

**Investigating sodium reduction
in the UK diet through salt
particle design and
understanding contributions of
food oral processing on
perception**

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Abstract

The global food industry urgently requires effective sodium reduction solutions to increase health credentials and align with changing salt reduction targets. Previously investigated strategies for sodium reduction in potato crisps (e.g. salt enhancers, replacers and direct salt removal) have proven limitations and challenges. Therefore, studies presented in this thesis focused on redesigning salt particles to deliver optimum saltiness in snacks by developing and validating a range of physicochemical design rules to achieve effective salt reduction. It is also important to consider variations in consumer perception due to inter-individual differences in oral processing parameters and their impact on dietary intake. Therefore, the relationship between salivary parameters, saltiness perception and dietary intake, and the impact of mouth behaviour type on the perception and liking of potato crisps were also investigated.

Physicochemical design rules for salt particles were established by measuring adhesion properties, dissolution and temporal saltiness perception of a diverse range of salts ($n = 8$) with varying physical and chemical properties (size, density, shape, hydrophobicity and flow properties). Findings determined that salt particles should be small in particle size, have a low density, low hydrophobicity and an optimised particle shape. Optimised model salts identified in this study were used to validate the design rules when topically applied to potato crisps.

Inter-individual differences in salivary flow rate and sodium concentration affected salt taste threshold but did not predict the perceived saltiness intensity of supra-threshold concentrations. Furthermore, the high salt taste sensitivity group consumed significantly more salt than the low salt taste sensitivity group, suggesting that 'salt-responsive' individuals seek more salt-containing foods, or high salt consumers may develop these behaviours due to their diet.

The selected optimised model salts and established particle design rules enabled a 30 % salt reduction in potato crisps while maintaining saltiness perception and consumer acceptance. Comparatively, only 15 % salt reduction was achieved without sensorial impact by directly removing salt. Other potential strategies investigated did not enhance saltiness perception or product liking, highlighting the utility of design rules to develop new optimised salt particles. Mouth behaviour type did not impact saltiness liking or perception of potato crisps. However, the 'suckers' group liked the texture of the whole product set significantly less than the other mouth behaviour classifications (crunchers, chewers, smooshers).

This research contributes to the knowledge of salt reduction strategies in snack foods, specifically around the developed physicochemical design rules and the importance of considering individual salivary parameters when assessing the perception of saltiness. The physicochemical design rules developed within this research could also be utilised in other flavour particles to facilitate sugar reduction and optimise flavour delivery.

List of publications and presentations

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Chapter 1

1. Introduction to thesis and literature review

1.1 Brief project background and rationale

Sodium chloride (NaCl) salt, referred to as table salt, is an ionic compound composed of sodium and chloride ions. First used in ancient times for food preservation, it is now one of the cheapest commodities and most commonly used ingredients within processed foods due to its appealing unique taste quality and a plethora of functionalities. For clarification, throughout this introduction and the rest of the thesis, 'salt' without stating otherwise refers to sodium chloride. It is well known that overconsumption of sodium, usually consumed in the form of NaCl, considered the prototypical salty tastant, is a major risk factor for high blood pressure and cardiovascular diseases (CVD)(World Health Organisation, 2021). An estimated 2.5 million preventable deaths are caused each year globally by salt-associated diseases, including hypertension and heart disease (He and MacGregor, 2003). Because of this, the World Health Organisation (WHO) currently recommends no more than 5 g of salt per day for adults and even less for children (World Health Organisation, 2021). However, most individuals do not achieve this recommendation and consume an average salt intake of 8-14 g per day in developed countries, including the UK, Australia, USA and China (Thout et al., 2019, Zhou et al., 2019). Specifically, in the UK, it has been

estimated that reducing salt intake by just 1 g per person per day could prevent over 4,000 avoidable deaths per year while saving the public health service provider around £288 million (Department of Health, 2017).

Since 75-85 % of salt consumed is from that contained within processed foods (Wardlaw, 2004), food manufacturing companies are responsible for adhering to salt targets implemented in several countries, including in the UK (Trieu et al., 2015). Salt reduction programmes for processed foods and restaurants in the UK are controlled by the Food Standards Agency (FSA). The FSA established a number of salt reduction targets which started in 2005, with the most recent targets being published in 2020 to be achieved by 2024 (Public Health England, 2020b). Current salt targets are outlined for 84 specific food categories (8 more than the previous set in 2017), including; meat products, bread, butter and spreads, baked beans, ready meals, soups, pizzas, crisps (also known as potato chips) and snacks, cakes and sweet desserts, shop-bought sandwiches, sauces and condiments, biscuits, pasta, cereals, puddings, quiche, canned vegetables, meat alternatives and stocks and gravies (Public Health England, 2020b). More specifically, in snack foods, targets are set for standard potato crisps made from sliced fried potato, extruded and sheeted snacks like tortillas or puffed snacks, pelleted snacks and salt and vinegar flavours crisps. Meanwhile, the first-ever targets were brought in for other topically applied snacks such as savoury and sweet popcorn and nuts. Table 1.1 on page 23 outlines the current and previous

targets set for the category of snack foods along with the proportion of products that met the previous targets in each sub-category.

Table 1.1. Salt reduction targets for crisps and snacks to be achieved by 2024 with reference to the proportion of products meeting previous salt targets for 2017. All information sourced from: (Public Health England, 2020b).

Sub-category of crisps and snacks	Definition	Previous salt target for 2017 (set in 2014) g or mg per 100g	Proportion of products at or below maximum targets (%) (2018 data)	New salt targets for 2024 (set in 2020) g or mg per 100g
Standard potato crisps	<ul style="list-style-type: none"> All standard potato crisps (sliced potato or vegetable only). All flavours except salt and vinegar. Includes crisps aimed at a more adult market. 	Average: 1.31g salt / 525mg sodium Max: 1.45g salt / 580mg sodium	85	Average: 1.25g salt / 500mg sodium Max: 1.38g salt / 550mg sodium
Extruded and sheeted snacks	<ul style="list-style-type: none"> All extruded or sheeted snacks e.g. cheese flavour corn puffs, potato hoops, pretzels, formed crisps, sheeted crisps, tortillas all flavours except salt and vinegar 	Average: 1.7g salt / 680mg sodium Max: 2g salt / 800mg sodium	81	Average: 1.61g salt / 645mg sodium Max: 1.90g salt / 760mg sodium
Pelleted snacks	<ul style="list-style-type: none"> All snacks made from pellets e.g. prawn cocktail flavour shells, crispy bacon flavour corn snacks, curly cheese snacks, and mini poppadum's All flavours except salt and vinegar 	Average: 2.13g salt / 850mg sodium Max: 2.88g salt / 1150mg sodium	89	Average: 2.03g salt / 810mg sodium Max: 2.73g salt / 1090mg sodium
Salt and vinegar products	<ul style="list-style-type: none"> All crisps, snacks etc. salt and vinegar flavour only. Includes salt and vinegar popcorn and nuts. 	Max: 2.5g salt / 1000mg sodium	90	Max: 2.25g salt / 900mg sodium
Savoury popcorn	<ul style="list-style-type: none"> All savoury and salted popcorn. Includes 'sweet and savoury' popcorn, and coated popcorn. Excludes no added salt popcorn and salt and vinegar popcorn. 	N/A New target for 2024	n/a	Average: 1.23g salt / 490mg sodium Max: 1.44g salt / 575mg sodium
Sweet popcorn	<ul style="list-style-type: none"> All sweet popcorn, including coated popcorn. Excludes 'sweet and savoury popcorn' and popcorn kernels 	N/A New target for 2024	n/a	Average: 0.76g salt / 305mg sodium Max: 1.00g salt / 400mg sodium
Flavour nuts	<ul style="list-style-type: none"> Salted and flavoured nuts. Includes salted and flavoured dried seeds, beans, peas and corn e.g. dried wasabi peas, broad beans, edamame beans. Excludes coated nuts, fruit and nut mixes, and plain/unflavoured nuts. 	N/A New target for 2024	n/a	Average: 1.00g salt / 400mg sodium Max: 1.20g salt / 480mg sodium

The success of previous salt targets which were first introduced in 2005 has been evidenced by the steady reduction of salt across product categories in the UK. Examples include; bread products which on average were reduced by 20 % between 2001-2011 (Brinsden et al., 2013), ready meals on average were reduced by 45 % in just 4 years (Consensus Action on Salt and Health, 2007), and breakfast cereals decreased by 57 % between 2004 and 2011, among others (He et al., 2014). There is also evidence that this reduction of salt in processed foods has had its desired positive effect on reducing salt intakes across the UK population, as the average salt intake decreased from 9.5 g per day in 2000-2001 to 8.4 g per day in the UK in 2018-2019 (Public Health England, 2020a, Henderson et al., 2003). Some success may be attributed to the research and implementation of salt reduction strategies, which are approaches based on scientific principles that facilitate reducing salt in foods. Most of these strategies are based on; the chemical stimulation of another ingredient, optimisation of salt release through product or salt particle structure re-design, or cognitive mechanisms to increase awareness or alter preference or perception of saltiness (Busch et al., 2013). Further details on salt reduction strategies are outlined in section 1.8.

Still, there is concern within the food industry regarding the barriers to reducing salt further to meet the continual targets. Challenges faced by the food industry in reaching targets include maintaining organoleptic acceptability and technical limitations, including impact on shelf-life and,

therefore, food safety (Lacey et al., 2016). Further details on the effects of salt reduction on product properties are discussed in section 1.7. Despite some evidence of success, not all products are meeting salt reduction targets. For example, up to 20 % of products in the crisp and snack food category (Table 1.1) did not meet salt targets in 2018, and therefore are also unlikely to meet new targets set for 2024, and even products currently complying with the 2018 targets will find meeting new targets challenging. Thus, category-specific salt reduction strategies need to be researched and implemented. In order to continue to reduce salt while maintaining consumer acceptance, further research is necessary to find novel ingredients and technologies to further capabilities in the food industry.

The top contributors to salt intake within the UK diet determined by household purchases are; table salt (22.6%), bread (9.6%), sauces and stocks (9.2%), bacon (7.5%), milk (5.8%), cheese (4.9%), breakfast cereals (3.3%) and snack foods such as crisps (3.2%) (Ni Mhurchu et al., 2010). With extensive research previously conducted on salt reduction in bread, cheese and processed meats (Inguglia et al., 2017, Jaenke et al., 2017), much less research has been conducted on snack products. Salt reduction is a major challenge across many categories; however, an additional challenge for snacks such as crisps and peanuts is that the main driver of liking is saltiness (Meullenet et al., 2003). Additionally, snacking has become increasingly common in the last 30 years with an increase of 20 % prevalence (Bellisle, 2014), and in some countries, up

to half of young people's daily sodium intake comes from salty snacks in diets (Ponzo et al., 2015, Timic et al., 2020). More recently, snacking in the home has increased even more substantially due to lockdowns during the COVID-19 pandemic, boosting sales of crisps, savoury snacks, nuts and popcorn, causing an increase of £173 million being spent on these products and 25 million kg more being purchased in 2020 compared to 2019. In 2020 the crisps, savoury snacks and nuts market was valued at nearly £4.47 billion (Mintel, 2021). Therefore, considerable effort is required to achieve further salt reductions to advance previous work in this category. Due to this important gap in research, the current thesis focuses on reducing salt content across snacks products that use salt as a topical seasoning application.

There is also some evidence that food oral processing parameters (saliva and mouth behaviours) can influence individual perception (Heinzerling et al., 2011, Feng et al., 2018, Lawrence et al., 2012b, Jeltama et al., 2015) and thus potentially impacting salt intake (Martinelli et al., 2020, Cattaneo et al., 2019, Veček et al., 2020). However, there is a lack of published literature considering the link between saltiness perception and saltiness acceptance with mouth behaviour classifications (Jeltama et al., 2015). Furthermore, the intrinsic relationships between salivary parameters, salt taste perception and intake are not fully understood. Therefore, in addition to understanding and developing food-centred approaches to reducing salt within foods, it is also vital to understand individual differences in consumer salt perception, sodium intake, table

salt behaviours, and potential reasons behind these variations. These factors are also focussed on within the various chapters presented in this thesis. Therefore, findings could also help inform and develop consumer centred salt reduction interventions and further inform food and ingredient design for sodium reduction.

The next section in this chapter introduces sodium chloride and its functions in the body, discusses the different stages of saltiness perception and presents the influence of individual factors on saltiness perception. It also discusses the role of salt in processed foods and explores the literature on salt reduction strategies.

1.2 Sodium chloride salt, its function within the body and health implications related to overconsumption

NaCl salt is commonly known as 'table salt' due to its familiar presence at the dinner table during meals. It is a mineral that comprises sodium

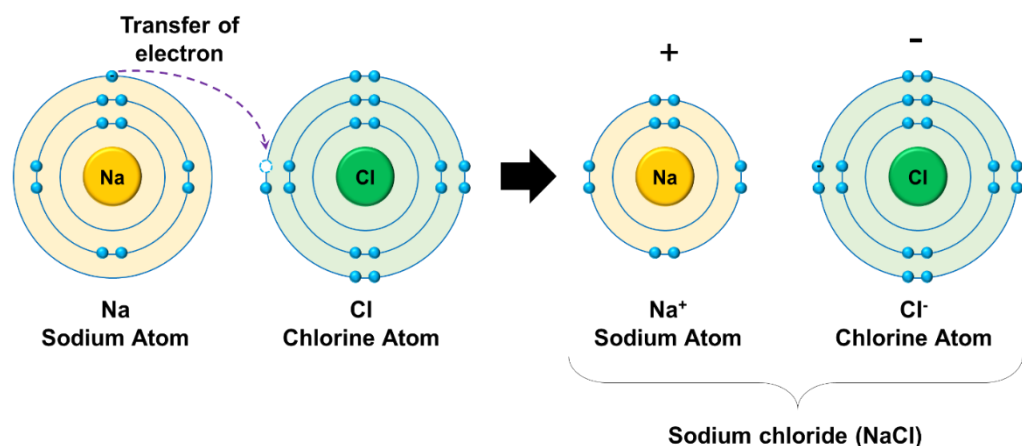


Figure 1.1 Formation of sodium and chloride ions through the transfer of electrons from the valence shells in the sodium atom to the chlorine atom forming a complete octet configurations.

and chloride ions to form a crystal lattice (defined as a symmetrical three-dimensional arrangement of atoms inside a crystal). This is formed when a sodium atom donates an electron to a chlorine atom to form an octet configuration (shown in Figure 1.1), forming a sodium cation (Na^+) and a chloride anion (Cl^-). Due to the opposite charges, electrostatic forces form between the ions, and complete valence shells (outermost shell of an atom) are formed and thus are more energetically stable than when the elements are in the atomic state.

