *International Dairy Journal,* 13, 631-641.
LI, E. T., TSANG, L. B. & LUI, S. S. 1999. Menstrual cycle and voluntary food intake in young Chinese women. *Appetite,* 33, 109-18.
LI, X. D., STASZEWSKI, L., XU, H., DURICK, K., ZOLLER, M. & ADLER, E. 2002. Human receptors for sweet and umami taste. *Proceedings of National Acadamic Sciences of United States of America,* 99, 4692-4696.
LIBUDA, L. & KERSTING, M. 2009. Soft drinks and body weight development in childhood: is there a relationship? *Current Opinion in Clinical Nutrition and Metabolic Care,* 12, 596-600.
LITTLE, T. J., MCKIE, S., JONES, R. B., D'AMATO, M., SMITH, C., KISS, O., THOMPSON, D. G. & MCLAUGHLIN, J. T. 2014. Mapping glucose-mediated gut-to-brain signalling pathways in humans. *Neuroimage,* 96, 1-11.
LORET, C., WALTER, M., PINEAU, N., PEYRON, M. A., HARTMANN, C. & MARTIN, N. 2011. Physical and related sensory properties of a swallowable bolus. *Physiology & Behavior,* 104, 855-864.
LUDWIG, D. S., PETERSON, K. E. & GORTMAKER, S. L. 2001. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. *Lancet,* 357, 505-508.
MALIK, V. S., SCHULZE, M. B. & HU, F. B. 2006. Intake of sugar-sweetened beverages and weight gain: a systematic review. *American Journal of Clinical Nutrition,* 84, 274-288.
MALNIC, B., GODFREY, P. A. & BUCK, L. B. 2004. The human olfactory receptor gene family. *Proc Natl Acad Sci U S A,* 101, 2584-2589.
MALNIC, B., HIRONO, J., SATO, T. & BUCK, L. B. 1999. Combinatorial receptor codes for odors. *Cell,* 96, 713-723.
MARCIANI, L., ELDEGHAIDY, S., SPILLER, R. C., GOWLAND, P. & FRANCIS, S. 2010. Brain imaging. *In:* TAYLOR, A. J. & LINFORTH, R. S. (eds.) *Food flavour technology.* 2nd ed. UK: Wiley-blackwell.
MARCIANI, L., PFEIFFER, J. C., HORT, J., HEAD, K., BUSH, D., TAYLOR, A. J., SPILLER, R. C., FRANCIS, S. & GOWLAND, P. A. 2006. Improved methods for fMRI studies of combined taste and aroma stimuli. *Journal of Neuroscience Methods,* 158, 186-194.

MARGOLSKEE, R. F., DYER, J., KOKRASHVILI, Z., SALMON, K. S. H., ILEGEMS, E., DALY, K., MAILLET, E. L., NINOMIYA, Y., MOSINGER, B. & SHIRAZI-BEECHEY, S. P. 2007. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proc Natl Acad Sci U S A,* 104**,** 15075-15080.

MASSOLT, E. T., VAN HAARD, P. M., REHFELD, J. F., POSTHUMA, E. F., VAN DER VEER, E. & SCHWEITZER, D. H. 2010. Appetite suppression through smelling of dark chocolate correlates with changes in ghrelin in young women. *Regulatory Peptides,* 161**,** 81-86.

MATTES, R. D., HOLLIS, J., HAYES, D. & STUNKARD, A. J. 2005. Appetite: Measurement and manipulation misgivings. *Journal of the American Dietetic Association,* 105**,** S87-S97.

MATTES, R. D. & POPKIN, B. M. 2009. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. *American Journal of Clinical Nutrition,* 89**,** 1-14.

MATTES, R. D. & ROTHACKER, D. 2001. Beverage viscosity is inversely related to postprandial hunger in humans. *Physiology & Behavior,* 74**,** 551-557.

MAYER, J. 1953. Glucostatic Mechanism of Regulation of Food Intake. *New England Journal of Medicine,* 249**,** 13-16.

MCCRICKERD, K., CHAMBERS, L., BRUNSTROM, J. M. & YEOMANS, M. R. 2012a. Subtle changes in the flavour and texture of a drink enhance expectations of satiety. *Flavour,* 1, 618-638.

