Redesigning nutritional supplements for older adults: a flavour chemistry and nutrition approach.

By Sophie Lester

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Division of Food, Nutrition and Dietetics

School of Biosciences

University of Nottingham

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Abstract

Within the increasing older population, there is a burden of undernutrition. The prescription of oral nutritional supplements (ONS) for those who are undernourished, or at risk of undernutrition, can help improve nutritional status, but the patient must consume an adequate quantity of ONS to gain the clinical benefits.

This research first reviewed the literature to identify factors that influence adherence to ONS. Good palatability is crucial for adequate intake of ONS, but palatability challenges stem from both the product (undesirable sensory properties) and the consumer (age-related sensory changes). The contribution made by aroma compounds to the palatability of ONS was recognised as a comparatively underresearched area. Therefore, this work aimed to fill the evidence gap by investigating the role of intrinsic flavour quality (with emphasis on aroma) and age-related changes in oronasal physiology and sensory abilities on sensory perception and palatability of ONS.

The characterisation of aroma-active compounds in a commonly prescribed ONS was a fundamental stage in the research. Esters (sweet, fruity) and diacetyl (sweet, buttery) were found to make a large contribution to the perceived flavour of the studied ONS. Sulfurous aroma compounds, likely stemming from the heat-treatment of protein ingredients, were also found to contribute to the perceived flavour of ONS. An orthonasal hedonic evaluation established that sulfurous

aroma compounds are primarily unpleasant, whereas the fruity ester aroma (isoamyl acetate) and diacetyl are primarily pleasant. However, sulfurous aromas were rated more pleasantly by older adults who also had impairments in their ability to detect aromas at threshold concentrations.

When aromas were combined in a mixture within a real-food matrix (a flavoured dairy beverage), sulfurous aroma compounds were shown to negatively impact consumer acceptance (for consumers aged 18 – 79 years). However, these sulfurous flavours were less objectionable for older adults. This is a novel finding because it suggests that olfactory impairments may benefit older consumers who need to consume protein-rich foods containing off-flavours. Furthermore, the addition of diacetyl increased the acceptability of the sulfurous flavours, demonstrating partial masking abilities of this compound.

Older adults are also known to experience age-related changes in their oral and nasal physiology, such as reduced salivary flow rates which may influence the way flavours are released in the mouth. Therefore, the next stage of the research investigated differences in the temporal consumption experience (comprising in-mouth aroma release, sensory perception and subjective appetite) of a clinically relevant portion of ONS for groups differing in saliva flow rate, in which repeated measurements were made between sips. This study demonstrated that a lower saliva flow rate is associated with significantly more intense in-mouth aroma release ($p=0.015$), significantly higher aftertaste intensity

($p<0.001$), and greater increase in mouth drying over sips ($p=0.02$), compared to a medium- and high- saliva flow rate. These findings occurred concurrently with relatively lower hunger sensations in the low- and medium-flow rate groups.

This research adds to the growing body of evidence on how best to optimise food and beverage palatability for older consumers. Many older patients who are prescribed ONS are likely to experience reduced salivary flow rates and olfactory impairments. The unique sensory experience of these individuals should be considered in both product development and clinical practice to optimise palatability, hence maximising nutritional intake from ONS and other nutritional foods and beverages whilst minimising wastage.

List of Abbreviations

ONS	oral nutritional supplements
HS-SPME	headspace-solid phase microextraction
GC-MS	gas chromatography-mass spectrometry
GC-O-MS	gas chromatography-olfactometry-mass spectrometry
APCI-MS	atmospheric pressure chemical ionisation-mass spectrometry
CAS	chemical abstracts service registry number
LRI	linear retention index
ppb	part per billion
3-AFC	3-alternative forced choice
DF	detection frequency
I	intensity
MF(%)	modified frequency
BET	best estimate threshold
OAV	odour activity value
DT	detection threshold

RjT	rejection threshold
DMS	dimethyl sulfide
DMDS	dimethyl disulfide
DMTS	dimethyl trisulfide
ANOVA	analysis of variance
SFR	saliva flow rate
LF	low flow
MF	medium flow
HF	high flow
PRPs	proline rich proteins
TPC	total protein concentration
PSR	protein secretion rate
AA	alpha-amylase
Tmax	time to reach maximum intensity
AUC	area under the curve
Imax	maximum intensity
CFB	change from baseline

TIC total ion count

List of Publications

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Lester, S., Cornacchia, L., Corbier, C., Hurst, K., Ayed, C., Taylor, M.A. and Fisk, I. (2021). Age group determines the acceptability of protein derived off-flavour. *Food Quality and Preference*, 91:104212 doi.org/10.1016/j.foodqual.2021.104212

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Chapter 1

1 General introduction

The motivation for this thesis came from the prevalence of undernutrition in the older adult population. Oral nutritional supplements (ONS) are a recommended nutritional intervention to supplement the diet of those older individuals who are at risk of or experiencing undernutrition, however adequate adherence to ONS is crucial for clinical effectiveness. The remainder of this chapter will introduce anorexia of ageing, explore age-related changes that occur in oronasal physiology and sensory perception, give a general introduction to ONS and their use and finish by detailing the thesis aim and objectives.

1.1 The anorexia of ageing

There are an increasing number of older adults in the worldwide population; by 2050 one in five people will be aged 60 years and older (World Health Organisation, 2020). Within this older population, there is a high prevalence of protein-energy undernutrition (PEU) (Margetts et al., 2003, Kaiser et al., 2010, Gandy, 2019) which is defined as an inadequate intake of energy and protein relative to requirements. Intake of other dietary components, such as vitamins and minerals may be compromised too (Balboa-Castillo et al., 2018). Many age-related factors are involved in the aetiology of this undernutrition, including limited finances, social isolation, poor health, appetite changes and reduced sensory function (Guigoz et al., 2002, Donini et al., 2003, World Health Organization, 2015) and these factors can be encompassed into the single term “the anorexia of aging” (see Figure 1-1) (Donini et al., 2003, Landi et al., 2016).

The undernutrition resulting from “the anorexia of aging” has severe consequences which have been established for some time. For example it may deplete the immune system increasing infection risk, lead to muscle breakdown and subsequently an increased risk of falls and fractures, create higher hospital admissions and increase length of hospital stay, and ultimately create an overall greater risk of morbidity and mortality (Elia, 2015, Stratton et al., 2018). Unsurprisingly this undernutrition puts considerable pressure on healthcare costs; the financial consequences of malnutrition in England was estimated to be

>19 billion/year, and older adults were estimated to account for over half of this total cost (Elia, 2015).

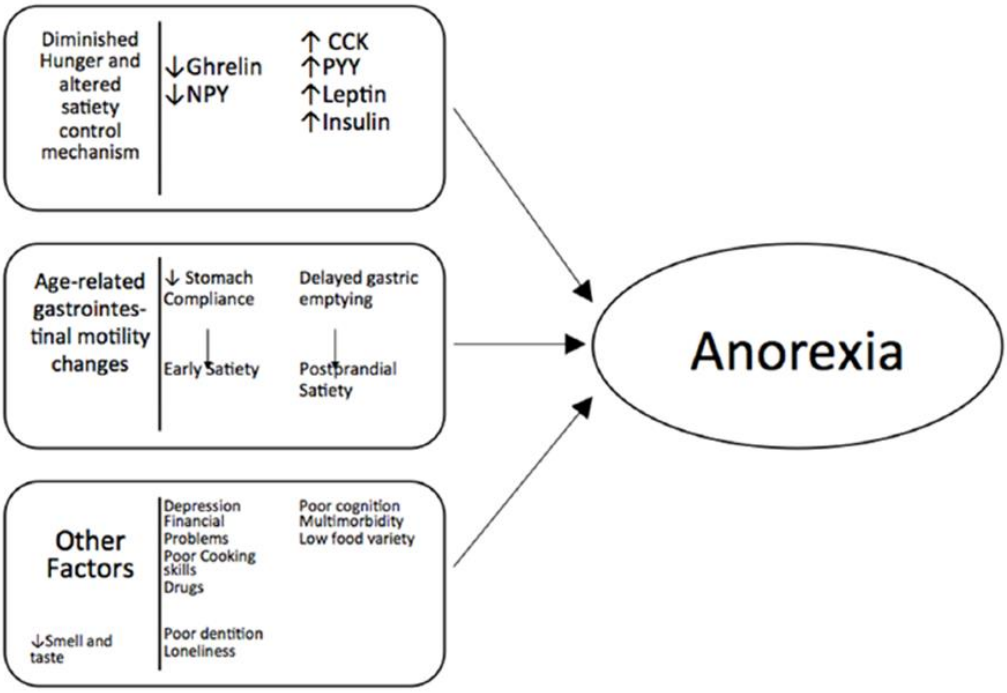

Figure 1-1: Major mechanisms involved in the development of anorexia of ageing by Landi et al (2016).

With ageing, adults experience a decline in sensory capacity (World Health Organization, 2020). Sensory losses in sight and hearing are well recognised; these can be managed effectively with strategies such as hearing aids and glasses (World Health Organization, 2020). Relatively less is known about the loss in sense of smell and taste with age, the impact this can have on health and life quality, or how older people can compensate for this sensory loss. The World Health Organisation (2020) has identified sensory impairments, such as a decreased sense of taste and smell, as a risk factor for undernutrition in the population of older adults.

1.2 Age-related changes in oronasal physiology and sensory perception

It is known that older adults experience age-related changes in their oronasal physiology (relating to the mouth and nose) which can directly impact their sensory perception of foods and beverages (Field and Duizer, 2016, Doets and Kremer, 2016). The human senses play a major role in the food enjoyment (Landi et al., 2016), along with mediating the type and quantity of food consumed (Delahunty, 2010), so it is understandable that age-related sensory impairments can affect appetite, food enjoyment, food choice and nutritional intake in older adults (Somekawa et al., 2017, Schiffman and Graham, 2000, de Jong et al., 1999, Duffy et al., 1995, Griep et al., 1996).

Many different oronasal parameters are involved in the perception of foods and beverages during food and beverage consumption (see Figure 1-2). This review will first provide a summary of the contribution made by each parameter to food and sensory perception, before discussing the changes which can develop in older age.

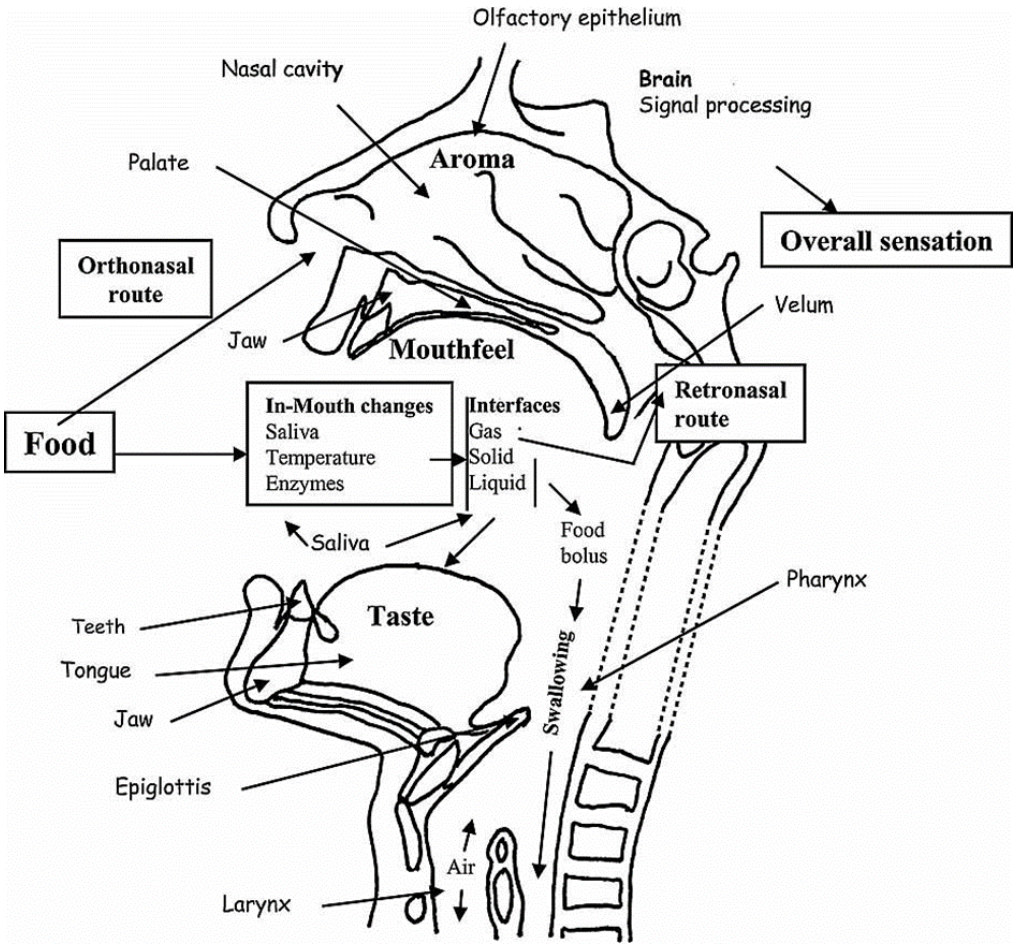

Figure 1-2: Mechanism of release and perception of sensory stimuli during food consumption (Gierczynski et al., 2011).

1.2.1 Olfaction (Smell)

The sense of smell contributes greatly to our appetite, food perception and enjoyment due to the significant contribution it makes to food evaluation prior to consumption, along with food flavour perception (Delahunty, 2010, Stevenson, 2010). In fact, our sense of smell makes the largest contribution to the diversity of food flavours (Delahunty, 2010). Not only this, but our sense of smell is crucial for food safety by permitting our ability to detect dangerous stimuli in foods (Stevenson, 2010, Doty and Kamath, 2014).

Foods contain thousands of aroma compounds which, if they become volatile, can act as stimuli and be sensed by our olfactory system. Two types of olfaction contribute to food perception: orthonasal olfaction, which occurs when smelling food or inhaling before it is placed in our mouths, or retronasal olfaction, which occurs during food consumption (Delahunty, 2010, Yang, 2012). During retronasal olfaction, volatile aromas are released from the food matrix into the gaseous airspace and travel to the back of the nose where they can bind with olfactory receptors. It is reteronasal olfaction which combines with the perception of taste and trigeminal stimuli to drive the perception of food flavour (Yang, 2012).

Olfactory receptors are the olfactory cells which detect volatile aroma compounds. These cells are true nerve cells which are located in the olfactory epithelium; a region of tissue in the upper part of two nasal

cavities (see Figure 1-3) (Delahunty, 2010). Olfactory cells contain cilia (hair-like projections) on their membranes which project from the cells into a mucous layer; these cilia serve to enlarge the surface area of the cell whereas the mucus provides protection and regulates transport of stimuli (Delahunty, 2010). Once a stimulus stimulates the olfactory receptors, neural activity is produced, and a signal travels to the olfactory bulb in the brain, before being sent to the olfactory cortex and the orbitofrontal cortex in the brain, resulting in perception.

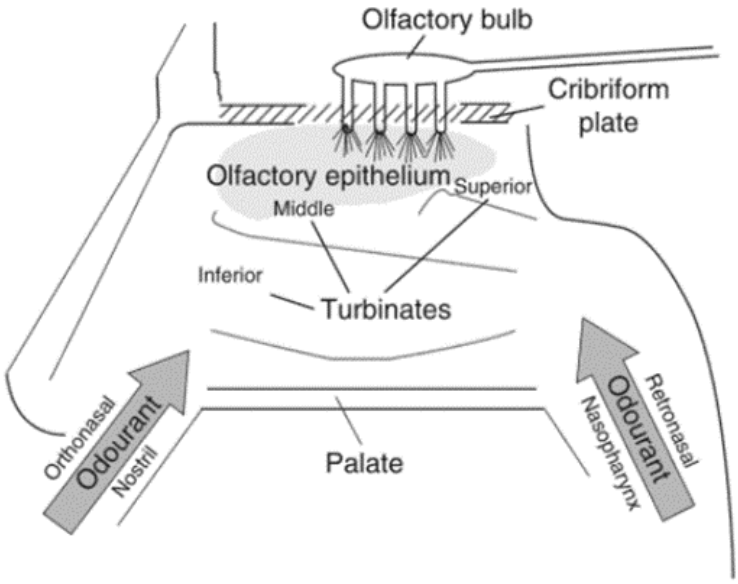

Figure 1-3: The human nasal cavity, illustrating the olfactory receptors, the olfactory epithelium, and the different means of entry for volatile compounds. By Delahunty (2010).

In nature, a perceived aroma is usually made up of a combined mixture of several volatile compounds, but sometimes particular volatiles can be associated with a particular smell, such as isoamyl acetate and banana/pear drops (Kemp et al., 2011).

Olfactory ability is known to decline with age and the prevalence of olfactory impairments that occur with age is high. Murphy et al (2002)

conducted a population-based study with 2491 individuals aged 57-93 years. They found the prevalence of olfactory impairment to be 24.9% but also that the prevalence increases with age; within the population of 80- to 97-year-olds, 62.5% were experiencing olfactory impairments. A recent meta-analysis has found that the age-related decline in aroma identification starts as early as the fifth decade of life (Zhang and Wang, 2017).

It has been found that aroma recognition/identification, aroma detection and aroma discrimination are all affected (Hummel et al., 2007, Doty and Kamath, 2014). Though, it has been suggested that recognition thresholds are relatively more impaired by the ageing process because identification of aroma is also influenced by cognitive factors such as semantic (long-term) memory (Calhoun-Haney and Murphy, 2005, Seow et al., 2016).

Some researchers have found ageing to impair the perceived strength or intensity of aroma at suprathreshold concentrations. In a large survey involving 1.2 million subjects, Wysocki and Gilbert (1989) found age-related impairments in perceived intensity of six odorants, but the rate of decline was not consistent across different odorants. For example, the decline was most noticeable for mercaptans (sulfur aroma) and isoamyl acetate (banana aroma) and less pronounced for rose and eugenol (clove-like aroma) (Wysocki and Gilbert, 1989). Only a limited number of researchers have investigated aroma-specific olfactory impairments and ageing (Wysocki and Gilbert, 1989, Larsson et al.,

2000, Sinding et al., 2014, Seow et al., 2016). Seow et al (2016) suggested that this age-related, aroma-specific loss may distort the perception of aroma mixtures, such as the aroma present in foods.

The aetiology of olfactory impairments is likely to be multi-factorial, and involve age-related alterations within the nose, olfactory epithelium, and olfactory bulb (Doty and Kamath, 2014), but also encompasses the known influence and higher rate of disease and medication on olfaction within the older age group (Schiffman and Zervakis, 2002). There is also a strong association between olfactory impairment and age-related neurodegenerative disease, such as Alzheimer's disease and Parkinson's disease. Olfactory impairments are an early symptom of these diseases, which is due to expression of aberrant proteins within the brain (Wilson et al., 2011, Doty and Kamath, 2014).

The complex aetiology of age-related olfactory impairment are discussed in detail in a review by Doty and Kamath (2014). These authors summarised the contributing factors to include: altered nasal engorgement and airflow, increased propensity for nasal disease, cumulative damage to the olfactory epithelium from viral and other environmental insults, decrements in mucosal metabolizing enzymes and changes in neurotransmitter and neuromodulator systems. A genetic predisposition to age-related impairments in smell has also been identified (Doty and Kamath, 2014).

1.2.2 Gustation (Taste)

Taste receptors are located in the taste buds which lie within papillae on the surface of the tongue, but also in other area of the mouth or throat (Kemp et al., 2011). Taste buds are supplied by a number of nerve cells (see Figure 1-4) which transmit signals by a variety of mechanisms.

There are five distinct taste modalities, and each modality has a variety of different stimuli (tastants) that can elicit the particular taste. For example, salty can be stimulated by sodium chloride or potassium chloride, sweet can be stimulated by sucrose or glucose, sour can be stimulated by citric acid or phosphoric acid, bitter can be stimulated by quinine or caffeine and umami by monosodium glutamate (Kemp et al., 2011). Emerging evidence suggests that, in addition to the five traditional taste qualities, there are further tastes qualities, including a metallic taste and a fat taste (Delahunty, 2010).

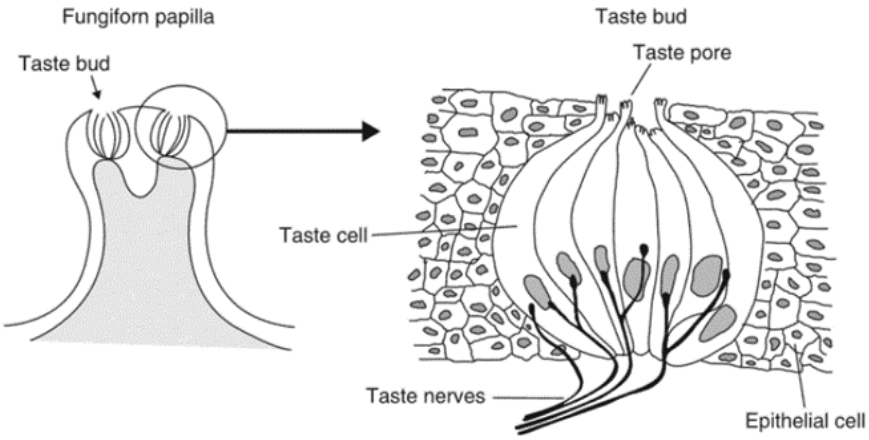

Figure 1-4a (left): Cross section of a fungiform papilla showing the location of taste buds. Figure 1-4b (right): Cross section of a taste bud, showing the taste pore where the taste enters, the taste cells, and the taste nerve fibres (Delahunty, 2010).

Tastants are non-volatile and they must dissolve in an aqueous medium, such as saliva, to be detected by taste receptors. Transduction of a taste nerve impulse may involve a variety of mechanisms depending on the specific tastant involved. It is believed that sweet, bitter and umami tastants transduce via G protein-coupled receptors which, when stimulated, initiate a cascade of events including changes to membrane permeability to cations (K^+ and Ca^{2+}) and production of a nerve signal. Whereas salt and sour taste transductions involve the movement of the taste active ions (Na^+ or H^+) through ion channels, which produces depolarising potentials that create a nerve signal (Delahunty, 2010).

It is well known that taste sensitivity declines with age. In a large meta-analysis of sixty-nine articles, Methven et al (2012) found that all five tastes are negatively affected by ageing and encompassed both detection thresholds and taste identification. However, it was noted that the effect of sensory decline depended largely on the taste modality and specific tastant; detection of umami was impacted most consistently by ageing. Methven et al (2012) also found that in sixteen out of twenty-five studies, the perception of taste intensity at suprathreshold concentrations (such as that in food) was significantly lower for older adults. Although the sweet tastant sucrose was relatively less affected by ageing, with six out of nine studies found intensity not to decline with age (Methven et al., 2012).

The aetiology of taste sensitivity loss may be due to factors such as environmental damage over time (Schiffman and Zervakis, 2002), reduced regeneration efficiency of taste buds (Field and Duizer, 2016) along with declines in oral health, mucosal diseases and bacterial growth which could produce noxious by-products (Ship, 1999). Taste may also be altered by dental status. Use of dental prosthesis which is more common in older age, may cover the palate and efficiency of food breakdown (Wayler et al., 1990, Methven et al., 2012). Schiffman and Zervakis (2002) state that taste losses in normal ageing may be due, in part, to altered functioning of ion channels and receptors. As with olfaction, medication and treatment use can disrupt the ability to taste. For example, many patients who undergo chemotherapy treatment not only experience alterations in their taste perception, but they can detect phantom tastes in their mouths (an unpleasant taste even in the absence of a stimulus in the mouth) (Schiffman and Zervakis, 2002, Galaniha et al., 2020) which could alter the taste of foods.

1.2.3 Saliva

Saliva is an aqueous fluid produced in the oral cavity. It is composed of 99% water with the remaining 1% constituents being a variety of ionic salts and proteins including enzymes and glycosylated mucins which are responsible for the physiochemical properties of saliva (Humphrey and Williamson, 2001, Dinu et al., 2018).

Saliva has a diverse and crucial role in food consumption. Firstly, saliva secretions are involved in the initial stage of food digestion, which occurs in the oral cavity, by providing enzymes which facilitate chemical digestion (Humphrey and Williamson, 2001). Moistening properties of saliva aid food oral processing, whereby consumed food is transformed into a form and consistency suitable for swallowing (Chen and Engelen, 2012, Salles et al., 2010). Saliva provides lubrication, allowing the newly formed food bolus to pass down the oesophagus to the stomach (Bongaerts et al., 2007, Chen and Engelen, 2012).

Whole saliva is produced by three major glands in the mouth (sublingual, parotid and submandibular). The consistency and constituents within whole saliva can change depending on whether the individual has received a stimulus (Humphrey and Williamson, 2001) and even by the type of stimulus (Engelen et al., 2003). This is because each gland differs in the quantity and consistency of saliva which they produce, and the relative contribution made by each gland can alter (Humphrey and Williamson, 2001). A stimulus can be either mechanical (the physical force of biting down), olfactory, gustatory, or visual and anticipatory (Engelen et al., 2003).

Upon rest (in the absence of stimuli), it is the submandibular which contributes the greatest quantity to whole saliva (65%) (Dinu et al., 2018). Saliva produced by these glands is viscous, mucin-rich and contains relatively lower levels of alpha-amylase (Humphrey and Williamson, 2001). Upon stimulation, the contribution made by the

parotid gland increases to around 50 - 70 % (Humphrey and Williamson, 2001, Gibbins and Carpenter, 2013, Dinu et al., 2018). Parotid saliva is known to be lower in mucins (Mosca and Chen, 2017) but higher in proteins such as proline-rich-proteins (PRPs) and amylase (Dinu et al., 2018). Upon stimulation, whole saliva therefore increases in volume and changes in consistency, becoming relatively less viscous with a lower concentration of mucins (Gibbins and Carpenter, 2013).

Saliva is essential in texture perception because of the contribution it makes to the initial hydration and breakdown of foods (Chen and Engelen, 2012). Moreover, some mouthfeel sensations, such as astringency, are suggested to be related to the type and quantity of salivary proteins such as PRPs (Horne et al., 2002, Dinnella et al., 2009, Dinnella et al., 2010). In addition, salivary viscosity can mediate the extent of food clearance from the mouth, with a low viscosity fluid working best (Chen and Engelen, 2012).

Saliva is also an essential medium for modifying the extent of flavour release during food consumption. Hydrophilic tastants must travel to receptors through the aqueous medium, and hydrophobic aroma compounds must partition from the aqueous salivary-food matrix into the ‘mouth space’ to reach the olfactory epithelium to be perceived (Ployon et al., 2017). Salivary components such as mucin and amylase can also bind with aroma compounds mediating their release (Pagès-Hélary et al., 2014). Volatile aroma compounds differ in chemical properties such as hydrophobicity, so the chemical nature of the volatile

aroma compound, and the subsequent interaction with aqueous saliva and salivary constituents, can determine the extent of the aroma release into the gaseous olfactory mouth-space (Ployon et al., 2017, Yang et al., 2020).

A normal unstimulated saliva flow rate (USFR) is anything above 0.1 mL/min, hyposalivation can be diagnosed when a USFR is below 0.1 mL/min. A stimulated saliva flow rate (SSFR) of 1.5 - 2.0 mL/min is considered normal and a SSFR ≤ 0.5 mL/min can be considered suggestive of hyposalivation (Edgar et al., 2004, Villa et al., 2014).

Due to factors such as loss of acinar cells and secretory tissue (Xu et al., 2019) ageing is generally associated with reduced saliva flow rate and increased occurrence of hyposalivation (Affoo et al., 2015, Muñoz-González et al., 2018b). The prevalence of salivary hypofunction in the older population is high and is estimated to range from 30 – 50% (Muñoz-González et al., 2018b). Though, there has been debate over whether hyposalivation is a direct consequence of ageing, or due to increased levels of medication use, disease, and poor hydration status in the older age group. One recent study has found that salivary flow rate decreases independently of dental status and medication use (Vandenberghe-Descamps et al., 2016).

Relatively less is known about how saliva quality and composition changes with ageing. Due to a decreased rate of protein synthesis, along with increased rate of mucin degradation, it has been proposed that the saliva of older adults contains a reduced number of salivary proteins such as mucins (see Figure 1-5) (Xu et al., 2019). However, it has also been proposed that a reduced saliva volume can lead to more concentrated whole saliva, containing a higher concentration of salivary proteins and salivary ions (Nagler and Hershkovich, 2005, Zussman et al., 2007). This has been found to be associated with a more viscoelastic saliva (Zussman et al., 2007, Xu et al., 2019). A highly concentrated and viscous saliva lacks the ability to flow freely in the oral cavity (Zussman et al., 2007, Xu et al., 2019) and may be more mucoadhesive (Pushpass et al., 2019).

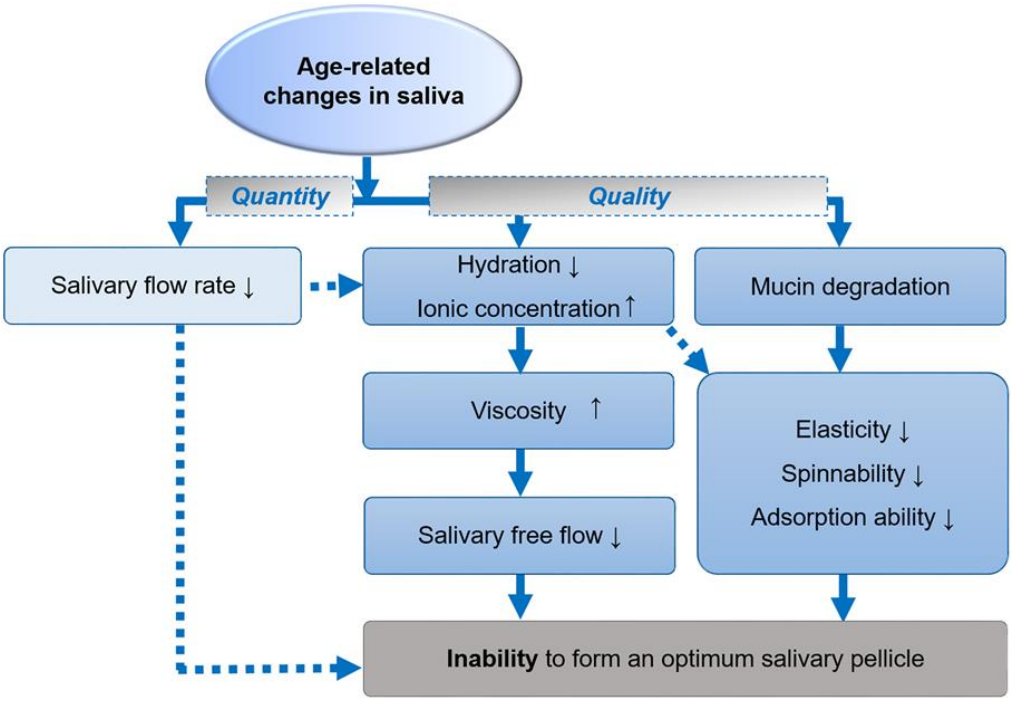

Figure 1-5: Summary of age-related changes in quantity and quality of saliva by Xu et al (2019)

More recently, one recent study found the viscoelasticity and mucin concentration of unstimulated saliva to reduce with ageing (Pushpass et al., 2019). In contrast, the authors found the stimulants menthol and caffeine to produce stimulated saliva with higher viscoelasticity in the older adult group, compared with the younger group (Pushpass et al., 2019).

Age-related salivary changes in the quantity and quality of saliva have large implications for the ability of the older adult to manipulate and breakdown food in the oral cavity. A reduced lubricating capacity of saliva could impair swallowing abilities and restrict adequate food consumption in the older population (Muñoz-González et al., 2018b). Due to the essential role of saliva in food flavour perception, changes could alter the ability of food stimuli to reach their receptors. In an ex-vivo study, Munoz-Gonzalez et al (2018b) recently observed lower aroma release of aroma compounds (octanal, ethyl hexanoate and 2-nonanone) in a group of older adults with hyposalivation compared to a control group. In contrast, a number of recent studies have found significant negative associations between stimulated saliva flow rates and the dynamic intensity and duration of aroma release and perception (Yang et al., 2020, Criado et al., 2021).

Age-related salivary changes could drive changes in saliva-food interactions, and therefore alter the perception of textural and mouthfeel attributes such as astringency. In addition, age-related salivary changes could affect how efficiently foods can be cleared from the oral cavity,

and thus have consequences in the perception of aftertaste and mouthfeel sensations that linger post-consumption.

1.2.4 Trigeminal sensations

The trigeminal somatic sensory system is crucial in food perception and the third important sensory modality which contributes to flavour perception. The trigeminal nerve, or fifth cranial nerve, communicates most of the information perceived in mouth for tactile, proprioceptive, temperature and painful stimuli (Chen and Engelen, 2012). Therefore, it is the trigeminal system which is responsible for perception of food texture and mouthfeel sensations along with changes in temperature and the perception of chemical irritation, such as that from capsaicin in chilli pepper.

Texture is perceived by a number of different types of mechanoreceptors which line the oral cavity. When food is manipulated, deformed, or moved across the oral receptor sheath, an action potential is initiated which transmits an impulse to the central nervous system (Chen and Engelen, 2012). Chemesthesis (defined as the sensations that arise when chemical stimuli activate receptors) is believed to occur due to stimulation of free nerve ending of afferents from the trigeminal nerve in the oral region whereas temperature is perceived by thermoreceptors in the oral cavity (Chen and Engelen, 2012).

Compared to other sensory modalities, relatively less is known about how the sensitivity of the trigeminal systems change with age. It is

generally believed that, as with olfactory and gustatory sensations, trigeminal somatic sensory system deteriorates with ageing, resulting in reduced sensitivity and reduced ability to discriminate between texture and chemical stimuli (Forde and Delahunty, 2002, Kremer et al., 2007a, Kremer et al., 2007b).

The contribution made by food texture to food liking appears to differ between the young and old, as it has been found that texture can play a greater role in food enjoyment and pleasantness for older adults, compared with the young (Forde and Delahunty, 2002, Forde and Delahunty, 2004, Kremer et al., 2007a, Kremer et al., 2007b).

Compared with other sensory modalities, it has also been proposed that the trigeminal somatic sensory system is better preserved through the ageing process, (Pushpass et al., 2019). For example, Fukunaga et al (2005) found significant age-related deterioration in ability to taste, but not for somatic sensations such as touch and irritation from capsaicin. Kremer et al (2007b) found no age effects in judgments of oral astringency or pungency of black pepper. Withers et al (2013) found that the perception of milk thickness and mouthcoating does not differ with age, and in fact they found that mouth drying perception was enhanced in older age. The mechanism behind this is unknown but was hypothesised to be due to altered physiology such as age-related changes in saliva flow rate or dentition. Many physiological changes, such as the age-related decrease in salivary volume and compositional changes, along with muscle forces and dentition, likely affect the

breakdown of food in the oral cavity, and thus the perception of food texture (Field and Duizer, 2016).

1.2.5 Dentition

Teeth are essential for effective food breakdown and oral processing, and hence also sensory and flavour perception. Ageing is associated both with tooth loss (edentulism) but also tooth wear (Field and Duizer, 2016). This can result in difficulties manipulating and chewing food into a form suitable for swallowing (Ship, 1999, Naka et al., 2014, Field and Duizer, 2016). Ageing is also strongly linked with increased use of prosthetic teeth such as dentures which can help to restore function, but these are frequently reported to be poor fitting (Sahyoun and Krall, 2003). Hence, chewing efficiency is not always fully restored and prosthesis can hinder breakdown of food particles (Mishellany-Dutour et al., 2008, Field and Duizer, 2016).

The use of dentures is associated with reduced oral clearance and poor oral hygiene (Kanlı et al., 2005) which will promote the development of noxious odours and mucosal infections (Ship, 1999). Dentures could also result in a reduced oral surface area for the perception of textural and taste stimuli and modify the perception of foods (Henkin and Christiansen, 1967, Veyrune and Mioche, 2000, Engelen et al., 2002).