Sodium ions make up 39 % of the sodium chloride crystal lattice, and once inside the body, sodium plays a vital role in maintaining osmotic pressure in blood and tissues. At the same time, the chloride ions that make up 61 % of salt are required for acid/base homeostasis in the body (Chen and Engelen, 2012). While a small amount of dietary sodium (around 500 mg/day of according to the American Heart Association) is vital for normal bodily physiological functions (American Heart Association, 2021), a surplus of sodium is associated with high blood pressure, increasing the risk of stroke, cardiovascular disease, kidney diseases, osteoporosis, and stomach cancers (He et al., 2010).

During digestion, sodium is absorbed and released into the blood. Due to the osmotic gradient, water diffuses into the bloodstream, increasing blood volume and blood pressure (Grillo et al., 2019). Over time, this increase in blood pressure contributes to damage to the blood vessel walls, subsequently increasing the risk of plaque deposits on the walls, leading to the heart needing to work harder (O'Rourke, 1990). In addition

to cardiovascular issues, this increase in blood pressure can also contribute to kidney disease since arteries around the kidneys can become damaged, weakened, narrowed and hardened, kidney function is reduced since there is a reduction in the efficiency of blood delivery to the kidneys. Since the kidney's role is to filter waste and remove extra fluid from the blood, this reduction in kidney function can further increase blood pressure (Safar et al., 2004). In terms of stomach cancer, it is thought that salt is a gastric irritant that damages gastric mucus facilitating the multiplication of epithelial cells, increasing the chance of mutations, with other mechanisms previously speculated (Wang et al., 2009).

The human body has evolved to use taste to identify nutrient-rich foods resulting in the consumption or avoidance of specific nutrients to maintain a constant balance for health (Breslin, 2013). For example, potential toxins are avoided in large quantities as they commonly have a bitter taste (Peyrot des Gachons et al., 2011). In the case of salt, the tongue's ability to perceive saltiness is an essential physiological mechanism to ensure rapid recognition of external sodium in case of a sudden deficit due to illness or dehydration (Kilcast and Angus, 2007), with salt appetite heightened during a sodium deficit, to ensure physiological balance (Daniels and Fluharty, 2004).

1.3 Gustatory (taste) physiology

Gustatory taste is just one of the senses which contributes to the overall perception of flavour. Taste, olfactory aroma perception and trigeminal sensations all combine at the cognitive level to provide an overall flavour impression of the food being consumed. Taste is comprised of 5 modalities detected by the tongue: sweet, salty, umami, sour, and bitter. Specific non-volatile chemical compounds (also called tastants) dissolve in saliva and stimulate the gustatory system through exposure to the papillae found on the tongue, throat and soft palate (Figure 1.2). There are three different types of these papillae responsible for taste perception, including circumvallate, foliate and fungiform (Chandrashekar et al., 2006). All have different characteristic structures shown in the centre of Figure 1.2.

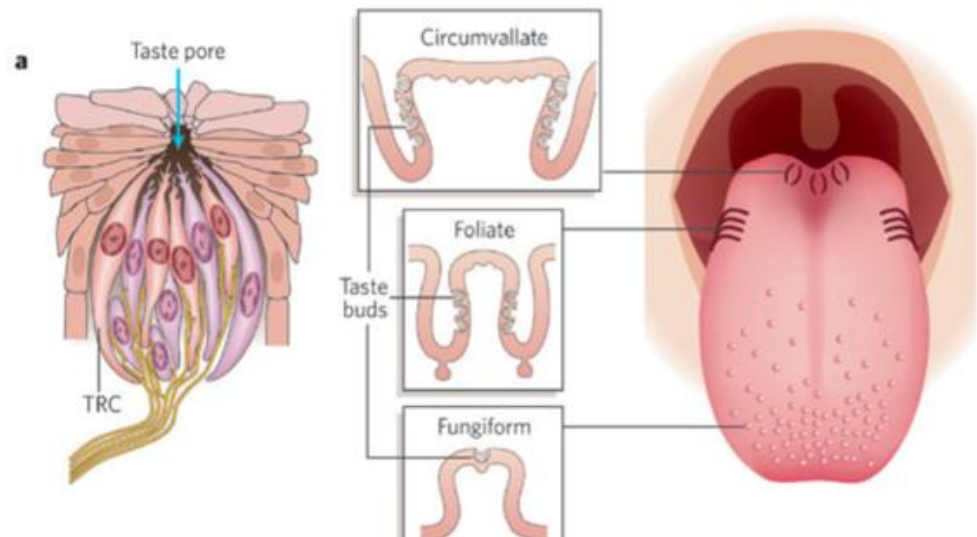


Figure 1.2. Diagrams show the location of taste buds within papillae (circumvallate, foliate and fungiform) on the mammalian tongue and the structure of the taste bud (left). TRC (left) stands for taste receptor cell. [Adapted with permission from “The receptors and cells for mammalian taste” by J. Chandrashekar et al., 2006, *Nature*, 444, p288. Permission conveyed through Copyright Clearance Center, Inc.].

Humans have an average of 5000 lingual taste buds distributed across the papillae structures on the tongue, however this number varies highly between individuals (taste bud structure found on the left in Figure 1.2) (Roper, 2017). Taste buds consist of up to 100 taste receptor cells (TRCs) with microvilli that protrude to the taste bud's apical surface, forming a taste pore (Kinnamon, 2012). Tastants require saliva for dissolution and delivery to the taste pore sites in order to bind to the receptors embedded within the TRC membranes. The proteins and channels found within these cell membranes facilitate taste transduction. They include; G-Protein Coupled Receptors responsible for the transduction of sweet, bitter and umami stimuli (Sanematsu et al., 2014); acid-sensitive membrane channels responsible for sour transduction

(Roper, 2007) and epithelial sodium ion channels (ENaC) control salty taste transduction (Chaudhari and Roper, 2010). Sweetness is stimulated by sugars such as sucrose or glucose, acids like citric acid stimulate the sour taste, bitter is typically perceived due to caffeine or quinine and umami by amino acids such as monosodium glutamate. Sodium-containing salts typically stimulate salty taste; however other ionic salts, containing cations such as potassium, calcium, magnesium and lithium also can elicit a salty taste, with sodium and lithium providing the highest saltiness with the lowest levels of bitterness compared to other salts (Murphy et al., 1981, Lawless et al., 2003). This thesis will focus on sodium (from NaCl) perception since it is the most commonly used salt in the food industry as it does not provide any off-notes like other ionic salts (further discussed in section 1.8.3). In the next section (1.4), the mechanism of salt taste perception is discussed in more detail.

1.4 The three stages of saltiness perception

A three-stage model of saltiness perception from a food matrix was first proposed by Kuo and Lee (2014) (Figure 1.3). For the final saltiness to be perceived by the consumer, sodium and chloride ions must first be released from the food matrix to be made available for dissolution into the saliva. Secondly, sodium must then be delivered through diffusion from the food structure or boli to the taste pore site where the TRCs are located on the tongue via the saliva. Finally, sodium must passively diffuse from the saliva in the oral cavity through the ENaC's to generate

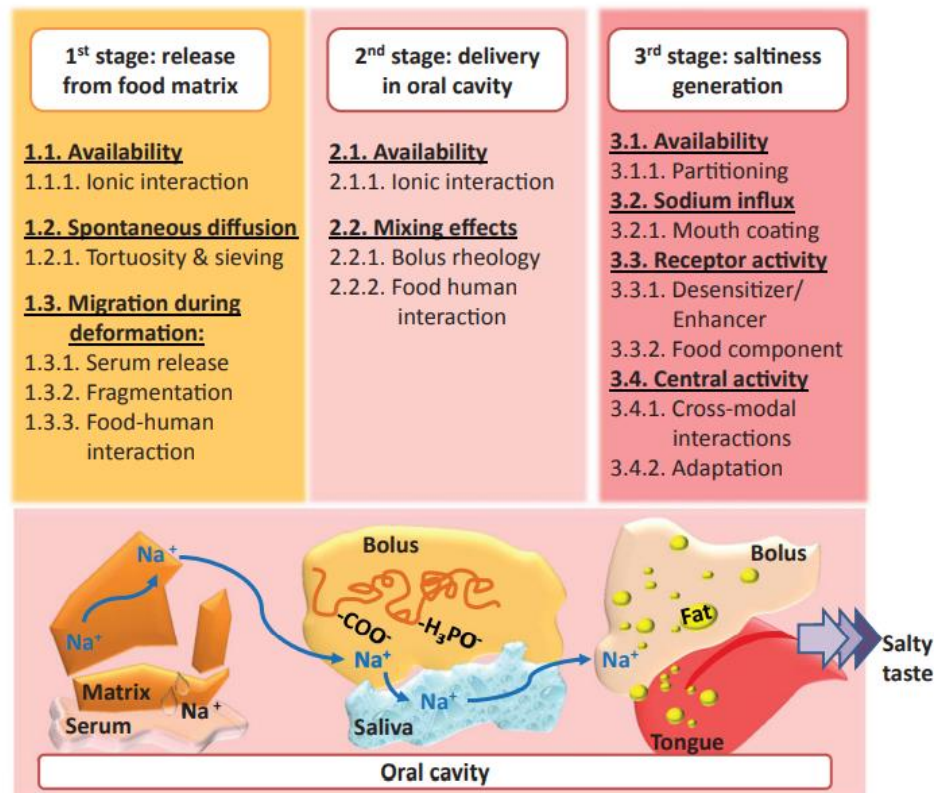


Figure 1.3. Schematic diagram showing the three stages of saltiness perception, including; 1) release of salt from the food matrix, 2) delivery of salt to taste receptor cells within the oral cavity and 3) generation of saltiness signals. [Republished with permission from “Effect of Food Matrix on Saltiness Perception—Implications for Sodium Reduction” by Y. Lee and W-Y. Kuo, 2014, *Comprehensive Reviews in Food Science and Food Safety*, 13, p906-923. Permission conveyed through Copyright Clearance Center, Inc.]

an action potential leading to signal transmission to the central nervous system (more details in section 1.4.3). This final impression of the saltiness of food depends on the efficiency at each stage in the model. The following sections will outline in more detail these three stages. Depending on the food’s physical state and location of salt within the food, some stages in the model are not applicable. For example, sodium found in liquid foods such as soup or model solutions is already dissolved within the water phase and therefore does not need to be released from the food matrix (stage 1), but it must diffuse from the liquid matrix to the saliva (stage 2). Thus saltiness perception of liquids is primarily driven

by the final two stages of the saltiness perception model (Kuo and Lee, 2014).

1.4.1 Stage one: Sodium release from the food matrix

Sodium is released in the mouth from the food matrix or its crystalline structure (depending on the method of application) through diffusion and/or convective transport. The rate of diffusion is mainly driven by the sodium concentration gradient across the food boundary layer, and sodium movement through convective transport is driven by outward serum flow due to the compression of the matrix during eating (Kuo and Lee, 2014). Serum is the liquid released from foods when compressed (either outside or inside the mouth); for example, liquid serum is released during compression of gel microstructures and meat containing tastants like sugars or salts, which helps facilitate the release of these tastants from the food matrix for perception. An increase in serum release will likely enhance saltiness perception (Sala et al., 2010, van den Berg et al., 2007). These findings were mainly determined in gel products, and therefore the effect of serum release on saltiness perception isn't applicable to dry snack foods.

Various factors have been found to impact the rate of sodium release from the food matrix, including; interactions between sodium and proteins (Boisard et al., 2014), structural properties such as tortuosity (Guinee, 2004), the surface area of food fragments (Koliandris et al., 2008) and salt crystals (Emorine et al., 2014, Rama et al., 2013) and food oral

processing parameters of the consumer such as salivary parameters and mastication (Phan et al., 2008).

For topically applied salt systems, e.g. salted potato crisps or salted peanuts, the salt is located mainly on the product's exterior in its original crystalline structure or modified salt crystal matrix. Therefore, sodium is more readily available to be released from the salt crystal for stage 2 than sodium bound within the food matrix. However, it is still a critical stage for determining the perceived salt level of foods as the release rate from the salt particle can influence saltiness perception. More details are outlined in section 1.8.4, which considers the optimisation of salt dissolution, as a salt reduction strategy.

1.4.2 Stage two: Delivery of sodium to the receptor cells

After the sodium has been released from the food matrix, sodium must be delivered to TRC's for the third stage of saltiness perception (taste transduction). Sodium delivery to TRC's is driven by convective mixing of saliva and food bolus (Ferry et al., 2006) and the migration of sodium via diffusion within saliva along the taste pores. During stage 2, saliva is becoming incorporated into food particulates to form a food bolus (see Figure 1.3). Similar to stage 1, interactions with other food components (e.g. polymers or proteins) within the bolus and interactions between matrix and food oral processing parameters impact the availability of sodium for passive diffusion to TRCs. At this stage, any resistance to bolus-saliva mixing caused by bolus rheology can impact the delivery of

sodium through saliva to TRCs, while salivary parameters are the major oral parameters that may affect sodium delivery (Kuo and Lee, 2014). Section 1.5 will cover the latter in more detail.

1.4.3 Stage three: Generation of saltiness signals

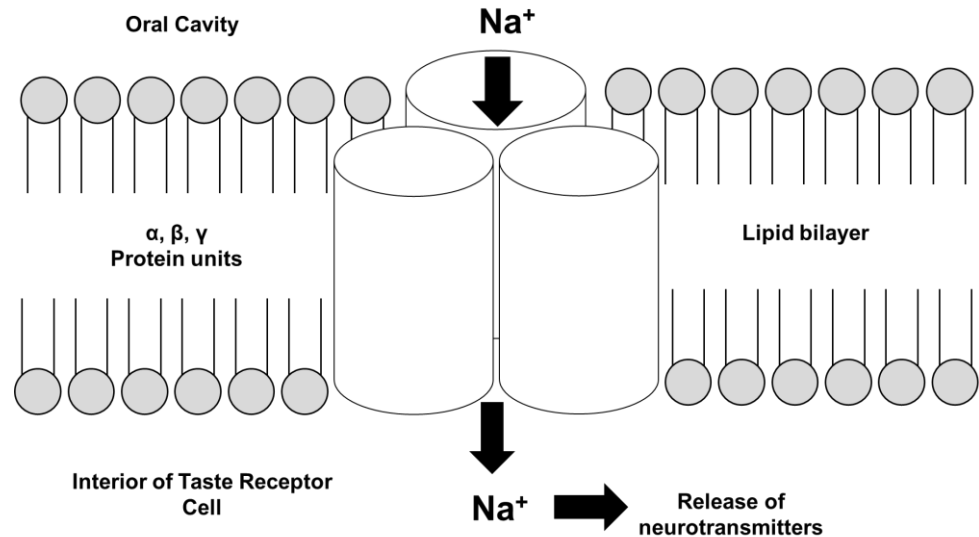


Figure 1.4. Schematic diagram of epithelial sodium ion channel within the epithelial membrane of taste receptor cell.