MCCRICKERD, K., CHAMBERS, L., BRUNSTROM, J. M. & YEOMANS, M. R. 2012b. Subtle changes in the flavour and texture of a drink enhance expectations of satiety. *Flavour,* 1, 618-638.

MCCRICKERD, K., CHAMBERS, L. & YEOMANS, M. R. 2014. Does modifying the thick texture and creamy flavour of a drink change portion size selection and intake? *Appetite,* 73**,** 114-120.

MCCRICKERD, K., LENSING, N. & YEOMANS, M. R. 2015. The impact of food and beverage characteristics on expectations of satiation, satiety and thirst. *Food Quality and Preference,* 44**,** 130-138.

MEILGAARD, M. C., CIVILLE, G. V. & CARR, B. T. 2006a. Attribute difference tests: how does attribute X differ between samples? *Sensory Evaluation Techniques.* 4th ed.: CRC Press, pp 97-108.

MEILGAARD, M. C., CIVILLE, G. V. & CARR, B. T. 2006b. *Sensory Evaluation Techniques*, CRC Press.

MELA, D. J. 1996. Eating behaviour, food preferences and dietary intake in relation to obesity and body-weight status. *Proceedings of the Nutrition Society,* 55**,** 803-816.

MELA, D. J. 2006. Eating for pleasure or just wanting to eat? Reconsidering sensory hedonic responses as a driver of obesity. *Appetite,* 47**,** 10-17.

MERRILL, E. P., KRAMER, F. M., CARDELLO, A. & SCHUTZ, H. 2002. A comparison of satiety measures. *Appetite,* 39**,** 181-183.

MINITAB. 2016. *Regression Analysis* [Online]. Available: http://blog.minitab.com/blog/adventures-in-statistics/regression-analysis-how-do-i-interpret-r-squared-and-assess-the-goodness-of-fit [Accessed April 10 2016].

MOHAMED-ALI, V., PINKNEY, J. H. & COPPACK, S. W. 1998. Adipose tissue as an endocrine and paracrine organ. *International Journal of Obesity,* 22**,** 1145-1158.

MORIARTY, P., DIMALINE, R., THOMPSON, D. G. & DOCKRAY, G. J. 1997. Characterization of cholecystokinin, and cholecystokinin, receptors expressed by vagal afferent neurons. *Neuroscience,* 79**,** 905-913.
MORTON, G. J., CUMMINGS, D. E., BASKIN, D. G., BARSH, G. S. & SCHWARTZ, M. W. 2006. Central nervous system control of food intake and body weight. *Nature,* 443**,** 289-295.
MUURAHAINEN, N., KISSILEFF, H. R., DEROGATIS, A. J. & PISUNYER, F. X. 1988. Effects of Cholecystokinin-Octapeptide (Cck-8) on Food-Intake and Gastric-Emptying in Man. *Physiology & Behavior,* 44**,** 645-649.
MUZY, T., AGUIAR, O. B. & FONSECA, M. J. M. 2013. Association between Obesity and Common Mental Disorders among Nutritionists, Rio De Janeiro, Brasil. *Annals of Nutrition and Metabolism,* 63**,** 1315-1315.
NAGELOVA, V., ZELEZNA, B. & MALETINSKA, L. 2014. Anorexigenic Neuropeptide CART in Food Intake Regulation. *Chemicke Listy,* 108**,** 354-357.
NHS. 2010. *The economic burden of obesity* [Online]. Public Health England. Available: http://www.noo.org.uk/uploads/doc/vid_8575_Burdenofobesity151110MG.pdf [Accessed 21/03 2016].
NISWENDER, K. D. 2008. Leptin and insulin as adiposity signals. *Neurobiology of Obesity***,** 83-126.
NOBLE, A. C. 1996. Taste-aroma interactions. *Trends in Food Science and Technology,* 7**,** 439-444.
NORGREN, R. 1983. The Gustatory System in Mammals. *American Journal of Otolaryngology,* 4**,** 234-237.
OLOFSSON, J. K. & NORDIN, S. 2004. Gender differences in chemosensory perception and event-related potentials. *Chemical Senses,* 29**,** 629-637.
OLSEN, N. J. & HEITMANN, B. L. 2009. Intake of calorically sweetened beverages and obesity. *Obesity Reviews,* 10**,** 68-75.
PARKINSON, J. R., CHAUDHRI, O. B., KUO, Y. T., FIELD, B. C., HERLIHY, A. H., DHILLO, W. S., GHATEI, M. A., BLOOM, S. R. & BELL, J. D. 2009. Differential patterns of neuronal activation in the brainstem and hypothalamus following peripheral injection of GLP-1, oxyntomodulin and lithium chloride in mice detected by manganese-enhanced magnetic resonance imaging (MEMRI). *Neuroimage,* 44**,** 1022-31.
PAYKEL, E. S. 1977. Depression and Appetite. *Journal of Psychosomatic Research,* 21**,** 401-407.
PECINA, S., SMITH, K. S. & BERRIDGE, K. C. 2006. Hedonic hot spots in the brain. *Neuroscientist,* 12**,** 500-511.
PERYAM, D. R. A. G., N.F. 1952. Advanced taste test method. *Food Engineering.*
PFEIFFER, J. C., HORT, J., HOLLOWOOD, T. A. & TAYLOR, A. J. 2006. Taste-aroma interactions in a ternary system: A model of fruitiness perception in sucrose/acid solutions. *Perception & Psychophysics,* 68**,** 216-227.
PFEIFFER, J. F., BOULTON, R. B. & NOBLE, A. C. 2000. Modeling the sweetness response using time-intensity data. *Food Quality and Preference,* 11**,** 129-138.
PHILLIPS, R. J. & POWLEY, T. L. 2000. Tension and stretch receptors in gastrointestinal smooth muscle: re-evaluating vagal mechanoreceptor electrophysiology. *Brain Research Reviews,* 34**,** 1-26.
PIQUERAS-FISZMAN, B. & SPENCE, C. 2012. The weight of the container influences expected satiety, perceived density, and subsequent expected fullness. *Appetite,* 58**,** 559-562.
POLIVY, J. & HERMAN, C. P. 1985. Dieting and Binging - a Causal-Analysis. *American Psychologist,* 40**,** 193-201.