In addition, it has been found that denture wear can result in elevated retronasal detection thresholds of olfactory stimuli (Duffy et al., 1999). This was hypothesised to be caused by interference to effective

chewing and mouth movements (Duffy et al., 1999) but could be related to alterations to the oronasal airflow and hence mediate the potential for volatile aroma compounds to interact with olfactory receptors.

1.2.6 Muscle forces

Muscle forces within the oral cavity encompass both the tongue and jaw muscles and are important during food sensory perception because their movement drives the manipulation and breakdown of foods. Upper and lower jaw muscles (the maxilla and mandible) must come together to allow the teeth to crush and grind food whereas tongue muscle is essential for bolus manipulation and swallowing (Field and Duizer, 2016).

Sarcopenia can be defined as the decline in function due to the loss of muscle mass (Morley, 2017). This muscle loss is generally associated with increased frailty and loss of functional ability and can be measured using outcomes such as walking speed and handgrip strength (Tsutsumimoto et al., 2018, Bhasin et al., 2020). However, sarcopenia is not confined to a single area of the body, so it similarly affects muscles within the oral cavity, a significant positive correlation between tongue strength and handgrip strength have been found (Butler et al., 2011). It has been found that older adults have reduced size of masticatory muscles (Palinkas et al., 2010) and have reduced bite force (Yeh et al., 2000, Palinkas et al., 2010). Older adults may compensate

for this loss by chewing for a longer duration and with more chewing strokes (Mishellany-Dutour et al., 2008).

Withers et al (2013) have drawn attention to how the clearing of unpleasant flavour could be a prominent problem in older adults with impaired muscle strength. Alterations in the extent of food manipulation and oral processing could influence in-mouth flavour release. de Wijk et al (2003a) found that more complex intraoral food manipulations trigger more intense perceptions of flavour of a range of semi-solid foodstuffs, something which the authors attributed to increased flavour release during food breakdown. Steele et al (2009) found that older adults have slower and longer duration of tongue movements during the oral processing of water, which could alter flavour release from liquid foods and beverages, but this is also likely to be product and matrix dependent.

1.3 Oral nutritional supplements

Oral nutritional supplements (ONS), sometimes referred to as sip-feeds, are commercially available products that are prescribed for patients who are undernourished, or at risk of undernutrition, due to not meeting their dietary requirements from normal food (Beck et al., 2009). The aim of ONS provision is to improve the patient's overall nutritional intake in order to improve their nutritional status and subsequently clinical outcomes (National Collaborating Centre for Acute Care, 2006). ONS typically contain a mixture of both macronutrients (carbohydrate,

protein, and fat) and micronutrients (vitamins and minerals) and, when consumed in a certain volume, some ONS are nutritionally complete meaning they contain sufficient macronutrients and micronutrients to meet daily requirements (Gandy, 2019). Nutritional information on the typical macronutrient composition of two commercially available ONS (Fortisip® and Fortisip Compact®, Nutricia) can be found in Table 1-1.

Table 1-1: A comparison of the macronutrient composition of two commercially available oral nutritional supplements.

	Fortisip (standard)		Fortisip Compact	
Nutritional information	Per 100 mL	Per bottle (200 mL)	Per 100 mL	Per bottle (125 mL)
Energy (kcal)	150	300	240	300
Carbohydrate (g)	18.4	36.8	29.7	37.1
Protein (g)	6.0	12.0	9.6	12.0
Fat	5.8	11.6	9.3	11.6

Being foods for special medical purposes (FSMPs), in Europe, ONS are regulated under the Commission Directive 1999/21/EC (European Commision, 1999). In the UK, they are prescribed by doctors and dietitians, according to medical judgement, using the British National Formulary (BNF) as guidance and come under the Advisory Committee on Borderline Substances (ACBS).

There are many different varieties of ONS marketed to meet the needs of different patient groups (Gandy, 2019). ONS can be puddings or powders, different types (high protein, fibre containing) and styles (juice, milkshake, yoghurt), but most ONS are prescribed in a sweet, long life, milk-based, liquid form (Gandy, 2019).

1.3.1 Clinical effectiveness of ONS

A Cochrane review by Baldwin et al (2011) reviewed randomised control trials that lasted between 18 days to 24 months and found that the prescription of ONS resulted in significant weight gain in patients (2.20 kg, $p < 0.001$) compared with patients receiving dietary advice alone. As part of the development of guidelines for nutritional support, a systematic review of ONS supplementation versus standard care found benefits from ONS supplementation such as significant weight gain, a significant reduction in the number of complications and a significant reduction in mortality rate (National Collaborating Centre for Acute Care, 2006).

Stratton et al (2007) conducted a 'review of reviews' with the aim 'to assess and consolidate the key findings'. They found that ONS consistently increased total nutritional intake along with a significant overall reduction in mortality and reduction in complications (e.g. infections, pressure ulcers) across patient groups.

1.3.1.1 Clinical effectiveness of ONS in undernourished older adults

It has been suggested that older people who are unwell and undernourished are expected to benefit more from supplementation and that the inclusion of candidates that do not meet the clinical criteria for nutrition support in trials may mask the benefits of treatment (Wolfe and Mathiesen, 1997, Milne et al., 2009).

One systematic review (Milne et al., 2009), has reviewed evidence on trials which exclusively include older adults as the study population. The authors brought together evidence from sixty-two trials with older participants in a variety of different settings (such as the community and hospital) and found benefits to weight gain (2.2%), a reduced number of health complications, and a reduction in mortality risk of older patients who are undernourished. Though the weight increase of 2.2% may seem small, the authors concluded that it was consistent and would equate to an average weight gain of 1.2 kg for a person weighing 55 kg (Milne et al., 2009). This weight gain is considerable in a population for whom weight loss is a significant problem and can lead to muscle wasting, decreased immunocompetence and increased rate of complications, along with being highly predictive of morbidity and mortality (Alibhai et al., 2005).

1.3.1.2 Clinical effectiveness of high-protein ONS

Due to an age-related anabolic resistance, and a greater occurrence of disease-related protein catabolism in older age, there is consensus amongst international bodies and researchers that the optimum daily requirements of protein for older adults (≥ 65 years) is higher, than for younger adults (< 65 years) (Deutz et al., 2014). Irrespective of age, in order to maintain nitrogen balance in adults, the UK has a Reference Nutrient Intake (RNI) of 0.75 g protein/kg body weight/day. Though, these requirements are believed to rise to 1.0 – 1.2 g protein/kg body weight/day in older age (Bauer et al., 2013, Deutz et al., 2014). If acute

or chronic illness is experienced in older age, requirements are believed to rise further to 1.2 – 1.5 g protein/kg body weight/day (Deutz et al., 2014).

A large proportion of older adults do not meet their recommended daily intake of protein from foods alone; a recent report found that 27 % of those aged 65 to 74 years, and 33 % of those aged 75 years and older, had protein intakes below the RNI (Scientific Advisory Committee on Nutrition (SACN), 2020). This emphasises the demand for palatable, high-protein foods to support protein intake for older adults. To be classified as a high protein food, EU regulations state that a product must contribute > 20% energy from protein.

A systematic review and meta-analysis by Stratton et al (2005) found that high-protein ONS, in particular, resulted in significantly lower incidence (25 %) of pressure ulcers when compared with routine care. A more recent systematic review and meta-analysis by Cawood et al (2012) evaluated whether high-protein ONS has beneficial effects on clinical, nutritional and functional outcomes on adults in any setting. They found a significant reduction (19 %) in a range of complications (such as healing of surgical wounds and pressure ulcers) and reduced infection rates along with increased handgrip strength, quality of life and a reduction in hospital stay and hospital re-admissions.

1.3.2 Cost-effectiveness of ONS

A recent comprehensive systematic review by Elia et al (2016) evaluated the cost-effectiveness of ONS use in any setting. They found significant cost-savings with both short-term (< 3 months) and long-term (≥ 3 months) of ONS use. They also found that investment into ONS use in the community produces cost savings in the hospital, through various means such as reduced hospital re-admissions and reduced complications.

1.3.3 Adherence to ONS

Adherence can be defined as “the extent to which a person’s behaviour – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider” (World Health Organization, 2003). To meet requirements, reduce wastage and gain the cost- and clinical effectiveness of ONS, supplementation relies on patients consuming a high percentage of the total ONS that is prescribed. Adherence to the ONS prescription to ensure adequate nutritional intake is critical and, in most cases, this will need to be long-term adherence.

Some authors have identified that adherence to ONS is a problem which can prevent clinical benefits of the intervention. Bruce et al (2003) reported that poor adherence to ONS was the major factor which limited the prevention of weight loss after hip fracture in elderly patients. Kayser-Jones et al (1998) found that patients who were prescribed

ONS following weight loss continued to lose weight, something which they attributed to the low adherence of 7 % (only 2 out of 29 participants consumed the full amount of ONS).

However, there is a lack of consensus as to what is considered “good” adherence. In one study 55-83 % adherence was considered “very good” (Nolan, 1999) and in another study, 50-79 % has been considered reasonable (Roberts et al., 2003). Nonetheless, it could be questioned how a shortfall affects clinical outcomes. Jobse et al (2015) found that even medium adherence within the range of > 30 % - < 80 % leads to markedly lower positive effects in clinical outcomes such as body weight and arm circumference than high adherence (≥ 80 %).

The most comprehensive data came from Miller et al (2005) who conducted a randomised, controlled trial of ONS supplementation in older, nutritionally at-risk patients. To our knowledge, it is the only study to attempt to take into account variability in individual energy requirements in the ONS prescription rather than a ‘one dose fits all’ approach and hence evaluate adherence relative to requirements. They concluded that adherence to prescribed ONS was two-thirds of the volume prescribed, and as a result, patients were at risk of not meeting their nutritional requirements.

Not only does low adherence limit positive clinical outcomes in patients but the cost to healthcare of wasted ONS could be considerable. Gosney (2003) obtained costings for the wastage of sip feeds during a 24-hour period on four elder care wards (n = 24). Adherence was

measured to be 37 %, and subsequently the cost of this waste was approximately £50.12 a day. The authors further extrapolated this cost to a net loss of £18,294 per annum on these four wards alone. Although this study only gave a “snapshot” of ONS use over a limited period of time, this study gives a suggestion that, when the worldwide prescription and entire potential wastage of ONS is considered, this figure may be dramatically high.

1.4 Summary

In this chapter, anorexia of ageing, incorporating changes in oronasal physiology and sensory perception of foods, has been shown to occur in older age, which may impact intake, amplifying the risk of undernutrition resulting in potentially serious health consequences. ONS are an effective strategy to overcome undernutrition, but adherence issues have been identified. This is an important area for future research to improve health outcomes.

A comprehensive review of the available literature on factors affecting adherence to and perceived palatability of ONS would be beneficial to identify potential areas for improvement and to guide the focus of future research.

1.5 Research motivation, aim and objectives.

Oral nutritional supplements (ONS) offer a clinically effective way to increase nutritional intake in older adults who are at risk of undernutrition, however poor long-term adherence to prescribed volumes limits their clinical effectiveness.

Aim of research:

The overarching aim of this research was to investigate the role of intrinsic flavour quality, and age-related changes in oronasal physiology and sensory abilities, on sensory perception and palatability of ONS. Due to a lack of knowledge in the area, an emphasis was placed on the contribution made by volatile aroma compounds.

Objectives:

Chapter 3

- To review the available literature on factors affecting adherence, intake and perceived palatability of ONS to identify areas for improvement, uncover gaps in the available evidence and guide the focus of the experimental research.

Chapter 4

- To use gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry-mass spectrometry (GC-O-MS) to characterise the volatile profile of a commonly prescribed

ONS and identify the major aroma-active compounds which contribute to flavour perception of ONS.

- To assess the hedonic impact of these aroma compounds in isolation at suprathreshold concentrations and differences between older and younger adults.
- To use detection thresholds to evaluate differences in olfactory sensitivity between older and younger adults for these major aroma-active compounds and predict how this may affect global flavour perception of ONS.

Chapter 5

- To determine the hedonic impact (positive, negative or no impact) of potential off-flavours when added in increasing concentrations to a flavoured dairy beverage.
- To identify the concentrations at which consumer rejection occurred and evaluate whether human age was a factor influencing consumer acceptance of potential off-flavours.
- To uncover interactions between potential off flavours compounds in a flavoured dairy beverage.
- To compare the suitability of two separate rejection threshold methodologies (graphical approach (RjT_{50}) and best estimate thresholds (BET)) and the impact of each on our conclusions.

Chapter 6

- To investigate differences in the temporal consumption experience (comprising sensory perception, in-mouth aroma release and subjective appetite) of a clinically relevant portion of ONS, for groups differing in SFR, in which repeated measurements were made between sips.
- To elucidate the role of salivary quality and constituents (saliva protein content and saliva viscosity) in potential group differences.

Research Hypothesis

I hypothesise that the studied ONS contains inherent flavour characteristics that influence product acceptability, and that individual differences in consumer perception (resulting from human ageing) interact to modulate the perceived flavour and palatability.

Chapter 2

2 Factors affecting adherence, intake, and perceived palatability of ONS: A literature review

Sophie Lester[1], Marleen Kleijn[3], Leonardo Cornacchia[3], Moira A. Taylor[2], Ian Fisk[1]

[1]Division of Food Nutrition and Dietetics, School of Biosciences, University of Nottingham, United Kingdom

[2]National Institute for Health Research (NIHR), Nottingham Biomedical Research Centre, Division of Physiology, Pharmacology and Neuroscience, School of Life Sciences, University of Nottingham, United Kingdom

[3]Danone Nutricia Research, Uppsalalaan 12, 3584 CT Utrecht, the Netherlands

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2.1 Abstract

Good adherence to a nutritional intervention is a primary determinant of effectiveness. Older adults experience unique barriers which can hamper their ability to adhere to nutritional interventions. Oral Nutritional Supplements (ONS) are one nutritional intervention where adherence is lower than optimal. This review aimed to bring together the major factors involved in a patient's adherence to ONS, detect areas for improvement and identify gaps for future research. Contextual factors included healthcare staff and timing of administration, and personal factors stemming from the older consumer, such as sensory changes and motivation, which alter their experience and desire to consume ONS. The product's sensory characteristics determine palatability and intake, but many undesirable attributes stem from nutritional ingredients. One comparatively under-researched area is the contribution made by aroma to older adults' experience of ONS. Further research should address this evidence gap to optimise the flavour profile and palatability for older consumers, thereby optimising intake. A combined multidisciplinary effort involving strategic expansion of research efforts, industry development and clinical practice should simultaneously address identified factors to provide the best possibility at improved adherence.

Key words: Healthy ageing, Oral nutritional supplements, Adherence, Palatability.

2.2 Introduction

The worldwide population is ageing markedly. Global life expectancy has doubled since 1900, and it is estimated that every month there is an increase of 800 000 older people in the world population (World Health Organization, 2003, World Health Organization, 2020). Maintaining adequate nutritional status in older age has been identified as a key factor to achieve healthy ageing, which is defined as the process of developing and maintaining the functional ability that enables wellbeing in older age (World Health Organization, 2020). Interactions have been suggested between undernutrition and sarcopenia, the latter having major detrimental impacts on quality of life (Vandewoude et al., 2012).

Many barriers stand in the way of achieving adequate nutrition in older age. For example, older individuals may experience poverty and isolation, changes to appetite mechanisms, swallowing difficulties and sensory changes; these factors can be encompassed under the common term “anorexia of ageing” (Landi et al., 2016). This ‘anorexia of ageing’ can predispose older individuals to undernutrition, particularly protein-energy undernutrition (PEU), which is defined as an inadequate intake of energy and protein compared to requirements. PEU is associated with delayed recovery from disease, lower quality of life, and increased morbidity and mortality risk (Leij-Halfwerk et al., 2019). Oral nutritional supplements (ONS) are a clinically effective and relatively inexpensive way to supplement the diet of those older individuals who

are undernourished or at risk of undernutrition (Gandy, 2019). Elia et al (2018) estimated that the appropriate provision of ONS could result in net cost savings of £172 - £229 million, mainly due to reduced healthcare costs.

Nevertheless, adherence is a primary determinant of the effectiveness of a nutritional intervention or treatment (World Health Organization, 2003) and can be defined as "the extent to which a person's behaviour – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider". Adherence to ONS has been found to be lower than adequate (Gosney, 2003, Miller et al., 2005, Jobse et al., 2015), limiting the potential effectiveness.

An increased understanding of the factors contributing to poor food and supplement intake in older adults should enable the development of appropriate strategies to support the health of older persons (Lad et al., 2005). Therefore, to gain an understanding of factors that influence adherence to ONS, identify areas for improvement and further research, this review explores the factors that affect adherence to, intake of, and perceived palatability of a prescribed dosage of oral nutritional supplement, with an emphasis on research with older individuals.

2.3 Factors affecting patient adherence, intake, and perceived palatability of ONS.

Adherence to ONS has multifaceted causes; however, they can be broadly categorised into contextual (environmental), person and product factors (Nieuwenhuizen et al., 2010, den Uijl et al., 2015). Under the categorisation of these headings, the main factors influencing ONS adherence will be discussed.

2.3.1 Contextual factors

Contextual factors are those not directly related to the food or subject (Nieuwenhuizen et al., 2010); they may include the physical environment of the patient, along with the interaction with those around them, such as family members and healthcare staff.

2.3.1.1 Setting

Few studies have compared adherence to ONS by the setting. Miller et al (2005) found no difference in adherence to ONS between those who spent most of the intervention period in institutional care (supervised administration via the drug cart as opposed to the community (self-administration). A systematic review by Hubbard et al (2012) found the mean percentage adherence to ONS in the community studies was 80.9 %; significantly greater than the mean percentage adherence to ONS in hospital (67.2 %). However, no significant differences in adherence were found across settings when weighted for sample size.

2.3.1.2 Healthcare staff

Healthcare staff have a significant influence on patient's nutrient intake, but some authors report that within a medical context, nutrition can have a low priority (Brosnan et al., 2001, Nieuwenhuizen et al., 2010)

Lad et al (2005) suggested that the provision of ONS is not well regulated. A qualitative study by Lambert et al (2017) into patients and healthcare professionals' views on the efficacy of a specialised ONS programme, found that some staff made an individual judgment when dispensing the prescription, which led to more or less ONS being dispensed. Over two consecutive days of observation in a nursing home, Simmons and Patel (2006) found fewer than 10 % of patients received ONS consistent with their prescription. A longer prospective study by Kayser-Jones et al (1998) found that only nine of the twenty-nine residents (31 %) receiving supplements were served the correct type and number of supplements as ordered by their physicians. If patients are not provided with their correct prescription, adherence and clinical efficacy are restricted.

The opinions of healthcare staff on ONS could also influence patient adherence to ONS. Lad et al (2005) collected self-completed questionnaire data from healthcare staff and found that a small number of staff had sampled the ONS themselves, and of these, over 50 % gave unfavourable remarks such as "horrible", "too sickly", and "not appetising to look at or smell". The importance of expectation on sensory perception is well known (Blackmore et al., 2020), so

healthcare staff's perception of ONS may indirectly influence patient perception and adherence. This effect could be utilised for a positive benefit if staff played an active role in endorsing the product to the patient to enhance their expectations before it is tasted.

Nursing home residents need at least an average of 38 minutes of assistance per meal to encourage adequate food and fluid intake (Simmons et al., 2001). However, it has been found that nurses spent less than one minute per patient encouraging consumption of ONS between meals (Simmons and Patel, 2006). Prompting and physical assistance with feeding is recognised to increase nursing and care staff time demands (Simmons and Schnelle, 2004) and in reality there is uncertainty over the feasibility of adjustments with limited resources (finance and time).

2.3.1.3 Social factors

Due to reduced social networks and physical mobility, greater social isolation in older adults has been credited as a contributing factor to undernutrition in this population (Guigoz et al., 2002, Roberts, 2002, Nieuwenhuizen et al., 2010). The importance of social company during eating on nutritional intake in older adults has been well documented (Shahar et al., 2001, Hughes et al., 2004). For example, several researchers have found that serving food in a social dining room setting versus at the bedside of older patients is beneficial to nutritional intake and clinical outcomes such as body weight (Remsburg et al., 2001, de Graaf et al., 2006, Wright et al., 2006). With relevance to ONS

products, McAlpine et al (2003) assessed snack intake (including ONS intake) in twenty-one adults aged between 60 and 79. All participants underwent both “alone” and “group” conditions. Participants were asked to invite two friends to the study in group conditions. They found that overall, energy intake was generally higher in the group condition for all items.

The views and attitudes of family members on ONS use could be important to adherence, especially if they become the healthcare proxy for the patient. Simmons et al (2003) used forced-choice questionnaires to investigate family members preferences for nutritional interventions that aimed to improve their relatives' nutritional intake. ONS was reported to be the most frequently used nutritional intervention, but it was placed second to last (out of seven nutritional interventions) in order of preference, with preference given to improving quality of food and quantity of eating support. Educating family members about the benefits of ONS and providing greater confidence in the consequences of their use could have potentially positive effects on patient adherence to ONS.

2.3.1.4 Timing of administration

Due to the potential impact on appetite, the timing of ONS administration, and the relationship to their administration with meals may affect adherence to ONS and overall nutritional intake. National Institute for Health and Care Excellence (NICE) currently recommend that ONS are prescribed between meals; hence it is recommended to

administer one ONS after breakfast and one after lunch (Volkert et al., 2006). In agreement with these recommendations, Hubbard et al (2012) found that most ONS were administered between meals rather than with meals and subsequently reported that overall energy and protein intake significantly increased. The impact of ONS on satiation and satiety is discussed further in section 2.3.3.2.

ONS administration with medicine rounds (such as the drug cart) could also affect adherence because it establishes the supplement as part of medical treatment and encourages intake (Miller et al., 2005). This hypothesis is supported in studies by van den Berg et al (2015) who found benefits to adherence and Potter et al (2001) who found a reduction in weight loss and mortality risk after providing ONS in smaller volumes with medical rounds.

2.3.2 Personal factors

Personal factors include patient's attitudes and motives towards consuming ONS, their consumption behaviour, health status and age.

2.3.2.1 Attitudes and motives

Den Uijl et al (2015) highlighted the lack of research into the personal factors which drive ONS consumption in older frail adults. They suggested that in some subgroups, ONS might be used for the benefits of the product (such as improved health status and prolongation of independence) rather than for the product attributes (e.g. taste,

volume). Using a means-end chain (MEC) method, they conducted a study to elucidate the personally relevant factors related to ONS consumption in two groups of older nutritionally frail ONS users: community-dwelling persons and care home residents. Two hierarchical value maps (HVM) were rendered, revealing that the community-dwelling group took ONS to prolong their independence and strength. In contrast, the care home group reported values related to improvements in quality of life. It would be intriguing to explore the benefit of using these consumer derived and relevant values in ONS communication strategies.

An advantage of this study was the use of in-depth interviews covering a wide range of topics, including perceived benefits of ONS. Participants were also physically exposed to their product and asked to share experiences, reducing reliance on retrospective memory. Interestingly, 80 % of participants viewed ONS as a snack food rather than a medicine, something which the authors attributed to the participants preferring to be regarded as consumers rather than patients.

2.3.2.2 Consumption behaviour

Small changes in how foods and drinks are served can affect nutritional intake. For example, simply serving food and drinks to people with Alzheimer's disease on red coloured crockery, compared with white, significantly increased the amount of food and drink consumed (Dunne

et al., 2004). Despite this, little research has investigated the effects of consumer behaviour on ONS adherence.

Den Ujil et al (2015) found that older adults living in the community preferred to drink the ONS from the bottle using a straw (85 %) because it was more convenient and more manageable (65 %). However, in care home settings, almost an equal proportion of participants used a straw (45 %) as drunk from a glass (30 %). Variations in drinking behaviour are likely to influence adherence. Allen et al (2014) found that a greater amount of ONS was consumed by undernourished older adults in a variety of settings (care homes and hospitals) when it was supplied to them in their usual drinking method (glass or beaker) versus with a straw inserted directly into the container. Authors attributed this to greater familiarity, as for older people, drinking from a straw may be less familiar than drinking from a glass or beaker. However, drinking from a straw requires lip muscle strength and suction pressure and it is important to note that with ageing, older adults can experience systemic wasting of muscle (sarcopenia), which impedes suction ability (Kubo et al., 2013) and may be a contributing factor in preference for a glass over a straw.

Another factor that may be important in adherence to ONS is the temperature at which patients consume them. It is recommended that ONS be chilled to fridge temperature before consumption, which has been reported to improve flavour perception (den Uijl et al., 2015). Den Uijl et al (2015) have reported that most older adults in the community

consumed ONS preferably at fridge temperature (60 %). However, within hospitals and care homes, there is doubt over the availability of resources for chilling large amounts of products for residents; Den Uijl et al (2015) found that only 45 % of ONS were drunk at fridge temperature in care homes. The temperature at which ONS are drunk may impact ONS palatability because ONS served at lower temperatures are less sweet (Methven et al., 2010a). Furthermore, chilled ONS may be more 'mouth wetting' (Nieuwenhuizen et al., 2010) and thus have implications on various mouthfeel effects (discussed in section 2.3.3.4.1). Serving temperature may also alter the partitioning of volatile aroma compounds released from the matrix. This may mediate the intensity and quality of the ONS aroma and potentially alter the perceived flavour (Delwiche, 2004), with ONS serving at fridge temperature reported to be preferred (den Uijl et al., 2015).

2.3.2.3 Age and sensory decline

Hubbard et al (2012) found a significant negative correlation between adherence to ONS and mean patient age ($p = 0.01$), suggesting that adherence is worse in older patients. These findings are supported by Miller et al (2005) who found that participants aged 70 – 84 years were able to consume 78 % of the prescribed volume compared with 45 % in those aged 85 years and above. Studies have also reported lower adherence in undernourished patients (Lawson et al., 2000, Lammel et al., 2013). Taken together, it would seem that those most in need of ONS support have the lowest intake (Allen et al., 2014).

These findings are expected considering the many factors involved in the “anorexia of ageing”, such as sensory changes, which can impair nutritional intake and could impair adherence to ONS and attempts to improve their nutritional status (Bruce et al., 2003). For example, olfactory impairments are present in a quarter of older adults, which rises further to 62.5 % in 80 – 97-year-olds (Murphy et al., 2002). Older adults may also experience changes to their oronasal physiology, such as a reduced salivary flow rate (Affoo et al., 2015) and impaired dentition (Ship, 1999, Field and Duizer, 2016), which may alter their ability to consume and enjoy foods and beverages. Age-related sensory impairments are proposed to impede appetite, nutritional intake and negatively affect food enjoyment (Duffy et al., 1995, Mattes et al., 1990, de Jong et al., 1999, Schiffman and Graham, 2000, Aschenbrenner et al., 2008) and several studies have shown impact on perception of ONS (IJpma et al., 2016, Kennedy et al., 2010).

2.3.2.4 Familiarity

Laureati et al (2006) suggested that one of the main factors influencing institutionalized elderly food preference may be familiarity and food tradition with Gosney (2003) noting that when older adults are offered a choice of drinks, are more likely to choose cups of tea. However, several studies provide somewhat contradictory evidence indicating ONS are well accepted and selected from various high-energy foods and drinks (Harper et al., 2001, McAlpine et al., 2003).

2.3.3 Product factors

Food palatability is defined as the positive hedonic evaluation of foods sensory characteristics, is strongly determined by sensory properties inherent to the food, and correlates strongly with product intake (Sørensen et al., 2003). Therefore, if ONS adherence is to be good, the product properties creating the product's sensory experience must be acceptable to the consumer. Appetite sensations also govern nutritional intake, so ideally, the product should minimally impact satiation, defined as the process that leads to termination of eating (Benelam, 2009), which could hinder adherence to ONS in the short-term (the consumption period). Additionally, the product should have minimal impact on satiety, defined as the feeling of fullness that persists after eating (Benelam, 2009), and subsequently not restrict food and nutrient intake in the longer term (e.g. over the course of a day).

2.3.3.1 ONS type

Most ONS are liquid feeds, although pudding and powders are available. To meet a wide range of patient preferences and needs, there are various flavours and a range of ONS styles, such as juice, milkshake, yoghurt and savoury ONS (Gandy, 2019).

Darmon et al (2008) conducted a study to investigate preferences for different varieties of ONS with undernourished in-patients (mean age 64.8 years). It was found that overall pleasantness was significantly

better for milk-based than for sweet fruit-juice typed ($p < 0.01$) and salty fruit-juice typed ONS ($p < 0.0001$).

Several authors have commented on the differences in acceptability for supplements prepared freshly with milk against ready to use, long-life supplements, with preference given to fresh, milk-based supplements (Rahemtulla et al., 2005, Auty et al., 1983, Bolton et al., 1990). Not all freshly prepared supplements are nutritionally complete or prescriptible but can be useful when patients' dietary intake is poor (Gandy, 2019).

It is possible this preference is due to the generation of unfavourable mouthfeel sensations and aroma compounds generated in the high-temperature processing of dairy proteins that exist in heat-treated ONS products (see section 2.3.3.4). Regardless, the suitability of using fresh, milk-based supplements in hospitals and care homes may be limited as a consequence of the time and resources required for their preparation and because they are not always nutritionally complete, whilst long-life ONS are ready-made, easy and instant to use involving no preparation time (Hubbard et al., 2012).

2.3.3.2 Volume and energy density

Consumption of ONS could generate feelings of “fullness” in the patient (Kayser-Jones et al., 1998, den Uijl et al., 2015) which may not only impact on complete adherence to an ONS portion (satiation) but also negatively impact on patient’s intake of food throughout the day (satiety). Early satiation, during consumption of a meal or supplement,

is undesirable for undernourished elderly consumers who need to increase their intake of macronutrients and energy to improve their nutritional status.

As Nieuwenhuizen et al (2010) has indicated, it is well established that the degree of satiation per calorie caused by isolated macronutrients is in the order protein > carbohydrate > fat. As some ONS products are complete forms of nutrition containing each macronutrient, it can be challenging for manufacturers to develop ONS without inducing a satiating effect and potentially leading to early termination of a prescribed portion.

Perception of satiety is linearly associated with postprandial gastric volume (Goetze et al., 2007, Nieuwenhuizen et al., 2010). Studies have found that, when comparing milk-based drinks with identical nutritional contents, the incorporation of air and water reduced subjective appetite and nutritional intake at a meal served 30 minutes later (Rolls et al., 1998, Rolls et al., 2000).

Older adults are more sensitive to this effect of volume and some older people report that the total volume of ONS is too high and that they feel bloated and full after drinking them (Gosney, 2003). Wilson et al (2002) found that ONS administration with meals induced satiation in older subjects. The authors suggested this was because the appetite of older adults is more dependent on gastric reservoir function than post-absorptive regulatory signals and may be due to a dysfunction in appetite signalling mechanisms with age (Rolls et al., 1995, Wilson et

al., 2002, Popper and Kroll, 2003). Young et al (2004) suggested this could be beneficial for seniors at risk of undernutrition if appropriately exploited, for example, by providing small volume energy-dense foods more regularly.

ONS' have been developed to offer the same nutritional content but in a smaller volume and condensed form to reduce feelings of fullness, and hence increase subsequent voluntary energy intake (Nieuwenhuizen et al., 2010). Indeed, evidence indicates administrating ONS in a smaller volume but higher energy density effectively increases ONS adherence (Hubbard et al., 2012).

2.3.3.3 Thickness

Thickness is a textural attribute of liquids, which can be defined as the perceived viscosity of a liquid when in the mouth (Kokini et al., 1977).

Den Boer et al (2019) found a 33.3 % increase in total volume consumed of a nutritionally-matched thin ONS compared to a thick ONS, without differences in subjective fullness sensations. The authors commented that this finding might have been due to greater orosensory stimulation due to slower consumption or consumer expectations about the food's satiating properties (den Boer et al., 2019). The role of perceived thickness on the perceived satiation of high protein drinks has been demonstrated previously (Bertenshaw et al., 2013).

To reduce the thickness and maintain the essential macronutrient content without increasing the total volume poses a challenge for ONS

manufacturers. Compacting ONS into a smaller volume will typically result in a thicker product as the macronutrients become concentrated. Product developers must keep in mind that both volume and thickness can increase satiation and potentially decrease ONS intake.

2.3.3.4 Flavour

The overall flavour of a food product is a complex combination of three main sensory modalities: olfaction (the perception of aroma compounds), gustation (the perception of tastants) and trigeminal sensations (Yang, 2012); trigeminal sensations combine perception of texture, mouthfeel, temperature and chemesthesis (irritation by chemical stimuli such as capsaicin in chilli).

The quality of flavour is one of the most critical factors determining consumers' acceptance of foods. The perception of flavour is a fundamental survival instinct that allows humans to evaluate foods' nutritional value and safety (Clark, 1998, Delahunty, 2010) and plays a central role in the sensory enjoyment of foods in addition to governing appetite and food intake (Sørensen et al., 2003).

Poor flavour quality of ONS is reported consistently both anecdotally and in the literature and is frequently linked to low levels of adherence (Gosney, 2003, Miller et al., 2005, Darmon et al., 2008, Kennedy et al., 2010, Methven et al., 2010b, Thomas et al., 2016, Lambert et al., 2017, Thomas et al., 2018), with numerous studies proposing that perception of poor flavour is, in fact, the most important factor in product liking and

adherence to ONS (Bolton et al., 1990, Bruce et al., 2003, Lad et al., 2005, Darmon et al., 2008, Kennedy et al., 2010, Glencorse et al., 2010, ÖZÇA et al., 2013).

Several undesirable sensory attributes, such as taints and mouth-effects, stem from nutritional ingredients used in ONS formulations (Methven et al., 2016b, Bull et al., 2017). Furthermore, age-related changes in sensory abilities and physiology (discussed in section 2.3.2.3) likely further modulate the perceived palatability and consumer experience of ONS, demonstrating, a complex interaction between factors inherent to the product (undesirable sensory attributes) and factors intrinsic to the consumer (sensory abilities), which influence the overall flavour and palatability of ONS.

For the purpose of this review, we focus on the separate contribution made by trigeminal stimuli, tastants and aroma compounds to the palatability of ONS. However, it is vital to remember that flavour is a complex construct and that interactions will occur between modalities that drive the overall consumer experience (Delwiche, 2004).

2.3.3.4.1 Trigeminal stimuli

The trigeminal system is responsible for the perception of food texture, mouthfeel sensations and temperature changes and the perception of chemical irritation, such as that from capsaicin in chilli pepper (Delwiche, 2004). Texture and mouthfeel are important tactile sensations mediated by mechanoreceptors in the oral cavity which play

a crucial role in sensation, perception and the safe manipulation of food (Chen and Engelen, 2012).

Mouth drying

Mouth drying is defined as ‘drying sensation in the mouth’ (Norton et al., 2021) and is a negative driver of liking in ONS products (Thomas et al., 2016).

The perception of mouth drying during ONS consumption is not static but ‘builds up’ over multiple sips during ONS consumption concurrently with self-reported thirst (Methven et al., 2010b, Thomas et al., 2018). Methven et al (2010b) compared the perception of a mineral-free and sweet-suppressed ONS with a standard ONS. They identified mouth drying increased over sequential sips, but there was no difference between mineral-free and standard ONS; however, the sweet-suppressed ONS elicited more intense mouth drying. They concluded minerals were not the primary source of mouth drying and that multimodal interaction between sweetness and drying plays a role, a finding supported by Norton et al (2021).

Methven et al (2010b) hypothesised that the source of mouth drying in ONS products might be milk proteins. Withers et al (2014) found that fortification of milk with both casein and whey protein concentrates significantly increased the perception of mouth drying over repeated sips, which confirmed that the source of mouth drying is dairy protein ingredients. Heat-treatment of dairy proteins play a role in the extent of

this effect; Bull et al (2017) found higher perceptual intensities of mouth drying with an increased heating time of whey protein beverages.

Mucoadhesion, the binding of milk proteins to the oral mucosa, has been proposed to explain the phenomenon of dairy-protein derived mouth drying in the oral cavity (Bull et al., 2017, Norton et al., 2020b, Norton et al., 2021).