Finally, sodium passively diffuses through ENaC's (Figure 1.4) into TRCs down the sodium concentration gradient. This sodium influx causes depolarisation within the cell, causing a cascade of transduction pathways, sending signals conveyed by the chorda tympani nerve, finally forming a neural response in brain regions (Lee and Owyang, 2017). This channel, highlighted as the primary transduction mechanism of salty taste, is known as the amiloride-sensitive pathway (Heck et al., 1984). However, there is some uncertainty around whether amiloride-sensitive ENaC channels are the only mechanism explaining salt taste perception in humans since amiloride does not block all salty taste (Ossebaard et

al., 1997, Ossebaard and Smith, 1995). Therefore, an amiloride-insensitive pathway has also been proposed (Lewandowski et al., 2016) and is suggested to be dependent on the size of the anion present. It is thought that anions (the negative ions, e.g. chloride in sodium chloride salt) contribute to the salty taste in terms of intensity. For example, sodium chloride has a stronger intensity than sodium sulphate due to the size of the anion reducing the movement of sodium ions to the taste bud (Roper, 2007).

At this third and final stage, factors that can impact the generation of saltiness signals include; availability of sodium (a common factor throughout all three stages) (Metcalf and Vickers, 2002), sodium influx driven by concentration gradient across the TRC membrane and inhibition by fat barriers (Metcalf and Vickers, 2002), cellular activity (Melis and Tomassini Barbarossa, 2017) and central activity at the brain level (Nasri et al., 2011)(Figure 1.3). Once a signal is initiated within the TRC, the transmitter is relayed along the chorda tympani nerve or the glosso-pharyngeal nerve up to the central nervous system (Taylor and Roberts, 2004). However, this signal is not final in the perception of saltiness; the perception can still be altered at the cellular or cognitive level. For example, activity at the cellular level, chemical desensitizers or enhancers may block or trigger further saltiness perception (Melis and Tomassini Barbarossa, 2017). More information on cross-modal interaction at the cognitive level affecting saltiness perception is discussed in sections 1.7.1 and 1.8.3.

1.5 Impact of consumer oral processing parameters on salt taste

Section 1.4 introduced the idea that oral processing parameters, including salivary properties and mastication, can impact salt taste perception (Lawrence et al., 2012b) with the impact of these factors extending to aroma perception (Salles et al., 2010). Furthermore, the texture or addition of other tastants in foods can manipulate chewing activities and salivary parameters, thus impacting flavour perception, including salt taste perception. There is high variability in food oral processing parameters between individuals due to differing masticatory efficiencies, denture status, oral physiology, salivary composition and flow rate or even individual preferences in the way individuals like to manipulate their foods (Ketel et al., 2020, Jeltema et al., 2015, Gittings et al., 2015, Feron, 2019). Individual variations in these parameters may also impact the efficiency of flavour, tastant release and thus overall flavour perception. This current section will introduce saliva as a biological fluid and its role within the oral cavity, specifically its role in taste transduction, then, it will go into further detail on specific salivary parameters and their influence on saltiness perception. Similarly, the role of mastication on food oral processing and its effect on saltiness perception will also be discussed.

1.5.1 Saliva

Saliva is an aqueous fluid secreted into the oral cavity. It has various functions (Figure 1.5) including; lubrication of the mouth for comfort and ease of swallowing, buffering capacity to maintain pH balance preventing acidic build-up, which could contribute to tooth decay; protection against infection through its anti-viral, anti-fungal and anti-bacterial properties; initiates the digestive process through the activity of enzymes such as

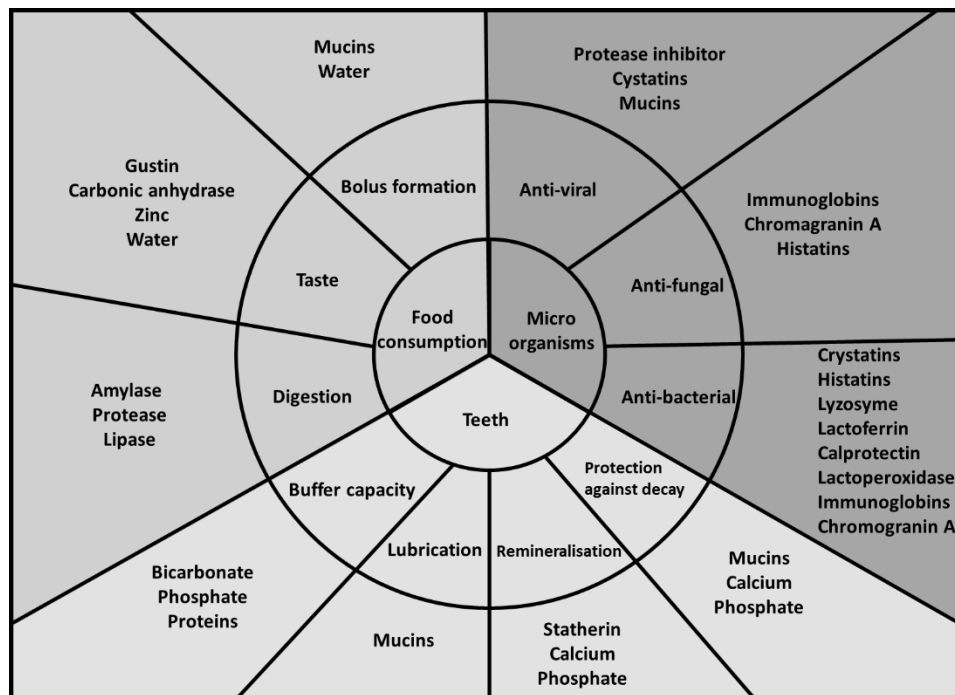


Figure 1.5 Diagram displaying the many functions of saliva in the oral cavity (the middle ring) split into three main areas; food consumption, microorganisms and teeth. The outer boxes outline the constituents of saliva that contribute to each specific function. Information obtained from Uneyama et al. (2009).

alpha-amylase and ensuring tooth integrity through remineralisation, among others (Edgar et al., 2012).

Saliva is produced and secreted from many locations within the oral cavity, including three major glands; the parotid (20%), sublingual (5%)

and submandibular (65%). Minor glands can also be found in the tongue and cheeks mucosa lining, which secrete as little as 10 % of the total saliva (Chen and Engelen, 2012). The saliva secreted from different glands vary slightly in their composition (Taylor and Roberts, 2004), however, on average, saliva is composed of 98 % water and 2 % inorganic and organic substances, e.g. electrolytes, mucus, glycoproteins, proteins, antibacterial compounds, enzymes. Electrolytes found in saliva, including; sodium, potassium, calcium, chloride, magnesium, bicarbonate and phosphate (Chen and Engelen, 2012) form a hypotonic medium to facilitate taste perception (Edgar et al., 2004). The hypo tonicity of saliva is created at the salivary ducts (Figure 1.6). Primary isotonic solution is obtained from the blood plasma and has a very similar composition to blood plasma. As the primary saliva secretion travels through the lumen of the salivary duct, changes are made to the concentration of the saliva through ion exchange at the striated and excretory ducts to make it hypotonic to enable detection of salt at low concentrations. Sodium and chloride ions are removed by the striated duct cells, and potassium is pumped into the lumen to form the final hypotonic saliva (lower osmotic pressure than plasma) (Figure 1.6) (Proctor, 2016).

There are typically two states of saliva, unstimulated and stimulated saliva. Unstimulated saliva typically refers to the saliva at rest, i.e. when

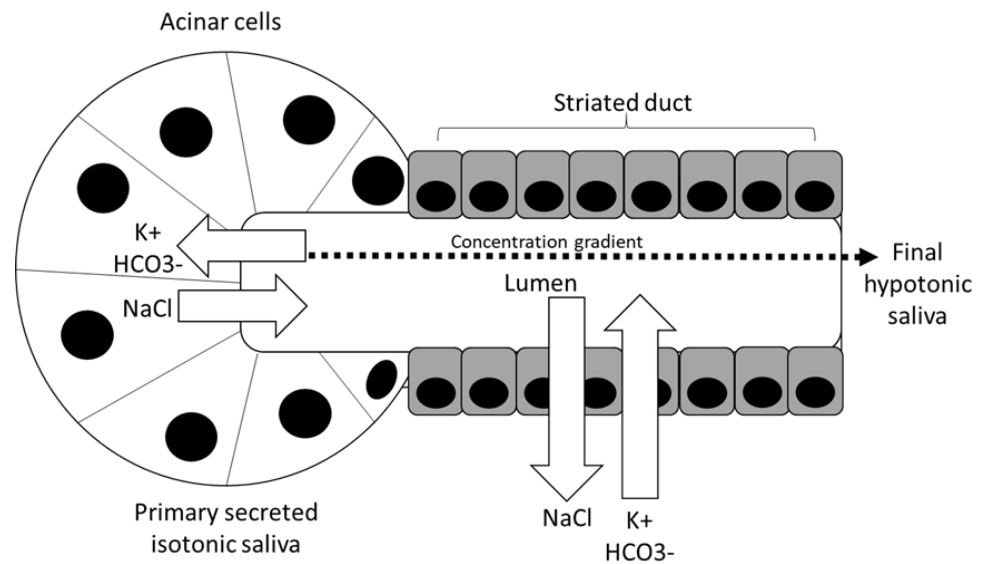


Figure 1.6. Schematic diagram of salivary duct.

no mechanical (chewing) or chemical (trigeminal, taste or visual) stimulation occurs. Reabsorption of sodium by glandular ductal cells at rest provides typically a sodium concentration of 29.9-373.3 ppm, according to sources detailed in a review by Feron (2019). Salivary secretion is controlled by the autonomic nervous system and is altered by both the sympathetic and parasympathetic systems, but predominantly by the latter, at rest. Secretion of saliva is a reflex reaction stimulated most commonly by chewing foods or chemical stimulation by compounds in foods to prepare for eating. Chewing (i.e. the movement of the jaw and tongue to manipulate food) stimulates the receptors in the periodontal ligament, and cues are sent to the brain and integrated along with taste and smell cues from the tongue and nose, signals are then sent to the salivary glands to initiate secretion (Chen and Engelen, 2012). Due to this reflex, the salivary flow rate increases to around 0.5-3.5 ml/min upon mastication (Gittings et al., 2015), and a consequence of

this increase in flow rate is that sodium concentration is also impacted. Sodium concentration increases during chewing, becoming less hypotonic (more similar to blood plasma levels) due to the reabsorption of salts not being upregulated during stimulation (Chen and Engelen, 2012). While saliva volume changes are similar across type of stimulation (chewing vs acidic solution), changes to saliva composition depend on the type of stimulation. For example, chewing stimulation provides a relatively inelastic saliva and stimulation by acidic solutions like citric acid provides a saliva secretion that is highly elastic (Stokes and Davies, 2007).

Since saliva facilitates food oral processing and acts as a solvent for tastants to allow for perception at the taste receptor cells, saliva is also an important component to evaluate when studying saltiness perception and could contribute to the individual variation in perception and potentially sodium intake. The following section will discuss the approaches to saliva collection for subsequent saliva analysis in order to be able to relate salivary parameters to taste perception. Subsequently, evidence will be discussed around the impact of salivary properties and constituents on saltiness perception.

1.5.1.1 Methods of saliva collection for analysis

Since saliva has an essential role in food oral processing, various methods of salivary collection have been described previously that can be used depending on researcher's aims, budget, time and expertise.

Table 1.2 describes each method with information on the advantages and limitations. Considering these points, in chapter 3, this thesis obtained unstimulated saliva using the passive drool method and stimulated saliva using mastication of inert parafilm (as it is readily available) and spitting. These methods were primarily chosen as they were not invasive and did not require extensive training for use like some of the devices used in the suction methods (Table 1.2). They also ensured that flow rate can be calculated easily without contamination of other materials and allows for the collection of the whole saliva rather than from a selected gland.

Table 1.2. Approaches to saliva collection for analysis.

Method	Method outline	Advantages	Limitations	Sources
Passive drool/ draining	<ul style="list-style-type: none"> Participant leans over the collection vessel while sitting with their mouth slightly open, allowing the saliva to drool into the vessel, without any stimulation. This method provides a sample of unstimulated saliva (resting) 	<ul style="list-style-type: none"> Collection of whole saliva Non-invasive Researcher able to alter collection time depending on amount of saliva required for analyses 	<ul style="list-style-type: none"> Long sampling duration due to low unstimulated flow rates Participants must understand protocol and not force saliva to spit out Some contamination of micro-organisms and debris from the mouth 	(Bellagambi et al., 2020)
Mastication of inert substance and spitting	<ul style="list-style-type: none"> Participant leans over the collection vessel while chewing a piece of inert substance such as parafilm or dental wax, exporating into the collection vessel when they feel the need to 	<ul style="list-style-type: none"> Materials are widely available Collection of whole saliva Non-invasive Researcher able to alter collection time depending on amount of saliva required for analyses Mimics the oral movements of the mouth during consumption; therefore researcher can estimate the parameters during eating 	<ul style="list-style-type: none"> Some contamination of micro-organisms and debris from the mouth Participants must comply with the chewing of parafilm in the correct way 	(Bellagambi et al., 2020)
Expectoration after food oral processing	<ul style="list-style-type: none"> Participant chews sample for a determined amount of time and boli is spat out Saliva incorporation is determined by gravimetrical analysis and drying of the boli in an oven 	<ul style="list-style-type: none"> Amount of saliva incorporated into food bolus can be determined during actual eating of real-life food 	<ul style="list-style-type: none"> Considerable contamination of saliva from food material Requires extensive extraction from bolus for further analysis Invasive and may be unpleasant for panellists 	(Devezeaux de Lavergne et al., 2017, Devezeaux de Lavergne et al., 2015)
Simple suction method	<ul style="list-style-type: none"> Saliva is allowed to accumulate in the oral cavity and continuously removed using a micropipette, syringes, saliva ejector or an aspirator 	<ul style="list-style-type: none"> Collects whole saliva This suction method requires less training and expertise by the researcher to attach the collecting device to the opening of the ducts 	<ul style="list-style-type: none"> Some contamination of micro-organisms and debris from the mouth Fairly invasive 	(Bellagambi et al., 2020)
Suction (use of suction device)	<ul style="list-style-type: none"> A device such as a Lashley cup (Figure 1.8) or modified Carlson Crittenden device. The device cup is placed over the ductal opening, and saliva is sucked out using vacuum pumps, dental suction unit or similar. 	<ul style="list-style-type: none"> Isolates and collects saliva secreted from the parotid gland (selective sampling) Saliva less contaminated by any residues or debris or microorganisms in 	<ul style="list-style-type: none"> Complex, slow and Invasive sampling Requires training and expertise by the researcher to attach the collecting device to the opening of the ducts Longer sampling times Possible salivary gland injuries 	(Bellagambi et al., 2020, Heinzerling et al., 2011)