POLONSKY, K. S., GIVEN, B. D. & VANCAUTER, E. 1988. 24-Hour Profiles and Pulsatile Patterns of Insulin-Secretion in Normal and Obese Subjects. *Journal of Clinical Investigation,* 81**,** 442-448.
POORTVLIET, P. C., BERUBE-PARENT, S., DRAPEAU, V., LAMARCHE, B., BLUNDELL, J. E. & TREMBLAY, A. 2007. Effects of a healthy meal course on spontaneous energy intake, satiety and palatability. *British Journal of Nutrition,* 97**,** 584-590.
POULTON, E. C. 1979. Models for Biases in Judging Sensory Magnitude. *Psychological Bulletin,* 86**,** 777-803.
POWLEY, T. L. & PHILLIPS, R. J. 2004. Gastric satiation is volumetric, intestinal satiation is nutritive. *Physiology & Behavior,* 82**,** 69-74.
RAMAEKERS, M. G., BOESVELDT, S., LAKEMOND, C. M. M., VAN BOEKEL, M. A. J. S. & LUNING, P. A. 2014. Odors: appetizing or satiating? Development of appetite during odor exposure over time. *International Journal of Obesity,* 38**,** 650-656.
RITTER, R. C. 2004. Gastrointestinal mechanisms of satiation for food. *Physiology & Behavior,* 81**,** 249-273.
ROLLS, B. J. 1986. Sensory-Specific Satiety. *Nutrition Reviews,* 44**,** 93-101.
ROLLS, B. J. 2009a. The relationship between dietary energy density and energy intake. *Physiology & Behavior,* 97**,** 609-615.
ROLLS, B. J., BELL, E. A. & WAUGH, B. A. 2000. Increasing the volume of a food by incorporating air affects satiety in men. *American Journal of Clinical Nutrition,* 72**,** 361-368.
ROLLS, B. J., ROE, L. S., KRAL, T. V. E., MEENGS, J. S. & WALL, D. E. 2004a. Increasing the portion size of a packaged snack increases energy intake in men and women. *Appetite,* 42**,** 63-69.
ROLLS, B. J., ROE, L. S., MEENGS, J. S. & WALL, D. E. 2004b. Increasing the portion size of a sandwich increases energy intake. *Journal of the American Dietetic Association,* 104**,** 367-372.
ROLLS, B. J., ROLLS, E. T., ROWE, E. A. & SWEENEY, K. 1981. Sensory Specific Satiety in Man. *Physiology & Behavior,* 27**,** 137-142.
ROLLS, E. T. 2009b. Functional neuroimaging of umami taste: what makes umami pleasant? *American Journal of Clinical Nutrition,* 90**,** 804s-813s.
ROLLS, E. T. 2012. Taste, olfactory and food texture reward processing in the brain and the control of appetite. *Proceedings of the Nutrition Society,* 71**,** 488-501.
ROLLS, E. T. 2015. Taste, olfactory and food texture reward processing in the brain and the control of appetite. *Chemical Senses,* 40**,** 216-217.
ROLLS, E. T. & BAYLIS, L. L. 1994. Gustatory, Olfactory, and Visual Convergence within the Primate Orbitofrontal Cortex. *Journal of Neuroscience,* 14**,** 5437-5452.
ROLLS, E. T. & GRABENHORST, F. 2008. The orbitofrontal cortex and beyond: From affect to decision-making. *Progress in Neurobiology,* 86**,** 216-244.
ROLLS, E. T., KRINGELBACH, M. L. & DE ARAUJO, I. E. T. 2003. Different representations of pleasant and unpleasant odours in the human brain. *European Journal of Neuroscience,* 18**,** 695-703.
ROLLS, E. T., ROLLS, B. J. & ROWE, E. A. 1983. Sensory-Specific and Motivation-Specific Satiety for the Sight and Taste of Food and Water in Man. *Physiology & Behavior,* 30**,** 185-192.
ROLLS, E. T. & ROLLS, J. H. 1997. Olfactory sensory-specific satiety in humans. *Physiology & Behavior,* 61**,** 461-473.
ROSENBLUM, J. L., CASTRO, V. M., MOORE, C. E. & KAPLAN, L. M. 2012. Calcium and vitamin D supplementation is associated with decreased abdominal visceral