Older adults appear more sensitive to the mouth drying sensation elicited by milk proteins than younger adults (Withers et al., 2013), possibly due to reduced salivary flow rates, which occurs with age, disease and medication use (Affoo et al., 2015). However, the influence of saliva flow rate on mouth drying perception is yet to be confirmed. Interestingly, Norton et al (2020b) recently demonstrated unstimulated saliva flow rate had no statistically significant influence on the perception of mouth drying. However, as the greatest contribution to salivary volume in the oral cavity during consumption comes from stimulated saliva, and mouth drying builds over repeated exposure, a temporal approach may elucidate the influence of natural variations in stimulated saliva flow rate on perceived mouth drying and palatability of high protein dairy beverages such as ONS.

Mouthcoating

Mouthcoating, a textural attribute, defined as the residual food that sticks to the oral surface after food ingestion (Repoux et al., 2012) has been studied in ONS products (Methven et al., 2010b, Thomas et al.,

2016). However, mouthcoating is not necessarily a negative attribute. If the sensory qualities of the ONS are well liked, then mouthcoating may be considered desirable (Withers et al., 2013). Conversely, if the ONS' sensory properties are unpleasant, mouthcoating becomes undesirable. This may be especially relevant for older adults who can experience reduced salivary flow rates and impaired muscle strength for clearing product from the oral cavity (Withers et al., 2013, Thomas et al., 2016).

Chemesthesis

One recent study investigated the acceptability of ONS prototypes that had the addition of chemical agents that elicit chemosensations, such as cooling menthol and warming/spicy ginger and mango, in patients who were undergoing cancer treatment (de Haan et al., 2021). Patients rated three flavours (cool red fruits, hot mango and hot tropical ginger). Interestingly, one flavour (cool red fruits) was rated significantly higher for liking by patients with taste and smell alterations, compared to patients without taste and smell alterations. Hence, adding chemosensory stimuli to ONS may be an effective way to improve the flavour and palatability of ONS for the older consumer (Forde and Delahunty, 2002), compensating for sensory losses, and warrants further investigation in longer-term clinical studies to evaluate the impact on adherence.

2.3.3.4.2 Tastants

Several studies have provided evidence suggesting the high sweetness of ONS may be a factor limiting palatability and adherence (Gosney, 2003, Kennedy et al., 2010, Lambert et al., 2014).

However, the hypothesis that high sweetness drives a dislike of ONS is not consistently supported by evidence. Methven et al (2010b) found that higher sweetness led to higher mean initial liking compared with an ONS in which the sweetness was suppressed. In addition, compared to an ONS that was not sweetness-suppressed, liking of a sweetness-suppressed ONS decreased more significantly over the consumption of consecutive aliquots (Methven et al., 2010b). Den Boer et al (2019) found that participants consumed 8 % more of a sweeter ONS, which also had significantly greater product pleasantness, liking and wanting. Individuals have wide-ranging variation in their optimally preferred concentration of sweetness and can be categorised as ‘sweet likers’ or ‘sweet dislikers’ depending on their tolerance for sweetness in foods (Methven et al., 2016b). This may be a factor that could play a part in the discrepancies between findings and more research is needed to uncover the role of sweetness on ONS acceptance and adherence.

It has been hypothesised that minerals added to ONS during manufacture, such as iron sulphate, may contribute to metallic tastes (Methven et al., 2010b) and are negative drivers of liking in ONS (Thomas et al., 2016). Methven et al (2010b) investigated the impact of mineral content of ONS and found the mineral free ONS was rated

significantly less metallic compared to the control, however the difference was small, and the authors concluded that although the minerals added to the ONS formulation contribute, this cannot be the only source of metallic taste in the products.

The authors suggested that calcium salts may hold some responsibility because they can exhibit metallic taste properties (Methven et al., 2010b). However, metallic ions can oxidise salivary proteins in the oral cavity resulting in the production of aromatic carbonyl compounds (Lawless et al., 2004, Ömür-Özbek et al., 2012, Delompré et al., 2019). This metallic off-taste is an example of how the addition of essential nutritionally functional ingredients can create sensory challenges in food products (for a review, see Delompre et al (2019)).

2.3.3.4.3 Aroma compounds

Aromas are volatile compounds released from foods which stimulate the olfactory receptors in two distinct ways and in doing so play significant roles in food intake. Firstly, orthonasal olfaction (the perception of food aroma before it is placed in the mouth) is critical for evaluating the suitability of food for ingestion (Stevenson, 2010) and therefore is an essential gate-keeper to food choice and intake. Orthonasal smell drives food acceptance as it occurs prior to consumption, thereby setting our expectations of food palatability (Spence, 2015), modulating appetite (Yeomans, 2006, Yin et al., 2017) and stimulating physiological response (such as salivary flow) in preparedness for food digestion (Spence, 2015). To promote appetite

and nutritional intake, positive orthonasal cues are crucial for older individuals that are already experiencing blunted appetite sensations (Section 2.3.3.2). Positively perceived aromas may be a way to stimulate saliva flow before food consumption in older individuals experiencing hyposalivation. On the contrary, an unpleasant aroma can limit consumers' willingness to consume foods (Pelchat, 2000). Consequently, ONS should have a palatable, product-congruent and enticing aroma (for example, aroma associated with freshness) to stimulate the patient's appetite and promote the desire to consume ONS.

However, research shows that the aroma of ONS is not optimal. In a series of focus groups conducted with health professionals, Lambert et al (2017) identified 'unpleasant odour' as a barrier to ONS consumption in patients. In a separate study, through use of a questionnaire, Uí Dhuibhir et al (2019) found the sensory attribute 'smell' to be one of the least favourite sensory characteristics of ONS as rated by dietitians, and some participants reported a 'medicinal' or 'synthetic' smell. Healthcare staff who dispense ONS perceive aroma because it is noticeable without consuming ONS. Staff have reported that ONS are 'not appetising to look at or smell' (Lad et al., 2005), which could influence patient expectations before consumption.

The source of unpleasant aromas within ONS has not been fully elucidated. However, it could stem from essential nutritional ingredients within ONS, such as proteins, and/or processing conditions during ONS

manufacture. For example, most ONS are heat-treated to ensure consumer safety and prolong the shelf-life but heat-treatment of protein ingredients can cause a greater intensity of 'cooked' flavours in high-protein dairy beverages (Bull et al., 2017). Recent research identified eggy, rancid and sulfate flavours within baked protein-fortified foods (Norton et al., 2020a). Undesirable smelling sulfurous aroma compounds, such as dimethyl sulphide and methanethiol, are known to form in dairy products at high temperatures from essential amino acids present in milk proteins (such as methionine) (Al-Attabi et al., 2008, Jo et al., 2018). The generation of unpleasant aroma during heat-treatment could explain the difference in acceptability between 'fresh' ONS products and those processed by high-temperature treatment to become long-life ONS (Section 2.3.3.1).

Although researchers have identified poor perceived flavour quality as a key factor in ONS adherence (Miller et al., 2005, Darmon et al., 2008, Kennedy et al., 2010, Methven et al., 2010b, Thomas et al., 2016, Lambert et al., 2017, Thomas et al., 2018) no research has investigated the contribution made by aroma to perceived flavour and palatability during ONS consumption. Retronasal olfaction refers to the release of volatile aromas from foods, which travel through the gaseous airspace, and bind with olfactory receptors in the back of the nose *during* food consumption. It is retronasal olfaction that combines with the perception of taste and trigeminal stimuli to drive the perception of food flavour, and hence the palatability of food (Yeomans, 2006).

Simultaneously, a greater intensity or duration of stimulation by aroma during food consumption can be associated with greater satiation (Ruijschop et al., 2008, Ramaekers et al., 2014). Therefore, an appetising combination and optimal intensity of aroma is crucial for good palatability, adequate intake, and best possible adherence.

Furthermore, both orthonasal and retronasal perception of aroma is also likely to be distorted by impairments in the consumer's sensory abilities, which can occur with ageing, disease state, and medication use (as discussed further in section 2.3.2.3). One study found a correlation between reduced smell function in cancer patients with a decreased liking of vanilla-flavoured, milk-based ONS, which the authors attributed in part to a higher smell threshold influencing the palatability (IJpma et al., 2016). Many studies have linked age-related declines in olfactory perception to dietary changes and reduced nutritional intake (Duffy et al., 1995, Mattes et al., 1990, de Jong et al., 1999, Schiffman and Graham, 2000, Aschenbrenner et al., 2008). No researcher has investigated how modifying the aroma of ONS can affect palatability, although aroma enhancement of foods for the elderly has produced conflicting results (Boesveldt et al., 2018).

Little is known about the contribution made by aroma compounds to the flavour and palatability of ONS or how age-related changes in physiology and sensory abilities of older adults further distort the perception and adherence to ONS. Due to the importance of aroma in

food acceptance, palatability and intake, this gap in the evidence requires further investigation.

2.4 Conclusion

With the identification of key factors influencing adherence and strategic expansion of research effort, there are exciting opportunities to develop next-generation ONS products. Increased palatability, through modification of texture and use of flavour profiles explicitly designed for aged sensory abilities, will contribute to increased end-consumer adherence. One comparatively under-researched area is the contribution made by aroma to older adults' experience of ONS. Further research should address this gap with the aim to optimise the flavour profile, stimulate appetite and salivation, thereby optimising the palatability of ONS and intake. Thus, the clinical benefit of nutritional supplementation in the ageing population can be maximised.

Chapter 3

3 Identification of aroma active compounds in a commonly prescribed oral nutritional supplement (ONS) and associated changes in olfactory abilities with human ageing

Sophie LESTER[1], Leonardo CORNACCHIA[3], Camille CORBIER[3], Moira A TAYLOR[2], Charfedinne AYED[1], Ni YANG[1], Mui LIM[1], Rob LINFORTH[1], Ian FISK[1]

[1]Division of Food Nutrition and Dietetics, School of Biosciences, University of Nottingham, United Kingdom

[2]National Institute for Health Research (NIHR), Nottingham Biomedical Research Centre, Division of Physiology, Pharmacology and Neuroscience, School of Life Sciences, University of Nottingham, United Kingdom

[3]Danone Nutricia Research, Uppsalalaan 12, 3584 CT Utrecht, the Netherlands

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Identification of aroma active compounds in a commonly prescribed oral nutritional supplement (ONS) and associated changes in olfactory abilities with human ageing

3.1 Abstract

Undernutrition is prevalent in the older adult population. Oral Nutritional Supplements (ONS) are a clinically effective nutritional intervention, however, patient acceptance of ONS can be limited by their palatability. While sensory attributes such as sweetness and mouthfeel have been investigated, the contribution made by aroma to the perceived flavour of ONS has not been studied. Firstly, this research aimed to identify the aroma-active compounds within a commonly prescribed ONS using estimated odour activity values (OAV) and gas chromatography-olfactometry-mass spectrometry (GC-O-MS). Secondly, age-related differences in olfactory detection were explored. Eight aroma-active compounds were identified within the ONS, including diacetyl (sweet), isoamyl acetate (banana), dimethyl trisulfide (sulfur) and methanethiol (sulfur). When compared with younger adults (n=24, 18-44 years), older adults (n=24, 62-80 years) had higher detection thresholds for all aroma compounds and this was significant for isoamyl acetate (sweet, fruity) and methanethiol (sulfur) ($p=0.01$ and $p=0.03$, respectively). Thus, a decline in olfactory sensitivity was present in the older subjects included in the study, and this reduced detection sensitivity was aroma-specific. Thus, older adults' flavour perception of ONS likely depends on the combined effect of product factors (the aroma profile) along with age-related consumer factors (the degree of impairment in perception). This is a fundamental study which will aid future research into how the aroma profile, and associated age-related impairments in perception,

shape the global perception of ONS for nutritionally at-risk older individuals.

Key words: Healthy Ageing, Olfaction, Oral Nutritional Supplements (ONS), Aroma, Flavour perception, GC-MS.

3.2 Introduction

Undernutrition is commonly experienced in the older population (Stratton et al., 2018) and the aetiology includes a multitude of age-related physiological, social and environmental factors, encompassed under the common term “The Anorexia of Ageing” (Cox et al., 2019). The consequences include many comorbidities and adverse outcomes including disability and poor quality of life (Landi et al., 2016, Cox et al., 2019) and results in major healthcare costs (Stratton et al., 2018). Undernutrition is estimated to cost at least £23.5 billion in the UK; with older adults accounting for 52% of this cost (Stratton et al., 2018). Considering this, it is critical to identify effective nutritional interventions to prevent and treat undernutrition in the older population.

Foods for special medical purposes, such as oral nutritional supplements (ONS), are prescribed to supplement, or replace the diet of individuals who are undernourished, or at risk of undernutrition. Most prescribed ONS are dairy-based, protein and energy-dense drinks, which are ready-to-drink, and as such they are easy to consume by individuals with poor mobility, cognition and/or dentition. Liquids are less satiating than nutritionally equivalent solids (Zijlstra et al., 2008) facilitating consumption of a larger quantity before termination of intake. The clinical effectiveness of ONS, when consumed, is well established (Stratton et al., 2018). Stratton et al., (2007) conducted a ‘review of reviews’ and found that ONS consistently increased total nutritional intake concurrently with a significant overall reduction in mortality and a

reduction in complications (such as infections and pressure ulcers) across patient groups.

In order to gain the clinical benefits associated with ONS, sufficient long-term intake is essential. After ‘nutritional value’, Uí Dhuibhir et al., (2019) found that ‘patient palatability’ was the most important factors affecting ONS prescription in clinical practice. However, ONS are poorly tolerated by patients (Cox et al., 2020) and many may not consume their full prescription; Gosney (2003) found average adherence to a prescribed course of ONS on an elder care ward was as low as 37 % (average quantity consumed as a proportion of the quantity provided). Gosney (2003) found that the greatest waste was seen in those patients who disliked the taste of ONS and poor palatability has been proposed as a key factor limiting sufficient intake (Bolton et al., 1992, Lad et al., 2005, Kennedy et al., 2010, den Boer et al., 2019).

ONS typically contain adequate levels of nutritionally essential macronutrients (carbohydrates, protein, and fat) and micronutrients (vitamins and minerals) to produce a nutritionally complete product. However, the composition of ONS plays a crucial role in palatability (Kokkinidou et al., 2018, Galaniha et al., 2020). For example, the type and quantity of carbohydrate chosen in an ONS formulation (usually a simple sugar which are easy to digest and absorb (Kokkinidou et al., 2018)) must ensure sufficient quantities of glucose and calories to meet the nutritional needs of the patient, but the sweetness level must be considered; an overly sweet taste is undesirable for patients (Gosney,

2003). The protein choice is also crucial in the palatability of the final product. For example, whey proteins (a high-quality protein source) elicit a ‘mouth drying’ phenomenon during consumption, which can build,up in intensity over multiple intakes or sips (Methven et al., 2010b) and becomes more intense with heat-treatment of the protein ingredients (Bull et al., 2017). During manufacture, ONS are typically heat-treated to ensure adequate shelf-life, but this treatment likely affects the sensory properties and palatability of the final product, including mouthfeel but also the generation of new flavours and aromas (Kokkinidou et al., 2018). Some micronutrients have also been proposed as important in the generation of taste sensations in nutritional supplements (Delompré et al., 2019).

Olfaction is a sensory modality with great importance in food flavour. because it is olfaction, which combined with the perception of taste during food consumption, creates the overarching perception of flavour. To the authors knowledge, no research has yet investigated the contribution made by volatile aroma compounds to the flavour and palatability of a commercial ONS. However, heat-generated Maillard reactions products with undesirable sensory properties have been identified in heat-treated dairy products, such as pungent, sulfur-containing aroma compounds with low detection thresholds (Whetstine et al., 2005, Al-Attabi et al., 2008, Zabbia et al., 2012, Newton et al., 2012, Jo et al., 2018). Uncovering the aroma which are present in a

commonly prescribed dairy-based ONS is a fundamental first-area of investigation.

In addition to factors inherent to the product, older consumers experience unavoidable age-related changes in their oro-sensory physiology which are likely to alter the sensory experience. It is well known that gustatory sensitivity declines with age, however, the extent and significance of this decline varies between taste modalities, tastants and studies (Methven et al., 2012). Olfactory sensitivity declines considerably with age too, though, only a limited number of studies have investigated how ageing affects olfactory sensitivity to single aroma compounds (Wysocki and Gilbert, 1989, Sinding et al., 2014, Seow et al., 2016). Impairments in olfactory abilities likely involve age-related alterations within the nose, olfactory epithelium, bulb, and higher brain structures (Doty and Kamath, 2014). In addition, it should also be noted that reduction in olfactory sensitivity is associated with early stages of some neurodegenerative diseases, for example Alzheimer's disease and sporadic Parkinson's disease (Doty and Kamath, 2014). Considering these age-related changes, it is understandable how the palatability of foods, including ONS, may change over the life course.

Considering the importance of aroma compounds in flavour perception, this study firstly aimed to characterise the volatile profile of a commercial ONS and estimated odour activity values (OAV) aimed to predict the volatiles with the potentially highest aroma-activity. Gas

chromatography-olfactometry-mass spectrometry (GC-O-MS) used human participants to validate these findings and confirm the aroma-active volatiles which contribute most greatly to the flavour of ONS. Lastly, detection threshold tests were used to investigate the association between ageing and perceptual sensitivity to aroma-active compounds.

3.3 Materials and methods

This study was approved by the School of Bioscience Ethics Committee at the University of Nottingham (SBREC160140A) (Appendix J). All experiments were performed in accordance with relevant guidelines and regulations.

3.3.1 Gas chromatography-mass spectrometry (GC-MS)

3.3.1.1 Sample preparation

Commercial samples of a banana flavoured milk-based oral nutritional supplement (ONS) were obtained from Danone Nutricia Research (Utrecht, The Netherlands) and stored at ambient temperature until analysis. Banana flavoured products were chosen as they are one of the most commonly prescribed flavours in the UK. For each sample, 7 mL of the ONS was placed into an opaque vial and 15 µl of a freshly prepared internal standard (0.1% 3-heptanone in ultra-pure water) was added. The samples were analysed in triplicate.

3.3.1.2 GC-MS parameters

A trace 1300 series Gas Chromatograph coupled with a Single-Quadrupole Mass Spectrometer (Thermo Fisher Scientific) was used. The sample was incubated at 37°C for 15 min with shaking. Solid phase microextraction (SPME) was used to extract volatile aroma compounds from the sample headspace (extraction for 15 min, desorption for 1 min). The fibre used was a 50/30µm DVB/CAR/PDMS

SPME Fibre (Supelco, Sigma Aldrich, UK). The separation was carried out on a ZB WAX Capillary GC column (30 m x 0.25mm x 1µm). The column temperature was initially held at 50°C for 1 min and then increased by 8°C/min until the temperature reached 190°C. The temperature was then increased by 15°C/min until 250°C was reached and then held for 0.5 min. Helium was used as a carrier gas and spitless mode was used at a constant carrier pressure of 30 psi. Full scan mode was used to detect volatile compounds (m/z 35 to 300). Volatile compounds were tentatively identified by comparison of each mass spectrum with spectra in reference libraries (NIST/SPA/NIH Mass Spectral Library, version 2.0, Faircom Corporation, U.S.) and by comparing calculated Linear Retention Indices (LRI) with those from either the literature or an internal database generated using authentic standards.

The relative abundance of each volatile compound present in the headspace was calculated by comparing the GC peak area to the peak area of the IS (of known abundance).

Odour activity values (OAV) give an indication of the relative contribution made by each aroma to the overall flavour and were calculated by dividing the volatile abundance by the odour thresholds (OT).

3.3.2 Gas chromatography-olfactometry-mass spectrometry (GC-O-MS)

3.3.2.1 Participant recruitment

Six young adults (24-38 years, 4 male 2 female) and six older adults (67-81 years, 3 male 3 female) were recruited from Sutton Bonington Campus, University of Nottingham and local villages via posters and flyers. Interested participants were informed they were being recruited for a “smelling study” and must fit the criteria of being in good general health (healthy BMI, absence of physical or mental illness) and no known anosmia unrelated to ageing. Due to the high occurrence of medication use in older age, and to maintain ecological validity, medication use was not a criterion for exclusion, but the details of medication use were recorded (see Appendix B). All participants were new to sensory and flavour analysis.

3.3.2.2 Participant screening and training

Written informed consent was obtained and, to ensure they met the recruitment criteria, participants were asked to complete a questionnaire obtaining health, lifestyle and demographic information. In a training session (1 hour), the 12 participants were familiarised with the GC-O-MS procedure and given the opportunity to practice smelling using the GC-O-MS equipment with a model blend of aroma compounds.

3.3.2.3 Sample preparation

Sample preparation was exactly as stated in the GC-MS analysis apart from the exclusion of the internal standard.

3.3.2.4 GC-O-MS parameters

The volatile compounds passing through the column were split in a 1:1 ratio, part was directed to the mass spectrometer and part was directed out of the oven via a transfer line (200ºC) and exited at a glass sniffing port.

GC-O-MS analysis took place over 23 minutes. Data were collected by a combination of detection frequency (DF) (number of participants that detect the aroma) and posterior intensity rating (I) (participant rating of aroma intensity). The intensity rating used a simple 1-3 scale, which has been shown to be effective in GC-O-MS analysis using untrained participants (Ferreira et al., 2003). Participants were asked to breathe normally through their nose in the sniffing port and state (i) exactly when they smelt an aroma (ii) give the intensity of the aroma (scale of detection: 1= weak, 2= intermediate, 3= strong) (iii) give a description of the aroma. Participants were allowed to describe the aroma using their own terminology. To limit disruptions during smelling, participants verbally stated their ratings and descriptions, and these were recorded electronically by the experimenter. The detection time was used to identify odiferous regions of the chromatogram.

3.3.3 Detection threshold and hedonic tests

The threshold testing methods were based on the ATSM (2011) E679 3-alternative forced choice (3-AFC) method of limits. This method is suited for “best estimate” thresholds of populations or groups, which naturally include the large variation in threshold values. In addition, this method was especially suitable for naïve groups, such as untrained older adults, due to its relative simplicity (Methven et al., 2016a).

Oral nutritional supplements are a complex matrix of macronutrients and micronutrients. Thus, ONS can be a challenging matrix to reproduce, particularly whilst ensuring consistent aroma between samples or batches. Therefore, in line with existing literature values (Leffingwell and Associates, 2020), this initial study opted to use water as the aroma dilution medium.

Four aroma compounds were chosen from those detected during GC-O-MS (section 3.3.2) to represent a variety of hedonic, chemical and sensory characteristics (sensory descriptors taken from Good Scents Company (1980-2021). The aroma compounds and concentration ranges chosen were: isoamyl acetate (sweet, fruity) (25 – 6000 ppb), diacetyl (sweet, buttery) (1 – 200 ppb), methanethiol (cabbage, garlic) (0.025 - 6 ppb), dimethyl trisulfide (sulfurous, onion) (0.002 - 0.5 ppb). Suitable concentration ranges were determined by pilot threshold studies, with both age groups, leading up to the experiment. A total of six ascending concentration steps were chosen for each aroma and to

encompass large variances in threshold values, but limit the number of samples, concentrations steps were increased by a factor of 3.

3.3.3.1 Materials

Three of the aroma compounds (isoamyl acetate (>95%), 2,3-butandione (diacetyl) (97%) and dimethyl trisulfide (>98%)) were purchased from Sigma-Aldrich, U.S., and methanethiol (methyl mercaptan) (1 mg/mL in H_2O) was purchased from Fisher Scientific UK Ltd.

3.3.3.2 Sample preparation

Aroma concentrations were prepared in ultrapure water using the serial dilution technique and blanks were water only. To prevent the loss of volatiles, dilutions were prepared no more than 24 hours before the study day and stored in 500 mL Duran bottles, without headspace, and refrigerated until the day of the study session. On the day of a study session, for each sample, 5 mL of each dilution was pipetted into 28 mL glass screw-top bottles and labelled with a randomised 3-digit code.

3.3.3.3 Participants

Using the same recruitment procedures and criteria the GC-O-MS analysis (section 3.3.2.1), 24 healthy young adults (18-44 years, 8 male 16 female) and 24 healthy older adults (62-80 years, 9 male 15 female) were recruited to take part.

3.3.3.4 Threshold screening and training session

Interested participants were invited to a group screening and training session where they were informed about the purpose and nature of the study. Participants provided informed consent before completing a health, lifestyle and demographic questionnaire.

To familiarise participants with the sensory procedure, participants were invited to practice the 3-AFC technique, for each aroma compound, by identifying an odour containing bottle from the two blanks. The aroma compounds were presented at the next highest theoretical concentration step in the threshold range (concentration step seven). Participants were trained to smell the bottles in a standardised way: smell the bottles from left to right, shake the bottle and count to five before removing the lid, hold the bottle close to the nose without touching.

During this practice session, a small number of participants were unable to detect the odour containing bottle at the higher concentration (isoamyl acetate: 1 younger, diacetyl: 1 younger 1 older, methanethiol: 2 younger, dimethyl trisulfie: 2 younger, 2 older). These participants were possibly anosmic to the aroma (aroma-specific anosmia) and subsequently excluded from testing for that particular aroma. Additionally, during the threshold testing, two older participants experienced acute respiratory illness which affected their normal smelling abilities. Subsequently, they were not included in the section

they were scheduled to attend. To see final group sizes for each threshold experiment, see Appendix A.

3.3.3.5 Aroma compound pleasantness

As an additional measure, during the 3-AFC practice session, participants who correctly identified the odour containing bottle were asked to rate the pleasantness of each aroma compound using a 3-point categorical scale: Pleasant, Neutral, or Unpleasant, a recommended scale for hedonic testing with older adults due to the ease of use of category scales (Methven et al., 2016a).

3.3.3.6 Threshold testing procedure

All threshold study sessions took place in sensory booths designed to ISO Standards (ISO8589:1988) within The Sensory Science Centre (Sutton Bonington Campus, University of Nottingham). There were two study sessions per day (morning or afternoon) and sessions were mixed with both older and younger participants. Participants completed 2 different aromas per session with a compulsory 15 min break between different aroma compounds. The order that the different aroma compounds were presented was randomised and sample presentation order (1 aroma, 2 blanks) was randomised using Compusense Cloud (Compusense, Ontario, Canada).

Participants were presented with the 3 samples and asked one question “Which one sample smells different to the other two?” Between each set of 3 samples, participants were given a compulsory 1

min break and asked to neutralise their nose using a damp cloth. Answers were recorded electronically using Compusense Cloud® (Compusense, Ontario, Canada).

3.3.4 Statistical analysis

All statistical analysis was carried out using the software XLSTAT® statistical and data analysis solution (version 20.6.01, Addinsoft, Long Island, NY, USA) or GraphPad Prism software (version 7.0, San Diego, CA, USA).

3.3.4.1 Gas Chromatography-Olfactometry-Mass Spectrometry

Detection frequency (DF) and posterior intensity ratings (I) were combined into a single value using the modified frequency (MF(%)) method proposed by Dravnieks (1992) and used effectively in recent GC-O-MS studies (Brattoli et al., 2013, Kortesniemi et al., 2018, Corsini et al., 2019, Osorio et al., 2019). MF (%) can be calculated using the following formula:

$$MF\ (\%) = \sqrt{DF(\%)I(\%)}$$

Whereby DF (%) is the total detection frequency, expressed as a percentage of total participants, and I (%) is the total intensity rating, expressed as a percentage of the maximum possible sum of intensity ratings. MF(%) was calculated both for age groups and for data pooled from both groups. To control for noise a MF 50% cut-off was used within each age group (Brattoli et al., 2013).

3.3.4.2 Threshold tests and pleasantness ratings

Differences in aroma compound pleasantness ratings, between the older and younger groups, were analysed by Chi-Squared (X^2) test at a significance level of $p < 0.05$.

Individual best estimate thresholds (BET) were determined by taking the geometric mean of each individual's highest incorrect concentration and the correct concentration above this. If an individual's threshold was above or below the highest possible, or lowest possible concentration, the next theoretical concentration step was used. Age-group BET were determined by taking the geometric mean of all individual BET within an age-group. Due to the data not being equally distributed, Mann-Whitney U test was used to compare threshold values between age-groups, at a significance level of $p < 0.05$.

3.4 Results and Discussion

3.4.1 Gas chromatography-mass spectrometry (GC-MS)

GC-MS detected twenty-nine volatile compounds, with wide ranging chemical and sensory properties in the headspace of the ONS (See Table 3-1). The main functional groups were: esters (8), aldehydes (6), sulfur-containing (4), furans (4), ketones (3), alcohols (2), phenylpropenes (1) and monoterpenes (1).

Table 3-1: Volatile compounds detected in the headspace of the ONS beverage, ordered by functional group and relative abundance.

					GC-MS-SPME			
LRI[1]	Literature LRI[2]	Volatile compound name	m/z[3]	CAS number[4]	Relative abundance (ppb ± SD)[5]	DT in water (ppb)[6]	OAV[7]	Sensory descriptions (GoodScents®)[8]
		esters						
1147	1141[g]	Isoamyl acetate	130[a]	123-92-2	53000 ± 9000	2	26,500	Sweet, fruity, banana, solvent
1319	1285[d]	Isoamyl isovalerate	172[a]	659-70-1	40000 ± 8000	19.9	2,010	Sweet, fruity, Green, Ripe
1213	1181[e]	Isoamyl propionate	144[a]	105-68-0	5000 ± 700	8.6	581	Sweet, fruity, banana
1096	1053[e]	Butyl acetate	116[a]	123-86-4	80 ± 12	66	1	Ethereal, solvent, fruity, banana
1058	1055[g]	Ethyl butyrate	116[a]	105-54-4	50 ± 6	1	50	Fruity, juicy, pineapple,
1300	1263[e]	Isoamyl butyrate	158[a]	106-27-4	33 ± 6	210	0.2	Fruity, green, apricot, pear
1297	1293[g]	Hexyl acetate	144[a]	142-92-7	25 ± 3	2	13	Fruity, green, apple, banana
1033	1012[f]	Isobutyl acetate	116[a]	110-19-0	13 ± 1	66	0.2	Sweet, fruity, ethereal, banana
		Aldehydes						
1574	1573[a] 1520[b]	Benzaldehyde	106[a]	100-52-7	600 ± 80	350-3500	2	Almond, fruity, powdery, nutty
2173	2196[g]	Vanillin	152[a]	121-33-5	500 ± 430	20-200	25	Sweet, vanilla, creamy
646	707[a]	Acetaldehyde	44[a]	75-07-0	25 ± 4	15-20	2	Pungent, ethereal, fresh

1109	1083[b]	Hexanal	100[a]	66-25-1	8 ± 1	4.5-5	2	Fresh, green, fatty, aldehydic
1005	988[a]	Pentanal	86[a]	110-62-3	4.20 ± 0.4	1004	$4x10^{-3}$	Fermented, bready, fruity
902	884[a]	Butanal	72[a]	123-72-8	1.20 ± 0.2	9-37.3	0.1	Pungent, cocoa, musty, green
		sulfur compounds						
1105	1096[a] 1077[b]	Dimethyl disulfide	94[a]	624-92-0	41 ± 6	12	3.4	Sulfurous, vegetable
637	689[a]	Methanethiol	48[a]	74-93-1	21 ± 4	0.02	1050	Vegetable, oily, alliaceous, eggy
1425	1426[a] 1430[g]	Dimethyl trisulfide	126[a]	3658-80-8	17 ± 2	0.005-0.01	3,400	Sulfurous, onion, meaty, savoury
680	758[a]	Dimethyl sulfide	62[a]	75-18-3	2.8 ± 0.6	0.3-1	9	Sulfurous, onion, sweet corn
		Furans						
1258	1241[a]	2-Pentylfuran	138[a]	3777-69-3	12 ± 2	6	2	Fruity, green, earthy, beany
817	799[b]	Furan	68[a]	110-00-9	3.6 ± 0.9	U	U	Ethereal
896	877[a] 869[b]	2-Methylfuran	82[a]	534-22-5	3.4 ± 0.4	U	U	Ethereal, acetone, chocolate
976	970[c]	2-Ethylfuran	96[a]	3208-16-0	2.0 ± 0.9	U	U	Sweet, burnt, earthy, malty
		Ketones						
835	819[b]	Acetone	58[a]	67-64-1	190 ± 40	500000	$4x10^{-4}$	Solvent, ethereal, apple, pear
923	910[a] 907[b]	2-Butanone	72[a]	78-93-3	110 ± 10	50000	$2x10^{-3}$	Ethereal, fruity, camphoreous.
1004	980[a] 979[b] 973[d]	Diacetyl	86[a]	431-03-8	118 ± 1.6	2.3-6.5	51	Sweet, buttery, creamy, milky.
		Alcohols						

1409	1405[g]	Cis-3-Hexanol	100[a]	928-96-1	290 ± 50	70	4	Fresh, green, grassy, foliage
1697	1690[g]	Furfuryl alcohol	98[a]	98-00-0	230 ± 20	U	U	Alcoholic, chemical, musty
		Phenylpropenes						
2120	2157[d]	Eugenol	167[a]	97-53-0	69 ± 4	6-30	11.5	Sweet, spicy, clove woody
		Monoterpenes						
1227	1223[g]	D-Limonene	136[a]	5989-27-5	420 ± 60	10	42	Citrus, orange, fresh, sweet

[1] LRI: linear retention indices
[2] Literature LRI [a](Villière et al., 2015), [b](Thammarat et al., 2018), [c](Olaoye, 2016), [d](Ricci et al., 2018), [e](Schubert et al., 2013), [f](Ubeda et al., 2012), [g]Internal database generated using authentic standards
[3] m/z: molecular ion peak [a]mass spectra identified by comparison to NIST database
[4] CAS: Chemical Abstracts Service unique numerical identifier
[5] Abundance values are relative to an internal standard (15 uL 0.1% 3-Hepanone)
[6] DT: detection thresholds from literature (Leffingwell and Associates, 2020), U=unknown
[7] OAV: Odour Activity Value
[8] Sensory descriptions from literature (Good Scents Company, 1980-2021)

Odour activity values (OAV) are important when considering the potential contribution of a volatile compound to the aroma or flavour of a food product. OAV can be used to estimate aroma potency in terms of the ratio of the concentration of a volatile to its odour detection threshold (Cadwallader, 2007) and help to translate the quantitative data gained from GC-MS into sensorial information (Belitz et al., 2004). As estimated using OAV, Figure 3-1 shows the relative contributions (%) made by volatiles to the overall ONS flavour (as a proportion of total OAV). This diagram therefore identifies the volatiles which are potentially aroma-active and make the greatest perceptual contribution to the ONS flavour.

OAV require an accurate representation of OT as calculated from the same matrix (Cadwallader, 2007). The matrix of the studied ONS is a complex aqueous mixture of fat, carbohydrate, protein in addition to micronutrients, so precise OT weren't available in the literature for each volatile. Therefore, in line with previous investigations on milk beverages (Vazquez-Landaverde et al., 2005), we used OT values determined in water, to estimate the relative contribution made by volatiles to the flavour and translate the quantitative data into sensorial information (Belitz et al., 2004). It is important to note that relying on OAV calculations alone could lead to an over- of underestimation of the perceptual importance of these aroma and hence were considered estimates at this stage in our investigations.