Chapter 1: Introduction to thesis and literature review

		<p>the mouth as it is collected straight from the salivary gland</p> <ul style="list-style-type: none"> • Device can be modified to remove saliva and replace saliva using a controlled composition and flow rate when a researcher wants to control these parameters in an experiment. 	<ul style="list-style-type: none"> • Complex procedure 	
Swabbing	<ul style="list-style-type: none"> • A synthetic gauze sponge, pre-weighed swab or cotton pads are placed into the mouth where major salivary glands are. • Participants may be asked to chew on the material to soak it with saliva. 	<ul style="list-style-type: none"> • Low cost, easily available and easy to use and handle for both researcher and for participant • Possible to estimate flow rate if using gauze or cotton pad • Versatile use of cotton bud swab– can be performed in most places within reason for the collection of unstimulated or stimulated saliva or collection of saliva during food oral processing 	<ul style="list-style-type: none"> • Not able to calculate flow rate if using a pre-weighed cotton swab • Potential retaining of salivary components by the swab • Risk of swallowing part of the gauze or cotton pad • Unpleasant for participants if using gauze or cotton pad • Maximum absorption of the swab might be exceeded 	(Bellagambi et al., 2020)
Filter paper (periopaper)	<ul style="list-style-type: none"> • Filter paper is placed on an area of the mouth (usually in an area where minor glands are) and soaks up saliva until paper is saturated. 	<ul style="list-style-type: none"> • Non-invasive sampling • Low cost, readily available and easy to use and handle for both researcher and participant 	<ul style="list-style-type: none"> • Small volume collected • Potential contamination from the collection paper • Maximum absorption of the swab might be exceeded 	(Bellagambi et al., 2020)

1.5.1.2 Salivary flow rate and saltiness perception

Since a functional role of saliva is to act as a solvent for tastants to dissolve and to facilitate transportation of sodium to the TRCs for detection, the presence of saliva is vital for taste perception. Individuals vary highly in their resting and stimulated salivary flow rates both

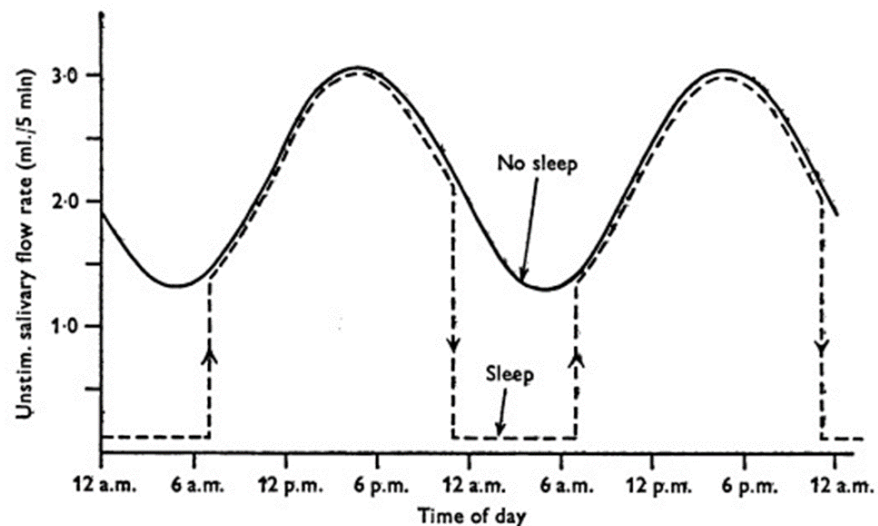


Figure 1.7. Unstimulated salivary flow rate over a 24 hour period. Source: (Dawes, 1972). [Republished with permission from “Circadian rhythms in human salivary flow rate and composition” by C. Dawes, 1972, *The Journal of Physiology*, 220, 3, p529-545. Permission conveyed through Copyright Clearance Center, Inc.].

between and within individuals (Dawes, 1972, Gittings et al., 2015). Intra-individual variation of unstimulated (resting) salivary flow rate is mainly influenced by circadian rhythms (Dawes, 1972), displayed in Figure 1.7. During sleeping hours (11 pm to 6 am), unstimulated saliva is reduced to almost 0.2 ml/min and increases from 6 am to almost 6 pm (late afternoon) when it reaches its peak (average of 0.6 ml/min). Salivary flow rates range from 0.3 to 0.6 ml/min when unstimulated and between 0.5 and 3.5 ml/min when stimulated (Gittings et al., 2015). Other factors apart

from circadian rhythms which can influence unstimulated salivary flow rate include; reduced hydration levels decreasing salivary flow rate (Ship and Fischer, 1997), gender (since males have larger gland sizes contributing to a higher flow rate) (Ono et al., 2007), and ageing, which is commonly thought to reduce salivary flow rate. However, studies on ageing show contradicting results, with many age-related disease states and medications also reducing saliva flow rate (Ship and Fischer, 1997).

Hyposalivation can be defined as a salivary flow rate of $\leq 0.5-0.7$ ml/min (Sreebny and Vissink, 2010) and taste and texture perception is often altered negatively with hyposalivation since foods cannot be manipulated in the usual way in the mouth (Mese and Matsuo, 2007). Sjogren's syndrome is an autoimmune disorder where salivary glands are destroyed by the body's immune system, causing hyposalivation (Mese and Matsuo, 2007). In these cases, patients show a reduction in taste function assessed by salt taste thresholds (Weifenbach et al., 1995). It is hypothesised that this is due to sodium being unable to be dissolved and therefore unable to reach the TRC for passive diffusion resulting in no action potential or nerve signals transmitted to the central nervous system.

Heinzerling et al. (2011) determined that the intensity of saltiness perception significantly decreased when salivary flow rate was increased artificially in humans by removing naturally secreted saliva using two modified Lashley cups attached to parotid salivary glands (Figure 1.8)

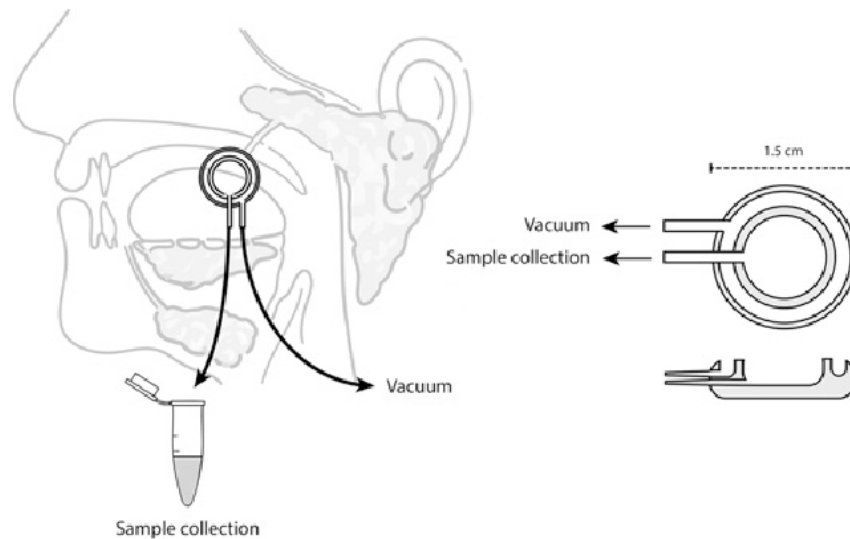


Figure 1.8 Schematic diagram of the use of a Lashley cup to collect saliva from the parotid gland. Source: (Michalke et al., 2016). [Republished with permission from “Saliva as a matrix for human biomonitoring in occupational and environmental medicine” by M. Bernhard, R. Bernd, G. Thomas, S. Anja and S. Gerhard, 2014, *International Archives of Occupational and Environmental Health*, 88, p1-44. Permission conveyed through Copyright Clearance Center, Inc.]

and adding artificial saliva to the oral cavity at a controlled rate. However, a limitation of the study was that the artificial flow rate was set at two times faster than a ‘normal’ flow rate and, therefore, may not be typical of ‘normal’ consumption parameters. In agreement with these findings, Feng et al. (2018) found a trend between individual unstimulated flow rates and salt taste thresholds, meaning that those with a higher unstimulated flow rate have a lower salt taste sensitivity (a higher threshold). These studies provide some evidence that individual differences in saltiness perception can be attributed to variations in

salivary flow rate. This may be explained by the increase in saliva volume available in the mouth to dilute the chemical tastants (sodium chloride and citric acid in this study), therefore reducing the concentration of tastants in saliva, providing a lower concentration gradient across the TRC membrane and subsequently perceived as less intense.

Not only does saliva facilitate taste perception, but it also has long term benefits within the oral cavity as it maintains the health and function of the TRCs, e.g. protecting receptors from infection (Ship et al., 1991). The long term effects of the loss of salivary gland function, resulting in the reduction or loss of saliva in mouth, causes an increase in taste thresholds (the minimum concentration of a tastant required for perception) and reduces taste nerve responses (Matsuo et al., 1997).

1.5.1.3 Salivary ion levels and saltiness perception

As mentioned in section 1.4.3, saltiness perception depends on the concentration gradient across the taste receptor cell membrane. This is because the sodium ions passively diffuse through the ENaC from the saliva found in the taste pore into the intracellular fluid of the TRC. An increase in salivary sodium concentration elevates salt taste threshold reducing the individuals' sensitivity to salt taste which could in turn decrease their perception of saltiness in foods. Delwiche and O'Mahony (1996) established that detection thresholds for salt are slightly above salivary sodium concentration. This is thought to be an adaption to the natural environment (saliva) that the receptor is exposed to in order to

elicit a response. However, evidence was based on; animal studies (Matsuo and Yamamoto, 1992, Rehnberg et al., 1992), patients with xerostomia (Weifenbach et al., 1995) or by artificially changing the natural environment of the oral cavity by oral rinses (O'Mahony, 1972, Heinzerling et al., 2011) and by artificial chewing to raise Na⁺ levels (Delwiche and O'Mahony, 1996) or by depleting sodium levels purposefully (Beauchamp et al., 1990). Therefore may not be truly representative of consumers in a normal environment and food situation, which this thesis explores in Chapter 3.

1.5.1.4 Salivary proteins and saltiness perception

There are up to 3000 proteins that have been identified in saliva, with their primary functions being: the first stage of digestion (i.e. alpha-amylase, proteases and lipases) and protection against infections (i.e. immunoglobulins) (Chen et al., 2012). These proteins in saliva can impact sensory and flavour perception through modulating trigeminal sensations (such as astringency), aroma perception, and taste perception (Canon et al., 2018). Specifically, some proteins identified as potentially important to saltiness perception are; alpha-amylase, endoprotease enzymes, and carbonic anhydrase VI (Ferry et al., 2006, Lamy et al., 2021, Stolle et al., 2018, Stolle et al., 2017).

Firstly alpha-amylase is the most abundant protein within saliva. It has a role in oral clearance and the initial digestion of starch in foods. The presence of alpha-amylase causes fast digestion through hydrolysis of

starch, converting it to maltose which can cause an initial impact on perceived viscosity in the mouth (Sharma et al., 2020). As a result of its role in food digestion, alpha-amylase affects the perception of food texture, but it has also been found to impact saltiness perception. Research has shown that an increase in alpha-amylase activity reduced the saltiness in starch thickened foods (Ferry et al., 2006) and bread (Lamy et al., 2021), the opposite of what was expected (Ferry et al., 2006). This reduction in saltiness with increased alpha-amylase activity was explained in Ferry et al. (2006) by the reduction in mixing efficiency between product and saliva which is caused by high alpha-amylase as mixing efficiency in the mouth highly impacted ion transport between viscous product and saliva, and therefore the TRCs. Conflictingly, Tournier et al. (2014) assessed the impact of alpha-amylase concentration on the salt release from the bolus to saliva phase and determined that the inter-individual differences in alpha-amylase activity did not impact salt release from the bolus; however, it is stated this could be as a result of individuals having similar salivary alpha-amylase concentrations.

The presence of protease enzymes in the saliva is also suggested to influence human salt taste sensitivity. It has been found that protease activity increases salt sensitivity and salt liking (Stolle et al., 2017, Stolle et al., 2018). It is believed that proteases cleave the γ -subunit of the sodium specific channels enhancing trans-epithelial sodium transport (Picard et al., 2008, Hughey et al., 2007, Kleyman et al., 2009). On the

other hand, the enhanced salt sensitivity could be due to protease enzymes breaking down salivary proteins releasing salt taste-enhancing peptides such as arginyl peptides or tetrapeptides (Schindler et al., 2011, Harth et al., 2018, Stolle et al., 2018). However, proteolysis also increases consumer taste sensitivity to other tastants (Dsamou et al., 2012, Mounayar et al., 2013). Thus, another possible mechanism has been hypothesised whereby the presence of the mucosal pellicle, caused by the absorption of salivary components onto the epithelial cells, acts as a barrier to taste receptors, reducing the accessibility of tastants. Proteolysis breaks down proteins within the pellicle, causing it to be thinner/looser, facilitating an increase in taste receptor interactions and therefore increasing taste sensitivity to tastants (Dsamou et al., 2012). Previously, Feng et al. (2018) found consistently negative correlations between tongue film weight and taste sensitivity meaning that those with a high film weight (thicker pellicle film on the tongue) are less sensitive to saltiness on the wafer, which could be a result of a reduction in diffusion through the thicker film as it acts as a barrier to the receptors on the tongue.

Carbonic anhydrase VI (CA6) is a zinc enzyme (also named gustin) that catalyses carbon dioxide's reversible hydration into carbonic acid, vital for regulating pH balance in the body and for the removal of carbon dioxide (Peres et al., 2010). The role of saliva extends to protecting dental tissues through remineralisation, preventing caries, with some indication that it helps the formation of dental calculus in periodontal

disease. In terms of taste, there is some evidence that this isoenzyme participates in the process of taste perception by enhancing taste bud function and salivary buffer capacity (Peres et al., 2010). For example, in a recent publication by Lamy et al. (2021), CA6 expression levels in saliva were positively correlated with the saltiness perception of bread, which could be because CA6 has been described as a trophic factor for taste bud development (Henkin et al., 1999). Similarly, a previous study by Feeney and Hayes (2014) determined that the variation of single nucleotide polymorphism of the CA6 gene responsible for encoding the CA6 protein is associated with saltiness perception, e.g. individuals with specific alleles (AA) of the CA6 SNP (rs3737665; chr 1) perceived salt solutions as more intense, furthering the evidence for individual variability in saltiness perception.