adipose tissue in overweight and obese adults. *American Journal of Clinical Nutrition,* 95**,** 101-108.
ROSSI, M., KIM, M. S., MORGAN, D. G. A., SMALL, C. J., EDWARDS, C. M. B., SUNTER, D., ABUSNANA, S., GOLDSTONE, A. P., RUSSELL, S. H., STANLEY, S. A., SMITH, D. M., YAGALOFF, K., GHATEI, M. A. & BLOOM, S. R. 1998. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. *Endocrinology,* 139**,** 4428-4431.
ROTH, K. A., KIM, S. & GORDON, J. I. 1992. Immunocytochemical Studies Suggest 2 Pathways for Enteroendocrine Cell-Differentiation in the Colon. *American Journal of Physiology,* 263**,** G174-G180.
ROZIN, P. 1982. Taste-Smell Confusions and the Duality of the Olfactory Sense. *Perception & Psychophysics,* 31**,** 397-401.
RUIJSCHOP, R. M., BOELRIJK, A. E., DE RU, J. A., DE GRAAF, C. & WESTERTERP-PLANTENGA, M. S. 2008a. Effects of retro-nasal aroma release on satiation. *British Journal of Nutrition,* 99**,** 1140-8.
RUIJSCHOP, R. M., BOELRIJK, A. E. M., BURGERING, M. J. M., DE GRAAF, C. & WESTERTERP-PLANTENGA, M. S. 2010. Acute Effects of Complexity in Aroma Composition on Satiation and Food Intake. *Chemical Senses,* 35**,** 91-100.
RUIJSCHOP, R. M., BOELRIJK, A. E. M., DE GRAAF, C. & WESTERTERP-PLANTENGA, M. S. 2009a. Retronasal Aroma Release and Satiation: a Review. *Journal of Agricultural and Food Chemistry,* 57**,** 9888-9894.
RUIJSCHOP, R. M. A. J., BOELRIJK, A. E. M., DE GRAAF, C. & WESTERTERP-PLANTENGA, M. S. 2009b. Retronasal Aroma Release and Satiation: a Review. *Journal of Agricultural and Food Chemistry,* 57**,** 9888-9894.
RUIJSCHOP, R. M. A. J., BOELRIJK, A. E. M., DE RU, J. A., DE GRAAF, C. & WESTERTERP-PLANTENGA, M. S. 2008b. Effects of retro-nasal aroma release on satiation. *British Journal of Nutrition,* 99**,** 1140-1148.
SAINT-EVE, A., KORA, E. P. & MARTIN, N. 2004. Impact of the olfactory quality and chemical complexity of the flavouring agent on the texture of low fat stirred yogurts assessed by three different sensory methodologies. *Food Quality and Preference,* 15**,** 655-668.
SAINT-EVE, A., MARTIN, N., GUILLEMIN, H., SEMON, E., GUICHARD, E. & SOUCHON, I. 2006. Flavored yogurt complex viscosity influences real-time aroma release in the mouth and sensory properties. *Journal of Agricultural and Food Chemistry,* 54**,** 7794-7803.
SAVANT, L. & MCDANIEL, M. R. 2004. Suppression of sourness: A comparative study involving mixtures of organic acids and sugars. *Perception & Psychophysics,* 66**,** 642-650.
SAWCHENKO, P. E. 1983. Central Connections of the Sensory and Motor Nuclei of the Vagus Nerve. *Journal of the Autonomic Nervous System,* 9**,** 13-26.
SCHIFFERSTEIN, H. N. J. & VERLEGH, P. W. J. 1996. The role of congruency and pleasantness in odor-induced taste enhancement. *Acta Psychologica,* 94**,** 87-105.
SCHWARTZ, G. J., MCHUGH, P. R. & MORAN, T. H. 1993. Gastric Loads and Cholecystokinin Synergistically Stimulate Rat Gastric Vagal Afferents. *American Journal of Physiology,* 265**,** R872-R876.
SCOTT, K. 2005. Taste recognition: Food for thought. *Neuron,* 48**,** 455-464.
SHAH, M. & VELLA, A. 2014. Effects of GLP-1 on appetite and weight. *Review of Endocrine & Metabolic Disorders,* 15**,** 181-7.