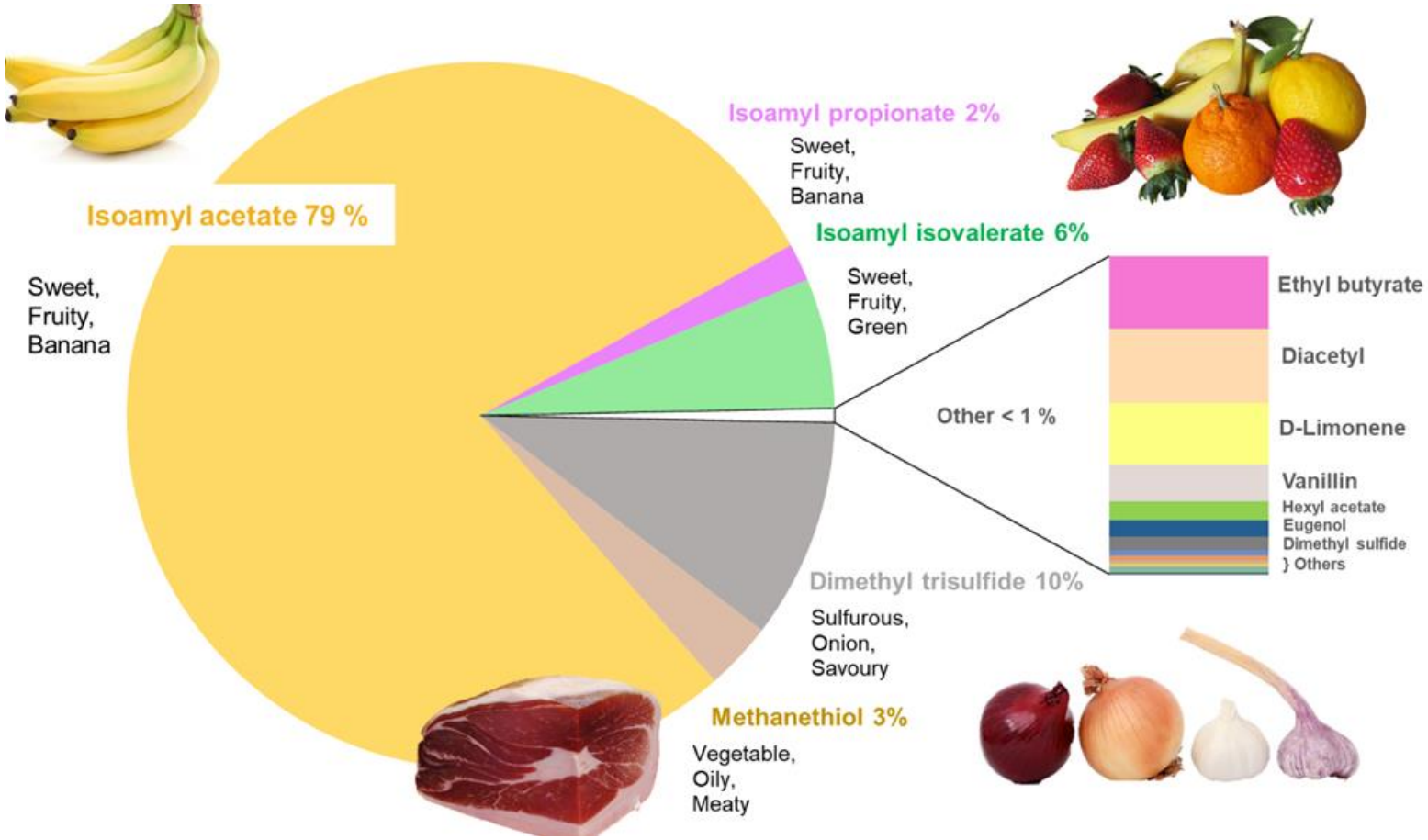

Figure 3-1: Relative contribution (%) made by volatiles to studied ONS flavour as determined by calculated odour activity values (OAV).

3.4.2 Gas chromatography-olfactometry-mass spectrometry (GC-O-MS)

GC-O-MS is an alternative approach which combines both the human nose and a mass spectrometer, to confirm which volatiles are aroma-active and contribute to the perceived flavour.

Eight aroma-active compounds were detected by both the younger and older adults during the GC-O-MS study. These aroma compounds, along with calculated MF(%) scores, are shown in Figure 3-2 (data pooled from all participants (n=12)). In agreement with the GC-MS findings (Figure 3-1), these compounds also had the highest calculated OAV, and are therefore confirmed as the volatiles which make the greatest perceptual contribution to the flavour of the studied ONS. The

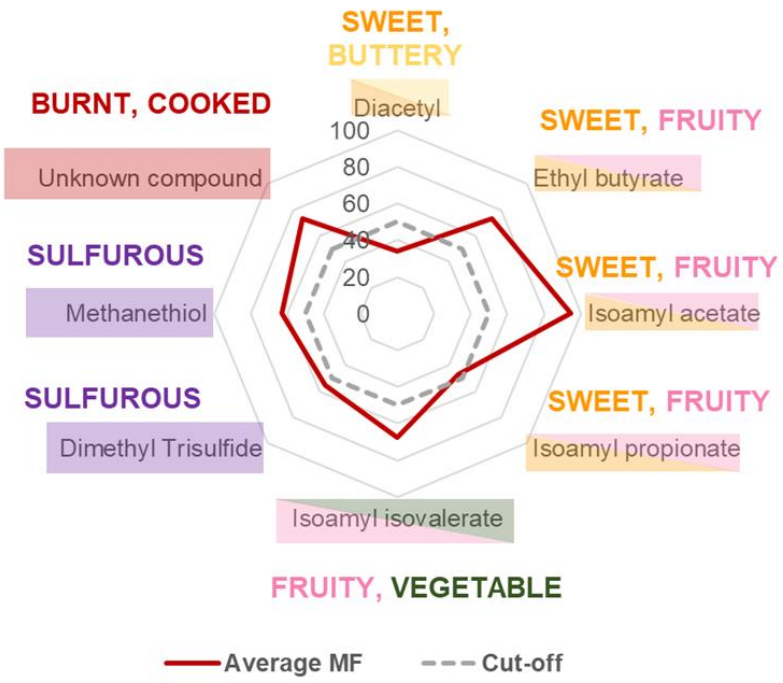

Figure 3-2: Aroma-active compounds detected by all participants (n=12) during the GC-O-MS analysis. Score shows average Modified Frequency values (%) for each aroma.

sensory descriptions generated by participants for the detected aroma compounds ranged from sweet and fruity (isoamyl acetate, ethyl butanoate and isoamyl isovalerate) to sulfurous and unpleasant (methanethiol and dimethyl trisulfide) to sweet and buttery (diacetyl) (see Appendix C for all descriptions generated).

As measured by GC-MS, fruity esters had a large relative abundance within the ONS (Figure 3-1) and several esters were detected by participants during the GC-O-MS analysis. This is not a surprising finding considering that it is a fruit (banana) flavoured product. For example, isoamyl acetate is an essential flavouring in both natural and artificial banana flavoured foods; in the current study this compound was detected in the highest abundance in the headspace and was detected by all 12 participants (MF% = 94).

A number of volatile compounds were detected, such as sulfur containing compounds (methanethiol and dimethyl trisulfide) and diacetyl, which are frequently detected in heat-treated milk and dairy products (Whetstine et al., 2005, Al-Attabi et al., 2008, Zabbia et al., 2012, Newton et al., 2012, Jo et al., 2018). Diacetyl is a rich, buttery, sweet aroma and can form through Maillard reactions and the thermal degradation of sugars (Al-Attabi et al., 2008, Zabbia et al., 2012, Jo et al., 2018). At a concentration of 38 ppb, it has previously been suggested that diacetyl contributes excessively to the “heated” flavour of Ultra-High Temperature (UHT) treated milks (Scanlan et al., 1968). To protect patients who are potentially immunocompromised, and

enable long-term storage without reliance on refrigeration, many ONS products are sterilised by high-temperature treatments during manufacture. It is likely that this heat-treatment contributes to the formation of volatiles and may be influential in forming the flavour of the final ONS product.

Volatile sulfur compounds are an important class of aroma compounds, due to their low detection thresholds, high-odour impact and wide distribution in food products, however, they are often overlooked because they are present at trace levels in foods (Du et al., 2015). GC-MS analysis detected four sulfurous compounds in trace concentrations within the headspace of the ONS (Figure 3-1) (methanethiol, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide). However, during the GC-O-MS study, the sulfur compounds detected by participants were methanethiol and dimethyl trisulfide (Figure 3-2); most likely due to their relatively low odour detection thresholds and relatively high estimated OAVs, as shown in Figure 3-1. A frequent source of sulfurous aroma compounds are amino acids with sulfur-containing side chains (cysteine and methionine) present in milk-proteins, and are liberated during heat-treatment (specifically through Strecker degradation) (Al-Attabi et al., 2008, Wong et al., 2008, Zabbia et al., 2012, Jo et al., 2018).

Although they can be an essential component of many flavours, sulfurous volatiles can be associated with undesirable off-flavours such as cooked and eggy flavours (Jo et al., 2018) and concentrations of these sulfurous, heat-generated compounds have also been found to

correlate positively with “heated” flavours of milk-products (Christensen and Reineccius, 1992). Vazquez-Landaverde et al., (2005) quantified sulfur compounds in heat-treated milk and proposed that, due to the high calculated OAVs, methanethiol and dimethyl trisulfide are the most important contributors to the undesirable sulfurous note in UHT milk. Considering this previous research, and the relatively high OAV values calculated for methanethiol and dimethyl trisulfide in the current study (OAV = 1,050 and 3,400 respectively (Table 3-1)). It is likely that these two aroma compounds contribute to the ONS flavour and could possibly be ‘off-flavours’ which negatively influence the sensorial experience of ONS. Though, considering that these aroma compounds originate from nutritionally essential amino acids, which cannot simply be removed from a product, it may be more within the interest of manufacturers to prioritise flavour masking, or changes to processing conditions, if aiming to hide or reduce the formation of these high-impact aroma compounds.

Despite the “unknown compound” having a high MF(%) value of 73% (Figure 3-2), it did not produce a peak on the GC-MS chromatogram, so was challenging to identify. Further investigation, using authentic standards and Linear Retention Indices (LRI), suggested the sulfurous compound “2-acetyl thiazole” (CAS: 24295-03-2, calculated LRI: 1699, literature LRI: 1666 (Culleré et al., 2013)) may be responsible for this aroma. When key ions for 2-acetyl thiazole (m/z 43, 58, 85, 99, 112, 127) were searched in the chromatogram they were not present on the

chromatogram and thus may not have been detected by the GC-MS. Participants described this aroma as having particularly “Burnt”, “Cooked” and “Nutty” qualities, thus also matching the organoleptic properties of 2-acetyl thiazole (Good Scents Company, 1980-2021).

3.4.2.1 Difference in GC-O-MS perception between older and younger participants

Although the same eight aroma compounds were detected by the both age-groups, there were some differences between groups, as shown in Figure 3-3 (individual and group scores are shown in Appendix D). The younger adult group had higher MF(%) values for the sulfurous compounds. For example, dimethyl trisulfide reached MF 68% for the younger adult group, whereas the older adult group had a MF(%) value of 43%, which is below the cut-off of MF 50% (Figure 3-3). However, in contrast to what was expected, older adults had higher MF(%) values for the compound Diacetyl; older adults had a MF(%) value of 58%, while younger adults had a much lower MF(%) value of 14%, below the 50% cut-off value (Figure 3-3).

Although recognition thresholds were not directly measured in this study, it was apparent that the ability to recognise and describe an aroma was superior in the younger group. At multiple times during the GC-O-MS study, older adults detected a stimulus but were unable to describe it, so they used terms such as “Indescribable” (ethyl butyrate and methanethiol), and “Indistinct” (isoamyl isovalerate) (see Appendix C). This suggests that some older adults had more difficulty in

recognising and describing the detected stimuli delivered by GC-O-MS. This is supported by other research that found recognition thresholds to be more sensitive to the effects of ageing than detection thresholds (Hummel et al., 2007, Seow et al., 2016) and makes reasonable sense considering that the humans' ability to recognise and describe odours is considered less of an evolutional priority than the ability to detect odours (Hummel et al., 2007, Seow et al., 2016).

Humans exhibit considerable variation in the perception of odorants (Mainland et al., 2014), and due to the relatively small group sizes used in the GC-O-MS analysis, group differences in olfactory ability cannot be explained in-depth by the scope of the current method. Factors such as stronger motivation to perform or greater familiarity with the aromas, may have also contributed to differences between age-groups. We therefore conducted threshold tests (section 3.4.3), with larger group sizes (24 younger adults, and 24 older) to better explore differences in perceptual sensitivity between age groups.

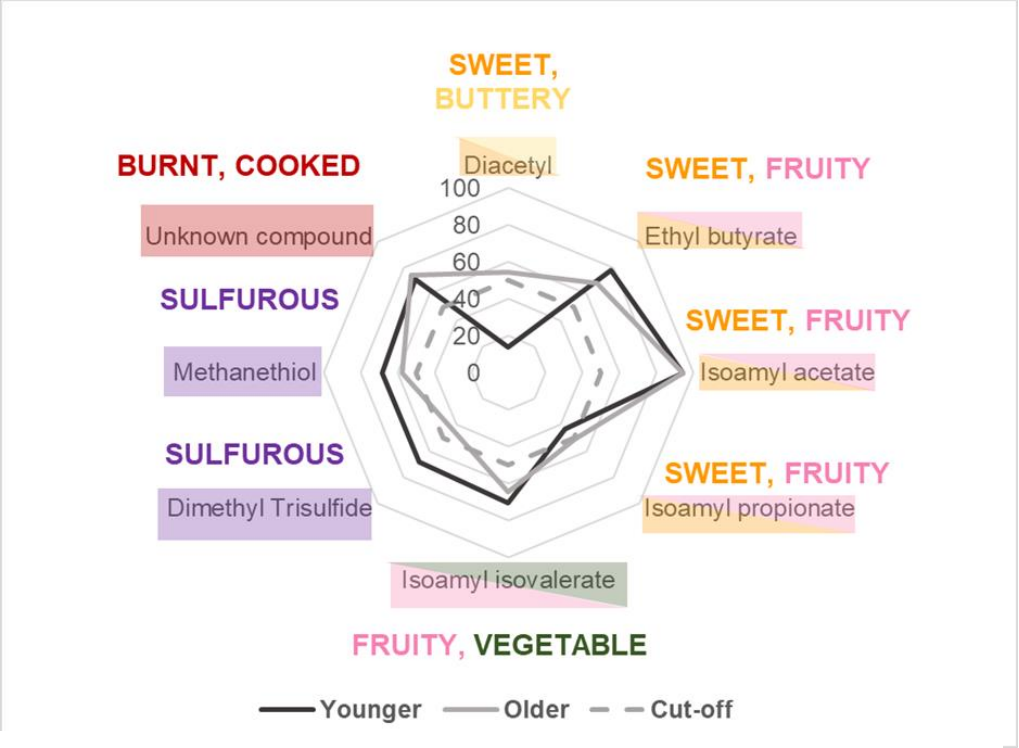

Figure 3-3: Modified frequency values (%) of younger participants (n=6, black) and older participants (grey, n=6). The 50% cut off value is indicated.

3.4.3 Age-related differences in perception of aroma-active aroma compounds.

This study used detection threshold tests to investigate the association between ageing and perceptual sensitivity to aroma-active compounds. Four aroma compounds were chosen from those detected in the gas chromatography-olfactometry-mass spectrometry analysis (Section 3.4.2) to represent a variety of hedonic, chemical and sensory characteristics. As an additional investigation, the pleasantness of the aroma-active compounds, at suprathreshold concentrations, was first assessed by each age-group.

3.4.3.1 Aroma compound pleasantness

Participants were asked to rate the pleasantness of each aroma compound using a categorical scale: ‘Pleasant’, ‘Neutral’, or ‘Unpleasant’. Isoamyl acetate and diacetyl were categorised as predominantly pleasant compounds or neutral compounds (Figure 3-4). Contrary to this, methanethiol and dimethyl trisulfide were rated as predominately unpleasant compounds (Figure 3-4). A greater proportion of younger adults found the sulfurous aromas to be unpleasant, though, there were not many significant differences in pleasantness ratings between the older and younger groups. The only significant difference found was that significantly more older adults rated methanethiol as a neutral compound (X^2=10.27, df=2, p=0.006) compared with younger adults, demonstrating that this compound

becomes less unpleasant with older age. Similar trends can be observed for the unpleasant sulfurous compound dimethyl trisulfide. These findings are supported by Wysocki and Gilbert (1989) who found that sulfurous mercaptans became less unpleasant with ageing, along with a deterioration of a negative intensity-pleasantness correlation, leading to the interpretation that a reduced perceived intensity of this aroma leads to a reduction in perceived unpleasantness.

It is important to note that, pleasantness of aroma is influenced by concentration (Moskowitz, 1977); an aroma may be perceived pleasant in low concentrations but unpleasant at high concentrations. This research is the first stage of understanding the hedonic response to aroma-active compounds in an ONS; the next stages of research should consider the compounds within a mixture, and a real food

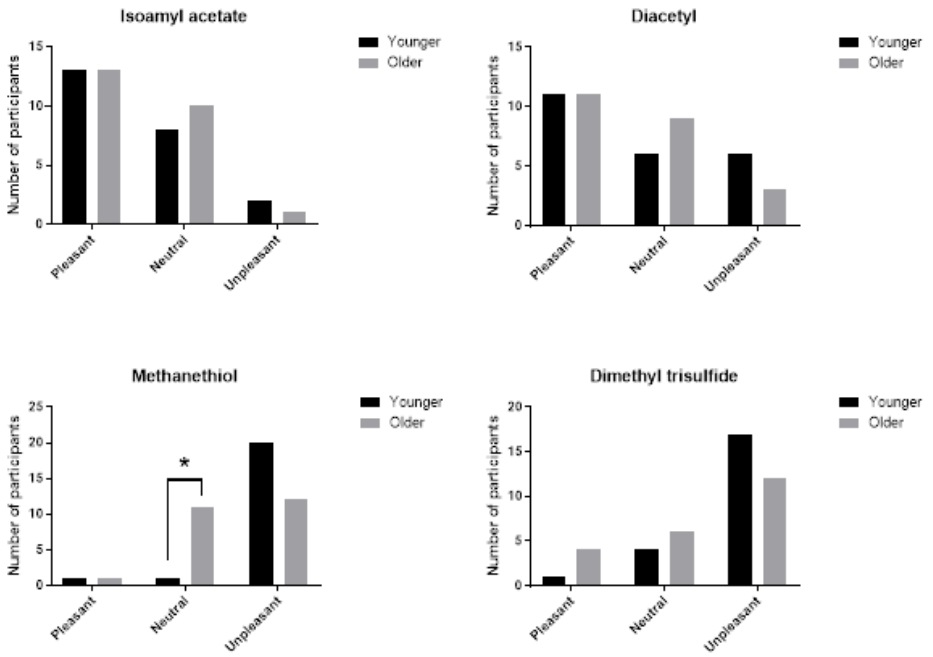

Figure 3-4: Pleasantness ratings of the aroma compounds, separated by both older and younger adults, for the four aroma compounds studied. Statistically significant differences, as analysed by the Chi-squared test, are marked with * for p < 0.05.

matrix, to draw conclusions about the hedonic influence of these compounds on ONS perception.

3.4.3.2 Difference in detection sensitivity between younger and older participants

In line with previous research on age-related differences in olfactory sensitivity (Wysocki and Gilbert, 1989), older adults had significantly higher detection thresholds for two aroma compounds (isoamyl acetate ($p=0.01$) and methanethiol ($p=0.03$)) (Figure 3-5) demonstrating that older adults need a greater quantity of aroma stimuli in water to detect the aroma.

Age-related olfactory impairments have been shown to lead to alterations in dietary habits and food choices (Mattes et al., 1990, Duffy et al., 1995), poor appetite (de Jong et al., 1999, Schiffman and Graham, 2000, Somekawa et al., 2017), reduce nutritional intake and status (Griep et al., 1996) and play a role in the development or sustainment of undernutrition and frailty (Schiffman and Graham, 2000, Gopinath et al., 2012, Somekawa et al., 2017). Across the increasing lifespan, Wysocki and Gilbert (1989) found an almost linear decline in ability to detect isoamyl acetate concurrently with a decline in the willingness to eat a banana flavour food. Considering this previous research, it may be the case that impairments in olfactory sensitivity to identified aroma-active compounds alters the perceived flavour or palatability of the studied ONS. Previous research has found differences in flavour preferences for ONS between patients and

healthy controls (Gallagher and Tweedle, 1983, Brown et al., 1986, IJpma et al., 2016, de Haan et al., 2021). Though, it is important to note that the current study recruited self-reportedly healthy older adults as participants. It would be informative to assess this hypothesis with older participants who fall into the ONS consumer group of undernourished older patients, with a greater magnitude of age-related diseases and likewise greater medication use. Within this group, age-related decreases in olfactory sensitivity may be further impaired.

The cause of this change in olfactory ability is likely to be multifactorial and involve changes in the nose, olfactory epithelium and higher brain regions that receive olfactory input (Doty and Kamath, 2014). The complex causes of age-related olfactory impairment are discussed in detail in a review by Doty and Kamath (2014) who summarised potential contributing factors to be: altered nasal engorgement and airflow, increased propensity for nasal disease, cumulative damage to the olfactory epithelium from viral and other environmental insults, decrements in mucosal metabolizing enzymes, ossification of cribriform plate foramina, loss of selectivity of receptor cells to odorants and changes in neurotransmitter and neuromodulator systems. It would also not be appropriate to discuss age-related olfactory loss without a discussion of the influence of medication within this population. Over 250 medications used to treat age-related conditions, such as antihypertensive medications and statins, are known to affect both taste and smell (Schiffman and Zervakis, 2002). In the current study, older

participants reported taking six times more medications than those in the younger group, including those medications with a recognised effect (a list of the medication classifications that participants were regularly taking are listed in Appendix B). This is likely to have been a factor contributing to the decreased olfactory acuity of the older group. A comprehensive discussion of the influence of medication and diseases on sensory abilities can be found in Schiffman and Zervakis (2002).

3.4.3.3 Age-related impairments in olfaction are aroma specific.

This research has found that age-related olfactory loss is not uniform across aroma compounds: detection thresholds were most different

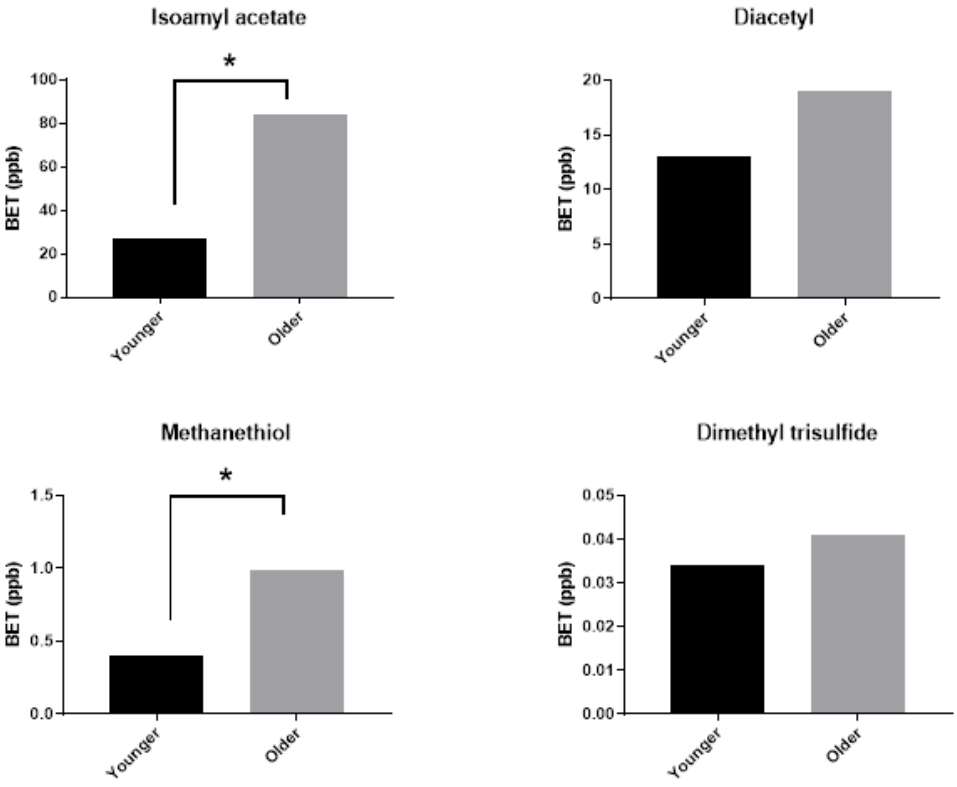

Figure 3-5: Best estimate detection threshold values (BET), for both younger and older adults, for the four aroma compounds studied. Statistically significant differences, as analysed by The Mann-Whitney U test, are marked by * for $p < 0.05$.

between age groups for isoamyl acetate, being over 3 times higher but almost identical for dimethyl trisulfide (see Figure 3-5). These findings are supported by previous research. For example, Wysocki and Gilbert (1989) found that age-related deficits in olfaction were not uniform across aromas, concentrations or across the life span. In agreement with the current study, they found that reduction in intensity ratings were most pronounced for isoamyl acetate and sulfur-containing mercaptans. Interestingly, Seow et al., (2016) also found that detection thresholds for isoamyl acetate (amongst others) were most significantly impeded by age from the 6th to 8th decade (51-80 years).

The trigeminal sense, responsible for the sensation of tactile, proprioceptive, temperature and painful stimuli (Chen and Engelen, 2012) is also vulnerable to age-related decreases in sensitivity (Hummel et al., 2003). Alongside olfaction, many aroma-active compounds are also known to stimulate the trigeminal nerve (Hummel et al., 2003, Doty et al., 1978) but at a different range of intensities. In particular, diacetyl (sweet, buttery) has been found to modify perception of trigeminal and textural sensations in foods (De Wijk et al., 2003b), elicit nasal pungency and can be an irritant at high concentrations (Jin, 2015, Hubbs et al., 2002). Therefore, the extent of the trigeminal component present for each studied aroma-active compound may somewhat help explain the aroma-specific variation in olfactory sensitivity between the age-groups.

This aroma-specific loss may also be related to the molecular structure and conformation of olfactory receptors, with some receptors preserved more superiorly across the life course than others. In-line with this theory, Sinding et al., (2014) found that older adults experience olfactory loss more specifically to heavier molecules. This was not supported by the current study; however, it is likely to be a complex story as humans have ~400 different types of olfactory receptor (Mainland et al., 2014) and volatile-aromas have wide ranging physicochemical properties.

Seow et al., (2016) suggested that unbalanced loss for specific aroma compounds may lead to a distorted perception of aroma mixtures. The current findings are novel because they are the first to focus specifically on aroma-specific, age-related decline in olfaction to aroma identified within a real product, which can have low adherence in older consumer groups. The new findings from this study generate the new hypothesis that significant age-related impairments in the ability to detect pleasant aroma such as isoamyl acetate (sweet, fruity) may negatively alter the perceived flavour of the studied ONS for older adults. Though on the contrary, age-related impairments in the ability to detect undesirable sulfurous compounds could be an advantage to the older consumer, because the potentially undesirable off-flavour may become less prominent within the overall perceived flavour.

3.5 Conclusions

Oral nutritional supplements (ONS) have the potential to improve the nutritional status of undernourished older individuals however, adequate adherence to the prescribed course of ONS is paramount. Poor palatability, particularly the flavour, has been identified in the literature as an important factor limiting acceptance of ONS. This research is the first to characterise the volatile aroma profile of a commonly prescribed ONS and secondly, to measure age-related differences in sensitivity to associated aroma-active compounds. Estimated odour activity values (OAV) and gas chromatography-olfactometry-mass spectrometry (GC-O-MS) identified aroma-active compounds in the ONS which were a combination of pleasant fruity esters (isoamyl acetate) and aroma compounds rated as unpleasant (sulfur containing compounds), which are proposed to originate from heat-treatment of milk proteins. Older adults had some impairments in their ability to detect aroma compounds at threshold concentrations; in particular these impairments were greatest for the pleasant aroma-active compound isoamyl acetate (sweet, fruity) and unpleasant aroma-active compound methanethiol (sulfur). We hypothesise that these impairments may alter the perceived flavour profile of ONS for older adults. Future research should consider aroma-active compounds in a mixture and the impact of complex food matrices on ONS perception. This is a fundamental study which will aid further research into how the aroma profile, and associated age-related impairments in perception,

shape the global perception of ONS for nutritionally at-risk older individuals. Considering the marked rate at which the population is ageing, and the associated risk of undernutrition, it is vital to understand how to refine flavour formulations for specific age groups.

Chapter 4

4 Age group determines the acceptability of protein derived off-flavour

Sophie Lester[1], Leonardo Cornacchia[3], Camille Corbier[3], Katherine Hurst[1], Charfedinne Ayed[1], Moira A. Taylor[2], Ian Fisk[1]

[1]Division of Food Nutrition and Dietetics, School of Biosciences, University of Nottingham

[2]National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, Division of Physiology, Pharmacology and Neuroscience, School of Life Sciences, University of Nottingham

[3]Danone Nutricia Research, Uppsalalaan 12, 3584 CT Utrecht, the Netherlands

Highlights

- Heat-treatment of protein ingredients can impart sulfurous flavours into beverages.
- Sulfurous flavours negatively impacted consumer acceptance of a dairy beverage.
- Older adults had greater acceptance of sulfurous flavours compared to younger adults.
- Diacetyl reduced the negative impact of these compounds for both age groups.
- Best estimate thresholds give a conservative estimate of off-flavour acceptability.

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4.1 Abstract

Many older adults fail to meet their daily protein requirements, potentially due to social, physical and medical factors, including sensory and appetite changes. Additionally, our previous research has identified potential sulfurous off-flavours, originating from heat-treatment of protein ingredients, which could play a role in consumer acceptance. This study aims to determine the hedonic impact of these potential off-flavours when added to a dairy beverage, identify the specific off-flavour concentrations which cause rejection by consumers, and lastly investigate difference in acceptance between older and younger consumers. A rejection threshold (RjT) protocol was used, in combination with best estimate thresholds (BET), whereby sulfurous flavours (dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide), and diacetyl were added to create a range of concentrations. 95 participants (younger n = 49, 18–38 years; older n = 46, 60–79 years) tasted 7 pairs of samples (one blank and one with ascending off-flavour concentration) and selected their preferred samples. Sulfurous flavours negatively impacted consumer acceptance, however, the extent to which they impart a negative effect differs between age groups. Younger adults rejected samples containing low concentrations of sulfurous off-flavours (1.55 ppb), however, older adults rejected samples with concentrations over 3 times higher (5.08 ppb). When combined with sulfurous flavours, diacetyl increased the rejection threshold for both groups. In conclusion, these observations imply that

a greater quantity of off-flavour may be present before acceptance is reduced in the older consumer group. Moreover, diacetyl demonstrates partial masking abilities of sulfurous off-flavours, and BET gave a more conservative estimate of acceptability. This knowledge will help guide sensory innovation of high-protein beverages for older consumers to support product acceptance and optimal intake.

Key words: Healthy ageing, Protein, Flavour perception, Off-flavours, Older adults.

4.2 Introduction

Daily protein recommendations for healthy adults range from 0.75 g protein/kg body weight/day in the United Kingdom (Department of Health, 1991) to 0.8 g protein/kg body weight/day in Europe and The United States (EFSA Panel on Dietetic Products Nutrition and Allergies, 2012). These recommendations are set irrespective of age, however, there is strong consensus amongst international bodies and researchers that daily protein requirements for healthy adults aged 65 years and above rise to 1.0–1.2 g protein/kg body weight/day (Deutz et al., 2014, Bauer et al., 2013). The increased requirement is due to an age-related resistance to the positive effects of dietary protein on body protein synthesis (known as anabolic resistance) along with a greater occurrence of disease-related protein catabolism (protein breakdown). In fact, if acute or chronic illness is experienced in older age, requirements are thought to rise further to 1.2–1.5 g protein/kg body weight/day (Deutz et al., 2014). The higher requirement could equate to a dietary protein increase of around 27 g protein a day for a typical 60 kg older adult, which is considerable.

The World Health Organisation defines malnutrition as deficiencies, excesses or imbalances in a person’s intake of energy, and/or nutrients (World Health Organisation, 2020). Two broad groups of malnutrition are identified: over-nutrition, such as in overweight, obesity and non-communicable diseases such as heart disease and undernutrition, such as in stunting, wasting, underweight and micronutrient deficiencies

(World Health Organisation, 2020). Protein-energy undernutrition (PEU), defined as an inadequate intake of energy and protein compared to requirements, is associated with delayed recovery from disease, poorer life quality and increased risk of morbidity and mortality (Leij-Halfwerk et al., 2019).

Many older adults fail to consume sufficient protein to meet their requirements (Ten Haaf et al., 2018), increasing their risk of muscle loss, sarcopenia and ultimately an increased risk of falls, fractures and hospital admissions (Lim et al., 2012, Bauer et al., 2013, Deutz et al., 2014). The prevalence of undernutrition risk in the older population has been estimated to be 14 %, and rises further to 21–35 % for those living in institutions and care environments (Margetts et al., 2003, Schilp et al., 2012). Currently in the UK, malnutrition is estimated to cost at least £23.5 billion; with older adults accounting for 52 % of this cost (Stratton et al., 2018).

To help combat undernutrition, the development of foods and beverages which are both nutritious and acceptable for older consumers, is an ongoing and crucial challenge for the food industry. Factors inherent to the older consumer may generate challenges which limit the acceptability of high-protein foods and beverages. Older consumers experience oro-sensory changes which may alter their food sensory experience. Age-related reduction in gustatory sensitivity is known to occur in the older consumer group (Kälviäinen et al., 2003, Methven et al., 2012, Sergi et al., 2017) along with olfactory function

(Ekström et al., 2019, Fluitman et al., 2019), which has found to be relatively more impeded by the ageing process (Stevens et al., 1984). Olfactory impairments can contribute to altered food choices and reduced nutritional intake and status (Duffy et al., 1995, Griep et al., 1995, Kremer et al., 2014, Somekawa et al., 2017).

Foods and beverages which are high in protein are particularly vulnerable to poor consumer acceptability as the protein molecules can be a source of undesirable sensory properties (Bull et al., 2017, Smith et al., 2016). Subjective mouth-feel sensations, such as mouth drying and mouthcoating, can be caused by proteins (Withers et al., 2014, Bull et al., 2017) and are negative drivers of liking in dairy-based Oral Nutritional Supplement (ONS) (Thomas et al., 2016). Proteins may also impart new flavours to food and beverages, through interactions with other ingredients, degradation and/or processing induced chemical reactions (Al-Attabi et al., 2008, Zabbia et al., 2012, Cadwallader, 2016, Smith et al., 2016)

Our previous research has identified sulfurous volatile flavour compounds in a commonly prescribed dairy-based ONS, some of which were rated as unpleasant by younger and older consumers. Dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) are a group of closely related sulfurous volatile flavour compounds, formed through Maillard reactions, from sulfurous essential amino acids, during high-temperature processing (Al-Attabi et al., 2008, Zabbia et al., 2012, Smith et al., 2016). The pungent character and

high-impact of these flavour compounds means they contribute to the cooked, heated and sulfurous flavour notes in thermally processed milk (Al-Attabi et al., 2008, Vazquez-Landaverde et al., 2006).

Another flavour compound of interest, which often occurs concurrently with sulfides in dairy foods (Zabbia et al., 2012), is the volatile flavour compound diacetyl (2,3-butandione). Diacetyl is noted for its appealing butter-like aroma and flavour (Antinone et al., 1994, Clark and Winter, 2015), and can be present naturally in many dairy products such as butter and cheese (Clark and Winter, 2015). In common with sulfides, diacetyl can be formed through Maillard reactions during thermal treatment of dairy products (Zabbia et al., 2012).

Previous studies investigating the hedonic impact of diacetyl in dairy products have found that sour creams with the greatest perceivable intensities of diacetyl had the greatest consumer acceptability, compared to sour creams with lower perceivable intensities of diacetyl (Shepard et al., 2013). Antinone et al (1994) found an increase in liking for attributes of cottage cheese as a function of diacetyl concentration, with the mean flavour score peaking at 1000 ppb. Drake et al (2009) identified diacetyl flavour to be a driver of liking in full-fat cottage cheese.