1.5.2 Mastication

Mastication, commonly known as chewing, involves the cutting or crushing of whole food pieces into smaller fragments alongside the incorporation of saliva. The process forms a food bolus that is cohesively mixed and lubricated by saliva. This occurs in preparation for swallowing and for digestion, including the absorption of nutrients from our food. Mastication and the simultaneous incorporation of saliva and lubrication ensure that the bolus can be swallowed safely and comfortably (Prinz and Lucas, 1995). Alongside this, mastication enhances the release of taste and aroma compounds from food structures (Neyraud et al., 2003, van Ruth and Roozen, 2000), thus optimising the experience and

hedonic responses to foods. The chewing process also increases food's surface area, increasing the rate of diffusion of sodium or other tastants. Since mastication increases salivary flow rate, chewing incorporates saliva into the food producing a food bolus, allowing for sodium ions to be released into the saliva and be transported to the TRCs for transduction and perception (stage 2, Figure 1.3). Therefore, individual differences in mastication may alter the amount of saliva incorporation, break down speed and food breakdown capacity, which could cause changes in surface area of bolus or bolus particles, and ultimately affect the perception of taste. For example, Lawrence et al. (2012) assessed mastication of a solid lipoprotein matrix by recording the muscle response on the jaw during chewing of the product using electromyography. This study found that chewing activity is a determinant for sodium release from foods. Individuals who displayed a higher chewing force showed the highest sodium release in the mouth and the highest saltiness perception values than those with a low chewing force. Similarly, when assessing bread products, Tournier et al. (2014) found that increased chewing muscle activity caused a quick initial sodium release rate while a prolonged chewing length initiated a later sodium release. It is suggested that these findings are due to the increase in product breakdown and, therefore, an increase in surface area of the food matrix caused by the increase in chewing force.

Electromyography and particle size of dental silicone after a set number of chewing cycles are common ways to assess mastication behaviour separately. However, another way to evaluate mastication behaviour is the determination of individual mouth behaviour preferences through a validated self-report questionnaire using a series of questions and a graphical tool such as that shown in Figure 1.9. Research suggested that individuals choose a chewing/mouth behaviour type that provides them with flavour intensity and texture that they seek from a product (de Wijk et al., 2003). Thus the JBMB typing tool™ was developed to classify these individuals into four different key mouth behaviour groups, which are; chewers (prefer to chew foods), crunchers (prefer to crunch foods), smoothers (prefer to smooch foods) and suckers (prefer to suck on



Figure 1.9 Graphic Mouth Behaviour Tool (JBMB™) used to help classify individuals into four mouth behaviour groups (chewers, crunchers, smoothers and suckers). Graphic sourced from: (Jeltema et al., 2015). [Republished with permission from “Model for understanding consumer textural food choice” by M. Jeltema, J. Beckley and J. Vahalik, 2015, *Food Science & Nutrition*, 3(3), p202-212. Permission conveyed through Creative Commons].

foods) (Jeltema et al., 2015). For some foods, a particular way of eating may be required, e.g. for meat, it needs to be chewed extensively before chewing. Therefore it is thought that each mouth behaviour type brings their mouth preference to said food. In this case, crunchers may typically chew with a harder force than a chewer, or when a fruit needs to be chewed, a sucker may spend more time sucking to release the juice before chewing (Jeltema et al., 2015). Since individual's vary extensively in the way they manipulate food, including snack foods in their mouth (shown by both measurements of electromyography and assessed qualitatively through focus groups and online questionnaires), it is interesting to investigate whether individual differences contribute to the saltiness perception and thus consumer liking of products (Franks et al., 2020). When assessing chewing behaviours, the use of electromyography is a complex procedure and requires extensive training for the researcher and necessary time to train assessors on the procedures with relatively high cost. Meanwhile, using the validated mouth behaviour typing tool allows the researcher to conduct large-scale consumer segmentation online compared to direct mastication measures. To also reduce close contact with assessors due to the COVID-19 pandemic, the mouth behaviour typing tool was used as a technique to classify consumers by their masticatory behaviours in chapter 4.

1.6 Other influences of individual variation in salt taste perception

It should be mentioned that the factors presented in the previous sections are not the only factors that determine individual taste perception (including saltiness perception). Other factors that influence taste perception and sensitivity include; differences in physiology in the gustatory system, cognitive processing differences, genetics and environment (Puputti et al., 2019). Intrinsic factors also can affect individual sense of taste, for example:

- Gender: males are generally less sensitive to tastants (Puputti et al., 2019); however, this is not always true for salty taste (Martin and Neyraud, 2021).
- Age: decline in taste sensitivity with ageing due to physiological changes in the sensory organs, factors related to ageing such as disease status and behavioural factors such as medications (Ogawa et al., 2017).
- Taster status: Sensitivity to the bitterness of 6-n-propylthiouracil (PROP) results from a single nucleotide polymorphism (SNP) on the TAS2R38 bitter receptor gene (Un-kyung et al., 2003). Intriguingly, individuals classified as PROP super-tasters also have elevated oral sensations to other tastes (Yang et al., 2014), including saltiness (Hayes et al., 2010).

- Ethnicity: some ethnicities have been found to perceive taste sensations as higher than others, e.g. in Williams et al. (2016), Hispanics and African Americans rated taste sensations higher than non-Hispanic Whites, with the reasons behind this remaining unclear.
- Extrinsic factors such as smoking, disease, weight, and medication can also play a role. However, the relationship between these factors and taste function is controversial, and some studies show they do not have an effect (Fischer et al., 2013, Konstantinidis et al., 2010, Methven et al., 2012a, Mojet et al., 2003, Pepino et al., 2010).

The consumption level of dietary sodium sources also has been found to affect consumer sensory perception. For example, in Nguyen and Wismer (2019), individuals were separated into three dietary sodium consumption groups (low, medium and high), and on average, those with the highest intake of sodium sources had the highest detection thresholds of sodium chloride. The low intake group had the lowest detection thresholds. Since a low detection threshold indicates a high sensitivity to salt taste, this suggests that those exposed to lower amounts of sodium-containing foods have a higher sensitivity to salt taste, or vice versa (high sensitivity leads to a lower salt intake). However, these findings on the association between salt intake and salt taste sensitivity (which can be assessed either by threshold levels or saltiness intensity scales) are contradictory (Pangborn and Pecore,

1982, Martinelli et al., 2020, Veček et al., 2020). Furthermore, it is also difficult to decipher which factor is the cause and effect, i.e. does eating a higher number of high sodium-containing foods impact salt taste thresholds and perception or does your salt taste threshold or level of perception influence the amount of sodium-containing foods consumed? One study suggested that a low sodium diet over several weeks (8-12 weeks) enhances the liking towards reduced-sodium foods (Mattes, 1997); however, there is limited evidence linking salt taste sensitivity and intake (Tan et al., 2021).

1.7 The function of salt in processed foods

As previously mentioned, 75-80 % of salt consumed is from processed foods (Gibson et al., 2000), with the largest contributors of sodium in the UK market being: processed meat, bread and bakery products, dairy, snack foods and sauces and spreads (Ni Mhurchu et al., 2010). On the other hand, only 20-25 % of salt consumed is contributed by discretionary salt use and the rest is found naturally in foods (Gibson et al., 2000).

Salt is a functional ingredient that has major roles in processed foods, such as: controlling yeast growth and fermentation rate, improving product texture, reducing spoilage by controlling water activity and food preservation (Kilcast and Angus, 2007). Regarding flavour, salt enhances flavour, masking specific unpleasant notes that impact the palatability of bland foods, providing consumer acceptance and often driving liking of products (Li et al., 2015).

The following sections will further explore the functional role of salt in various processed foods, focusing on its contribution to flavour perception, aroma generation, textural properties and food preservation, its functionality in seasonings mixes, and driving consumer acceptance of products.

1.7.1 Role of salt in flavour perception

In conjunction with imparting its characteristic salty taste to foods, salt can also modify the perception of other tastants and enhance overall flavour intensity through tastant interactions (Keast and Breslin, 2003). For example, salt enhances sweetness and sourness perception at specific concentrations (Van der Heijden et al., 1983, Keast and Breslin, 2003) while suppressing bitterness at all concentrations and intensities (Breslin and Beauchamp, 1997). The suppression of bitterness is due to sodium altering the transduction mechanism of bitter taste before the taste signal is transmitted to the central nervous system to be processed; this is called an oral peripheral interaction (Keast and Breslin, 2005). Similarly, findings when assessing interactions between three or more taste qualities; sodium again suppresses bitterness when a bitter-sweet mixture was assessed using sodium as the salt taste, sucrose for sweetness and urea for bitterness (Breslin and Beauchamp, 1997). As a subsequence, the perception of sweetness is also enhanced due to the suppression of bitterness. It is thought that these interactions happen at

the cognitive level and subsequently provide a pleasant and enhanced intensity of overall flavour (Figure 1.10).

The fact that sodium salts, specifically NaCl, can enhance favourable tastes (sweetness) and suppress typically unpleasant tastes (bitterness) is advantageous when food developers attempt to enhance pleasant notes like sweetness or mask typically unpleasant notes like bitterness.

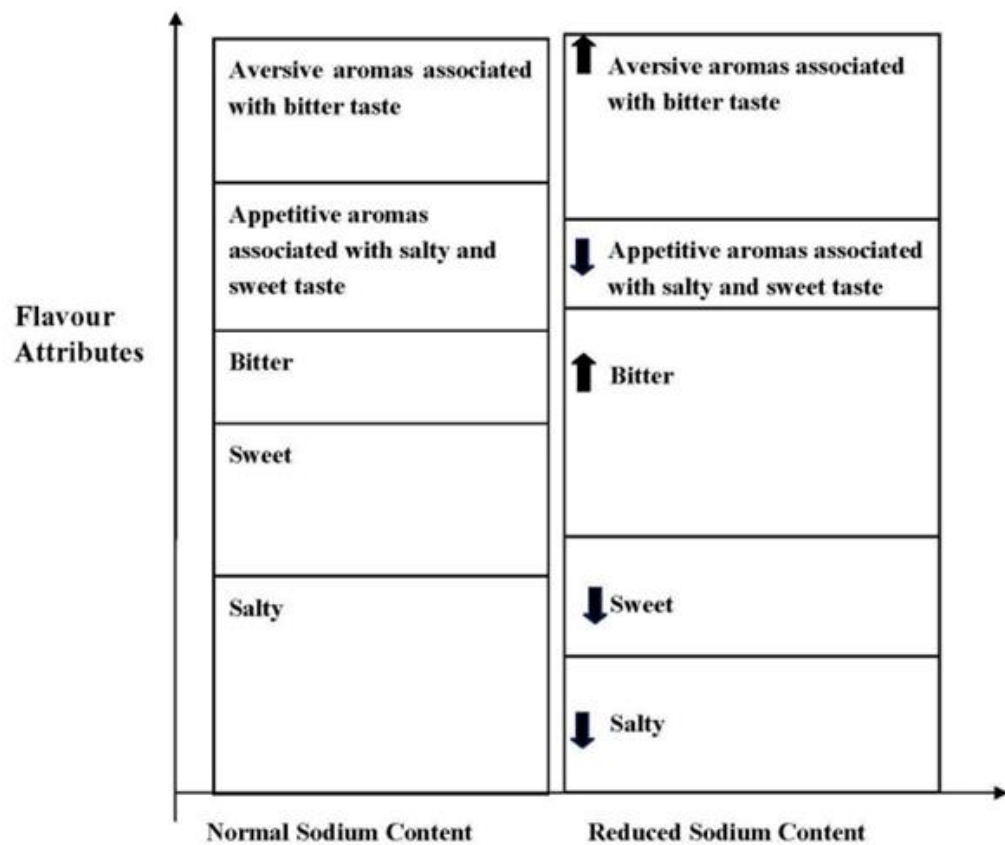


Figure 1.10 The consequence of sodium reduction on other flavour attributes. Source: Liem et al. (2011). [Republished with permission from “Reducing Sodium in Foods: The Effect on Flavor” by D. Liem, F. Miremadi and R. Keast, *Nutrients*, 3(6), p694-711. Permission conveyed through Creative Commons].

As a consequence, attempts to reduce salt in foods often have a deleterious impact on consumer on consumer acceptability and experience, for example; there are a number of implications of sodium reduction on products (Figure 1.10), including a reduction in sweetness,

saltiness and pleasant appetitive aromas associated with these attributes and increased bitterness and aversive aromas associated with bitterness (Liem et al., 2011).

Cross-modal sensory perception is a term used to describe the mechanism of the 5 senses (sight, smell, taste, touch and hearing) interacting in a complex way within the brain as they are simultaneously processed. It is the process of the brain interpreting the mixture of these signals and converging this information into one overall taste sensation (Knoop, 2011). This process explains how the taste of salt can enhance perceived aroma intensity and overall intensity and enhance other taste modalities, and vice versa. In a cheese matrix, salt significantly enhances the perception of cheese flavour intensity (Niimi et al., 2014) and the overall flavour intensity, broth-like intensity, and mushroom flavour of a broth model system (Ventanas et al., 2010). These cross-modal interactions can be leveraged as a sodium reduction strategy since savoury aromas can also enhance the perception of saltiness (see section 1.8.3).

1.7.2 Role of salt in aroma generation and release

Separate from the impact salt taste has on aroma and flavour perception through cross-modal interactions, it is also known that salt contributes to both the generation and/or the release of aroma compounds in some foods, such as fermented sausages (Corral et al., 2013), bread (Belz et al., 2017, Raffo et al., 2018), fermented soy sauce (Devanthi and

Gkatzionis, 2019) cheese (McMahon, 2010, Saint-Eve et al., 2009) and sweet biscuits (Ayed et al., 2021).

As an example, in the case of sweet biscuits, this product is not necessarily regarded as 'salty' and therefore, the salt found within this product is hidden but is still required to maintain properties of flavour (sweetness) and texture (brittleness) (Ayed et al., 2021). One study (Ayed et al., 2021) found that salt reduction of sweet biscuits decreases the concentration of hydrophobic volatiles in the headspace available for perception, explained by the change in the physical association of the fat in the biscuit (Ayed et al., 2021).

Regarding reactions in foods, it has been found that in bread, salt inclusion enhances the formation of Maillard volatile products and suppresses the formation of 2-phenyl ethanol, which contributes to a yeast note in the crumb of the bread. These findings were confirmed by sensory analysis since a less intense yeasty note in the crumb was perceived (Raffo et al., 2018). Similarly, in another study, Belz et al. (2017) found the formation of this aroma compound is significantly affected by salt level; however, contrary to Raffo et al. (2018) study, samples with differing salt contents and, therefore, an abundance of this aroma compound were not sensorially discriminable. This could be due to the level of 2-phenyl ethanol in the samples being lower than the odour threshold. The impact of salt level on the generation of associated yeast aromas (2-phenyl ethanol) is explained by the inhibition of yeast activity by salt (Lynch et al., 2009), while the promotion of Maillard reaction

products can be explained through several different mechanisms, including; alteration in water activity, moisture content, dough pH and the increase in mobility of reactants due to salt having a plasticising effect in bread. Alternatively, the increase in sugars available for the Maillard reaction as a subsequence of them not being used in the fermentation process due to salt inhibition could promote the formation of Maillard reaction products (Moreau et al., 2009).