SMALL, D. M., GERBER, J. C., MAK, Y. E. & HUMMEL, T. 2005. Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. *Neuron,* 47**,** 593-605.
SMITH, D. V. & MARGOLSKEE, R. F. 2001. Making sense of taste. *Scientific American,* 284**,** 32-39.
SORENSEN, L. B., MOLLER, P., FLINT, A., MARTENS, M. & RABEN, A. 2003. Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. *International Journal of Obesity,* 27**,** 1152-1166.
STANLEY, B. G., KYRKOULI, S. E., LAMPERT, S. & LEIBOWITZ, S. F. 1986. Neuropeptide-Y Chronically Injected into the Hypothalamus - a Powerful Neurochemical Inducer of Hyperphagia and Obesity. *Peptides,* 7**,** 1189-1192.
STATISTICAL SOLUTIONS, L. 2016. *Power & Sample Size Calculator* [Online]. Available: http://www.statisticalsolutions.net/pssZtest_calc.php [Accessed April 5 2016].
STONE, H., SIDEL, J. L. & BLOOMQUIST, J. 1980. Quantitative Descriptive Analysis. *Cereal Foods World,* 25**,** 642-644.
STUNKARD, A. J. & MESSICK, S. 1985. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research,* 29**,** 71-83.
SUDO, N., SEKIYAMA, M., WATANABE, C., BOKUL, A. T. M. M. H. & OHTSUKA, R. 2004. Gender differences in food and energy intake among adult villagers in northwestern Bangladesh: a food frequency questionnaire survey. *International Journal of Food Sciences and Nutrition,* 55**,** 499-509.
SUGITA, M. 2006. Taste perception and coding in the periphery. *Cellular and Molecular Life Sciences,* 63**,** 2000-2015.
SWINBURN, B., SACKS, G. & RAVUSSIN, E. 2009. Increased food energy supply is more than sufficient to explain the US epidemic of obesity. *American Journal of Clinical Nutrition,* 90**,** 1453-1456.
TARREGA, A., MARTINEZ, M., VELEZ-RUIZ, J. F. & FISZMAN, S. 2014. Hydrocolloids as a tool for modulating the expected satiety of milk-based snacks. *Food Hydrocolloids,* 39**,** 51-57.
TAYLOR, A. J. & LINFORTH, R. S. T. 2003. Direct mass spectrometry of complex volatile and non-volatile flavour mixtures. *International Journal of Mass Spectrometry,* 223**,** 179-191.
TAYLOR, A. J., LINFORTH, R. S. T., HARVEY, B. A. & BLAKE, B. 2000. Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release. *Food Chemistry,* 71**,** 327-338.
TENK, J., VARGA, E., RIMAI, T., ROSTAS, I., SOOS, S., SZEKELY, M., PETERVARI, E. & BALASKO, M. 2014. Peripheral CCK-1 receptors in age-related regulatory alterations affecting energy balance. *Acta Physiologica,* 211**,** 131-131.
TOMLINSON, B., HU, M., ZHANG, Y., CHAN, P. & LIU, Z. M. 2016. An overview of new GLP-1 receptor agonists for type 2 diabetes. *Expert Opin Investig Drugs,* 25**,** 145-158.
TONG, J. & D'ALESSIO, D. 2014. Give the Receptor a Brake: Slowing Gastric Emptying by GLP-1. *Diabetes,* 63**,** 407-409.
TOURNIER, C., SULMONT-ROSSE, C., SEMON, E., VIGNON, A., ISSANCHOU, S. & GUICHARD, E. 2009. A study on texture-taste-aroma interactions: Physico-chemical and cognitive mechanisms. *International Dairy Journal,* 19**,** 450-458.
TRUDE, A. C. B., KHARMATS, A., JOCK, B., LIU, D., LEE, K., MARTINS, P. A., PARDILLA, M., SWARTZ, J. & GITTELSOHN, J. 2016. Patterns of Food Consumption are Associated with Obesity, Self-Reported Diabetes and Cardiovascular Disease in