It is not yet known how the combination of sulfurous flavours and diacetyl affect the acceptability of foods and beverages. This study firstly aimed to examine the hedonic impact (positive, negative or no impact) of potential off-flavour compounds when added in increasing

concentrations to a flavoured dairy beverage. Secondly, we aimed to identify the concentrations at which rejection occurred by consumers (the rejection threshold). Thirdly, by comparing rejection threshold concentrations for each age group, we investigated whether human age was a factor influencing consumer acceptance of these flavours. Lastly, we aimed to compare suitability of two separate rejection threshold methodologies (graphical approach (RjT_{50}) and best estimate thresholds (BET)) and the impact of each on our conclusions. Flavour compounds were studied both alone, and in combination, to ensure any flavour-interactions were captured.

4.3 Materials and methods

This study was approved by Faculty of Medicine and Health Sciences Research Ethics Committee at the University of Nottingham (Reference No. 156-1810) (Appendix K).

4.3.1 Participants

Forty-nine younger participants and forty-six older participants were recruited to take part in the study from The University of Nottingham and local villages via an email invitation and poster advertisements. Inclusion criteria were: age between 18 and 40 years or 60–80 years, male or female and smokers or non-smokers. These age ranges were chosen to incorporate a large range with a defined age gap between the younger and older group. The World Health Organisation has previously defined older age as 60 years and older (Mathers et al., 2015). 80 years was chosen as an upper age limit to minimise risk of harm due to increasing prevalence of frailty with age. Exclusion criteria were: food allergies or intolerance to dairy (or other ingredients used in the beverage), pregnancy or breastfeeding, or known sensory impairments (unrelated to ageing) in taste or smell. A questionnaire was used to collect health, lifestyle, and demographic information, including habitual milk consumption, and confirmed their eligibility to take part (this data can be found in Table 4-1). Informed consent was collected from all participants, but participants were not informed that the study was investigating differences in flavour.

Table 4-1: Health and demographic information, and milk beverage consumption behaviour and preference, for the younger and older groups of participants included in the study.

	Younger	Older
Health and demographic information		
n	49	46
Mean age in years (range)	23 (18-38)	69 (60-79)
Male (%)	24.5	39.1
Female (%)	75.5	60.9
Mean no. regular medication taken by each participant (daily)	0.3	2.3
Percentage of participants with chronic health condition	6	20
Percentage of participants currently regularly smoking	2	0
Percentage of participants who previously regularly smoked (> 5 years)	6	33
Percentage of participants with previous experience in food sensory analysis	57	37
Milk consumption behaviour and preferences		
Percentage preferring pasteurised milk	90	91
Percentage preferring UHT milk	4	9
Percentage preferring dairy alternatives	6	0
Percentage who find the flavour of UHT enjoyable	49	52
Percentage regularly consuming UHT milk (once a month or more)	47	65
Regularly consume flavoured dairy beverages (milkshakes)	80	41
Enjoy banana as a flavour	88	98

4.3.2 Materials

Pasteurised whole milk was purchased from a national supermarket in the UK. It was essential to use milk that had only undergone a gentle heat-treatment such as pasteurisation, rather than Ultra High Temperature (UHT), in order to limit the presence of heat-associated flavours. Each bottle of milk also had the same production date.

Maltodextrin (DE 19) and banana flavourings were gifted by Danone Nutricia Research®, NL. Food-grade diacetyl (2,3-butandione) was supplied by De Monchy Aromatics Ltd®, UK, and food-grade dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) were purchased from Sigma-Aldrich®, US.

4.3.3 Dairy beverage preparation

The banana flavoured dairy beverage was produced in a single batch to limit batch-to-batch variations in flavour. Banana flavourings (0.05 g/ L) and maltodextrin (300 g/L) were incorporated into the milk by electric hand mixing at room temperature (20 °C ± 1). The beverage was separated into 4 L milk bottles and stored frozen (− 18 °C) until the evening before a study day when the desired quantity was defrosted in a refrigerator (3 °C) overnight. The beverage was stored for no longer than 3 weeks. No perceivable changes in flavour occurred during this time and no separation was observed.

On the morning before a study session, flavour compounds were ‘spiked’ into the beverage to create the desired concentrations (Table 4-2). Propylene glycol (PG) was used as the flavour carrier and the same volume of PG was also spiked into blank samples to ensure matrix uniformity between blank samples and flavour ‘spiked’ samples. The concentrations of flavours used were determined by concentrations previously quantified in a commercial product used as a reference (data not shown). For Experiment 1, three closely related sulfide compounds

(DMS, DMDS, DMTS) termed the common name ‘sulfurous flavours’, were spiked into the beverage. For Experiment 2, diacetyl alone was spiked into the beverage. For Experiment 3, both sulfurous flavours and diacetyl were spiked into the beverage, in the same concentrations used in the previous experiments.

Once the concentrations were prepared, the beverage was kept refrigerated until being pipetted into individual 10 mL samples. For all experiments, the first concentration was chosen to be 0 ppb, in an effort to obtain a RjT closer to 50 % (assuming that there would be an equal chance of participants choosing either of two samples) (see section 4.3.5 for statistical methods). Following this, concentrations of sulfurous flavours and diacetyl increased by set increments (approximately 33 % of the concentration quantified in the commercial product at Level 4, Table 4-2). This increment in flavour concentration is smaller than those used in previous rejection threshold studies (for example, Prescott et al (2005) increased concentrations of TCA by a factor of 2, or 100 %). In the present study, this smaller concentration increment was chosen because the high impact of sulfurous flavours was known prior to the experiment.

Table 4-2: Flavour concentrations (ppb) used in the rejection threshold experiments.

Level	**Experiment 1: Sulfurous flavours**				**Experiment 2: Diacetyl**	**Experiment 3: Mixture**	**Equivalent concentration in commercial product**
	DMS	DMDS	DMTS	**Total**			
1	0	0	0	0	0	0	0%
2	0.18	0.71	0.18	1.07	42	43.07	33%
3	0.37	1.42	0.36	2.15	85	87.15	66%
4	0.56	2.13	0.54	3.23	128	131.23	~100%
5	0.75	2.84	0.72	4.31	171	175.31	133%
6	0.94	3.55	0.90	5.39	214	219.39	166%
7	1.13	4.26	1.08	6.47	257	263.47	~200%

4.3.4 Protocol on study days

All sensory testing took part in The University of Nottingham's Sensory Science Centre (Sutton Bonington Campus) in sensory booths designed to ISO standards (ISO8589:1988). Study sessions were mixed with both older and younger participants and participants attended 1 session per week in a randomised order (each session consisting of Experiments 1, 2 or 3). Participants were instructed not to wear strong smelling cosmetics and not to eat, drink or smoke 2 h before attending a session.

In each study session, a rejection threshold design was employed whereby participants were provided with a series of 7 paired preference tests (each pair containing one blank sample, and one sample containing an ascending concentration of off-flavour). Paired preference tests have been recognised as an appropriate sensory test for use with older adults due to their relative simplicity (Methven et al., 2016a). Each sample was 10 mL in volume and served in 30 mL plastic cups, each labelled with a random 3-digit code. Samples were served chilled, in-line with the typical serving temperature for flavoured milkshake beverages. The order of presentation within a pair was randomised.

Participants were instructed to taste each sample within a pair, from left to right, and then were asked the question "Which sample do you prefer?". They were instructed to indicate their response by selecting the sample code. A 'no preference' option was not provided. To record

their responses, participants were given the choice to use a computer or a paper copy of the same test, of which 2 older and 1 younger participant chose to use paper. Online data was collected using Compusense Cloud® (Compusense, Ontario, Canada). In-between tasting each sample within a pair, participants were asked to rinse their mouth with water (Evian, Danone, France). In-between tasting of pairs, during a compulsory 2- minute break, participants were asked to cleanse their palate by chewing and swallowing one pre-prepared slice of green apple (Golden Delicious, Tesco, UK), and rinsing their mouth with water.

To gain an insight into the reasons for rejection, participants were provided with an open-ended question after each pair, where they were asked “Why did you choose this sample as your preferred sample?” and allowed to write freely.

4.3.5 Statistical analysis

All statistical analysis was conducted using the software XLSTAT® statistical and data analysis solution (version 20.6.01, Addinsoft, Long Island, NY, USA) or GraphPad Prism® (version 7.0, San Diego, CA, USA). Constant values of + 2 (sulfurous flavours) or + 100 (diacetyl and mixture) were added to the concentrations (ppb), to omit zero values and enable the statistical analysis. For example, at the first concentration of diacetyl (0 ppb), a constant value of 100 was added, to give a final value of 100 ppb. At the second concentration of diacetyl

(42 ppb), a constant value of 100 ppb was added, to give a final value of 142 ppb. These constant values were later subtracted (as described below).

At each concentration level, the percentage of participants preferring the blank sample in a pair (hence rejecting the 'spiked' sample in a pair), was plotted on the y-axis with the log concentration on the x-axis. The hedonic impact was thus indicated by consumer preference, relative to the blank, for the spiked sample across increasing concentrations of the flavour compounds of interest. To calculate rejection thresholds using a graphical approach, a sigmoidal variable slope dose–response function was fitted through the data points, using the Hill equation. The Hill equation, commonly used in pharmacology, describes four parameters: the top of the curve (max), the bottom of the curve (min), the spot halfway between min and max (EC_{50} or RjT_{50}) and the slope of the curve (the Hill coefficient).

The 2- AFC chance corrected probability (75% rejection) gave the rejection threshold ($LogRjT_{50}$) and this was automatically calculated by GraphPad Prism® (the $LogEC_{50}$ value, see Harwood et al (2012) for a concise description of statistical methods). To obtain the RjT_{50} concentration (ppb), the antilog of '$LogRjT_{50}$' is found and the constant values subtracted. Due to absence of data points at 50% (chance) for some of the investigations, some RjT_{50} values were ambiguous. Therefore, two of the four parameters within the Hill equation (the minimum and maximum) were constrained at values of 50 and 100, as

described in Harwood et al (2012), and the curve was re-fit. This constraint was applied to all investigations in order to compare accurately between them.

It has recently been recommended that when estimating consumer RjT, both a graphical approach (described above) and a best estimate threshold (BET) approach should be utilised (Murray et al., 2019). Thus, for a complementary comparison between age groups, BET were also calculated by using the adapted method presented by Murray et al (2019) by taking the geometric mean of the first concentration whereby individual participants preferred the blank, and the next lowest concentration where participants preferred the spiked sample. The geometric mean of individual BET within a group gave the age-group BET. Due to uneven distribution of this data, the non-parametric test Mann-Whitney U was used to statistically compare group values.

Qualitative data was interpreted by a method based on Ares et al (2008) and Ares et al (2010). Descriptive reasons consumers gave for rejecting the flavour spiked sample, at the concentration level immediately following the group rejection threshold (RjT_{50}), were compiled for both age groups. To generate categories, three researchers independently searched the data for recurrent and similar terms. Both personal interpretation and synonyms (as determined by an English dictionary) were employed to classify terms into categories. After the independent analysis, a meeting between the researchers resulted in consensus on the categories. For ease of interpretation by

the reader, categories were further categorised into sensory modalities. Within each age-group, category frequencies were determined by counting the number of individual consumers who mention each category. The percentage of consumers who mentioned each category, out of the number of consumers who rejected at this level (within each age-group), was calculated. Only categories which were mentioned by > 5% of consumers are shown.

4.4 Results

4.4.1 Hedonic effect and rejection thresholds determined by a graphical approach (RjT_{50})

4.4.1.1 Experiment 1: Sulfurous flavours

As the concentration of sulfurous flavours increased, higher percentages of participants preferred the blank samples (Figure 4-1), signifying that sulfurous flavours had a negative hedonic impact on consumer acceptance of the dairy beverage. This was true for both age groups, however, there were differences in the concentration at which rejection (75 % rejection) occurred. When combined into a single group, the group rejection threshold (RjT_{50}) was 2.47 ppb, however, when participants were separated into the respective age categories, younger adults reached rejection at 1.55 ppb and older adults reached rejection at 5.08 ppb (over 3 times higher). Importantly, unlike older adults, younger adults rejected sulfurous flavours at a concentration lower than the concentration in the commercial product (3.23 ppb, Level 4).

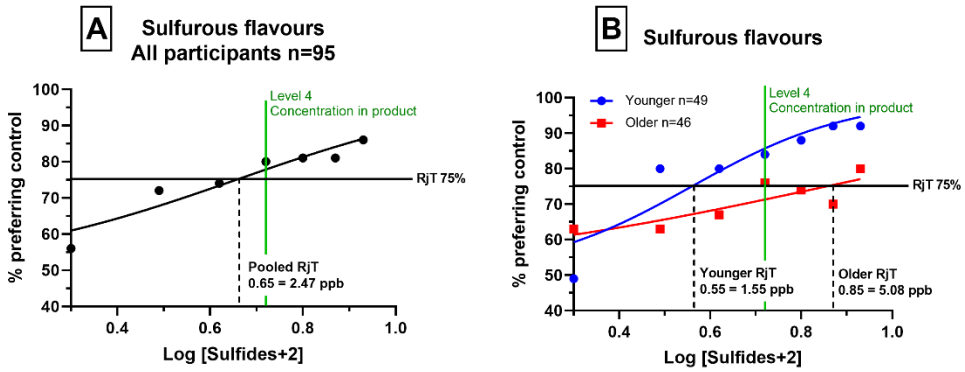

Figure 4-1: Consumer rejection thresholds of sulfurous flavours (experiment 1) as determined by a graphical approach (RjT_{50}). Proportion preferring the blank (y-axis) plotted against the log concentration of flavourings (x-axis), which were spiked into the beverage. A) shows global consumer rejection of all ages (n = 95), B) shows comparison of younger consumer (blue, n = 49) and older consumers (red, n = 46).

4.4.1.1.1 Qualitative reasons for rejection of sulfurous flavour-spiked sample.

As shown in Table 4-3, at the closest level to the RjT_{50} (concentration level 3), the main reasons given by the younger group for rejection of samples with sulfurous flavour included detection of 'Off-flavour' (18%) and 'Unpleasant aftertaste' (15%). In contrast to this, at the closest level to the RjT_{50} (concentration level 6) no older adults (0%) gave these as reasons for rejecting the samples. The main reasons given by the older adults to reject the samples with sulfurous flavour were 'Unpleasant aroma' (7%) and 'Weaker flavour' (7%). Older adults stated positive reasons for accepting the blank sample more frequently, such as 'pleasant aroma or 'good banana flavour', rather than explicitly state negative reasons for rejecting the sample containing sulfurous flavour.

Table 4-3: Frequencies (Freq), and percentages (%) of consumers who gave reasons within each category as a reason for rejecting the sample containing sulfurous flavour, counted at the concentration level immediately following the point of rejection for each age-group. % are the proportion of consumers who rejected at the specific concentration level.

Category	**Examples**	**Older** Concentration level 6		**Younger** Concentration level 3	
		Freq	**%**	**Freq**	**%**
Flavour and taste					
Unpleasant flavour	Less pleasant taste, Bad taste	2	3	6	8
Off-flavour	Metallic taste, sour taste, oniony taste	0	0	14	18
Stronger flavour	Less subtle taste, Full flavour, Too much banana flavour	2	3	4	5
Weaker flavour	Weak banana taste, Less flavour, Less strong	5	7	6	8
Less sweet	Not sweet enough, Sample was less sweet	1	1	7	9
Aroma					
Unpleasant aroma	Less pleasant aroma	5	7	7	9
Specific off-aroma	Bitter aroma, Sour aroma, Rancid smell	1	1	8	10
Texture					
Thinner texture	Watery texture, Less creamy, Less thick	2	3	5	6
Aftertaste					
Unpleasant aftertaste	Weird aftertaste, Tangy aftertaste, Less pleasant aftertaste	0	0	12	15

4.4.1.2 Experiment 2: Diacetyl

Over increasing concentrations of diacetyl, only marginally greater percentages of participants chose the blank samples over the flavour spiked samples (Figure 4-2), indicating a small negative hedonic impact. Although younger adults demonstrated greater rejection than

the older adults, neither age group reached 75% rejection. The Hill equation predicted that if concentrations of diacetyl did continue to increase rejection would have occurred at 592 ppb for younger adults and 1738 ppb for older adults (Log RjT_{50} values are 2.84 and 3.24 respectively). Qualitative data is not shown as a rejection threshold was not reached.

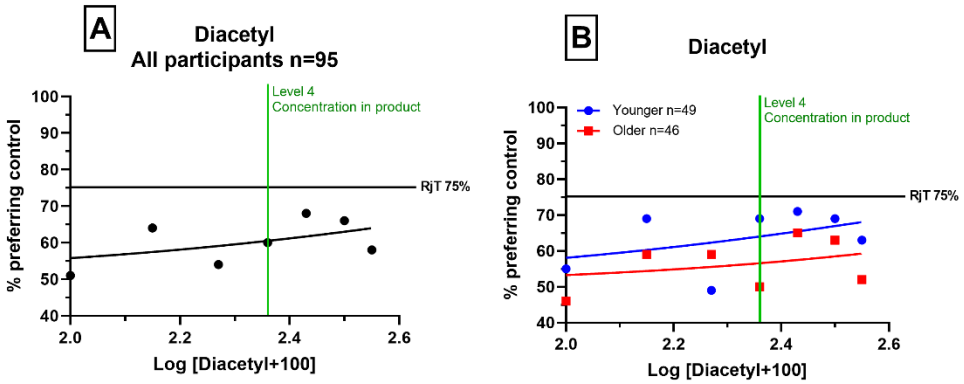

Figure 4-2: Consumer rejection thresholds of diacetyl (experiment 2) as determined by a graphical approach (RjT_{50}). Proportion preferring the blank (y-axis) plotted against the log concentration of flavourings (x-axis), which were spiked into the beverage. A shows global consumer rejection of all ages (n = 95), B shows comparison of younger consumer (blue, n = 49) and older consumer (red, n = 46) groups

4.4.1.3 Experiment 3: Mixture of sulfurous flavours with diacetyl

When the sulfurous flavours were combined with diacetyl, in a new series of paired preference tests (Experiment 3), we see that 75% rejection (RjT_{50}) is reached (Figure 4-3). The added flavours had a negative hedonic impact on consumer acceptance of this dairy beverage: as the concentration off 'off-flavours' increased, a greater number of consumers preferred the blank samples. If considering the participants as a single group, the rejection threshold occurred at 188 ppb. However, if again separated into their respective age categories,

younger adults reached 75% rejection at 163 ppb and older adults reached rejection at a higher value of 263 ppb. For both age-groups, when compared to Experiment 1 (sulfurous flavour alone), the point at which RjT_{50} occurred increased, with rejection occurring at higher concentration levels.

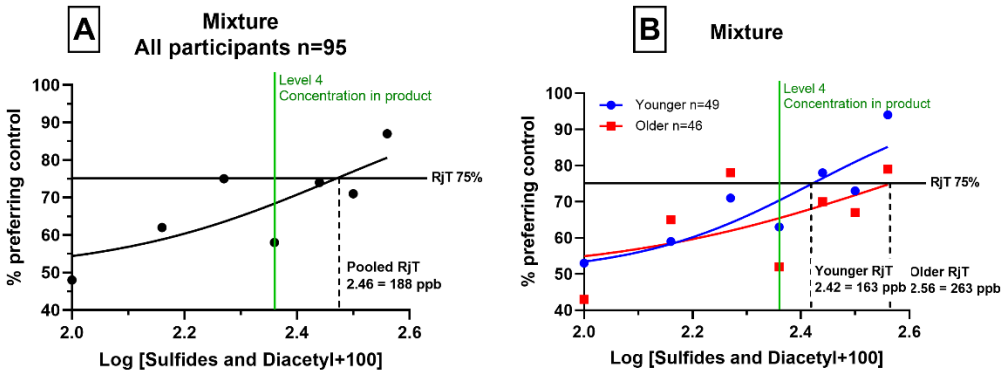

Figure 4-3: Consumer rejection thresholds of sulfurous flavours and diacetyl combined in a mixture (experiment 3) as determined by a graphical approach (RjT_{50}). Proportion preferring the blank (y-axis) plotted against the log concentration of flavourings (x-axis), which were spiked into the beverage. A shows global consumer rejection of all ages (n = 95), B shows comparison of younger consumer (blue, n = 49) and older consumer (red, n = 46) groups.

4.4.1.3.1 Mixture: Qualitative reasons for rejection of flavour-spiked sample

As shown in Table 4-4, at the closest level to the RjT (concentration level 5), the main reasons given by younger adults for rejecting the samples containing both sulfurous flavours and diacetyl included detection of ‘Off-flavour’ (20%) and ‘Unpleasant aroma’ (19%). In line with Experiment 1 (Section 2.1.1.1), ‘Unpleasant aftertaste’ (13%) was also a reason given frequently by younger consumers. In contrast, lower percentages of older adults gave detection of ‘Off-flavour’ (2.5%) and ‘Unpleasant aftertaste’ (0%) as reasons for rejecting the spiked

samples. In agreement with the data from Experiment 1 (Section 2.1.1.1), at the closest level to the RjT (concentration level 7), older adults stated a ‘Weaker flavour’ (10%) and ‘Unpleasant aroma’ (8.9%) as the main reasons for rejecting samples containing sulfurous flavour and diacetyl. In comparison, fewer younger adults stated a ‘Weaker flavour’ as a reason for rejecting the samples containing sulfurous flavour and diacetyl (7%). In agreement with Experiment 1, older adults stated positive reasons for accepting the blank sample more frequently, rather than state negative reasons for rejecting the sample containing off-flavours.

Table 4-4: Frequencies (Freq), and percentages (%) of consumers who gave reasons within each category as a reason for rejecting the sample containing sulfurous flavours and diacetyl, counted at the concentration level immediately following the point of rejection for each age-group. % are the proportion of consumers who rejected at the specific concentration level.

Category	**Examples**	**Older** Concentration level 7		**Younger** Concentration level 5	
		Freq	**%**	**Freq**	**%**
General					
Unpleasant	Less palatable, Less pleasant, Unpleasant	1	1	9	13
Flavour and taste					
Unpleasant flavour	Less pleasant flavour, Unpleasant taste	3	4	8	11
Off-flavour	Metallic taste, Sour taste, Less fresh taste	2	3	14	20
Stronger flavour	More potent flavour, Less subtle, More strong flavour	1	1	5	7
Weaker flavour	Weaker milk taste, Less strong taste, More watery taste	8	10	5	7
Less sweet	Less sweet	1	1	4	6
Artificial flavour	More synthetic flavour, More artificial taste	0	0	4	6

Aroma					
Unpleasant aroma	Off-putting aroma, Less pleasant smell	7	9	13	19
Texture					
Thinner texture	Watery mouthfeel, Less creamy texture	2	3	4	6
Aftertaste					
Unpleasant aftertaste	Nasty aftertaste, Less pleasant after taste, Weird aftertaste	0	0	9	13
Stronger aftertaste	More aftertaste, More strong aftertaste	1	1	5	7

4.4.2 Rejection thresholds as determined by best estimate thresholds (BET)

Best estimate thresholds (BET) were also calculated for each experiment (Figure 4-4). For Experiment 1 (sulfurous flavours alone), younger adults had significantly lower BET than the older adults ($p = 0.0009$). For Experiment 2 (diacetyl), although younger adults had lower thresholds, there is no significant difference between the age groups. When sulfurous flavours and diacetyl was again combined in Experiment 3, there is a significant difference between the age groups ($p = 0.0269$).

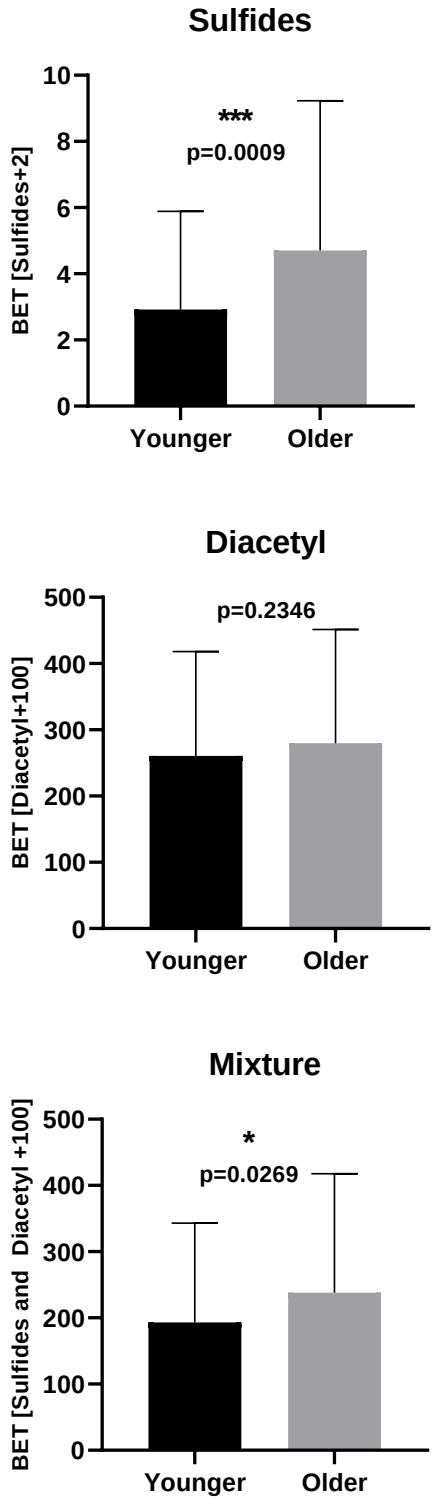

Figure 4-4: Best estimate thresholds (BET) of younger consumers (black) and older consumers (grey) for experiments 1–3. Values show geometric mean ± geometric standard deviation. Differences between groups were analysed by Mann Whitney U (p = 0.05). * indicates p ≤ 0.05, ** indicates p ≤ 0.01, *** indicates p ≤ 0.001.

4.4.2.1 Comparison between graphical approach (RjT_{50}) and best estimate thresholds (BET)

For both age-groups, all calculated BET values were lower than the threshold values calculated by a graphical approach (RjT_{50}) (Figure 4-5). This discrepancy between the methodologies led to important differences. For example, for Experiment 1 (sulfurous flavour), older adults had RjT_{50} values higher than the concentration in product. This finding contrasts with the BET values for this age group, which were below the concentration in product.

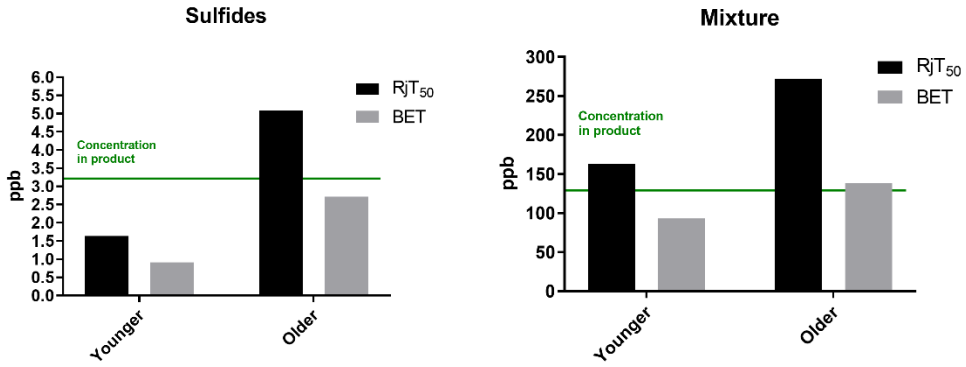

Figure 4-5: Comparison of the RjT_{50} (black) and BET (grey) values for both age groups. Left shows Experiment 1 (sulfurous flavours) and right shows Experiment 3 (mixture)

4.5 Discussion

This study firstly aimed to examine the hedonic impact of potential off-flavour compounds, when added in increasing concentrations to a dairy beverage. Secondly, we aimed to identify the concentration at which consumer rejection occurred and lastly, whether human age was a factor influencing consumer acceptance of these flavours. Flavour compounds were studied alone, and in combination, to ensure any flavour-interactions were captured.

Sulfurous flavours can impart essential flavours and background notes to some foods and beverages, such as vegetables and coffee (Buttery et al., 1976, Al-Attabi et al., 2008, Mishra et al., 2017, Kim et al., 2018). However, in other products, or at certain concentrations, they may become undesirable (Zabbia et al., 2012).

In the current study, for both age groups, as the concentration of sulfurous flavours increased in the dairy beverage, a greater percentage of consumers preferred the blank samples (Figure 4-1). This means that sulfurous flavours had a negative hedonic impact on consumer acceptance of the dairy beverage.

However, we observed that the impact was greatly dependent on age-group, as the rejection threshold occurred at much lower concentrations for younger adults, compared with older adults. Younger adults rejected samples at almost the lowest concentration of sulfurous flavour (Figure 4-1). This demonstrates that younger consumer acceptance of the dairy

beverage was affected strongly by the presence of sulfurous flavours, even at low concentrations. In comparison, older adults rejected samples at higher concentrations, demonstrating that sulfurous flavours have a less strong negative impact on older consumer acceptance of the dairy beverage. Best estimate thresholds (BET) were also significantly different between age groups for sulfurous flavours ($p = 0.0009$) and for sulfurous flavours in combination with diacetyl (mixture) ($p = 0.0269$) (Figure 4-4).

This age-related difference in consumer acceptance could be attributed to either i) sulfurous flavours are perceived more pleasantly or ii) older adults have an impaired ability to detect sulfurous flavours. There is some evidence to suggest that sulfurous flavour compounds become less unpleasant with human ageing (Wysocki and Gilbert, 1989). In addition, we hypothesise that impairments in olfactory sensitivity played a substantial role in the findings. This hypothesis is supported by a vast amount of research evidencing that olfactory ability decreases with ageing due to age-related alterations within the nose, olfactory epithelium, bulb, and higher brain structures (Doty and Kamath, 2014). In addition, many medications used to treat age-related conditions, such as hypertension, are known to alter taste and smell acuity (Schiffman and Zervakis, 2002). In the current study, the older adult group reported taking almost 8 times higher amounts of daily medication in comparison to the younger group (Table 4-1). Age-related impairments in taste and smell are known to affect older adults ability to

perceive flavour and have a negative influence on older adults dietary behaviour, nutritional intake and nutritional status (Duffy et al., 1995, Griep et al., 1995, Kremer et al., 2014, Somekawa et al., 2017). Age-related sensory impairments are often debilitating. Though, this current research is a unique example of how age-related changes may also offer benefits to the older consumer as beverages which have high or enhanced nutritional value, but undergo inevitable sensory changes, maintain greater acceptability. This current finding supports the hypothesis of Mattes (2012) who suggested that age-related sensory losses may diminish detection of undesirable flavour notes, thus promoting intake in older populations who are often presented with novel foods for therapeutic nutritional reasons. Our observations are also supported by previous research which found that older adults, particularly those with poor olfactory abilities, were more willing to accept novel foods with unpleasant odours than younger subjects (Pelchat, 2000).

In contrast, acceptance by younger consumers was reduced greatly by sulfurous flavours, even at low concentrations. Currently, many younger consumers aim to increase their protein intake for health or athletic reasons (Hartmann and Siegrist, 2016, Whitehouse and Lawlis, 2017, Sung and Choi, 2018). Dairy-protein ingredients such as dry powder concentrates, or ready to drink high-protein beverages, are a popular choice for many (Singh et al., 2019, Singh, 2020). Thermal treatment is frequently used to prolong shelf life and ensure consumer safety of

these high-protein products (Singh, 2020) but this can result in the formation of undesirable flavours (Al-Attabi et al., 2008, Zabbia et al., 2012, Cadwallader, 2016). It has previously been found that consumers are unlikely to compromise on taste for positive health outcomes (Verbeke, 2006). It is thus important that high-protein products deliver both palatable flavour and nutritious ingredients to ensure consumer satisfaction. The source of off-flavours, such as amino acids, are often essential nutrients which manufacturers cannot remove from a product. Flavour masking should therefore be prioritised, along with changes to processing conditions, to 'hide' or reduce formation of off-flavours whilst maintaining microbiological stability (Cadwallader, 2016).

Our observations demonstrate that diacetyl partially masked the undesirable effects caused by sulfurous flavours. This was observed for both age-groups as the combined effect of sulfurous flavours with diacetyl increased the point at which sulfurous flavours became objectionable (Figure 4-3). This finding demonstrates the importance of flavour interactions and may occur via a 'mixture suppression' mechanism, whereby the perceived intensity of an odorant mixture is less than that of the individual components (Cadwallader, 2016). When investigating off-flavours, future researchers should consider the combined effect of all aroma-active compounds which contribute to a flavour. When diacetyl was added to the beverage alone (Experiment 2, Figure 4-2), 75% rejection was not reached for either age-group, meaning diacetyl cannot be considered an off-flavour in the beverage at

the concentrations studied. Previous findings that diacetyl increased the acceptance of dairy products (Antinone et al., 1994, Drake et al., 2009, Shepard et al., 2013), for example in cottage cheese at concentrations of 1000 ppb (Antinone et al., 1994), are not supported by the current study. The concentration increments used in this present study were small (33 %) and therefore the objectionable concentration was not reached. The Hill equation predicted that consumer rejection would have occurred at concentrations of 592 ppb for younger adults and 1,738 ppb for older adults. These higher concentrations of diacetyl can occur in food and beverages; concentrations as high as 27,000 ppb have been reported in dairy products such as yoghurt (Clark and Winter, 2015) but the hedonic effects are likely to be product and matrix dependent.

The statistical methodology chosen to calculate rejection thresholds is important. Murray et al (2019) recently recommended that a complementary approach encompassing both RjT_{50} and BET methodologies would be beneficial when estimating the acceptability of sensory properties. We observed that all rejection thresholds calculated by the BET approach were lower than those concentrations calculated using the graphical approach (RjT_{50}) (Figure 4-5). Therefore, in agreement with Murray et al (2019), BET are a conservative approach to determine acceptability. It may sometimes be more appropriate to use the BET methodology for products where acceptance is particularly important. For example, Foods for Special Medicinal Purposes

(FSMPs), such as oral nutritional supplements, are typically prescribed to patients and not purchased out of ‘desire’ like most products. Subsequently, acceptability is particularly important for sufficient intake.

Younger adults stated ‘Off-flavour’ and ‘Unpleasant aftertaste’ as main reasons for rejecting samples containing sulfurous flavours. In contrast, very few older adults cited these as reasons for rejection (Table 4-3 and Table 4-4). Older adults may be less aware of unpleasant flavours lingering post-consumption, or perhaps due to reduced sensory acuity, older adults are less able to articulate specific sensory effects caused by off-flavours. Though older adults are more vulnerable to fatigue from multiple testing, along with difficulties writing and expressing themselves (Methven et al., 2016a), nevertheless, older adults were more inclined to state positive reasons for preferring the blank samples so perhaps reluctance to cause offence to the researchers contributed to the differences between age-groups. To mitigate this, future research could ask “What did you dislike about the rejected sample?”, however this approach could make the consumers aware of undesirable sensory properties which otherwise they may not have noted. It is also worth discussing that 10 % more older adults stated they ‘Enjoyed banana as a flavour’ compared to younger adults (Table 4-1) which may have driven a more positive sensory experience overall and increased their inclination to state positive reasons for choosing the control samples over the off-flavour samples.

Multimodal effects of sulfurous flavours were also observed: a number of participants reported perceived textural reasons for preferring the blank samples, such as ‘Less creamy’ (Table 4-3) and ‘Watery mouthfeel’ (Table 4-4). A number of participants also reported sweetness as reasons for preferring the blank samples, such as ‘Less sweet’ (Table 4-3). These reasons were stated despite the blank and ‘spiked’ samples having identical matrices and levels of macronutrients. It is known that volatile flavour compounds can influence the perception of both texture and sweetness (Delwiche, 2004). For example, Saint-Eve et al (2004) found that yoghurts containing fatty flavours were perceived as thicker, whereas a mixture of flavours were perceived as less thick. Aroma-taste interactions have also been found, for example, Saint-Eve et al (2004) found that yoghurts with the same sucrose content were perceived to be sweeter when flavoured with strawberry flavours. To the authors knowledge the reported effects of sulfurous flavours on tastant perception (sweetness) and texture perception have not been reported previously.