Salt also affects the release of aroma compounds from foods into the headspace for olfactory perception due to the 'salting out' process (Guichard, 2002). This phenomenon occurs when salt is added to foods reducing the solubility of certain molecules in a solution. In this scenario increasing the concentration of salt reduces the solubility of volatile compounds in the food matrix, driving them into the headspace (Poll and Flink, 1984). For example, a study by Flores et al. (2007) assessing the headspace concentrations of six aroma compounds in a model Spanish dry-cured meat system (solution-based) found that increasing the salt content in the system increased the concentrations of all aroma compounds in the headspace. These findings translated over to solid food matrices (model cheese systems) and odour perception since higher salt content cheeses had a higher perceived overall odour intensity than the lower salt cheeses (Saint-Eve et al., 2009). However, it should be noted that the extent of these effects depends on the composition of the product studied and the physiochemical properties of the aroma compounds studied (Saint-Eve et al., 2009).

1.7.3 Role of salt in food texture

Salt also assists in developing acceptable product texture in extruded snacks and breakfast cereals, sweet biscuits, cheese and meat, among others (Van der Sman and Broeze, 2014, Guinee and Fox, 2004, Desmond, 2006, Inguglia et al., 2017, Belz et al., 2012, Ayed et al., 2021). Salt affects the physicochemical properties and thermodynamics (the study of the relations between heat, work, temperature, and energy) of foods materials, including glass transition properties (Van der Sman and Broeze, 2014), ultimately impacting the properties consumers perceive, e.g. texture. For some products, salt reduction requires additional food reformulation approaches to compensate for the alteration in physical properties, to provide the same consumer acceptance, increasing cost and resources in the manufacturing process.

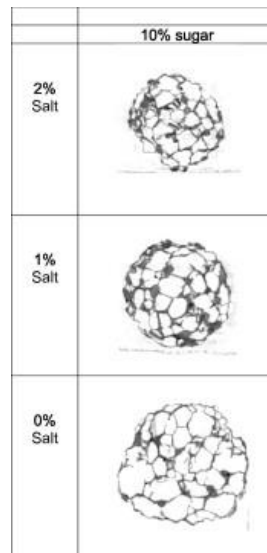


Figure 1.11. X-ray micro tomography diagrams sourced and modified from Pitts et al., 2014 to illustrate the change in expansion of extrudates upon the decrease in salt content and sugar content. [Republished with permission from “Co-effect of salt and sugar on extrusion processing, rheology, structure and fracture mechanical properties of wheat-corn blend” by K. Pitts, J. Favaro, P. Austin and L. Day, 2014, *Journal of Food Engineering*, 127, p58-66. Permission conveyed through Copyright Clearance Center, Inc.].

There is evidence that salt affects extruded snack products' texture and perceived quality. In one study, changing salt content (0-6 %) in extruded cassava and potato starches indicated expansion decreased concomitantly with decreasing salt (Farahnaky et al., 2009b). Hsieh et al. (1990) also agreed with this finding when assessing the impact of salt reduction (6-0 % salt content) on extruded cornmeal. The explanation behind the changes in expansion is due to the effect of salt reducing the glass transition temperature, thus optimising the potential for the formation of bubbles and snack expansion (Farahnaky et al., 2009a). Conversely, a separate study determined that decreasing salt content from 2 % to 0 % in an extruded wheat-corn blend increased extrudate expansion, as shown in Figure 1.11 (Pitts et al., 2014). With 2% being a

typical amount of salt found in this product and expanded products having a 2024 salt target of < 2 % salt (Table 1.1), it is important to understand the impact of salt reduction strategies, including direct removal and salt replacement within the matrix, while the topically applied salt could also be reduced simultaneously (see section 1.8 for discussion on salt reduction strategies).

Therefore, evidence on the effect of salt reduction on expansion properties is conflicting in published literature, which could be due to the many inter-related factors impacting expansion, different extrusion settings or differences between raw product material properties. Although the direction of change is not consistent in literature, it is probable that salt impacts the expansion of these snacks and requires further investigation since a reduction in expansion causes a decrease in brittleness (Robin et al., 2011a, Robin et al., 2011b) and crispness of the product (Beck et al., 2018, Day and Swanson, 2013). This potential impact of the product texture has implications on product fracture during transportation and consumer acceptance, as the organoleptic expectations of the product need to be met.

Salt reduction in extruded snacks requires further investigation since salt content is relatively high in these products due to the functionality of salt within the matrix and for a pleasant taste and consumer acceptance. Additionally, many new extruded products on the market use raw materials like pea, chickpea, bean and lentils, often having relatively high salt contents to mask any undesirable flavour notes from these materials.

Although out of the current scope for this thesis, research in this area should be considered for future work.

In meat products, salt assists in binding to meat proteins, altering protein charges, increasing hydration and water binding capacity due to the swelling of myofibrils and subsequently binding properties of proteins, thereby improving textural attributes, e.g. tenderness and juiciness increases (Desmond, 2006, Hamm, 1972, Hamm, 1986, Tobin et al., 2013).

Regarding textural attributes of bread, salt is required as a yeast inhibitor as it helps to limit the total gas production in the product during baking, which controls the yeast activity. Reducing salt leads to very gassy, acidic doughs with a poor texture and large crumb pore due to over fermentation (Matz and Matz, 1978). This role of salt during baking ensures the correct level of fermentation.

Alongside its role in cheese production as a preservative and for taste, salt is also required to establish an acceptable texture as it causes physical changes in the proteins within the matrix, including determining pH and whey percentage, improving casein hydration and combining the protein and fat networks (Guinee, 2004).

As mentioned previously, salt is used in baking to provide vital textural properties and thereby enhance consumer acceptance. As found in Ayed et al. (2021), reducing salt caused a more brittle texture in sweet biscuits,

which is explained by the larger and less uniform porous structure of the biscuits with less salt caused by the changes in water release. Therefore, salt is required to form a uniform porous texture since it alters the proteins surface charges reducing electro repulsion between proteins increasing interactions between proteins (McCann and Day, 2013). This mechanism helps facilitate the movement of proteins to hydrophilic interfaces (bubbles), providing surface activity that promotes bubble growth and formation (Ayed et al., 2021), determining the final texture profile of a biscuit.

1.7.4 Role of salt in food preservation

Meat is one of the first foods in which salt was used as a preservative traditionally. In the modern day, the use of salt is imperative to preserve and maintain microbial safety by preventing spoilage, including bread, packaged cakes, spreads, sauces and pickles, cheese and meat (Yigit and Korukluoglu, 2007, Kilcast and Angus, 2007). Salts main preservative effect is through decreasing water activity (the measure of water available for microbial growth), reducing oxygen tension and inhibiting enzyme action (Kilcast and Angus, 2007) and thus reducing the number of microorganisms that can survive, multiply/grow and spoil foods. These days, it is not just salt alone which preserves foods; there is also canning, freezing, refrigeration and modified atmospheric packaging. Therefore, salt is not the sole requirement for preservation today. However, it does have a role within the meat and remains one of the essential ingredients for preservation in cured meats along with

nitrite. More historical data shows that reducing salt levels in frankfurters and ground pork by 50-60 % caused an increase in bacterial growth (Terrell, 1983), creating the risk of harming consumers.

1.7.5 Role of salt in seasonings and topical application of snack foods

Salt remains within its undissolved crystalline form in dry topical seasonings until consumed, unlike in many other applications. Therefore, it has a very different role in these foods than what has already been discussed while still contributing to the overall flavour and appealing salty taste. Seasonings are usually a mixture of salt, herbs and spices or other natural and artificial flavour carriers to impart the desired flavour impact. Examples of seasonings may include; seasoning sachets added to fajita mixes or noodles, while topical applications include pretzels, peanuts, potato crisps, extruded snacks, popcorn and tortilla chips. Aside from the contribution to flavour while enhancing flavours of other ingredients present in the seasoning, salt is used in seasonings as a solid carrier for flavours to distribute it uniformly across the coated product (Matz, 1993, Brady, 2002) and improves the bulk seasoning and flowability of the seasoning (Titman, 2019). Although there is a lack of information in literature, Brady (2002) states that salt assists in the flowability and adherence of flavour powders to the product, thus allowing powders to flow freely and allowing the manufacturer to have more control over the application. They also state that for this function, there is no ideal replacement for salt. Furthermore, in some products, the salt flavour is

the prominent flavour note present, e.g. ready salted crisps and salted peanuts, and is required to provide consumers with that unique iconic flavour note they desire in these kinds of products, thus meeting consumer taste preferences. Low or no salt versions of these snacks, e.g. unsalted crisps and unsalted nuts, are not as popular or appealing to consumers since, without salt, they are a bland base with potentially any off-notes, such as bitterness, being tasted more predominately.

1.7.6 Role of salt in driving consumer acceptance

As evidenced, salt can improve the quality of food products in several ways. As well as its functional impact on the food matrix, salt can be added to provide a pleasant taste to an otherwise bland base while enhancing the flavour of other components. This is essential for consumer acceptance because it creates the desire to consume the product and re-purchase. Although safety and texture are important, the flavour must be most desirable to drive consumption. A review paper that examined drivers of liking of products found in the literature indicated that salty taste was a driver of liking for cheese, butter, and soybean paste out of the products they looked at (Li et al., 2015). Additionally, a study assessing drivers of liking using internal and external preference mapping of tortilla chips where salt is topically applied showed that flavour, particularly saltiness, was the main driver of overall consumer acceptance score (Meullenet et al., 2002). This has also been found to be the case in many other products such as; soups (Cox et al., 2019), cheeses (Ritvanen et al., 2005) and fish products (Quadros et al., 2015).

Therefore, it can be concluded that salt is essential to meet the taste preferences of consumers, required for the satisfaction and continual repurchase of products. It is also important to remember that when salt is used as a functional ingredient, i.e. development of texture, it is probable that even though the salt taste may not drive liking, the function of the salt to cause the particular texture profile may drive liking of that product. Overall there is limited literature exploring differences in sensory profiles and preferences between regular and sodium-reduced products such as; potato snacks (Nguyen and Wismer, 2019).

1.8 Salt reduction strategies

Section 1.7 evidenced that salt has a substantial impact on both the physical properties and the sensorial properties of products, including but not limited to saltiness. Therefore, several different salt reduction strategies have been researched, some extensively and some less so. It is important to note that some strategies for salt reduction are suited more to certain food products. Within this section, various product-level salt reduction strategies will be presented, the categories that the approach has been explored in, the types of products that the approach is most suited to, any advantages and disadvantages of each approach and the potential for success within certain products. This section will mainly focus on salt reduction strategies that compensate for the loss of saltiness rather than compensation for the alterations in physical properties.

1.8.1 Direct salt removal

It has been shown in some studies that salt can be simply directly removed without impacting saltiness perception and consumer acceptance; however, this is limited up to a certain amount depending on the food matrix (Jaenke et al., 2017). This is possible as long as the reduction is within the Just Noticeable Difference (JND), i.e. the amount something must be changed in order for a difference to be noticeable. The main product categories assessed for direct salt removal without an approach to compensate for the sodium reduction were typically high salted foods such as; bread, cheese and soup, with few studies evaluating potato and tortilla chips, biscuits, pickle and brined products (Jaenke et al., 2017). This approach is not exclusive to specific product types or states, e.g. liquid or solid foods. In this section, literature discussed focuses on the impact of direct salt removal on consumer acceptance; however, it is likely that there may be significant differences in sensory perception of saltiness, but this may not always be reflected by a drop in consumer acceptance.

Within a bread matrix, the removal of salt directly was determined to be successful up to 30 % in wheat bread without affecting consumer acceptance (La Croix et al., 2015), however further salt removal, e.g. up to 57 % in white pan bread, significantly reduced consumer acceptance (Miller and Jeong, 2014). Within cheese, similar salt reduction potential has been determined, with reductions through direct removal of 25 % without impacting consumer acceptance (Czarnacka-Szymani and

Jezewska-Zychowicz, 2015). However, the level of reduction is dependent on cheese type; for example, in cottage cheese, it was found that a reduction of even up to 20 % was perceivable by consumers (Drake et al., 2011). Similarly, it was concluded by Ganesan et al. (2014) that consumers could distinguish a 30 % reduction of mozzarella and cheddar cheese. Therefore, it was proposed that sodium reduction should be implemented using a gradual approach to ensure the acceptability of the product during the reduction steps (reduction by stealth covered in section 1.8.2). In a different matrix, liquid soup, the level of successful reduction depends on the flavour and complexity of the soup (Jaenke et al., 2017). Up to 48 % of salt was able to be removed from vegetable soup without affecting consumer liking (Mitchell et al., 2013), while others reported that even a lower reduction of 22 % by simple salt removal provided a significant decline in acceptability (Willems et al., 2011), showing a large discrepancy between studies on what can be achieved through simple salt removal.

In a topical application of potato crisps, Kongstad and Giacalone (2020) suggested that 30 % salt could be removed without impacting consumer acceptance. However, when observing just-about-right responses comparing the reference to the 30 % reduced salt potato chips, more people tended to rate the salt reduced sample as not salty enough rather than a just-about-right level of saltiness, as would be expected. Surprisingly these responses did not significantly impact consumer liking

but may cause consumers to opt for a saltier option on the market as they have noticed the change in saltiness.

Despite direct salt removal being the simplest approach since a reduction could be made without any other kind of reformulation, reducing time and cost while still maintaining consumer liking, the limitations and risks involved may outweigh this for some companies. There is a risk of consumer rejection of the low salt product causing consumers to choose an alternative, non-salt reduced product that provides the desired saltiness. In addition, consumers may compensate for the loss of saltiness by adding their own salt (Zandstra et al., 2016). To combat this issue and reduce the risk of consumer rejection of any new salt-reduced formulations by direct removal, a common approach is to use reduction by stealth (discussed in the next section), where gradual reduction over time can result in considerable reductions over an extended time period (Kilcast and Angus, 2007). Furthermore, there is also a risk that removing salt can affect the properties of a product such as textural profile, stability and shelf-life noted in section 1.7. Thus appropriate levels of reduction should be investigated, taking into account factors intrinsic to the quality and safety parameters of the particular food product.