Five American Indian Communities (vol 54, 437). *Ecology of Food and Nutrition,* 55**,** 110-110.
TSCHOP, M., WAWARTA, R., RIEPL, R. L., FRIEDRICH, S., BIDLINGMAIER, M., LANDGRAF, R. & FOLWACZNY, C. 2001. Post-prandial decrease of circulating human ghrelin levels. *Journal of Endocrinological Investigation,* 24**,** Rc19-Rc21.
VAN DER KLAAUW, A. A., KEOGH, J. M., HENNING, E., TROWSE, V. M., DHILLO, W. S., GHATEI, M. A. & FAROOQI, I. S. 2013. High Protein Intake Stimulates Postprandial GLP1 and PYY Release. *Obesity,* 21**,** 1602-1607.
VERDUIN, P., AGARWAL, S. & WALTMAN, S. 2005. Solutions to obesity: perspectives from the food industry. *American Journal of Clinical Nutrition,* 82**,** 259s-261s.
VICKERS, Z., HOLTON, E. & WANG, J. 1998. Effect of yogurt sweetness on sensory specific satiety. *Journal of Sensory Studies,* 13**,** 377-388.
VICKERS, Z., HOLTON, E. & WANG, J. 2001. Effect of ideal-relative sweetness on yogurt consumption. *Food Quality and Preference,* 12**,** 521-526.
VISKAAL-VAN DONGEN, M., KOK, F. J. & DE GRAAF, C. 2011. Eating rate of commonly consumed foods promotes food and energy intake. *Appetite,* 56**,** 25-31.
WALLACE, M. T. 2015. Multisensory Perception: The Building of Flavor Representations. *Current Biology,* 25**,** R986-R988.
WANSINK, B., PAINTER, J. E. & NORTH, J. 2005. Bottomless bowls: Why visual cues of portion size may influence intake. *Obesity Research,* 13**,** 93-100.
WEEL, K. G. C., BOELRIJK, A. E. M., BURGER, J. J., GRUPPEN, H., VORAGEN, A. G. J. & SMIT, G. 2003. A protocol for measurement of in vivo aroma release from beverages. *Journal of Food Science,* 68**,** 1123-1128.
WEENEN, H., STAFLEU, A. & DE GRAAF, C. 2005. Dynamic aspects of liking: post-prandial persistence of sensory specific satiety. *Food Quality and Preference,* 16**,** 528-535.
WEINSTEIN, K. J. 2013. Energy expenditure and physical activity levels in employees of an organic farm in central Pennsylvania. *American Journal of Human Biology,* 25**,** 280-280.
WESTERTERP-PLANTENGA, M. S., WIJCKMANS-DUIJSENS, N. E. G., VERBOEKET-VAN DE VENNE, W. P. G., DE GRAAF, K., HOF, K. H. V. & WESTSTRATE, J. A. 1998. Energy intake and body weight effects of six months reduced or full fat diets, as a function of dietary restraint. *International Journal of Obesity,* 22**,** 14-22.
WHO 2008. Waist circumference and waist-hip ratio: report of a WHO expert consultation. Geneva.
WHO. 2016a. *WHO/ Physical activity* [Online]. Available: http://www.who.int/dietphysicalactivity/pa/en/ [Accessed 22 Feb 2016].
WHO. 2016b. *WHO/Overweight and obesity* [Online]. Available: http://www.who.int/gho/ncd/risk_factors/overweight/en/ [Accessed 22 Feb 2016].
WHO. 2016c. *WHO/What is overweight and obesity?* [Online]. Available: http://www.who.int/dietphysicalactivity/childhood_what/en/ [Accessed 22 Feb 2016].
WITHROW, D. & ALTER, D. A. 2011. The economic burden of obesity worldwide: a systematic review of the direct costs of obesity. *Obesity Reviews,* 12**,** 131-141.
WOODS, S. C. 2007. Role of the endocannabinoid system in regulating cardiovascular and metabolic risk factors. *American Journal of Medicine,* 120**,** S19-S25.
YARMOLINSKY, D. A., ZUKER, C. S. & RYBA, N. J. P. 2009. Common Sense about Taste: From Mammals to Insects. *Cell,* 139**,** 234-244.
YEOMANS, M. R. 1998. Taste, palatability and the control of appetite. *Proceedings of the Nutrition Society,* 57**,** 609-615.