4.5.1 Strengths and limitations of research

To confirm our hypothesis that the higher rejection thresholds in the older adult group were driven by lower olfactory abilities it would have been advantageous to measure olfactory sensitivity alongside the rejection thresholds, for example through detection threshold testing. A strength of the study was the high participant compliance, which was 100 % for both age groups. To complete the additional olfactory

sensitivity investigation, a greater number of samples would have been required. This would have increased the risk of inducing fatigue in participants (of which, older adults are more vulnerable) but a greater number of study visits may have reduced participant compliance.

4.6 Conclusions

To support worldwide healthy ageing, the development of nutritious and acceptable high-protein foods and beverages is crucial. This study found that protein-originating sulfurous flavours negatively influenced consumer acceptance of a banana flavoured dairy beverage. The extent to which sulfurous flavours had a negative effect differed by age group. Compared with younger adults, sulfurous flavours were more acceptable for older adults, which was likely to have been driven by age-related impairments in sensory perception. This age-related effect may be a benefit to the older consumer, by increasing their willingness to accept protein fortified beverages, thus promoting nutritional intake. As a further finding, irrespective of age, the addition of diacetyl increased the concentration at which rejection occurred, subsequently providing masking benefits. This partial masking capability of diacetyl may be a solution to improve the palatability of beverages, a finding particularly relevant for younger consumers who were relatively less accepting of sulfurous off-flavours. Due to our findings, we recommend testing the acceptability of sensory properties with the consumer age-group of interest. In addition, compared with the graphical approach (RjT), we propose that BET is a more appropriate method to estimate consumer acceptance of nutritional food and beverage products where acceptability is essential for sufficient intake.

Chapter 5

5 The relation between stimulated salivary flow and the temporal consumption experience of a liquid oral nutritional supplement

Lester S[1], Hurst K[1], Cornacchia L[3], Kleijn M[3], Ayed C[1], Dinu V[1], Taylor MA[2], Fisk I[1]

[1]Division of Food Nutrition and Dietetics, School of Biosciences, University of Nottingham

[2]National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, Division of Physiology, Pharmacology and Neuroscience, School of Life Sciences, University of Nottingham

[3]Danone Nutricia Research, Uppsalalaan 12, 3584 CT Utrecht, the Netherlands

Highlights

- A full portion of ONS was evaluated by three groups differing in saliva flow rates.
- A sensory profiling method captured perceptual differences over repeated sips.
- Mouth drying built up most significantly for the low saliva flow group.
- Intensity of aftertaste and aroma release was highest in the low saliva flow group.
- Sensorial intensity of ONS may be associated with greater feelings of satiation.

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5.1 Abstract

Use of oral nutritional supplement (ONS) in undernourished patients has proven clinical benefits, but this can be hampered by low adherence due to poor experience of palatability. Many patients, particularly older patients, experience hyposalivation which can cause taste changes and reduce the enjoyment of foods. The aim of this study was to investigate differences in the temporal consumption experience (comprising sensory perception, in-mouth aroma release and subjective appetite) of a clinically relevant portion of ONS, for groups differing in stimulated saliva flow rates (SFR). The SFR (mL/min) of thirty healthy individuals was measured on three occasions. This data was used to categorise individuals into three groups using quartile analysis: low flow (LF) (0.3-0.6mL/min, n=5), medium flow (MF) (0.7-1.2mL/min, n=16) and high flow (HF) (1.3-1.8mL/min, n=9). Over the consumption of eight 15mL sips of ONS, individuals rated their sensory perception and subjective appetite perception using line scales. Additionally, in-mouth aroma release was measured for each sip, using atmospheric pressure chemical ionisation (APCI). Compared with the MF and HF group, the LF group reported a significantly greater increase of mouth drying over increased sips ($p=0.02$). The LF group also experienced significantly higher aftertaste perception ($p<0.001$), and more intense in-mouth aroma release ($p=0.015$), compared with the HF group. These findings occurred concurrently with relatively lower hunger sensations in the LF and MF group. Many patients who are prescribed ONS likely

experience reduced salivary flow rates. The unique sensory experiences of these individuals should be considered in order to optimise palatability and nutritional intake.

Key words: Saliva flow rate, hyposalivation, oral nutritional supplements (ONS), temporal perception, healthy ageing.

5.2 Introduction

During food oral processing, the in-mouth interaction between saliva and food is essential for perceiving sensory properties (Fischer et al., 1994, Horne et al., 2002, Condelli et al., 2006, Dinnella et al., 2009, Salles et al., 2010, Chen and Engelen, 2012, Mosca and Chen, 2017). Saliva is known to have a large influence on texture perception, for example, salivary enzymes facilitate the in-mouth digestion of macromolecules leading to a reduction in perceived thickness (Mosca and Chen, 2017). Some mouthfeel sensations, such as astringency, are suggested to be related to the type and quantity of salivary proteins such as proline-rich proteins (PRPs) (Dinnella et al., 2009, Dinnella et al., 2010, Horne et al., 2002). The viscosity of saliva (a measure of a fluids resistance to flow) may also be crucial in driving food perception, for example, a low viscosity saliva is known to be more effective in clearance of food residue from the oral cavity (Negoro et al., 2000, Chen and Engelen, 2012).

Flavour perception is also largely dependent on saliva secretions. Hydrophilic tastants from foods diffuse through the salivary aqueous medium to reach taste buds on the tongue (Salles et al., 2010) and in-mouth aroma release is largely dependent on the volume and constituents within the saliva (Odake et al., 1998, Van Ruth, 2000, Salles et al., 2010, Pagès-Hélary et al., 2014, Ployon et al., 2017, Yang et al., 2020). Volatile aroma compounds differ in chemical properties such as hydrophobicity, so the chemical nature of the volatile aroma

compound, and the subsequent interactions with aqueous saliva and the salivary constituents, can determine the extent of their release into the gaseous olfactory-space (Odake et al., 1998, Ployon et al., 2017, Yang et al., 2020). These factors ultimately determine the type and extent of volatile aroma compounds that are perceived retro-nasally.

Individual saliva flow rates (SFR) and salivary composition varies across the course of a day (Dawes, 1975, Humphrey and Williamson, 2001), under exposure to stress (Jemmott et al., 1983), or in response to different food stimuli (Engelen et al., 2003). There is also large variation between individuals, in quantity, properties, and concentrations of constituents within saliva secretions. Factors causing variations in healthy individuals include dietary intake (Dawes, 1970) smoking status (Edgar et al., 2004, Rad et al., 2010) and gender (Percival et al., 1994, Prodan et al., 2015).

Human ageing also is associated with chronic reductions in SFR and/or altered salivary compositions (Narhi et al., 1999, Edgar et al., 2004, Bossola et al., 2013, Villa et al., 2014, Iwasaki et al., 2016) and the cause of these changes are multifaceted. Older adults are more susceptible to dehydration, as thirst signalling mechanisms are impeded in older age (Schols et al., 2009), and dehydration has been proposed as one of the most important factors contributing to salivary hypofunction (Dawes, 1970, Narhi et al., 1999, Edgar et al., 2004). SFR and salivary compositions are also known to be strongly influenced by certain age-related diseases such as Parkinson’s disease, cancer,

stroke and diabetes (Edgar et al., 2004), in addition to the medications and treatments used to treat them (Edgar et al., 2004, Villa et al., 2014). For example, patients with cancer frequently experience long-term reductions in SFR or compositional changes due to radiotherapy treatment, particularly when administered in the head and neck region (Henson et al., 1999, Villa et al., 2014, Rogus-Pulia et al., 2016, Laheij et al., 2015).

It is not surprising therefore that many patients, particularly those of an older age, receive a clinical diagnosis of hyposalivation (Narhi et al., 1999, Villa et al., 2014). Hyposalivation is defined as a measurable decrease in the amount of saliva in the mouth, and objectively defined as a stimulated flow rate of ≤0.5 mL/min (Narhi et al., 1999, Nederfors, 2000, Edgar et al., 2004, Villa et al., 2014, Iwasaki et al., 2016).

Patients with hyposalivation frequently complain of taste changes (Villa et al., 2014) so it could be hypothesised that salivary variations may be a contributing cause. Furthermore, sensory perception occurs concurrently alongside physiological phenomena that regulate appetite establishing a sensorial feedback mechanism that notifies the consumer about the nutritional and satiating properties of foods (Gibson and Brunstrom, 2007, Ramaekers et al., 2014). Consequently, it has been proposed that hyposalivation may be a risk factor for reduced nutritional intake (Muñoz-González et al., 2018a) and could potentially contribute to undernutrition and involuntary weight loss (Sullivan et al., 1993, Iwasaki et al., 2016).

For individuals who are undernourished or at risk of a nutritional deficiency, oral nutritional supplements (ONS) are often prescribed to supplement or replace the oral nutritional intake. ONS are usually liquids, hence less satiating than nutritionally equivalent solids (Zijlstra et al., 2008) and easy to consume by those with poor dentition.

Although the clinical effectiveness of ONS has been proven (Stratton and Elia, 2007), patients must consume the prescribed volume in order to gain the nutritional benefits. However, adherence to the full prescription is known to be challenging and patients frequently terminate consumption before the prescribed volume is consumed (Gosney, 2003). Poor palatability has been proposed as a key factor limiting sufficient intake of ONS (Kennedy et al., 2010, den Boer et al., 2019). Food sensory properties known to be important to the palatability and intake of dairy-based ONS are thickness (den Boer et al., 2019), sweetness (Kennedy et al., 2010, Methven et al., 2010b, den Boer et al., 2019), off- tastes (Methven et al., 2010b), aftertaste (Regan et al., 2019) and mouth-feel effects, such as mouth drying and mouthcoating (Methven et al., 2010b, Withers et al., 2013, Thomas et al., 2016). The undesirable mouth drying phenomenon is known to build up over repeated sips of a consumed portion (Methven et al., 2010b).

For ONS to have the greatest clinical success, they must be palatable to the consumer to facilitate adequate intake. Considering that patients frequently experience hyposalivation, it is important to understand how

variations in saliva flow rate and composition influence the sensory perception of ONS.

We hypothesise that SFR and saliva composition may be associated with the consumption experience of ONS. Subsequently, findings may support our understanding of factors which potentially lead to early termination of ONS intake. As food experiences are known to change over repeated intakes, our overarching aim was to investigate differences in the temporal consumption experience (comprising sensory perception, in-mouth aroma release and subjective appetite) of a clinically relevant portion of ONS, for groups differing in SFR, in which repeated measurements were made between sips. Specific salivary parameters (such as saliva protein content and saliva viscosity) were also characterised for each group, as it was hypothesised that these may be crucial in our understanding of potential group differences. Unravelling the link between saliva composition and consumption experience is a fundamental step towards the design of nutritional formulations adapted to the specific consumer need.

5.3 Materials and methods

This study was approved by Faculty of Medicine and Health Sciences Research Ethics Committee at the University of Nottingham (Reference No. 207-1902) (Appendix L).

5.3.1 Participants

The study was conducted in the Food Flavour Laboratory on Sutton Bonington Campus at The University of Nottingham. Forty healthy adults were recruited to take part in the study via an email invitation. We chose to recruit healthy individuals with differing saliva rates, rather than patients, to limit the additional influences of medication and disease on sensory perception and appetite. Inclusion criteria were: aged between 18 and 40 years, self-reported health, healthy BMI within the range 18.5-24.9 kg/m^2, non-smoking and complete dentition. Exclusion criteria included food allergies or intolerances, physical or mental health problems, poor dental health, medication use (excluding oral contraceptives), pregnancy and lactation, and known sensory impairments in taste and smell.

5.3.1.1 Screening

All potential participants were electronically provided with information about the study, and then invited to a screening visit in order to assess their eligibility. On this screening visit, the study was explained to the participants and they were invited to complete a questionnaire

containing health, lifestyle and demographic questions. Height was measured to the nearest 0.1 cm using a stadiometer (Seca®, Germany). Body weight was measured using an electronic scale to the nearest 0.1 kg (Seca®, Germany) whilst participants were wearing light clothing with no shoes and an empty bladder. BMI was calculated from their height and weight as kg/m^2. Ten participants did not fit the criteria (their calculated BMI outside healthy range) and were therefore not invited to take part. Thirty participants met the inclusion criteria, so they were invited to take part in the study and informed, written consent was obtained.

5.3.2 Overview of study design

Participants attended three study sessions in total, which were one week apart, and occurred at the same time of day for each individual (between 9am and 6pm). Participants were required to not eat or drink for 2 hours prior to each session and not exercise strenuously or drink alcohol for 24 hours prior to each session.

At Session 1 (15 minutes), which immediately followed the screening session, participants were required to provide a stimulated saliva sample.

At Session 2 (1 hour), each participant provided their second stimulated saliva sample and following this underwent a 30-minute training session on sensory attributes and to standardise drinking behaviour.

At Session 3 (1 hour), each participant provided their third stimulated saliva sample (15 minutes) and following a short break, completed the ONS consumption study (section 5.3.3).

5.3.2.1 Protocol for saliva collection and determination of flow rate

Stimulated saliva was collected by asking individuals to chew continuously on a clean square of Parafilm® for 15 minutes. Every time the individual felt they needed to swallow they were asked to expectorate their saliva into a sterile polypropylene graduated collection tube. Once collected, the weight of saliva (g) was determined by weighing the collection tube before and after saliva collection. In line with previous research (Norton et al., 2020b) saliva volume (mL) was determined with the assumption that 1g of saliva is equal to 1 mL, and the stimulated salivary flow rate (SFR) calculated (mL/min).

The saliva was immediately separated into individual 1mL aliquots for further analysis. To prevent degradation during viscosity analysis (2.2.2) a protease inhibitor (2 uL protease inhibitor cocktail, Sigma Aldrich®) was added to the aliquots. The aliquots for the protein measurements (section 5.3.2.3) were immediately frozen at -80°C.

5.3.2.2 Rheological analyses

Salivary viscosity was measured immediately after collection.

A Modular Compact Cone-Plate Rheometer MCR 302 (Anton Paar GmbH, Germany) was used. The cone used was a CP50-2/TG with

diameter 49.957 mm, angle 2.006°, truncation 208 µm. Analysis was carried out at 37 °C. 5 points per decade were used for 3 decades with shear rate increasing logarithmically from 1 to 1000 s^{-1}. A total of 15 points were made, 1 point per minute. Rheoplus analysis software (Anton Paar GmbH, Germany) was used. The sample volume was 1.0 mL.

The viscosity at a sheer rate of 50^{s-1} was used in the data analysis as this closely represents the forces within the oral cavity during the movement of liquids (Chen and Engelen, 2012).

5.3.2.3 Protein concentration and α-amylase activity

Saliva samples were kept frozen at -80°C for a period no longer than 24 hours. Once removed from the freezer, the saliva samples were defrosted at room temperature for a period no longer than 5 minutes and then underwent a gentle centrifugation (1500 g for 15 min) to remove large cellular debris. Total protein content (TPC, mg/mL) was determined by using a colorimetric assay based on bicinchoninic acid (Pierce™ BCA Protein Assay Kit). Protein Secretion Rate (PSR, mg/min) was determined by multiplying the protein concentration by the saliva flow rate. The salivary activity of α-amylase (AA, U/mL) was determined using a colorimetric assay based on 2-chloro-p-nitrophenol linked with maltotriose (Salimetrics® Salivary Alpha-Amylase Assay Kit).

5.3.3 Study session protocol and procedures

Prior to the participants entering the lab, one full portion of a banana flavoured ONS (125 mL, Fortisip Compact Energy, Nutricia B.V., Zoetermeer, The Netherlands) was separated into 8 individual sips (15.6 mL per sip) by the experimenter. The total portion of ONS contained 12 g protein, 11.6 g fat and 37.1 g carbohydrate, comprising 300 kcal. The temperature of the sips was controlled using a water bath set to 20°C.

On arrival at the lab, a plastic tube was inserted into one of the participant's nostrils to sample their expired air. This tube was connected to the atmospheric pressure chemical ionisation (APCI) apparatus and allowed measurement of continuous in-mouth aroma release whilst the sips of ONS were consumed. Participants consumed the sips from a standard unbranded ONS bottle using a straw. Instructions were provided on an iPad (Apple, UK) using Compusense® and each total consumption event (8 sips) lasted approximately 15 minutes. Prior to the study session, in an effort to standardise drinking behaviour, participants were instructed and trained to consume each sip in a standardised way: consume as much of each pre-measured sip (15.6 mL) from the bottle as possible, control oral transit time to 1 second by counting and swallow in one mouthful (not multiple swallows).

Participants rated their subjective perception of sensory attributes and appetite during the consumption of the 8 sips (Figure 5-1). This data was collected electronically using Compusense®. Participants first gave a baseline appetite rating at sip 0 and following this, sensory and appetite ratings were made alternately between sips. Sensory ratings were thus made after sips 1, 3, 5 and 7 whereas hedonic perception and subjective appetite ratings were made after sips 2, 4, 6 and 8. A compulsory 1-minute break was given between each rating.

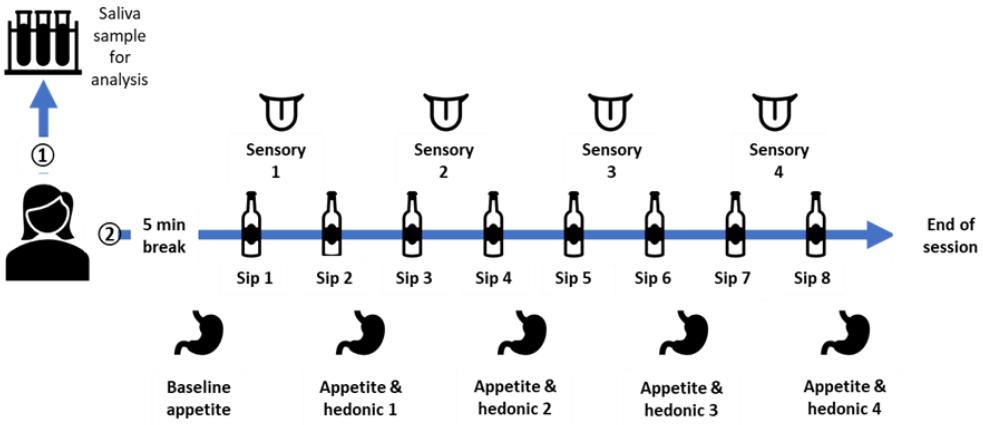


Figure 5-1: Outline of the study session whereby participants provided stimulated saliva sample (1) and proceeded to consume 8 sips of ONS whilst alternately rating sensory and appetite variables (2).

5.3.3.1 Sensory perception

Perceived intensity of sensory attributes was measured using an unstructured line scale with appropriate anchors. The methodology used to collect sensory data was based on the sequential profiling method developed by Methven et al (2010b), which permits sensory profiling of a number of attributes over the repeated consumption of ONS aliquots. The original method involves rating immediately after each aliquot, followed by another rating at 30 s and 60 s. In the current

study, instead of making 3 measurements per aliquot, the method was simplified to a single measurement taken immediately after each sip. This was to reduce participant fatigue from repeated tasting and scoring.

Four important sensory attributes to describe ONS were chosen from the literature (Methven et al., 2010b, Regan et al., 2019, Thomas et al., 2016). These attributes were Sweetness, Mouth drying, Mouthcoating and Aftertaste. In order to measure intensity of flavour, as the ONS was banana flavoured, Banana Flavour was also chosen to be an attribute. The description of these attributes is given in Table 5-1. The order of presentation of the first four attributes were randomised between individuals and between sips, but the attribute aftertaste was anchored to be asked last.

5.3.3.1.1 Training on sensory attributes

Prior to taking part, participants underwent a short training session to ensure they understood the meaning of each sensory attribute. Participants were provided with written definitions of each attribute and asked to read through them. After confirming they understood the meaning, participants were provided with physical references for each attribute and asked to taste them (Table 5-1). For aftertaste, no reference was provided but participants were asked to consider any lingering flavour in the mouth, considering both the attributes Sweetness and Banana flavour. Participants were also shown an example of the line scales.

Table 5-1: Sensory attributes including the description and physical references used to train participants to recognise sensations.

Attribute	Description	Reference
Sweetness	The intensity of sweet flavour	Semi-skimmed milk with 5% sucrose
Banana flavour	The intensity of banana flavour	Semi-skimmed milk with banana flavourings
Mouth drying	The intensity of a drying sensation in the mouth	Milk containing whey protein after being heated to 70 °C for 20 minutes
Mouthcoating	The intensity of milk clinging to the surface of the mouth	Full fat cream
Aftertaste	The intensity of flavour lingering in the mouth	No physical reference but participants asked to consider both sweetness and banana flavour

5.3.3.2 Hedonic perception and subjective appetite sensations

In-line with the sensory scales (5.3.3.1), hedonic perception and subjective appetite sensations were made on unstructured line scale with appropriate anchors. After providing their initial baseline appetite rating (sip 0 before any ONS consumption) participants were asked to rate their hedonic perception of ONS and subjective appetite (after sips 2, 4, 6 and 8) using a series of questions (Table 5-2). A baseline was not collected for the hedonic questions 1 or 6. Questions were not randomised and always anchored in the specific order shown.

Table 5-2: Appetite and hedonic questions asked at sips 3, 5, 7 and 9. Baseline scores (sip 0) were also collected for questions 2-5.

Hedonic and appetite questions
1) How pleasant would you rate the beverage?
2) How hungry do you feel right now?
3) How full do you feel right now?
4) How much do you think you could eat right now?
5) How strong is your desire to eat right now?
6) How strong is your desire to drink more of the beverage?

5.3.3.3 In-mouth aroma release

For the period which ONS was being consumed, the expired air of each participant was sampled at a flow rate of 45 mL/min, through a plastic tube inserted into the exterior opening of the nostril. A MS-Nose™ (Micromass, Manchester, UK) interface and a Quattro Ultima mass spectrometer (Waters Corporation, Milford, MA) was used to monitor the in-mouth release of four aroma molecules in real-time. The molecules were isoamyl acetate (m/z 130), isoamyl propionate (m/z 144), isoamyl isovalerate (m/z 172), ethyl butyrate (m/z 116). These flavour molecules have previously been identified as significant aroma-active contributors to the flavour of the banana flavour ONS.

5.3.4 Statistical analysis

All statistical analysis was conducted using GraphPad Prism version 8.1.2 (GraphPad Software, San Diego, CA, USA).

For each individual, the mean SFR of the three biological replicates was calculated. Quartile analysis of the mean SFR values was used in order to define three groups (Condelli et al., 2006, Dinnella et al., 2010, Shen et al., 2017). Individuals with a mean SFR less than the first quartile (Q1) determined the low salivary flow rate group. Individuals with an SFR the same as or greater than Q1, but less than the third quartile (Q3), defined the medium salivary flow group. Individuals with SFR the same as or greater than Q3 defined the high salivary flow rate group.

For each salivary variable (viscosity, TPC, PSR and AA), the individual mean of the three biological replicates was calculated in addition to group means and standard error. Linear regression assessed relationships between salivary variables and Pearson correlation coefficient was used to determine whether correlations were statistically significant ($p \leq 0.05$). Statistically significant differences between group salivary variables were assessed with one-way ANOVA ($p \leq 0.05$).

For each sensory, hedonic and appetite variable, the group mean and standard error values were calculated. A one-way ANOVA was used to assess whether statistically significant differences existed between groups for baseline appetite scores. Subjective appetite scores (Hunger, Fullness, Desire to eat, Prospective Consumption) were processed to generate change from baseline (CFB) scores by subtracting baseline scores from each score measured at Sips 2, 4, 6 and 8. Differences between groups, in sensory ratings and appetite CFB ratings, were analysed by Mixed Models ANOVA, with Group as a

between-subjects factor and Sip as a within-subjects factor. Sphericity was not assumed, therefore Greenhouse-Geisser corrections were used to adjust df and p-values of within-subject factors.

For in-mouth aroma release data, the Total Ion Count (TIC) was used for statistical analysis. Three release parameters were extracted from each ‘swallow breath curve’ generated for each individual sip: maximum intensity (Imax), Time to reach maximum intensity (Tmax) and Area under the curve (AUC). Group mean and standard error values were calculated. Release parameters were analysed by two-way ANOVA with Sip as a within-subjects factor and Group as a between-subjects factor.

Where any statistically significant effects were found ($p \leq 0.05$), post hoc pairwise comparisons were conducted (Tukey’s HSD test).

5.4 Results

30 participants completed the study, 9 males and 21 females, between the ages of 20 and 45 years. Mean characteristics of participants can be found in Table 5-3.

5.4.1 Participant grouping by salivary flow rate

Mean stimulated salivary flow rates of all participants (n=30) ranged from 0.3 mL/min to 1.8 mL/min (mean 1.0 mL/min). Individuals were categorized into the low flow rate group (LF group) if their flow rate was < Q1 (n = 5, 0.3 - 0.6 mL/min), medium flow rate group (MF group) if their flow rate ≥ Q1 or < Q3 (n = 16, 0.7 mL/min -1.2 mL/min), and high flow rate group (HF group) if their flow rate was ≥ Q3 (n = 9, 1.3 - 1.8 mL/min) (Figure 5-2).

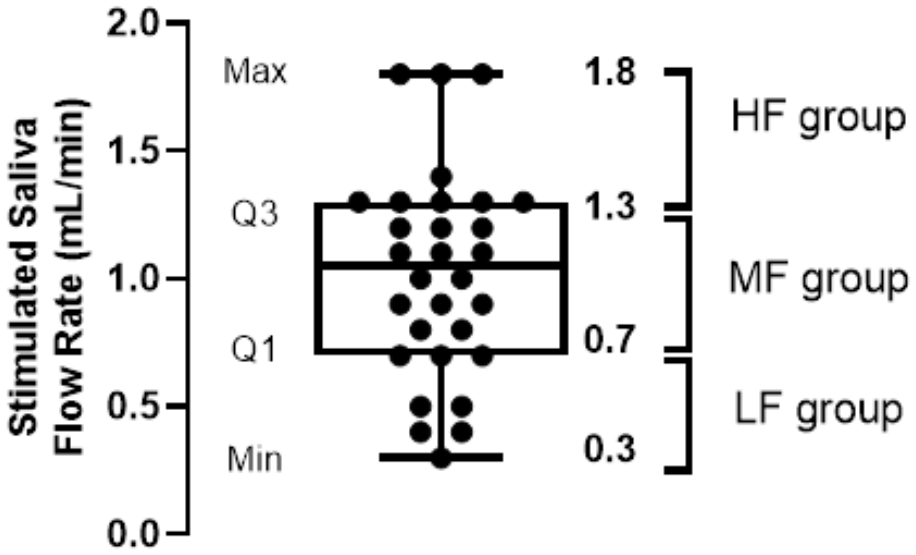


Figure 5-2: Quartiles of mean stimulated saliva flow rates for each participant. The minimum value (Min), Quartile 1 (Q1), Quartile 3 (Q3) and the maximum value (Max) are indicated.

Table 5-3: Demographic information for each group classified by saliva flow rate.

Group	n	Mean SFR (mL/min, ± SD)	Mean age (years, ± SD)	Mean BMI (kg/m^2, ± SD)	Male: Female
LF group	5	0.4 (0.1)	27 (3)	22.4 (2.7)	1:4
MF group	16	1.0 (0.2)	29 (7)	22.5 (2.1)	7:9
HF group	9	1.5 (0.2)	27 (4)	23.2 (1.9)	1:8
Average (mean)	30	1.0 (0.4)	28 (6)	22.7 (2.0)	9:21

5.4.2 Characterisation of salivary constituents

Table 5-4 reports correlations between mean salivary variables for pooled data. A significant negative correlation was observed between SFR and viscosity ($p = 0.04$), indicating that low-flow rate saliva is more viscous. A significant positive correlation was observed between viscosity and TPC ($p = 0.02$), indicating that a low viscosity saliva contains a higher concentration of proteins. As expected, a significant strong positive correlation was observed between SRF and PSR ($p < 0.01$), indicating that individuals with higher SFR secrete more proteins over time. AA was significantly positively correlated to TPC and PSR ($p < 0.01$).

Table 5-4: Correlations between salivary variables: Saliva Flow Rate (SFR), Saliva Viscosity, Total Protein Content (TPC), Protein Secretion Rate (PSR) and α-amylase activity (AA). Significant relationships (p ≤0.05) are highlighted in bold text.

	SFR	Viscosity	TPC	PSR	AA
SFR					
Viscosity	**r = -0.376** **p = 0.04**				
TPC	r = -0.235 p = 0.21	**r = 0.441** **p = 0.02**			
PSR	**r = 0.734** **p < 0.01**	r = -0.109 p = 0.57	**r = 0.431** **p = 0.02**		
AA	r = 0.168 p = 0.37	r = -0.026 p = 0.89	**r = 0.529** **p < 0.01**	**r = 0.511** **p < 0.01**	

When analysed by ANOVA, SFR was found to be significantly different between groups ($p < 0.001$) and pairwise comparisons revealed that the LF group had a SFR significantly lower than the MF ($p < 0.001$) and HF group ($p <0.001$), the MF group also had a SFR significantly lower than the HF group ($p < 0.001$).

From observing Table 5-4, it can be observed that the LF group had greater saliva viscosity and TPC, but lower PSR and AA, when compared with the MF and HF saliva groups. When analysed by ANOVA, PSR (mg/mL) was significantly different between groups and pairwise comparisons revealed that PSR was significantly lower in the LF group, compared with the MF ($p = 0.03$) and HF ($p < 0.001$) groups.

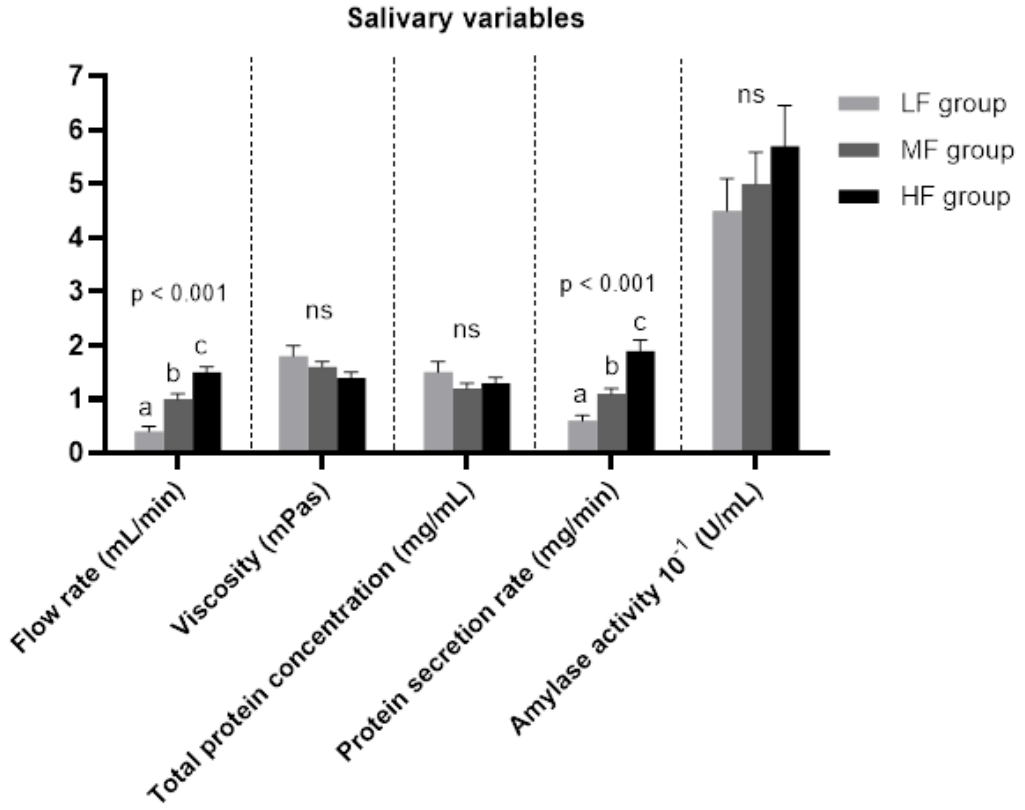


***Figure 5-3:** Mean values (± standard error) for each salivary variable (Flow rate (mL/min), Viscosity (mPas), total protein concentration (mg/mL), protein secretion rate (mg/min) and amylase activity (U/mL) for the Low flow (LF), Medium flow (MF) and High flow (HF) groups. Significant group differences were analysed by one-way ANOVA ($p \leq 0.05$). Means with statistical group difference are indicated by different letters, ns = not significant (Tukey post-hoc test).*

5.4.3 Temporal experience of ONS

5.4.3.1 Sensory perception

Immediately following consumption of sips 1, 3, 5 and 7 of the ONS, participants rated the intensity of five sensory attributes. Results are reported in Figure 5-4.

5.4.3.1.1 Sweetness

Figure 5-4 shows that all groups reported similar sweetness intensity over sips. No significant interaction effects were found between group and sip [F = (6,81) = 0.359, p=0.902]. No significant main effects of group [F = (2, 27) = 0.570, p=0.572] or sip [F = (2.33, 62.92) = 1.155, p=0.327] were found for sweetness.

5.4.3.1.2 Banana flavour

Figure 5-4 shows that all groups reported similar banana flavour intensity ratings over sips, though the LF group reported a small increase in intensity at Sip 7. Statistical analysis revealed that there were also no significant interaction effects between group and sip [F = (6,81) = 1.21, p=0.310]. There were no significant main effects of group [F = (2,27) = 0.335, p=0.718] or sip [F = (1.94, 52.33) = 0.134, p=0.869].

5.4.3.1.3 Mouthcoating

There were no significant interaction effects between group and sip [F = (6, 81) = 0.174, p=0.983]. From visually inspecting Figure 5-4, it can be seen that the LF group reported higher mouthcoating intensity than the MF and HF groups over sips, though this effect was not statistically significant [F = (2,27) = 0.806, p=0.457]. No significant main effects of sip were found [F = (2.45, 66.13) = 2.17, p=0.112].

5.4.3.1.4 Mouth drying

From visually inspecting Figure 5-4, the MF and HF group show little change in perception of mouth drying intensity over increasing sip numbers. This contrasts with the LF group, where an increase in mouth drying intensity is reported from sip 1 to sip 7, by 3.4 points on the scale. There was a significant interaction between group and sip [F = (6, 81) = 2.594, p=0.024]. Pairwise comparisons of the simple effects of groups within each time point revealed that, at sip 7, the LF group reported significantly higher mouth drying intensity than the MF (p=0.027) and HF group (p=0.017).

5.4.3.1.5 Aftertaste

From visually inspecting Figure 5-4, it is apparent that the LF group reported higher aftertaste intensity, particularly for the latter sips (Sip 5 and Sip 7). There were no interaction effects between group and sip [F = (6,81) = 1.210, p=0.310]. A significant main effect of group was found [F = (2, 27) = 4.009, p=0.030] and pairwise comparisons revealed that

the LF group rated significantly higher intensity of aftertaste compared with the MF group ($p<0.001$) and the HF group ($p<0.001$). No significant main effects of sip were found [$F = (2.331, 62.93) = 2.738$, $p=0.064$].