1.8.2 Salt reduction by stealth

Reducing salt by stealth is the process of gradual reductions in salt content of products over time to achieve final salt targets while going unnoticed by regular consumers (Liem et al., 2011). There is some

evidence showing the success of reducing salt by stealth by manufacturers. For example, the average salt content of bread sold in the UK was reduced by 20 % over ten years (He et al., 2014). However, it is difficult to determine whether this success is due to reduction by stealth method or a combination of various approaches such as product reformulation to compensate for the loss of saltiness. Previous reports support that historically reduction by stealth method was useful for UK food manufacturers such as Heinz and Kraft, who have achieved reductions of 33 % and 11-18 % in cereals and bread respectively over 7 years without loss of sales (Kilcast and Angus, 2007). There is a lack of information showing the more recent success of this method potentially due to reaching salt reduction limits without using other strategies. The success of this kind of approach was also exemplified in research papers. For example, 25 % reduction of salt in bread without affecting consumer acceptance was achieved using a series of 5 % salt reductions over 6 weeks (Girgis et al., 2003), and more recently, a gradual decrease of 35 % was achieved with limited impact of saltiness perception in Tunisian bread (El Ati et al., 2021).

This approach works since when reduced in small enough steps, the change in taste goes unnoticed by the consumer and this, in turn, may also adjust consumer preferences to the reduced level of salt (Mattes, 1997). For example, repeated exposure to a lower salt content soup to build familiarity with the taste quality allowed consumer liking to be

increased significantly, showing that preferences can be altered through this method (Methven et al., 2012b).

Interestingly, a study by Bobowski et al. (2015) was conducted to compare the success of direct salt removal (more abrupt) and the gradual reduction over time for tomato juice. Unsurprisingly, it was found that gradual salt reduction is more effective than suddenly reducing the salt level since gradually reducing the salt content maintained the acceptability over the time course of the study while there was a large drop in liking for the direct removal. Thus, it seems advantageous to reduce salt gradually where possible, although firstly, the impact of the final salt reduction level on food safety and other quality properties should be evaluated to ensure it is achievable.

1.8.3 Salt replacement, enhancers and cross-modal interactions

1.8.3.1 Salt replacers

In addition to sodium chloride, other ionic salts can elicit a salty taste and provide some similar level of functionality to certain product categories, therefore, they could be used as a salt replacer without contributing to sodium intake. The use of safe, ionic salts, such as potassium chloride (KCl), calcium chloride (CaCl₂) and magnesium chloride (MgCl₂), in cheese, bread and meat have been well documented (Pateiro et al., 2020, Barat et al., 2013, Grummer et al., 2012, Dunteman et al., 2021), with limited published studies on the application on snacks foods such

as potato crisps (Kongstad and Giacalone, 2020). The most commonly used salt replacer is potassium chloride, KCl. In addition to its salty taste characteristic, it is also characterised as metallic and bitter. When used as a partial replacement above 30 %, it leaves unpleasant off-notes when tested in solution (Sinopoli and Lawless, 2012); therefore, this approach may be better suited to complex semi-solid or solid systems with components that can mask these off-notes such as processed meats. KCl is also significantly lower in saltiness when comparing the same mass of NaCl (Sinopoli and Lawless, 2012). However, despite these two facts, KCl is still the most successful ionic salt replacer for NaCl salt. When used in a more complex food system where any off-notes may be masked, partial replacement using KCl has been successful in reducing NaCl salt by up to 25 % in some cheeses (Gomes et al., 2011), 50 % in processed meats (Carvalho et al., 2013) and frozen lasagne ready meals (Mitchell et al., 2009) and up to 30 % in pizza dough (Mueller et al., 2016) and potato crisps (Kongstad and Giacalone, 2020). CaCl_2 and MgCl_2 have also shown partial success for partial NaCl replacement; however, similarly to KCl; they also produce bitter, metallic and soapy off-notes (Grummer et al., 2012). Literature suggests that CaCl_2 and MgCl_2 have much more limited success in maintaining quality and sensory acceptability than KCl salt. For example, in sausages, only 5 % NaCl replacement with CaCl_2 and 5-25 % MgCl_2 replacement is suggested to be acceptable. In contrast, in the same product, 50 % substitution using KCl is thought to be acceptable (Kim et al., 2018).

The role of ionic salt replacers may maintain the saltiness perception and consumer acceptability. However, physicochemical properties are significantly affected upon replacement with other ionic salts. For example, KCl significantly increased product hardness and altered other physicochemical parameters measured for cheeses (Gomes et al., 2011) and sodium replacement with substitutes also significantly impacted quality parameters such as moisture, texture and yeast counts in dry-cured *lacón* (Lorenzo et al., 2015) however a number of studies have shown that partial replacement of NaCl by others salts maintain the microbiological stability of most meat products (Alino et al., 2010, Blesa et al., 2008).

Another issue with the use of KCl within processed products is that in the European Union KCl must be reported by the E number E508 (EFSA Panel on Food Additives and Flavourings et al., 2019), which many manufacturers may aim to avoid due to the negative connotations of E numbers by consumers who are more frequently seeking 'clean label' food products (Asioli et al., 2017).

1.8.3.2 Salt taste enhancers

The most well-known salt enhancer is an amino acid salt called monosodium glutamate (MSG), with others including yeast extracts and 5'nucleotides. Within MSG, glutamate is the amino acid responsible for the taste quality known as 'umami', with nucleotides also contributing, including inosine monophosphate (IMP) and guanosine monophosphate

(GMP). Umami can be described as savoury or meaty and can enhance low salt foods' perceived saltiness and palatability (Yamaguchi and Takahashi, 1984). The mechanism by which MSG or other umami related molecules do this is still unclear. However, some findings suggest that the taste enhancement is through taste-taste interactions at the cortical level of the brain rather than at the oral periphery (Yoshii et al., 1986, Onuma et al., 2018). MSG has been found to enhance saltiness across a range of products; potato crisps (Kongstad and Giacalone, 2020), soups (Wang et al., 2019), instant noodles, snacks, meat products and stocks and seasonings (Maluly et al., 2017). Therefore its use can be deemed relevant in a range of food categories.

Although MSG has been proven safe to consume with no adverse outcomes at levels added to foods (Walker and Lupien, 2000), and occurs naturally in a range of foods such as tomatoes and meat products, many consumers negatively favour and avoid food containing MSG (Wang and Adhikari, 2018) due to reports in the media claiming serious neurotoxicological effects. Therefore manufacturers, like with KCl, may avoid the use in their formulations. With this in mind, there has been a prevalence in research aiming to find alternatives to MSG to provide similar umami taste and enhance salty taste in the same way. Interestingly in a chicken soup, yeast extract high in umami substances enhanced salty taste to a higher degree than MSG (Wang et al., 2019).

Many other glutamate salts are commonly studied for sodium reduction: mono ammonium glutamate (MAG) and nucleotides, disodium inosinate

and disodium guanylate (Jinap and Hajeb, 2010). Previously, the use of 1 % of MAG, MSG, IMP or GMP provided a similar saltiness while allowing up to 40 % salt reduction compared to no salt reduction and no addition of flavour enhancer in aqueous solutions (Rocha et al., 2020).

The success of the use of MSG to reduce salt content is evidenced in complex multi-component dishes such as roasted vegetables, quinoa bowl, savoury yoghurt dip and pork cauliflower rice. Sodium was able to be reduced by between 31 % and 61 % within these products without the loss of consumer acceptance due to the addition of MSG (Halim et al., 2020). Glutamate salts like MSG seem to show better potential for sodium reduction than yeast extract since upon application of yeast extract to cooked ham, only a 20 % salt reduction was achieved without impacting consumer acceptance (Delgado-Pando et al., 2019). However, with this additive's negative consumer image, this approach is undesirable to food manufacturers, so other strategies that contribute to a clean label are required. Furthermore, MSG would also contribute to an umami taste, which may not be the desired taste in the flavour profile of some food products.

1.8.3.3 Salt-aroma cross-modal interactions

As mentioned previously in section 1.4.3, cross-modal interactions can enhance saltiness due to textural and aroma effects. Firstly, congruent aroma compounds have been extensively investigated to reduce salt content in a range of foods, mainly in; model solutions, soups and cream

products and cheese (see review by Thomas-Danguin et al. (2019) for more details). The approach is referred to as odour-induced saltiness enhancement (OISE). It is believed the mechanism behind the success of this method is due to the cognitive integration of smell and taste, increasing saltiness and overall flavour intensity (Thomas-Danguin et al., 2019). Extensive research using salty water solutions with added odours (such as sardine, bacon and soy sauce odour) evidenced that salt-related odours successfully enhance salty taste at low salt concentrations (Lawrence et al., 2009, Nasri et al., 2011, Manabe et al., 2020). In more complex liquid matrices (model soups and cream products), beef odour enhanced saltiness by up to 30 % (Batenburg and van der Velden, 2011) and soy sauce odour marginally enhanced saltiness perception of beef soup (Lee et al., 2015). Despite the evident success of enhancing salty taste through OISE, consumer acceptability was significantly reduced when using a soy sauce odour to compensate for the loss of saltiness in a beef soup due to an additional artificial taste (Lee et al., 2015), limiting this approach. In addition, Nasri et al. (2011) determined that the salt content of a solution could be reduced up to 25 % using a sardine aroma without significantly impacting perceived saltiness intensity. This finding was supported when assessing sardine odour in a model cheese system. However, the success depended on the cheese composition (optimum effect in low fat and low salt cheese) (Syarifuddin et al., 2016). In topical applications, soy sauce odour successfully enhanced saltiness when applied to roasted peanuts (Chokumnoyporn et al., 2016). Although there is a plethora of evidence

showing saltiness enhancement effects through aroma, there is minimal literature assessing the consumer acceptability of this approach for sodium reduction.

This strategy may be successful in markets where soy sauce and sardines are popular, and bacon odour could be advantageous for meaty flavoured products. Despite this, this method is limited because these very specific aromas may not apply to a wide range of food products, and the successful aromas may be undesirable to some consumers (e.g. sardine is a niche flavour) (Lawrence et al., 2011). Another disadvantage of OISE is that aroma additions to food products are typically more expensive than salt since salt is abundant and cheap to manufacture.

1.8.3.4 Salt-texture cross-modal interaction

Another cross-modal interaction that has the potential to enhance saltiness perception is texture-taste interactions. However, since most studies observed in this area either looked at the interaction between tactile touch texture and taste of the food (Biggs et al., 2016, van Rompay and Groothedde, 2019) or used compositional or process changes to alter the texture (Chabanet et al., 2013), it is difficult to decipher whether this is a successful technique for the enhancement of saltiness perception. Pflaum et al. (2013) assessed the impact of bread crumb texture on saltiness perception and determined that saltiness intensity of the bread crumb is not only influenced by sodium released during chewing but also by 'texture-induced tactile gustatory interactions in the

mouth or brain'. To observe these differing crumb textures visually, see Figure 1.12.

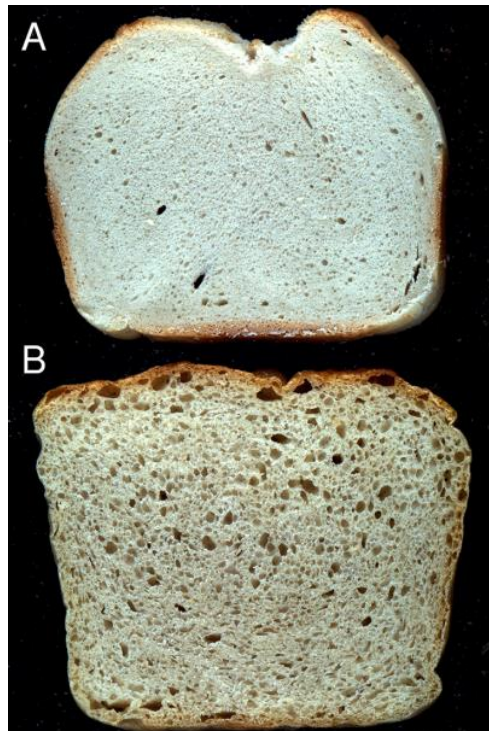


Figure 1.12. Bread crumb samples produced under no proving step (A) and with extended proving (B) to assess the differences in salt perception caused by alterations in crumb texture. Source: Pflaum et al. (2013). Reprinted (adapted) with permission from “Influence of Texture on the Perception of Saltiness in Wheat Bread” by T. Pflaum, K. Konitzer, T. Hofmann and P. Koehler, 2013, *Journal of Agricultural and Food Chemistry*, 61, 45, p10649-10658. Copyright (2013) American Chemical Society.

Interestingly, the eating implements which are used for the consumption process may alter the perceived saltiness. For example, when investigating the effects of different textures of plate ware, Biggs et al. (2016) found that biscuits were perceived as saltier when served from a rough plate. This was suggested to be due to the visual and haptic perception of the plate texture influencing judgements of the flavour of food. Similarly, van Rompay and Groothedde (2019) concluded that the saltiness perception of salty potato crisps is enhanced when consumed from a bowl with a rough texture compared to smooth. However, it is

unclear whether this impact demonstrated in both of these studies is due to the touch-taste interaction or the visual-taste interaction as plate ware was visually different.

It is also unclear whether this insight could be transferable to texture sensations within the oral cavity, e.g. does modifying the surface texture of a product detected in the mouth alter saltiness perception? Kongstad and Giacalone (2020) have investigated this in potato crisps. The saltiness of smooth and wavy crisps was compared, showing that perceived saltiness and liking were not significantly impacted by the change in surface texture.

1.8.4 Altering salt particle size and morphology to optimise salt release and dissolution

The release of sodium from the food structure or salt crystal is considered a limiting factor in saltiness perception (section 1.4). For example, when studying the topical application of salt, a large proportion is not perceived due to the interactions within the food bolus and the short length of time of the bolus in the mouth, limiting sodium from coming into contact with the taste receptors. Therefore some of the salt is not perceived but simply swallowed by the consumer (Tian and Fisk, 2012). Consequently, opportunities have been investigated to optimise the sodium release rate from the food matrix to maximise potential sodium available for perception before swallowing. The focus of reformulation or food macro- or micro-structure design to optimise sodium release has been on semi-

solid and solid foods rather than liquids. In a liquid, salt is already dissolved within an aqueous product and thus already available for perception. In semi-solid or solid foods, the salt is either incorporated into the food matrix, becoming 'locked' in through various interactions with other food components or applied topically to products. For systems using a topical application, e.g. undissolved salt crystal particles within the seasoning, it is imperative to optimise salt perception by increasing the rate of salt crystal dissolution in saliva. This ensures that the optimum amount of salt can be perceived.

To observe alterations in dissolution kinetics, a number of methods are typically used in literature and are summarised in Table 1.3 which includes discussion on each method's advantages and limitations. *In vitro* dissolution of ions can be determined using conductivity and dissolution media (example of a set up shown in Figure 1.13), and *in vivo* delivery rate of ions to the taste buds can be measured using a chew and swab method with subsequent sodium analysis or through in mouth conductivity measurements using electrodes fixed to the teeth (Rama et al., 2013, Linforth, 2000, Davidson et al., 1998). These methods measure the release and delivery of salt ions as a function of time since sensory perception and flavour/tastant release are temporal processes. Therefore, especially when studying this method of salt reduction of optimising salt release, it is important to observe the changes in the intensity of flavour and saltiness during consumption (temporally) using robust sensory methodology such as; time-intensity (TI). This method

ultimately quantifies the continuous changes in the intensity of a specific attribute assessed by a trained panel producing a TI curve similar to that in Figure 1.14. Rama et al. (2013) demonstrated that saltiness intensity, assessed using the TI methodology, directly followed the salivary sodium concentration levels tracked by saliva swabs during eating.