YEOMANS, M. R. 2006. Olfactory influences on appetite and satiety in humans. *Physiology & Behavior,* 87**,** 800-804.
YEOMANS, M. R. 2012. Flavour-nutrient learning in humans: An elusive phenomenon? *Physiology & Behavior,* 106**,** 345-355.
YEOMANS, M. R., DURLACH, P. J. & TINLEY, E. M. 2005. Flavour liking and preference conditioned by caffeine in humans. *Quarterly Journal of Experimental Psychology Section B-Comparative and Physiological Psychology,* 58**,** 47-58.
ZALD, D. H. & PARDO, J. V. 1997. Emotion, olfaction, and the human amygdala: Amygdala activation during aversive olfactory stimulation. *Proc Natl Acad Sci U S A,* 94**,** 4119-4124.
ZALD, D. H. & PARDO, J. V. 2000. Functional neuroimaging of the olfactory system in humans. *International Journal of Psychophysiology,* 36**,** 165-181.
ZEMANKOVA, K., MRAZKOVA, J., HOUDKOVA, R., PITHA, J. & KOVAR, J. 2015a. Glucose Addition Affects Postprandial Response of Glucagon-Like Peptide 1 (Glp-1) to a Test Meal. *Atherosclerosis,* 241**,** E34-E34.
ZEMANKOVA, K., MRAZKOVA, J., PITHA, J. & KOVAR, J. 2015b. The effect of glucose when added to a fat load on the response of glucagon-like peptide-1 (GLP-1) and apolipoprotein B-48 in the postprandial phase. *Physiological Research,* 64 Suppl 3**,** S363-9.
ZHONG, C. R., SONG, Y. L., WANG, Y. P., ZHANG, T. L., DUAN, M., LI, Y. M., LIAO, L. J., ZHU, Z. Y. & HU, W. 2013. Increased food intake in growth hormone-transgenic common carp (Cyprinus carpio L.) may be mediated by upregulating Agouti-related protein (AgRP). *General and Comparative Endocrinology,* 192**,** 81-88.
ZHU, Y., HSU, W. H. & HOLLIS, J. H. 2013. The Impact of Food Viscosity on Eating Rate, Subjective Appetite, Glycemic Response and Gastric Emptying Rate. *Plos One,* 8.
ZIJLSTRA, N., DE WIJK, R. A., MARS, M., STAFLEU, A. & DE GRAAF, C. 2009. Effect of bite size and oral processing time of a semisolid food on satiation. *American Journal of Clinical Nutrition,* 90**,** 269-275.
ZIJLSTRA, N., MARS, M., DE WIJK, R. A., WESTERTERP-PLANTENGA, M. S. & DE GRAAF, C. 2008. The effect of viscosity on ad libitum food intake. *International Journal of Obesity,* 32**,** 676-683.