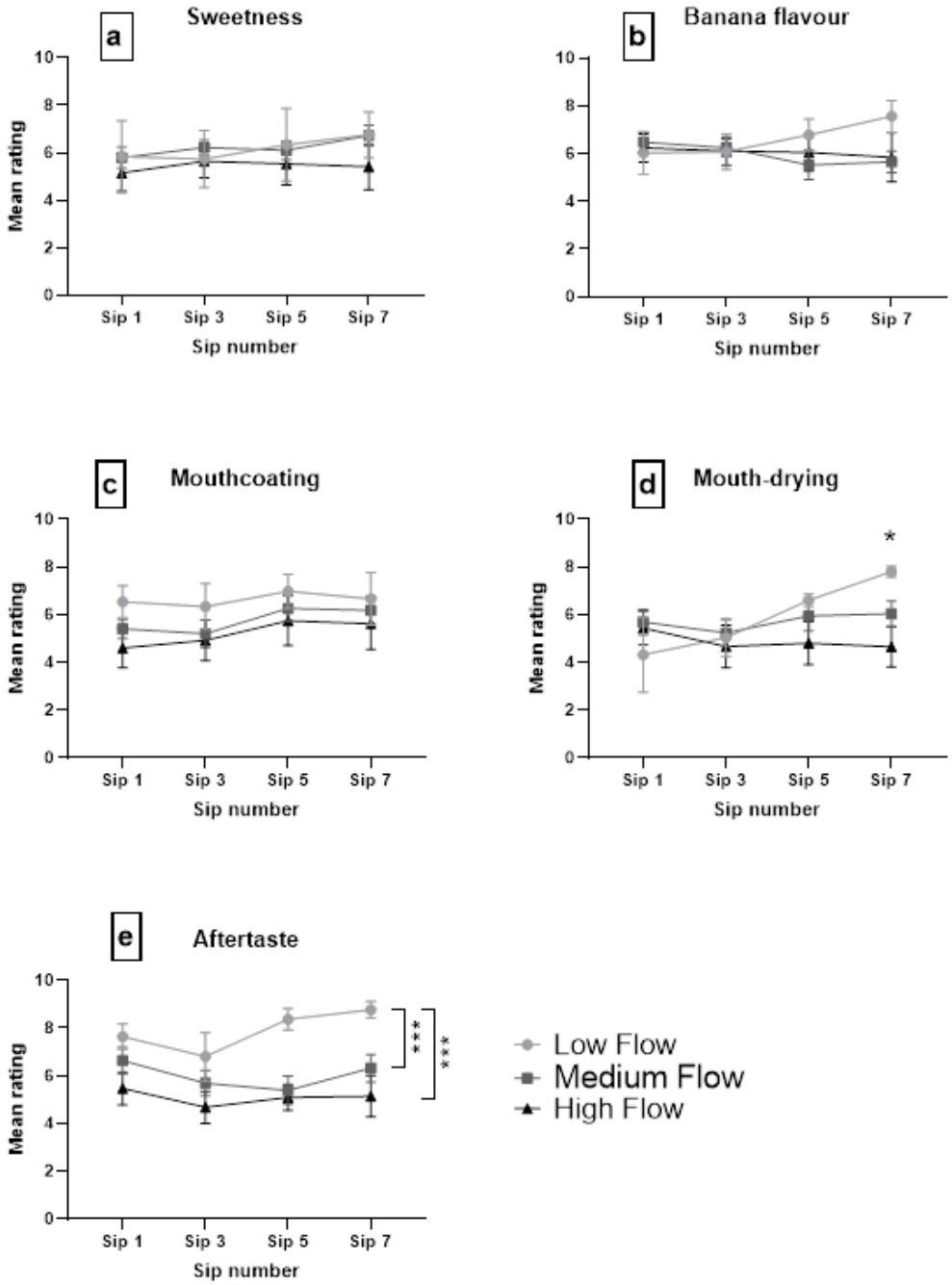


Figure 5-4: Mean group intensity (± standard error) of sensory perception, as rated by each group differing in saliva flow rate, for each separate sensory attribute (a-e) over the consumption of four sips (sip 1, 3. 5 and 7) of ONS. Main effects and simple main effects are indicated by *p<0.05, **p<0.01, ***p<0.001.

5.4.3.2 Hedonic perception and subjective appetite sensations

Immediately following consumption of sips 2, 4, 6 and 8 of the ONS, participants rated hedonic perception (comprising pleasantness and desire to drink more) along with subjective appetite sensations (hunger, fullness, desire to eat and prospective consumption).

For the appetite variables, as analysed by one-way ANOVA, no significant difference in baseline values between groups were found and change from baseline (CFB) values were calculated for use in subsequent ANOVA analyses.

5.4.3.2.1 Pleasantness of beverage

From visual inspection of Figure 5-5, the data suggests that the LF group rated pleasantness lower compared with the MF and HF group. No significant interaction effects were found [F = (6, 81) = 0.547, p=0.771] and likewise no significant main effect of group was found [F = (2, 27) = 0.8942, p=0.421]. A borderline insignificant main effect of sip

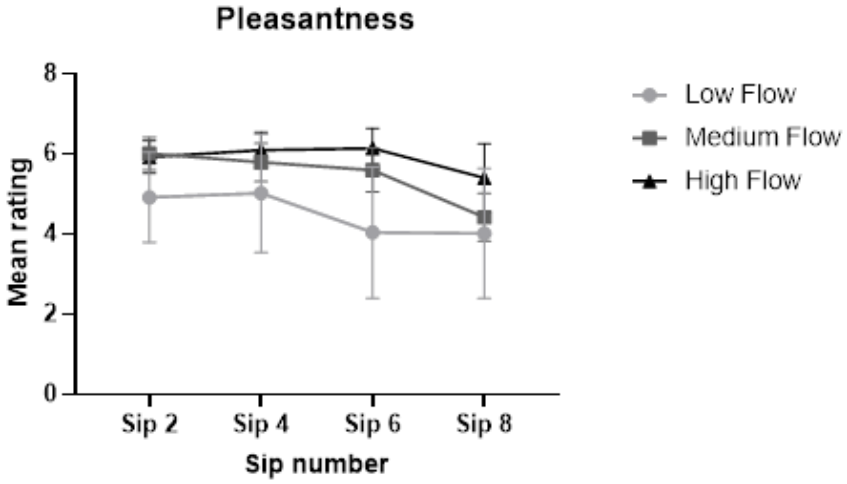


Figure 5-5: Mean group subjective ratings of pleasantness (± standard error) of sips 2, 4, 6 and 8 of the ONS, as rated by each group differing in saliva flow rate.

was found [F = (1.964, 53.03) = 3.049, p=0.057]. Pairwise comparisons revealed that the participants perceived the ONS as increasingly less pleasant during consumption (for pairwise comparisons see Appendix H).

5.4.3.2.2 Desire to drink more of beverage

Figure 5-6 shows there is little difference between groups in their desire to drink more of the ONS over increasing sips, though all groups show a decline in desire over increasing sips. No interaction effects were found between group and sip [F = (6, 81) = 1.053, p=0.398]. A significant main effect of sip was found [F = (2.231, 60.23) = 3.098, p=0.047] and pairwise comparisons revealed that participants desire to drink more of the beverage decreased significantly during consumption (for pairwise comparisons see Appendix H). No significant main effect of group was found [F = (2, 27) = 0.180, p=0.836].

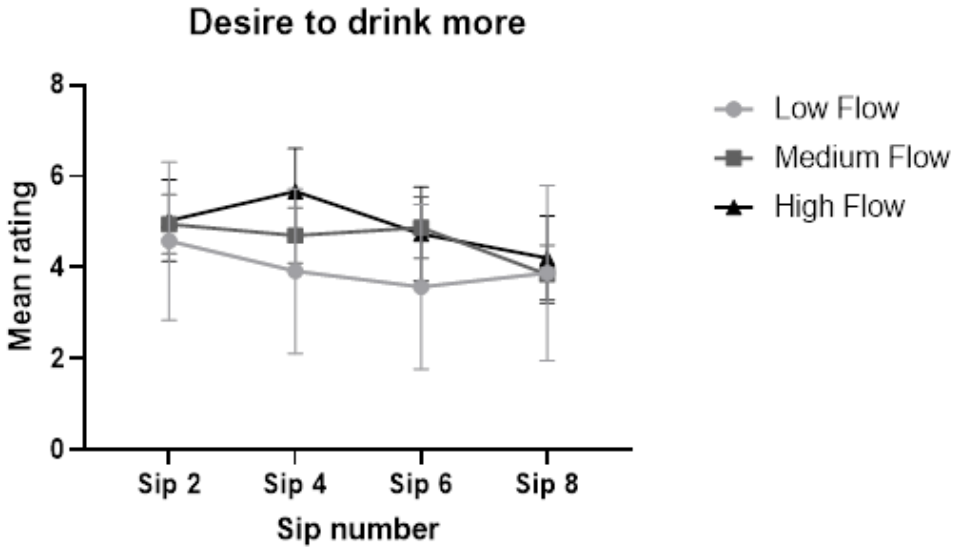


Figure 5-6: Mean group ratings of Desire to drink more of the ONS (± standard error), as rated by each group differing in saliva flow rate, over the consumption of four sips (sip 2, 4, 6 and 8) of ONS.

5.4.3.2.3 Hunger

From visually inspecting Figure 5-7, we can see that the LF and MF group decrease in hunger ratings over increased sips of ONS, whereas the HF group show little change in their hunger ratings. No significant interaction effects between sip and group were found [F = (6, 81) = 1.822, p=0.105]. A significant main effect of sip was found [F = (1.450, 39.16) = 14.17, p<0.001], and pairwise comparisons revealed a suppressive effect of ONS on hunger during consumption (for pairwise comparisons se Appendix I). There was a borderline insignificant effect of group [F = (2, 27) = 2.764, p=0.081] and pairwise comparisons revealed that the HF group has significantly higher CFB values, compared with the MF and LF groups (p<0.05), indicating relatively less decrease in hunger during ONS consumption.

5.4.3.2.4 Fullness

From visually inspecting Figure 5-7, we can see that all groups show an increase in fullness ratings over increasing sips of ONS. No interaction effects between sip and group were found [F = (6, 81) = 1.160, p=0.336]. A significant main effect of sip was found [F = (2.026, 54.71) = 6.247, p=0.004] and pairwise comparisons revealed a significant increase in subjective fullness ratings during ONS consumption (for pairwise comparisons see Appendix I). No significant main effect of group was found [F = (2, 27) = 1.029, p=0.371].

5.4.3.2.5 Desire to eat

Figure 5-7 shows that all groups decline in their reported desire to eat over increasing sips of ONS. No significant interaction effects between sip and group were found [F = (6, 81) = 0.620, p=0.714]. A significant main effect of sip was found [F = (1.506, 40.65) = 18.10, p<0.001] and pairwise comparisons revealed that participants reported desire to eat decreased significantly during consumption of the ONS (for pairwise comparisons see Appendix I). There was no significant main effect of group [F = (2, 27) = 0.5843, p=0.564].

5.4.3.2.6 Prospective consumption

Figure 5-7 shows that all groups reported a decline in the perception of the amount they could eat over increasing sips. There does not appear to be any major differences between groups, though the HF group rated marginally higher ratings at sips 4, 6 and 8, compared with the MF and LF groups. No significant interaction effects between sip and group were found [F = (6, 81) = 0.946, p=0.467]. A significant main effect of sip was found [F = (1.957, 52.84) = 15.06, p<0.001] and pairwise comparisons revealed that participants' perception of the amount which they could eat decreased significantly over increasing sips (see Appendix I for pairwise comparisons). No significant main effect of group was found [F = (2, 27) = 0.4804, p=0.624].

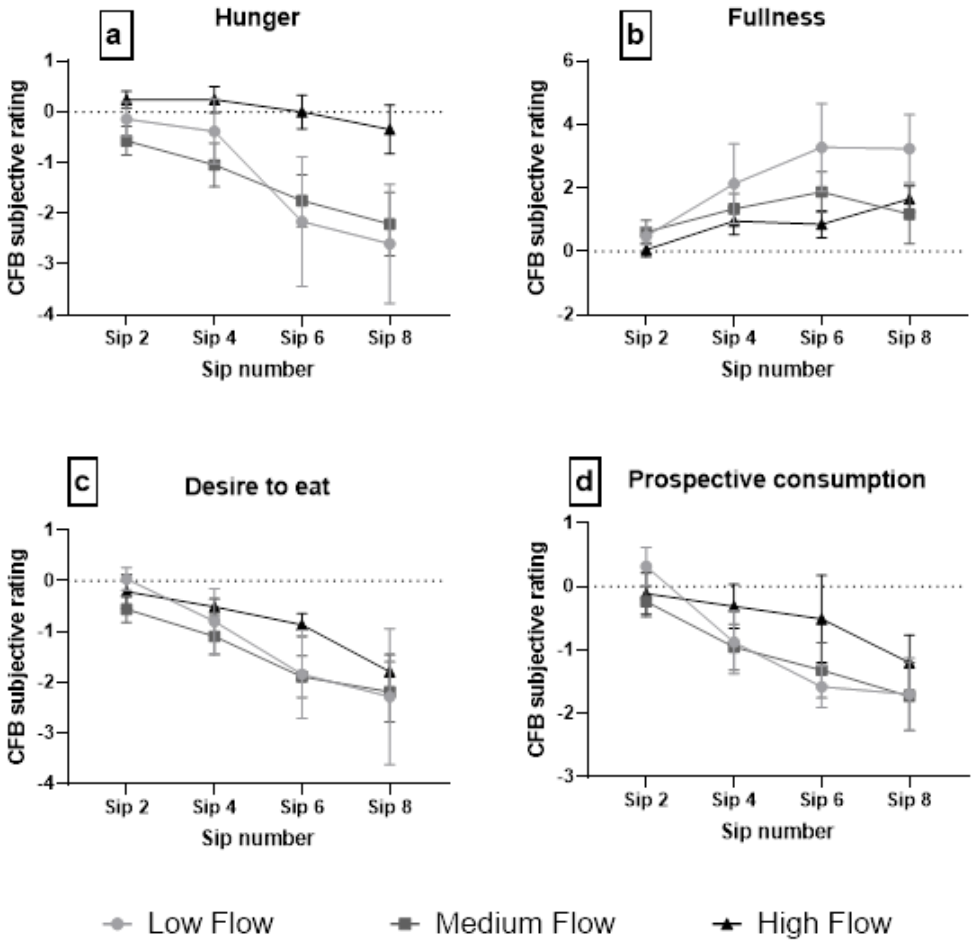


Figure 5-7: Mean group Change From Baseline (CFB, ± standard error) appetite scores, as rated by each group differing in saliva flow rate, for each appetite variable (a-e) over the consumption of four sips (sip 2, 4, 6 and 8) of ONS.

5.4.3.3 In-mouth aroma release

It can be observed in Figure 5-8 that no significant Sip*Group interaction effects were found for I max [F = (14, 216) = 0.3101, p=0.992], T max [F = (14, 216) = 0.6735, p=0.799] or AUC [F = (14, 216) = 0.1951, p=0.999]. A significant main effect of group was found for I max [F = (2, 216) = 4.025, p=0.019] and T max [F = (2, 216) = 7.445, p<0.001]. Pairwise comparisons revealed that the LF group had significantly higher intensity of aroma release (Imax) than the HF group (p=0.015). Pairwise comparisons also revealed that the LF group took significantly longer to reach maximum intensity (T max) compared with the MF (p=0.008) and HF group (p<0.001). No significant differences were found between group AUC values [F = (2, 216) = 0.6022, p=0.549]. No significant main effects of Sip were found for I max [F = (7, 216) = 0.3502, p=0.93], T max [F = (7, 216) = 0.2992, p=0.954] or AUC [F = (7, 216) = 0.3478, p=0.931].

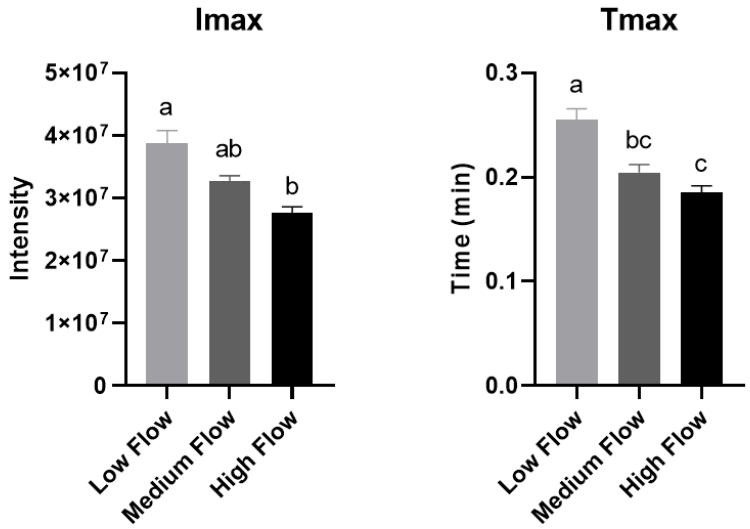


Figure 5-8: Mean Imax and Tmax values, averaged across eight sips of ONS, for each group. Means with statistical group difference are indicated by different letters, ns = not significant (Tukey post-hoc test).

5.5 Discussion

The aim of this study was to investigate the temporal consumption experience (comprising sensory perception, in-mouth aroma release and subjective appetite) of a clinically relevant portion of ONS, for groups of healthy individuals differing in their stimulated saliva flow rate (SFR). Specific salivary parameters, such as protein content and saliva viscosity, were characterised as it was hypothesised that these may be relevant in our understanding of potential group differences.

To classify individuals into groups, SFR was measured on three occasions, at the same time of day for each individual. Mean SFR of participants, averaged across the three replicates, were found to range from 0.3 – 1.8 mL/min. The three groups differed significantly in their SFR. Normal SFR are known to be within the range 1.5 – 2 mL/min (Villa et al., 2014), and a value ≤ 0.5 mL/min can be used as a cut-off value for diagnosis of hyposalivation (Narhi et al., 1999, Nederfors, 2000, Edgar et al., 2004, Villa et al., 2014, Iwasaki et al., 2016). We were therefore confident that individuals in the LF group, with a mean SFR of 0.4 mL/min (range 0.3 – 0.5 mL/min), had SFR characteristic of individuals with hyposalivation.

Significant differences between groups in perception of mouth drying over repeated sips of ONS were found. The LF group increased considerably in perception of mouth drying over increased sips of ONS, whereas the MF and HF groups remained relatively constant in their

perception of mouth drying (Figure 5-4d). This ‘build up’ of mouth drying has been found previously during ONS consumption by trained sensory panellists. Methven et al (2010b) found mouth drying to increase by around 30 points, on the 0-100 point scale, over the consumption of eight sequential 5 mL aliquots of an ONS; though, it must be noted that the contribution of participant saliva to mouth drying perception was not measured. More recently, Norton et al (2020b) found no significant differences in perception of mouth drying, after consumption of a whey protein beverage, between groups differing in SFR. Though, Norton et al (2020b) measured mouth drying after consumption of a single portion, and therefore it is likely that significant perceptual group differences were not able to ‘build up’ over repeated consumption events.

We propose that individuals with low saliva flow rates are most susceptible to a greater ‘build up’ of mouth drying over multiple intakes. For patients who experience hyposalivation, consumption of ONS could aggravate mouth dryness and may contribute to premature termination of ONS intake. Subsequently, to mitigate this build-up of undesirable mouthfeel sensations, it may be advantageous to provide patients with an acute saliva stimulating intervention alongside the administration of ONS. This may lead to improvements in adherence to ONS and a greater nutritional intake.

As far as the present authors are aware, the relationship between low SFR and greater mouth drying perception has not been found

previously. Though, there is evidence to show that low SFR is associated with greater astringency sensations (Fischer et al., 1994, Iwasaki et al., 2016, Horne et al., 2002, Condelli et al., 2006) of which, the mouth drying sensation may be caused by a similar mechanism. However, salivary volume does not seem to account by itself for differences in astringency perception (Horne et al., 2002, Dinnella et al., 2010) and may be more strongly related to variabilities in saliva composition, as described below.

Astringency sensations are thought to be caused by a disruption of the lubricating pellicle coating the oral tissues, by astringent food compounds such as tannins, leading to greater oral friction (Breslin et al., 1993, Prinz and Lucas, 2008) and ultimately a subjective dry, puckering sensation in the mouth. It has been suggested that salivary proline-rich proteins (PRPs), which contribute over 70 % of proteins in stimulated saliva (Kauffman and Keller, 1979, Dinnella et al., 2009), may play a protective role, due to their ability to strongly bind and eliminate astringent compounds from the oral cavity (Horne et al., 2002, Dinnella et al., 2009). In the current study, the LF group had a significantly lower rate of protein output (0.6 mg/min) compared with the HF group (1.90 mg/min), due to the higher stimulated flowrates. Therefore, we propose that in between sips, the HF group was able to efficiently replenish the proteins within the oral cavity, providing protection to their oral environment. In contrast, the LF group was relatively less able to replenish proteins between sips of ONS.

Therefore, the LF group had less protection of the lubricating pellicle over time and a gradual delubrication of oral tissues occurred, leading to the greater drying sensation. This is supported by Dinnella et al (2009) who observed that a group who is less able to rapidly restore their protein contents in the mouth perceived significantly higher astringency sensations. As an expansion of this finding, Dinnella et al (2010) found groups which were less able to restore protein contents, experienced higher build-up of astringency sensation over repeated samples.

Alternatively, it has been proposed that the mechanism behind dairy-originating mouth drying may not be the same as with true astringency, but could alternatively be explained by mucoadhesion of dairy proteins within the oral cavity (Withers et al., 2013, Cook et al., 2017, Norton et al., 2020b). In the context of food proteins, such as casein and whey, mucoadhesion is the binding of proteins to the mucosa surrounding the cheeks, gums and tongue, and occurs through electrostatic attraction, hydrophobic interactions and hydrogen bonding. It is known that for clearance of food from the oral cavity, a low viscosity fluid works best (Negoro et al., 2000, Chen and Engelen, 2012). In the present study, the saliva from the LF group was not only relatively lower in volume, but also more viscous. Sufficient oral clearance may have been hindered in the LF group, leaving behind a higher quantity of dairy protein on the oral mucosa between swallows, and the greater perceptual drying sensation. In support of this theory, Norton et al (2020b) recently found

higher amounts of mucoadhesion in a low saliva flow group after consumption of a whey protein beverage. In the same study, older adults were found to have significantly higher quantities of adhered protein post-whey beverage consumption. Older adults have also been found to be more sensitive to milk-protein elicited mouth drying, compared to younger adults (Withers et al., 2013).

It must be noted that if a greater extent of mucoadhesion was present in the LF group, the participants were relatively unaware, as there was no significant difference in the perceptual intensity ratings of 'mouthcoating' between groups (Figure 5-4). Though, the sensory standard used to train participants to recognise mouthcoating was full-fat cream (Table 5-1), which compared with the ONS in the current study, was relatively higher in fat but also relatively lower in protein. Fat in dairy products is also a source of mouthcoating (Aime et al., 2001, Prinz et al., 2006) and a focus group has previously defined 'Fatty mouthcoat' and 'Dry lingering mouthcoat' as separate sensations (Porubcan and Vickers, 2005). Therefore, for each product, perhaps the contribution made by each macronutrient to the oral coatings differed, and this resulted in different perceptual sensations, causing the discrepancies between findings.

A greater extent of mucoadhesion in the LF group could explain other sensory effects observed. The LF group perceived significantly higher aftertaste than the HF group (Figure 5-4). It is known that retronasal olfaction, along with gustation, can persist for a prolonged period after

food has been swallowed (Linforth and Taylor, 2000, Buffo et al., 2005). This is likely due to a ‘reservoir’ of tastants and aroma volatiles within residual ONS which is absorbed on the mucosa lining the mouth and/or pharynx mucosa by mucoadhesive forces after swallowing (Buettner et al., 2002, Cook et al., 2017). Buttner et al (2002) illustrated that between 30% - 40% of aroma compounds can be retained on the oral and pharyngeal mucosa. In fact, mucoadhesive polymers have been proposed as a method to prolong the residence time, and therefore perception, of flavour compounds on oral surfaces (Cook et al., 2017, Dinu et al., 2019a). In the same way the LF group may have been less able to clear the drying proteins from the mouth, flavour compounds are likely to have persisted to a greater extent in-between sips leading to the significantly higher aftertaste perception. These findings strengthen the crucial importance of ensuring adequate hydration is continuously accessible for patients with hyposalivation. Not only to fundamentally maintain adequate hydration status but also to enhance salivary flow and provide the opportunity to remove any lingering food flavours occurring post-consumption.

Significant differences in aroma release parameters (Tmax and Imax) were also found between groups. On average, the LF group reached Imax approximately 4 seconds later than the HF group (Figure 5-8). According to Linforth et al (1999) Tmax of volatile release is usually defined by the moment of swallowing and therefore, the longer food remained in the mouth (slower eating) the greater the value for Tmax

(Hollowood, 2002). Although we had aimed to standardise oral transit time in the present study, swallowing is a behaviour under both voluntary and reflex control (Ertekin et al., 2001) and is therefore challenging to control under experimental conditions. Aprea et al (2006) also found that participants who were asked to follow an oral processing protocol retained natural swallowing behaviour.

Interestingly, reduced saliva flow has been found to increase oral transit time and reduce swallowing efficiency (Hughes et al., 1987, Rogus-Pulia et al., 2016). It could therefore be the case that, after placing in the mouth, the HF group swallowed the 15 mL portion of ONS quickly, and it was efficiently cleared from the oral cavity. Whereas the LF group, with less saliva to facilitate swallowing, experienced a prolonged oral and/or pharyngeal stage of swallow, and subsequently delayed aroma release. In support of this hypothesis, Blissett et al (2006) found positive correlations between Tmax and swallowing time. As far as the author is aware, the relationship of SFR and Tmax has not been found in human participants previously. Though, in a mathematical model developed to understand aroma release from liquid food products, Tmax was found to decrease upon increasing SFR (Harrison and Hills, 1997). In line with the current hypothesis, the authors attributed this to the flavour being more quickly removed and therefore unavailable for release into the headspace (Harrison and Hills, 1997).

Averaged across sips, intensity of aroma release in the LF group was almost 30% more intense than the HF group (Figure 5-8). A negative

relationship between salivary flow rate and intensity of in-mouth aroma release has been found previously in model mouth systems (Odake et al., 1998, Van Ruth, 2000). Recently, Yang et al (2020) found that upon stimulation by capsaicin, saliva flow of participants increased by 75%, leading to a decrease in aroma release intensity in vivo. Yang et al (2020) suggested that enhanced saliva production is likely to dilute aroma compounds in the mouth, so aroma release from the liquid matrix was reduced and therefore volatiles were less bioavailable in the nose. This is supported by Hollowood (2002), who explained that when considering volatile release from a liquid product, dilution reduces the aqueous phase concentration, and hence lowers the breath volatile concentration.

In-mouth interactions between volatiles and salivary components are also known to occur which may influence extent of aroma release, through binding or enzymatic conversion (Ployon et al., 2017). Pagès-Hélary et al (2014) found that both mucin and α-amylase have the ability to retain aroma molecules within saliva, and these findings were recently supported by Dinu et al (2019b). A significantly greater PSR in the HF group, along with greater amylase activity, may have facilitated greater protein-aroma binding and therefore contributed to the relatively lower in-mouth aroma release in the HF group.

It could be anticipated that significantly higher aroma release intensity would drive differences in flavour perception, though no significant differences in ‘banana flavour intensity’ were found between groups.

However, from viewing Figure 5-4, we observe that the LF group reported a marginally higher perception of banana flavour at Sips 5 and 7. We hypothesise that with larger group sizes the significantly higher aroma release observed in the LF group would have translated into a significantly higher perception of banana flavour, thus this warrants further investigation.

All hedonic and subjective appetite measures had a significant (or borderline significant) effect of sip (see Appendix I) representing a decrease in enjoyment, and reduction in appetite, during ONS consumption. The decline in pleasantness ratings during ONS consumption agrees with previous findings on ONS consumption (Methven et al., 2010b, Thomas et al., 2016, Regan et al., 2019). The first sip or taste of any food is typically the most pleasant and this effect can be explained by the effect sensory-specific satiation (SSS) or satiety. SSS is defined as the decline in wanting or liking of a food as it is eaten relative to uneaten foods during a single eating episode (Nolan and Hetherington, 2009, Weijzen et al., 2009, Hetherington and Havermans, 2013).

Though not significant, averaged over the eight sips, the LF group rated pleasantness lower, compared with the HF group, by an average of 1.39 points on the scale (Figure 5-5). It has been proposed that certain attributes which are disliked in ONS, such as mouth drying and aftertaste, may contribute to the decline in liking over repeated consumption of ONS (Methven et al., 2010b, Thomas et al., 2016,

Regan et al., 2019). Hence, the greater perception of these attributes likely contributed to the lower hedonic ratings of the LF group. The increased feelings of fullness, and reduction in hunger, during ONS consumption has been found previously (Regan et al., 2019) and can be explained by the relatively high consumption of nutrients and energy within a full portion (125 mL) of ONS (Regan et al., 2019).

Compared with the HF group, we observed greater decreases in hunger ratings, for the LF and MF group (Figure 5-7), which reached borderline significance ($p=0.08$). In addition, though not significant, fullness ratings increased to a greater extent over the 8 sips for the LF group, compared with the MF and HF group. A wealth of data demonstrates how a more intense (Weijzen et al., 2008, Bolhuis et al., 2011, Ramaekers et al., 2014, Tang et al., 2020) or longer duration (Ruijschop et al., 2008, Zijlstra et al., 2009, Zijlstra et al., 2010, de Graaf et al., 2006, Ramaekers et al., 2014, Tang et al., 2020) of flavour release and/or oro-sensory perception is known to suppress appetite and can lead to reduced food intake. As described by Yin et al (2017) and Gibson and Brunstrom (2007) individuals may gradually learn that food with more intense or complex sensory profiles, may be more nutritionally rich, and therefore more satiating. It is a possibility that over increasing sips, more intense and prolonged aroma release and sensory perception, as a result of relatively low SFR, contributed to a greater satiation response in the LF group. These findings are important because, for patients with hyposalivation, a greater decrease

in subjective appetite sensations during ONS consumption may contribute to premature termination of ONS intake.

5.5.1 Limitations

To alleviate the influence of medication and disease on sensory and flavour perception, the present study chose to recruit healthy adults as participants, rather than patients with hyposalivation. Therefore, our sample contained few individuals with a low saliva flow rate (n=5), and therefore our study could be sensitive to the ‘small study effect’ phenomenon (Sterne et al., 2000). We therefore recommend findings are validated with larger group sizes to generate firm conclusions.

5.6 Conclusions

This study has found that group salivary differences are associated with variations in temporal sensory perception and in-mouth aroma release during consumption of a liquid ONS. Over repeated sips of ONS, a group with a low salivary flow rate, along with a low protein secretion rate, perceived greater aftertaste and a greater build-up of mouth drying; we propose that this may have been caused by reduced oral clearance and a greater extent of mucoadhesion. This group also experienced relatively higher intensity of in-mouth aroma release likely to be caused by a lower in-mouth dilution of volatiles. These factors may have contributed to greater appetite suppression and reduced subjective ratings of pleasantness, through sensory-specific satiety. Due to small group sizes, further research should i) validate significant and borderline findings with larger group sizes in healthy individuals with hyposalivation and ii) validate findings in patients with hyposalivation. The unique sensory experience and preferences of individuals with hyposalivation should be considered, both clinically and in product development, to ensure food palatability and adequate nutritional intake. To improve the sensory experience, it may be helpful to provide ONS alongside saliva-stimulating interventions, to mitigate the undesirable temporal sensations perceived during consumption. In addition, these findings strengthen the crucial importance of ensuring adequate hydration is continuously accessible for patients with

hyposalivation, to enhance salivary flow, and provide the opportunity to remove lingering flavour occurring post- consumption.

6 General discussion and conclusions

The relative proportion of older people in the worldwide population is rapidly increasing, and within this older population, there is a high prevalence of undernutrition. Prescription of oral nutritional supplements (ONS) is a clinically effective strategy to treat and prevent undernutrition in older adults. However, patients must consume an adequate quantity of ONS to gain the clinical benefits, which can be challenging.

The overarching aim of this research was to investigate the role of flavour quality, and age-related changes in oronasal physiology and sensory abilities, on the sensory perception and palatability of Oral Nutritional Supplements (ONS).

6.1 Key findings

Good palatability, defined as the positive hedonic evaluation of food's sensory characteristics, correlates strongly with nutritional intake. Therefore, if adherence to ONS is to be good, ONS must be palatable for the patient. Chapter 2 (section 2.3.3) identified many sensory characteristics which stem from essential ingredients within ONS formulations (such as proteins and minerals), which elicit undesirable taste and mouthfeel sensations and reduce the palatability of ONS. The review revealed that the contribution made by aroma to the palatability of ONS is a comparatively under-researched area. Volatile aroma compounds play a major role in food evaluation and acceptance before

food is even placed in the mouth (orthonasal olfaction) but also by driving the perception of flavour during food consumption (retronasal olfaction). Consequently, in Chapter 3 (section 3.4.2), this research characterised the aroma-active compounds within a commonly prescribed ONS. Unpleasant sulfurous aroma compounds contribute significantly to the perceived flavour of ONS, and in Chapter 4 (section 4.4.1.1), when added to a flavoured dairy beverage, sulfurous flavours negatively impacted consumer acceptance. However, a greater concentration of sulfurous flavour may be present before acceptance is reduced in the older consumer group, potentially due to age-related sensory impairments. Finally, Chapter 5 (section 5.4) focussed on saliva flow rate (SFR). As identified in section 1.2.3, SFR is essential for mediating the extent of in-mouth aroma release and sensory perception, but SFR is known to decline with ageing. This study demonstrated that a lower stimulated SFR is associated with more intense in-mouth aroma release and greater perceptual aftertaste intensity; these findings occurred concurrently with lower hunger sensations. The novel findings from these studies make an essential contribution to understanding how best to optimise food and beverage palatability for older consumers. Hence, they inform clinical practice and the development of ONS and other nutritional food products to potentially improve patient adherence and maximise the benefits of nutritional supplementation in the ageing population.

6.2 The flavour quality of ONS

Chapter 3 represents a fundamental step forward in the research area by establishing which volatiles are present in a commonly prescribed ONS (sections 3.4.1) and identifying aroma-active compounds which contribute most significantly to the perceived flavour (section 3.4.2). The gas chromatography-mass spectrometry (GC-MS) analysis in section 3.4.1 identified twenty-nine volatiles in the headspace of the studied ONS, which had wide-ranging chemical and sensory properties. In particular, it identified sulfurous volatiles, known to form from proteins during the heat-treatment of dairy products. Through the calculation of odour activity values (OAV) (section 3.4.1) it was hypothesised that these sulfurous volatiles contribute significantly to the perceived flavour of ONS. In section 3.3.2, gas chromatography-olfactometry mass spectrometry (GC-O-MS) analysis confirmed this hypothesis because these sulfurous aroma compounds had relatively high modified frequency (MF%) values.

Compared to usual food products, which are optimised for palatability, foods for special medical purposes (FSMPs) can be more prone to developing off-flavours and taints due to the essential nutritional ingredients. These two studies (3.4.1 and 3.4.2) established efficient analytical techniques which can be applied to examine the flavour quality of nutritional food products, such as ONS. The use of headspace-solid phase microextraction (HS-SPME) is a comparatively fast and straightforward aroma extraction technique that involves

relatively little sample preparation, which is often time-consuming. This extraction method also enabled the identification of important but low-concentration, unstable or highly volatile aromas, such as sulfur compounds, which can be often lost through other extraction processes (Vazquez-Landaverde et al., 2006). Therefore, this analytical technique can be applied to assess the aroma quality routinely during the development of FSMPs to identify, uncover the formation, and monitor changes in the relative abundance of important volatiles by modifying ingredients and manufacturing processes. This will support the optimisation of flavour to ensure the greatest possible palatability.

Furthermore, the combination of GC-MS with human participants in GC-O-MS enables the translation of chemical information into sensorial information. GC-O-MS can be a labour-intensive and time-consuming technique for both the researcher and participant because it usually only permits only one panellist at a time and requires the participant or panellist to concentrate for extended time periods. Compared to other GC-O-MS methods, such as dilution to threshold methods (Brattoli et al., 2013), the modified frequency (MF%) method requires fewer study visits. Furthermore, simple scales mean participants who are relatively less experienced in sensory analysis can adopt it more easily. Therefore, this technique has application for time-critical routine use in industry but also for researchers interested in understanding human sensitivity to aroma compounds that contribute to a specific flavour.

However, we recommend using large participant numbers due to the considerable variation in individuals' olfactory abilities.

In section 3.4.3, participants confirmed the sulfurous aroma to be undesirable through an orthonasal assessment. The orthonasal evaluation of food aroma plays a vital role in identifying the suitability of a food item for consumption and communicates to the consumer about the palatability and nutritional adequacy of food before it is placed in the mouth (Stevenson, 2010). Furthermore, positive aroma can stimulate salivation, promote appetite and increase food intake (Spence, 2015, Yeomans, 2006). Our findings hypothesise that these high-impact, unpleasant sulfurous aromas contribute to the poor reported orthonasal aroma quality of ONS (Lad et al., 2005, Lambert et al., 2017).

Furthermore, when sulfurous aroma compounds were combined as an aroma mixture within a model dairy beverage (Chapter 4, section 4.4.1.1), they contributed negatively to palatability and consumer acceptance. This confirmed sulfurous aroma compounds to be off-flavours in the model dairy beverage at the concentrations studied. Consumer acceptability is crucial for adequate adherence; thus, this finding has consequences for the nutritional acceptance of high-protein beverages. Reasons for the rejection of samples containing sulfurous off-flavours included those related to flavour 'Bad taste', identification of off-flavours 'Sour taste', identification of bad/off-putting smell 'Rancid smell' but also those related to texture 'Watery texture' and 'Less creamy' demonstrating multimodal effects of these aroma compounds.

Perceived textual differences can influence appetite through altering expectations, as identified in section 2.3.3.3, and warrants investigation in future research.

6.3 Perceptual differences between older and younger adults

Our review in Chapter 1 (section 1.2) and Chapter 2 (section 2.3.2.3) found that older adults experience impairments in sensory abilities, such as taste and smell. These impairments are proposed to lessen the palatability of foods, impede appetite, nutritional intake and negatively affect food enjoyment. In chapter 3 (3.4.3.1) an orthonasal assessment of sulfurous aroma compounds revealed that these aromas are significantly less objectionable in older age. These findings are supported by previous research and are hypothesised to be related to reduced perceptual intensity of unpleasant aroma in older groups (Wysocki and Gilbert, 1989). In the current research, the view that age-related impairments in olfactory abilities caused a reduction in perceived unpleasantness is supported by the finding that older adults had lower MF(%) values for sulfurous volatiles (section 3.4.2.1), and higher olfactory detection thresholds (section 3.4.3.2), compared to younger adults, implying a lower sensitivity for undesirable aromas.

In agreement with these findings, in Chapter 4 (section 4.4.1.1) it was revealed that older adults had higher rejection thresholds for sulfurous flavours, meaning older adults were relatively less susceptible to the

negative effect of these off-flavours. This finding is novel because it suggests that age-related sensory impairments, which are often proposed to negatively influence palatability of foods, reduce the negative sensory effects caused by heat-treated protein ingredients and thus enhance the palatability of protein fortified beverages for older adults, thus potentially promoting nutritional intake. This finding may explain why some research has found a greater intake of novel foods with unpleasant odours in older adults experiencing olfactory impairments (Pelchat, 2000). Further work is needed to confirm how olfactory impairments affect the long-term adherence to ONS in older patients.

Consequently, the findings from this thesis imply that it may be too straightforward to presume that older adults perceive aroma mixtures, such as those in food and beverages, as less palatable. In line with previous findings (Wysocki and Gilbert, 1989, Seow et al., 2016), our findings imply that olfactory loss is aroma-specific, which can distort overall flavour perception and alter the hedonic response. This may explain why previous attempts to enhance aroma in foods have not been consistently successful (Koskinen et al., 2003, Boesveldt et al., 2018). We suggest future research takes a more informed approach to flavour enhancement, which accounts for the extent of age-related loss to individual flavours, but also the impact of a range of flavour intensities on food acceptability.

A rejection threshold technique was an appropriate technique to study the hedonic impact of aroma on acceptance and palatability of foods such as ONS. Our research uncovered several important aspects that researchers should consider:

1. It is paramount to test attributes with the consumer age-group of interest who likely differ in sensory abilities and/or preferences.
2. Aroma-interactions alter the hedonic response; therefore, researchers should consider the combination of all aroma compounds that contribute to a specific flavour.
3. A best estimate threshold (BET) technique gives a more conservative estimate (a lower threshold), so this could be proposed as more suitable method for products where palatability may be particularly crucial, such as with FSMPs. However, the combination of the graphical approach (RjT) along with BET gives the most comprehensive view of acceptability and should be taken forwards to be used as a standard sensory method. The graphical approach can demonstrate the hedonic effect of a sensory attribute or aroma by the direction of the curve. Thus, the graphical approach should be used as an initial method, to determine the hedonic effect of a sensory attribute, and if shown to be undesirable, BET can give a conservative estimate of the lowest acceptable concentration.

6.4 Influence of age-related changes in physiology on aroma release and sensory perception of ONS

Chapter 1 identified that the impact of age-related changes in oronasal physiology on food perception spans further than differences in sensitivity to stimuli. For example, age-related changes in the mouth, such as reduced muscle forces (section 1.2.6), can alter the oral processing of foods and in-mouth flavour release. It was also identified that older adults experience a reduction in saliva flow rate (SFR) (section 1.2.3) which could directly influence aroma release and sensory perception in the oral cavity.

In chapter 5 (section 5.4.3.3), our research using young, healthy participants found that the intensity of in-vivo aroma release (Imax) of four aroma compounds identified as perceptually important to the flavour of ONS (chapter 3, section 3.4.2) was significantly greater, and lasted for a longer duration (Tmax), in the group with low SFR compared to the group with high SFR. These findings occurred alongside a greater intensity of aftertaste ($p<0.001$) in the low SFR group (combining perception of both taste and aroma). We hypothesise that a lower volume and more viscous saliva hindered oral clearance between sips, leading to a ‘reservoir’ of flavour in low SFR group’s oral cavity. If the flavour of ONS is disliked, a greater aftertaste intensity post-consumption is not beneficial for a good consumer experience.

Atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS) is a well-established technique to study the retronasal aroma profile of foods and beverages *in-vivo* during consumption. However, the limit of detection of aroma, defined as the concentration required to produce a signal (Hatakeyama and Taylor, 2019), is compound-dependent (Taylor et al., 2000). Therefore, certain aroma, such as sulfur aroma compounds, are beyond the sensitivity of APCI-MS (Yang, 2012). Consequently, this technique detected aroma present in high concentrations in the ONS, such as the fruity esters (detected in 3.4.1). However, it was not sensitive enough to detect aroma in trace concentrations, such as the sulfur aroma compounds (detected in 3.4.1). Therefore, future research should investigate the hedonic quality of the flavour 'reservoir' and measure the relative contribution made by different aroma to the perceived aftertaste.

Compared with the medium SFR and high SFR, we also observed a greater build-up in the perceptual intensity of mouth drying in the low SFR group (section 5.4.3.1.4). Mouth drying has been identified as a negative driver of liking in ONS products (Thomas et al., 2018), and many patients will experience hyposalivation due to disease, medical treatment or ageing. Hence, a greater perceptual intensity of mouth drying in individuals suffering from hyposalivation could be a significant factor contributing to palatability and adherence to ONS. These findings contrast with recent research, which found no effect of modulating SFR on perception of mouth drying induced by a high-protein beverage

(Norton et al., 2021). The discrepancies between findings may be due to many reasons, but notably, our research used a temporal approach that measured perception over multiple sips, which revealed differences between groups at higher sip numbers. Consequently, we recommend that future researchers use a temporal approach to study saliva's association with sensory perception, exemplifying the natural consumption process.

The choice of temporal technique used in the current study was an adapted (simplified) sequential profiling technique. We chose to use a simplified sequential profiling technique because previously employed temporal sensory techniques, such as temporal check all that apply (TCATA), were not sensitive enough to capture perceptual changes as consumption of ONS progresses (Regan et al., 2019). Furthermore, we felt it was appropriate to use a consistent scale over repeated rating events; therefore, sensory profiling on 100mm line scales occurred concurrently alongside appetite ratings on 100mm visual analogue scales (VAS). For greater sensitivity, we recommend that future research using semi-trained participants consider this simplified profiling technique to capture changes in perception and appetite over multiple intakes.

Findings from chapter 5 may have implications in appetite regulation because a higher intensity of sensory perception (Ramaekers et al., 2014, Tang et al., 2020) along with greater intensity and slower release of aroma (Ruijschop et al., 2008) are strongly associated with enhanced

feelings of satiation and may reduce food intake. In Chapter 5 (section 5.4.3.2.3), trends towards enhanced appetite signals (hunger) were found, but this did not translate into statistical significance. The lack of significance may be due to small sample sizes or techniques used to measure perceived appetite. The observed trends do warrant further investigation, as these may be crucial factors affecting appetite and contributing to long-term adherence to ONS in patients with hyposalivation.

Due to our findings, and the known age-related changes in orosensory physiology, we suggest that for greater validity, future researchers or clinicians interested in assessing older adults' olfactory abilities use a retronasal method (tasting of aroma volatiles). A retronasal olfactory method considers the influence of oronasal physiology along with olfactory sensitivity on aroma perception, whereas an orthonasal route by-passes essential oronasal physiological parameters that influence in-mouth aroma release.

6.5 Conclusions

The novel findings and techniques established in this thesis increase understanding of the impact of off-flavours, and other undesirable sensory attributes, on consumers acceptance of nutritional products such as ONS. Manufacturers should aim to produce an optimised aroma profile with minimal off-flavours and an appetising aroma, specifically tailored to the consumer group of interest. This can be

achieved by exploring changes to processing conditions to mediate off-flavour generation. Furthermore, this work demonstrated the importance of flavour-interactions on food acceptance; enhancing positive aroma (such as diacetyl) offers a solution to reduce the sensorial impact of off-flavour without altering the essential nutritional quality of the food or beverage.

The findings can also be applied to optimise the sensory attributes of ONS for individuals experiencing hyposalivation. Our research identified that positive orthonasal assessment of aroma could stimulate salivation, but existing literature along with our findings imply that the orthonasal aroma quality of ONS is not optimal. Improving the orthonasal quality of ONS, such as enhancing freshness, may help elicit salivation in patients. Olfactory priming, defined as a diffusion of aroma into the eating environment, may impact appetite, salivation and food intake (Proserpio et al., 2017), so it presents an exciting avenue to explore in future research with older adults. Furthermore, the incorporation of saliva-stimulating stimuli, which could induce saliva flow, should be investigated because they may reduce the build-up of undesirable mouth drying and aftertaste intensity. Trigeminal flavours are known to elicit salivation and have recently resulted in a high mean liking for ONS products by cancer patients (de Haan et al., 2021). However, these researchers did not consider the influence of saliva flow rate, so this is a crucial succeeding research area to explore.

While impaired olfaction and/or hyposalivation are more frequent with ageing, not every older adult will experience these, or to the same extent. Therefore, a personalised approach may be beneficial in clinical practice, such as providing artificial saliva products, along with adequate hydration, to limit lingering aftertaste and unpleasant mouthfeel sensations for susceptible individuals. In daily clinical practice, healthcare staff should make routine effort to assess older patients' sensory needs and preferences to optimise the palatability of ONS and other foods. Nevertheless, if ONS are palatable and thus spontaneously enjoyed by patients, it lessens the dependence on and encouragement from healthcare staff.

With the expansion of research efforts and investment in product development, adherence to oral nutritional supplements (ONS) can be improved to maximise the nutritional benefits and tackle the increasing burden of undernutrition in the ageing population.

6.6 Strengths, limitations and future work.

- The study presented in Chapter 4 was limited in matrix choice due to the need to avoid high-temperature processing (and resulting aroma generation) whilst creating a safe beverage for consumer tasting. Further work should develop a matrix that more closely represents the ONS matrix to study the impact of off-flavours and other sensory attributes that contribute to acceptance and palatability.

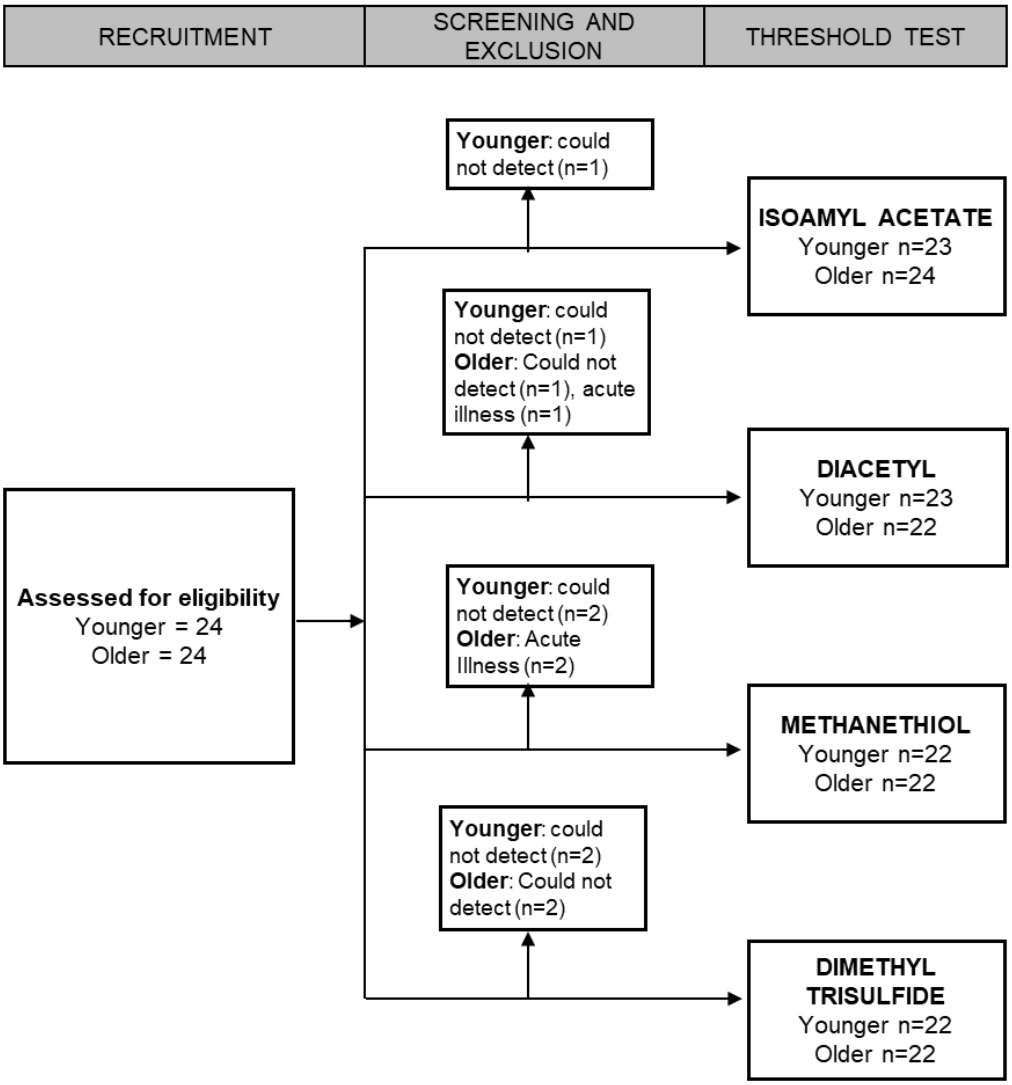
- As discussed in Chapter 1 (section 1.2.5), denture wearing is known to affect the oral processing of foods and the sensory abilities of older adults. Denture wearing was not screened for or controlled for in Chapter 3 or Chapter 4 and may have influenced the findings. It would have been interesting to find out what impact denture wearing had on the results, and we recommend future research considers recording this factor in research with older consumers.
- Our reviews in Chapter 1 and Chapter 2 identified that older adults have impaired sensory abilities, including trigeminal sensations. Our study (Chapter 5), which investigated the impact of saliva flow rate (SFR), used healthy younger adults. Therefore, future work should explore the objectives of Chapter 5 in a larger group of older individuals experiencing hyposalivation. This will reveal whether the greater intensities associated with low SFR transform into higher perceptual intensities in the older population who may be experiencing sensory impairments. Moreover, we hypothesise that a larger group size could translate significantly greater aroma-release into significantly greater flavour perception, which may have been limited in the current study due to the small group size of the low SFR group.
- Due to low concentrations of aroma in-vivo, and limits of detection with the APCI-MS technique, the current work was unable to evaluate the influence of saliva volume on the in-mouth release of trace volatiles (such as the undesirable sulfur

compounds identified in section 3.3.2) during ONS consumption. Future work should assess saliva's influence on the release of these trace compounds, perhaps through the development of a model mouth.

- Changes to processing conditions are likely to influence the formation of off-aroma compounds in ONS. When choosing processing parameters to sterilise dairy products, compared to a low-temperature long time (LTLT) method, a high-temperature short time (HTST) sterilisation has lower chemical change values (C*) whilst maintaining microbial lethality (F0). We hypothesise that HTST sterilisation may result in less off-flavour formation in heat-treatment of protein-containing dairy products, thus defining an important area for future investigation.
- Besides saliva flow rate, older adults experience many other changes in their oronasal physiology, which may affect how aroma is released from foods and beverages in the oral cavity (See review section 1.2). Compared with orthonasal olfaction, researchers know comparatively less in the field of retronasal olfaction, and any effects are likely to be product dependent. For example, age-related declines in jaw muscle force may significantly influence the aroma release from solid foods, but less so from a liquid beverage which requires relatively less oral processing. Future research should investigate how age-related changes in other physiological parameters affect in-mouth aroma

release to optimise foods for the growing population of older consumers.

Appendices



Appendix A (chapter 3): Flow diagram illustrating the total number of older and younger participants included in each section of the aroma pleasantness rating and aroma detection threshold test.

Appendix B (chapter 3): Classification of medications regularly taken by participants included in the gas chromatography- olfactometry and detection threshold tests. Numbers in brackets show total amount of participants who reported taking the specific medication class.

Age-group	Medication classification	Mean no. medication per participant
Gas chromatography-Olfactometry-Mass Spectrometry		
Younger	None	0
Older	Supplements (6), Calcium Channel Blocker (2), Proton pump inhibitor (2), Alpha blockers (2), 5α-reductase inhibitor (1), Beta-Blocker (1), Xanthine oxidase inhibitor, Angiotensin II receptor blocker (1) Serotonin reuptake inhibitor (1), Statins (1).	3
Detection threshold tests		
Younger	Supplements (6), Histamine H1 receptor antagonist (2), Hormonal contraceptive (2).	0.4
Older	Supplements (20), Statins (5), Thyroxine (T4) replacements (3), Proton pump inhibitors (4), Calcium channel blockers (4), Biguanides (2), Angiotensin II receptor antagonists (2), Angiotensin-converting enzyme inhibitors (2), Thiazide diuretics (1), Nonsteroidal anti-inflammatories (1), Serotonin reuptake inhibitors (1), Antimetabolites (1), Anticoagulants (1), Aromatase inhibitors (1), Anticonvulsants (1), Angiotensin receptor blockers (1), Dipeptidyl Peptidase 4 Inhibitors (1), Insulin replacement (1), Hormone replacement (1), Antimuscarinics (1), Bronchodilators (1), Synthetic nucleoside analogues (1), Antibiotics (1).	2.6

Appendix C (chapter 3): Pooled data showing aroma compounds detected by all participants (n=12) ordered into relative importance by greatest modified frequency (MF %) values. Aroma descriptions were generated by older and younger participants, the number in brackets.

Relative impact	Aroma compound name	LRI range	Functional group	Participants aroma descriptions		Pooled DF (%)	Pooled I (%)	Pooled MF% (n=12)
				Older	Younger			
1	Isoamyl acetate	1160-1201	Ester	Banana (3), Sweet (2), Fruity (2), Barley sugar, Unpleasant, Strong.	Banana (3), Fruity (3), Sweet (2), Pear, Estery.	100	89	94
2	Ethyl butyrate	1069-1088	Ester	Sweet (2), Fruity, Indescribable, Strong, Eggy.	Fruity (5), Sweet (3), Estery, Cherry.	92	58	73
3	Unknown compound	1684-1849	Unknown	Cooking (2), Burning, Donuts, Cake, Flour, Unpleasant, Savoury.	Burnt (3), Nutty (2), Bread, Milky, Sweet, Musty, Medicine, Iron, Bitter, Earthy, Dry.	83	64	73
4	Isoamyl isovalerate	1334-1368	Ester	Fruity, Vegetables, Not sweet, Not fruity, Indistinct.	Fruity, Cheesy, Sulfur, Bad, Not pleasant, Off-food, Ammonia-like, Not fruity, Not sweet.	92	50	68
5	Methanethiol	637-663	Sulfur	Indescribable, Unpleasant.	Sulfuric (3), Bad (3), Rotten (2), Faeces, Earth.	75	53	63
6	Dimethyl trisulfide	1431-1457	Sulfur	Sulfur, Bad, Squash, Non-descript.	Sulfur (2), Onion, Cooked, Cabbage, Air-freshener, Chemical.	75	42	56
7	Isoamyl propionate	1233-1240	Ester	Sweet (3), Fruity.	Fruity (3), Estery, Sweet.	67	33	47
8	Diacetyl	1018-1027	Ketone	Sweet (2), Fruity.	Sweet, Caramel.	42	28	34

Appendix D (chapter 3): Detection frequency (DF) and intensity ratings (I) of each participant, for each aroma, during the GC-O-MS study. DF of 1 is equal to detection, DF of 0 is equal to no detection. The Sum and percentage (%) for each age-group are shown.

	Aroma compound															
	Isoamyl acetate		Ethyl butyrate		Unknown compound		Isoamyl isovalerate		Methanethiol		Dimethyl trisulfide		Isoamyl propionate		Diacetyl	
	DF	I	DF	I	DF	I	DF	I	DF	I	DF	I	DF	I	DF	I
Younger adults																
Participant 1	1	3	1	2	1	3	1	1	1	3	1	3	1	2	1	2
Participant 2	1	3	1	2	1	2	1	2	1	1	1	1	1	1	0	0
Participant 3	1	2	1	2	1	3	1	2	1	2	1	2	0	0	0	0
Participant 4	1	2	1	1	0	0	1	1	0	0	1	2	0	0	0	0
Participant 5	1	3	1	1	1	1	1	2	1	2	0	0	1	1	0	0
Participant 6	1	3	1	2	1	2	1	1	1	2	1	2	1	2	0	0
Sum	6	16	6	11	5	11	6	9	5	10	5	10	4	6	1	2
%	100	89	100	61	83	61	100	50	83	56	83	56	67	33	17	11
Older adults																
Participant 7	1	3	0	0	1	2	1	1	0	0	0	0	0	0	1	2
Participant 8	1	3	1	3	1	3	1	3	0	0	1	1	1	2	0	0
Participant 9	1	2	1	1	1	2	1	1	1	1	0	0	0	0	0	0
Participant 10	1	3	1	3	1	3	1	2	1	3	1	1	1	2	1	1
Participant 11	1	2	1	2	0	0	0	0	1	2	1	1	1	2	1	2
Participant 12	1	3	1	1	1	2	1	2	1	3	1	2	1	1	1	3
Sum	6	16	5	10	5	12	5	9	4	9	4	5	4	7	4	8
%	100	89	83	56	83	67	83	50	67	50	67	28	67	39	67	44

Appendix E (chapter 5): Mean, Standard error (SE), Minimum (min) and Maximum (max) values of each salivary constituent for each group classified by saliva flow rate. Significant differences between groups are indicated in both, letters indicate statistical group differences as determined by Tukey's test.

Group	Mean saliva flow rate (mL/min)	Mean saliva viscosity (mPas)	Mean saliva total protein concentration (mg/mL)	Protein secretion rate (mg/min)	Amylase activity (U/mL)
LF group					
Mean	**0.4**[a]	1.8	1.5	**0.6**[a]	45.0
SE	0.1	0.2	0.2	0.1	6.00
Min	0.3	1.3	1.0	0.4	24.0
Max	0.5	2.3	1.9	0.8	76.0
MF group					
Mean	**1.0**[b]	1.6	1.2	**1.1**[b]	50.0
SE	0.1	0.1	0.1	0.1	5.90
Min	0.7	1.0	0.7	0.6	2.00
Max	1.2	2.3	2.1	1.7	118
HF group					
Mean	**1.5**[c]	1.4	1.3	**1.9**[c]	57.0
SE	0.1	0.1	0.1	0.2	7.60
Min	1.3	0.9	0.8	1.3	18.0
Max	1.8	2.4	1.9	3.1	126
ANOVA					
p value	**<0.001**	0.302	0.512	**<0.001**	0.759
F statistic	**58.166**	1.253	0.687	**17.725**	0.278

Appendix F (chapter 5): Mean (±SE) intensity ratings for all sensory attributes, over four sips (sip 1, 3, 5 and 7), for each group.

	Sip 1	**Sip 3**	**Sip 5**	**Sip 7**
Sweetness				
Low flow group	5.84 (1.36)	5.74 (1.07)	6.34 (1.36)	6.76 (0.86)
Medium flow group	5.79 (0.43)	6.22 (0.31)	6.11 (0.38)	6.73 (0.42)
High flow group	5.16 (0.72)	5.64 (0.67)	5.53 (0.83)	5.41 (0.91)
Banana flavour				
Low flow group	6.02 (0.80)	6.08 (0.67)	6.78 (0.59)	7.56 (0.59)
Medium flow group	6.48 (0.40)	6.24 (0.34)	5.52 (0.60)	5.66 (0.45)
High flow group	6.23 (0.56)	6.10 (0.56)	6.04 (0.73)	5.84 (0.97)
Mouthcoating				
Low flow group	6.54 (0.60)	6.34 (0.86)	6.98 (0.64)	6.66 (0.98)
Medium flow group	5.41 (0.39)	5.21 (0.56)	6.26 (0.53)	6.18 (0.56)
High flow group	4.60 (0.77)	4.92 (0.80)	5.74 (0.98)	5.62 (1.02)
Mouth drying				
Low flow group	4.32 (1.40)	5.04 (0.70)	6.60 (0.23)	7.72 (0.22)
Medium flow group	5.69 (0.51)	5.24 (0.55)	5.94 (0.60)	6.04 (0.52)
High flow group	5.43 (0.66)	4.67 (0.84)	4.80 (0.84)	4.66 (0.82)
Aftertaste				
Low flow group	7.64 (0.47)	6.80 (0.91)	8.36 (0.40)	8.76 (0.32)
Medium flow group	6.64 (0.54)	5.69 (0.51)	5.39 (0.58)	6.32 (0.56)
High flow group	5.47 (0.65)	4.68 (0.63)	5.09 (0.51)	5.13 (0.82)

Appendix G (chapter 5): Mean Baseline and Change from baseline ratings (±SE), over sips 2, 4, 6 and 8, for all appetite variables, for each group.

	Baseline	**Change from baseline**			
		Sip 2	**Sip 4**	**Sip 6**	**Sip 8**
Hunger					
Low flow group	6.42 (0.65)	-0.14 (0.30)	-0.38 (0.57)	-2.16 (1.14)	-2.60 (1.05)
Medium flow group	5.53 (0.56)	-0.57 (0.28)	-1.04 (0.41)	-1.75 (0.49)	-2.21 (0.61)
High flow group	4.90 (0.91)	0.24 (0.16)	0.24 (0.24)	0.00 (0.31)	-0.34 (0.45)
Fullness					
Low flow group	2.42 (0.64)	0.48 (0.24)	2.14 (1.12)	3.28 (1.24)	3.24 (0.96)
Medium flow group	3.69 (0.55)	0.62 (0.34)	1.34 (0.46)	1.88 (0.62)	1.18 (0.89)
High flow group	3.77 (0.78)	0.06 (0.21)	0.96 (0.40)	0.86 (0.40)	1.66 (0.38)
Desire to eat					
Low flow group	6.86 (0.44)	0.04 (0.20)	-0.80 (0.57)	-1.84 (0.78)	-2.28 (1.20)
Medium flow group	5.77 (0.70)	-0.56 (0.25)	-1.09 (0.35)	-1.89 (0.40)	-2.19 (0.59)
High flow group	5.80 (0.96)	-0.21 (0.31)	-0.51 (0.15)	-0.87 (0.21)	-1.79 (0.32)
Prospective consumption					
Low flow group					
Medium flow group	7.08 (0.54)	0.32 (0.27)	-0.88 (0.44)	-1.58 (0.30)	-1.70 (0.51)
High flow group	5.58 (0.51)	-0.24 (0.23)	-0.96 (0.35)	-1.32 (0.42)	-1.73 (0.52)
	5.19 (0.65)	-0.11 (0.31)	-0.31 (0.33)	-0.51 (0.65)	-1.20 (0.41)

Appendix H (chapter 5): Mean (±SE) hedonic ratings, over sips 2, 4, 6 and 8, for each group.

	Sip 2	**Sip 4**	**Sip 6**	**Sip 8**
Pleasantness				
Low flow group	4.92 (1.00)	5.02 (1.32)	4.04 (1.46)	4.02 (1.45)
Medium flow group	6.01 (0.39)	5.79 (0.46)	5.60 (0.53)	4.43 (0.57)
High flow group	5.92 (0.38)	6.10 (0.42)	6.14 (0.47)	5.40 (0.81)
Desire to drink more				
Low flow group				
Medium flow group	4.58 (1.55)	3.92 (1.61)	3.58 (1.61)	3.88 (1.71)
High flow group	4.95 (0.62)	4.71 (0.59)	4.88 (0.65)	3.85 (0.62)
	5.03 (0.85)	5.67 (0.89)	4.73 (0.97)	4.21 (0.87)

Appendix I (for chapter 5): Main effects and pairwise comparisons (Tukey) for each Sip effect of appetite variables as analysed by Mixed models ANOVA. Significant differences are highlighted in bold.

		Beverage pleasantness	Hunger	Fullness	Desire to eat	Prospective consumption	Desire to drink more of beverage
Main effect of Sip							
p-value		0.057	**<0.001**	**0.004**	**<0.001**	**<0.001**	**0.047**
F statistic		3.049	14.17	6.247	18.10	15.06	3.098
DFn, DFd		1.964, 53.03	1.450, 39.16	2.026, 54,71	1.506, 40.65	1.957, 52.84	2.231,60.23
p-values as determined by pairwise comparisons (Tukey)							
Sip 2	Sip 4	0.996	0.330	**0.011**	0.054	**0.006**	0.994
	Sip 6	0.654	**0.009**	**0.002**	**<0.001**	**0.003**	0.831
	Sip 8	**0.047**	**0.002**	0.107	**<0.001**	**<0.001**	**0.016**
Sip 4	Sip 2	0.996	0.330	**0.011**	0.054	**0.006**	0.994
	Sip 6	0.758	**0.007**	0.328	**<0.001**	0.229	0.848
	Sip 8	**0.041**	**0.001**	0.895	**<0.001**	**<0.001**	**0.003**
Sip 6	Sip 2	0.654	**0.009**	**0.002**	**<0.001**	**0.003**	0.831
	Sip 4	0.758	**0.007**	0.328	**<0.001**	0.229	0.848
	Sip 8	0.231	**0.032**	0.989	0.059	0.049	0.195
Sip 8	Sip 2	**0.047**	**0.002**	0.107	**<0.001**	**<0.001**	**0.016**
	Sip 4	**0.041**	**0.001**	0.895	**<0.001**	**<0.001**	**0.003**
	Sip 6	0.231	**0.032**	0.989	0.059	0.049	0.195

Email correspondence

26/07/2017 17:54

SBREC160140A RE: Sophie Lester - Study 1

Dear Ms Lester, Dr Fisk and colleagues,

Thank you for your submission (SB1617/44 PGR Lester (Fisk))

Your submission has now been through the ethical review process conducted by the School of Biosciences Research Ethics Committee (SB REC), and we are able to grant ethical approval for this project. Please note your approval code, which you may use on your forms (e.g. participant form, etc), is:

SBREC160140A

Please remember (as your research progresses) to inform the SB REC if there are any ethically relevant changes in your materials or protocols. This approval is valid for 12 months from the date of this email. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review. As appropriate, please inform any co-applicants of the outcome of this review.

We wish you all the very best for your study.

Best wishes

Kate Millar on behalf of the SB REC

Appendix J: Ethical approval notice for research conducted with human participants in section 3.3.

University of Nottingham
UK | CHINA | MALAYSIA

Email: FMHS-ResearchEthics@nottingham.ac.uk

Faculty of Medicine & Health Sciences
Research Ethics Committee
c/o Faculty PVC Office
School of Medicine Education Centre
B Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham, NG7 2UH

13 December 2018

Sophie Lester
PhD Student
c/o Dr Ian Fisk
Associate Professor
Food Science
School of Biosciences
University of Nottingham
Sutton Bonington Campus
Loughborough, Leics

Dear Miss Lester

Ethics Reference No: 156-1810 **– please always quote**	
Study Title: Do off-flavours in milk beverages lead to rejection by older consumers?	
Short Title: Milk Flavour Study	
Chief Investigator/Supervisor: Dr. Ian Fisk, Associate Professor, Food Science, School of Biosciences	
Lead Investigators/student:	
Other Key Investigators: Dr Moira Taylor, Associate Professor, Human Nutrition (Dietetics), Faculty of Medicine and Health Sciences	
Type of Study: PhD	
Proposed Start Date: 01/11/2018	**Proposed End Date:** 31/03/2019
No of Subjects: 100	**Age:** 18+years

Thank you for notifying the Committee of amendment no 1: 04.12.2018 as follows:

- Increased inconvenience allowance from **£15 to £25** upon completion of 3 sessions
- The addition of two aroma compounds (Dimethyl **sulphide** and Dimethyl **disulphide**) to the beverage to be used in Sensory Sessions 1 (this will not increase the amount of time/samples or effort that panellists are asked to give to the session)
- The addition of **two questions** to the participant questionnaire

Revised application and supporting documents V2 dated 04.12.2018 were received.

These have been reviewed and are satisfactory and the study has been given a favourable opinion.

A favourable opinion has been given on the understanding that:

1. The protocol agreed is followed and the Committee is informed of any changes using a notice of amendment form (please request a form).
2. The Chair is informed of any serious or unexpected event.
3. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Appendix K: Ethical approval notice for research conducted with human participants in section 4.3.

University of Nottingham
UK | CHINA | MALAYSIA

Faculty of Medicine & Health Sciences
Research Ethics Committee
Faculty Hub
Room E41, E Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham, NG7 2UH
Email: FMHS-ResearchEthics@nottingham.ac.uk

14 June 2019

Sophie Lester
PhD Student in Food Science
c/o Dr Ian Fisk
Associate Professor
Division of Food Science
School of Biosciences
Sutton Bonington Campus
University of Nottingham
Loughborough Leics, LE12 5RD

Dear Ms Lester

Ethics Reference No: 207-1902 **– please always quote**	
Study Title: The influence of physiological and behavioural consumption parameters on in-mouth aroma release, appetite and sensory perception of an Oral Nutritional Supplement (Fortisip Compact).	
Chief Investigator/Supervisor: Dr. Ian Fisk, Associate Professor, Food Science, School of Biosciences	
Lead Investigators/student: Sophie Lester, PhD Student, Katherine Hurst, PhD student in Food Science, School of Biosciences	
Other Key investigators: Dr Moira Taylor, Associate Professor, Clinical, Metabolic and Molecular Physiology, School of Life Sciences. Dr Charfedinne Ayed, Research Fellow, Division of Food, Nutrition and Dietetics, School of Biosciences.	
Proposed Start Date: 10.06.2019	**Proposed End Date:** 31.12.2019

Thank you for notifying the Committee of amendment no 1: 21.05.2019 as detailed in the Notice of amendment and the following documents were received:

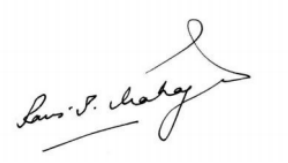
- Notice of Amendment dated 21.05.2019
- FMHS REC Application form and supporting documents version 2.0: 21.05.2019

These have been reviewed and are satisfactory and the study amendment no 1: 21.0.2019 has been given a favourable opinion.

A favourable opinion has been given on the understanding that:

1. The protocol agreed is followed and the Committee is informed of any changes using a notice of amendment form (please request a form).
2. The Chair is informed of any serious or unexpected event.
3. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely



Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Appendix L: Ethical approval notice for research conducted with human participants in section 5.3.

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