A few techniques have been explored to alter salt crystal structure in an attempt to optimise saltiness perception. For example, different drying methods and evaporation conditions such as spray drying to alter morphology and particle size (Yi et al., 2017), creation of oral disintegrating films (Rama, 2016) and grinding of salt to produce smaller particle size (Rama et al., 2013). Examples of modified salt particles can be found in Table 1.4. Modifying salt crystals aims to increase the surface area that will come into contact with saliva for dissolution, thus increasing dissolution rate, contributing to a higher saltiness perception.

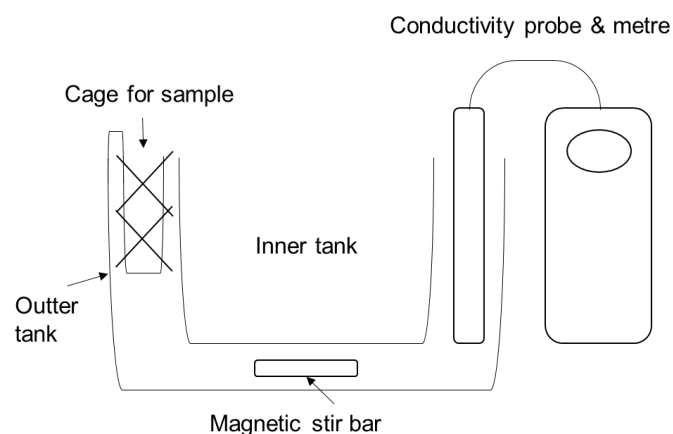


Figure 1.13 Schematic diagram of an example of the apparatus set up used for testing changes in the conductivity values over time to measure the release rate of salt ions from samples used by Rama (2016).

Table 1.3. *In vitro* and *in vivo* approaches used in literature studies to assess temporal salt release/dissolution from foods.

<i>In vitro analysis of salt release</i>				
Method	Method outline	Advantages	Limitations	Sources
Salt release using conductivity	<ul style="list-style-type: none"> Salt or ion release is measured by the change in conductivity of a dissolution media after the submersion of food into a cage or dissolution vessel (Figure 1.13). 	<ul style="list-style-type: none"> Do not need to rely on panellists like with <i>in vivo</i> methods below and therefore, no ethical implications to address. Tighter controls over chemical and physical environment, e.g. variation in oral physiology between individuals, will not impact results. Higher throughput of samples as panellists would get fatigued and could only assess a low number of samples in one sitting. Lower cost – do not need to pay panellists. 	<ul style="list-style-type: none"> Fails to replicate real-life in mouth conditions, e.g. absence of mastication using teeth and intermittent swallowing, absence of salivary properties if not using artificial saliva, absence of the continuous removal and secretion of saliva. 	(Rama, 2016, Chiu et al., 2015)
<i>In vivo analysis of salt release in mouth</i>				
Method	Method outline	Advantages	Limitations	Sources
In mouth sensors	<ul style="list-style-type: none"> Sensors are mounted in-mouth using a dental brace to measure the changes in conductivity associated with salts. 	<ul style="list-style-type: none"> Continually tracks the conductivity of saliva over time thus assessing the salt release kinetics from food in real-time. No need for further extraction of ions or subsequent analysis using other equipment. Sampling takes place within the same conditions as tasting would take place; therefore more accurately tracks the tasting experience than <i>in vitro</i>. 	<ul style="list-style-type: none"> Require an aqueous continuum for measurements to take place; therefore, dry snack foods are not an ideal sample to use in this method due to high capacity to absorb saliva, which cannot be assessed therefore by the sensors. Sensors may interfere with the natural way an individual chews and swallows their food. Complications of using panellists also include increased cost, lower throughput of samples and variation in salivary physiology between panellists may complicate interpretation of results. 	(Linforth, 2000, Davidson et al., 1998)
Chew and swab analysis	<ul style="list-style-type: none"> Sampling of saliva from the mouth at continuous time points of food oral processing using cotton buds. Ions are subsequently extracted from the cotton bud using solvent and sodium content assessed using flame photometer or other equipment. Specific chew and swab protocol are usually specified. 	<ul style="list-style-type: none"> Collection over time allows assessment of salt release kinetics from food in real-time. Less invasive to swab own tongue rather than having device in the mouth. Same as the last point above (in mouth sensor). 	<ul style="list-style-type: none"> It relies on participants to follow the chew and swab protocol correctly, i.e. swab and chew at the correct times. Challenging to coordinate chewing, swabbing while watching the timer. Significant training of participants is required. Same as the last point above (in mouth sensor). 	(Rama et al., 2013, Davidson et al., 2000).

Previously Rama et al. (2013) ground regular NaCl table salt into three fraction sizes to produce extra-fine, fine and coarse sized salt. When

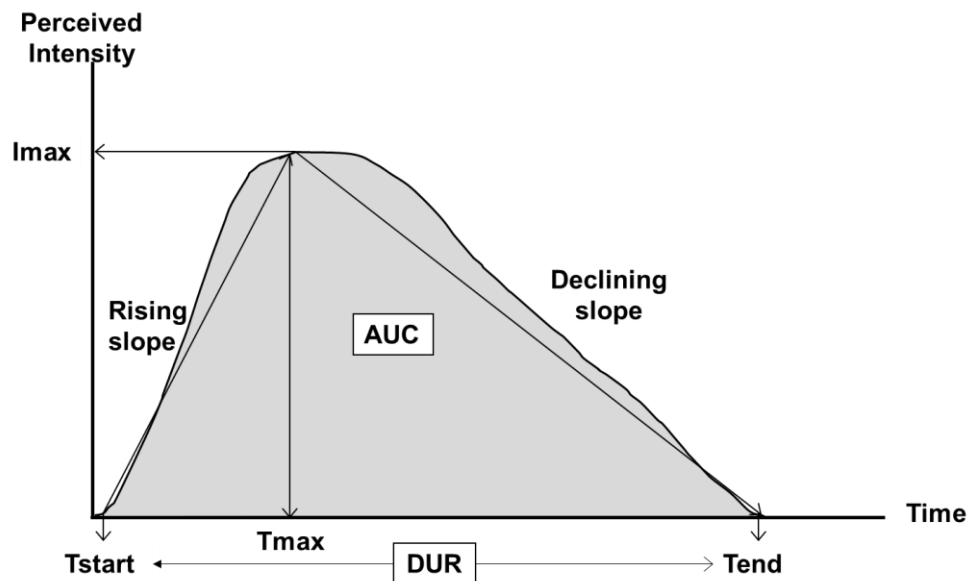


Figure 1.14. Example of a time-intensity curve of a specified attribute assessed by trained panellists. Possible curve parameters determined are shown in the diagram; Maximum intensity (I_{max}), time to I_{max} (T_{max}), duration of sensation (DUR), area under the curve (AUC), time until start if sensation (T_{start}), time to end of sensation (T_{end}).

applied to potato crisps, the extra-fine salt ($<106 \mu\text{m}$) enhanced the saltiness perception significantly. However, reducing salt particle size can often come with storage and shelf-life challenges since it increases the caking effect. Other drying methods such as spray drying can also control the size and morphology more efficiently than grinding. Spray-drying is a common technique that converts liquids into micro and nano-sized particles by pumping the fluid through an atomiser which sprays the liquid into a fine mist in a drying chamber, driving off moisture forming tiny particles (Figure 1.15). Some morphological examples of spray-dried particles are shown in Table 1.4.

The advantage of spray drying is it effectively produces very fine particles with particle diameter, size distribution and morphology that can be controlled through varying process parameters (Shoulders et al., 2016). Spray drying is used for a wide range of applications in the food and pharmaceutical industry, including; milk powder, coffee, spices, flavourings, and drug delivery (Ting et al., 2018), but has shown high potential for the production of salt particles for sodium reduction (Chindapan et al., 2018). The application of spray-dried salt is most commonly evaluated on topically applied products such as surface salted cheese crackers (Moncada et al., 2015) and popped kernels (Yi et al., 2017). However, some research has looked at the effect of replacing regular salt with spray-dried salt within the food matrix, such as meat products (Raybaudi-Massilia et al., 2019) and pizza dough (Mueller et al., 2016). When applying spray-dried salt to the surface of foods, saltiness and overall liking was enhanced for all spray-dried treated

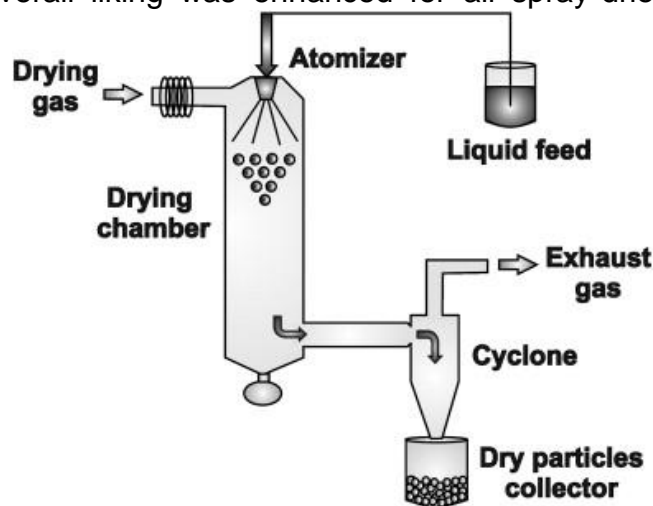


Figure 1.15. Schematic diagram of the spray-drying process. Source: (Sosnik and Seremeta, 2015). [Republished with permission from “Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers” by A. Sosnik and K. Seremeta, 2014, *Advances in Colloid and Interface Science*, 223, p40-45. Permission conveyed through Copyright Clearance Center, Inc.].

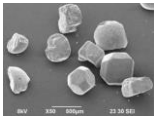
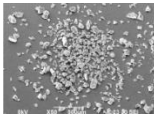

samples even when reducing the salt content by up to 50 % in surface-salted crackers (Moncada et al., 2015) and possible reductions of 19-54 % are achievable in popcorn while maintaining saltiness (Yi et al., 2017). With the evidenced success of spray-dried salt for enhancing saltiness perception in topical applications, spray-dried salt was commercialised as SODA-LO® under patent by Tate and Lyle (Shen et al., 2016). Compared to the use of spray-dried salt, including the use of commercialised SODA-LO®, there is limited literature presenting the sensory and salt release of Alberger flake and dendritic salts in food products (see Table 1.4 for more information). However, the manufacturer of Alberger flake salt claim that it has a faster dissolution rate and thus allows a 30 % reduction in sodium compared to a standard granulated salt while maintaining overall flavour and liking scores when applied to French fries (Cargill, 2021b).

A novel method to redesign particle shape and size is foam-mat drying. Foam-mat drying is often compared to spray-drying and freeze-drying as an alternative method of forming particulates due to its advantages; simplicity, enhanced product quality and the high drying rate due to the increased liquid-gas interface due to aeration (Teoh, Lasekan & Azeez, 2016). This approach has been researched extensively to dry products such as; juices, milk and fruits (Hardy and Jideani, 2017). However, only one reported study has used this drying process to produce salt particles. Chokumnoyporn, Sriwattana & Prinyawiwatkul (2016) first reported the potential use of foam-mat salt for salt reduction when used in conjunction

with an odour that induces saltiness enhancement (OISE). The use of foam-mat salt and OISE reduced the perceived saltiness intensity. However, the acceptance of saltiness was not affected. It is unknown how the foam-mat salt performed in terms of saltiness intensity and consumer acceptance alone without adding soy sauce aroma.

The modification of the NaCl structure is mainly applicable to topically applied salts as the mechanism in how it works relies on the dissolution rate impacting saltiness perception. It is doubtful that this strategy can be implemented within the matrix of many products due to the probability of liquid being present, the modified salt structure being dissociated into its respective ions, and any impact on saltiness perception as a consequence of structure being lost.

Table 1.4 Examples of modified salt particles available commercially or used in research articles include information on their production method, morphology and properties. Images republished with permission conveyed through Copyright Clearance Center, Inc.

NaCl salt particles	Brief production method outline	Morphology (no details on magnification)	Description of properties from literature or product webpages	Source(s):
Vacuum granulated (regular table salt)	Saturated salt solution evaporated under vacuum to form crystals		Concentric or cubic form of crystalline salt	(Rama et al., 2013).
Micronized or ground table salt (e.g. <math><106 \mu\text{m}</math>)	Grinding to a fine powder and use of nickel sieves to obtain a smaller particle size than regular table salt e.g. <math><106 \mu\text{m}</math>		Irregular shape due to physical damage from grinding. Free flowing but do not agglomerate.	(Rama et al., 2013)
Tate & Lyle SODA-LO Microspheres®	Spray-drying of a salt and malto dextrin or gum Arabic solution	Redacted	Free-flowing hollow microspheres. See source for images.	(Shen et al., 2016), (Tate and Lyle, 2017)
Alberger fine flake salt®	Saturated brine solution heated in a controlled evaporation process involving slow heating and gentle agitation	Redacted	Hollow-shaped crystal. See sources for images.	(Cargill, 2021a) (Cargill, 2021c)
Dendritic e.g. Morton Star Flake®	Saturated brine solution treated with sodium ferrocyanide then heated to remove water in vacuum pans	Redacted	Star-shaped crystal, porous, lower bulk density and greater surface area and slightly smaller particle size compared to regular vacuum pan salt. See sources for images.	(Mermelstein, 2014), (1961)
Foam-mat salt	Saturated brine solution with a foaming agent, mixed at a high speed to foam a stable form, sheeted and oven-dried		Lower bulk density, irregular shape with flake-type particles and relatively smaller particles size compared to regular vacuum pan salt	(Chokumnoyporn et al., 2016)

1.8.5 Inhomogeneous salt distribution

Salt is usually distributed throughout the product matrix homogeneously e.g. mixed evenly within the food matrix of a bread or biscuit dough. Alternatively, salt can be unevenly distributed when aiming for an inhomogeneous salt distribution by creating salty layers or salty 'spots' within a food matrix (see Figure 1.16 for examples). The contrasting salt concentrations in an inhomogeneous product are known to increase the intensity of perceived saltiness thus allowing sodium reduction (Noort et al., 2010b, Noort et al., 2012a, Monteiro et al., 2021). This approach has been heavily researched in bread products (Noort et al., 2010b, Noort et al., 2012a, Monteiro et al., 2021) and pizza dough (Diler et al., 2016, Guilloux et al., 2013, Mueller et al., 2016) with some research conducted

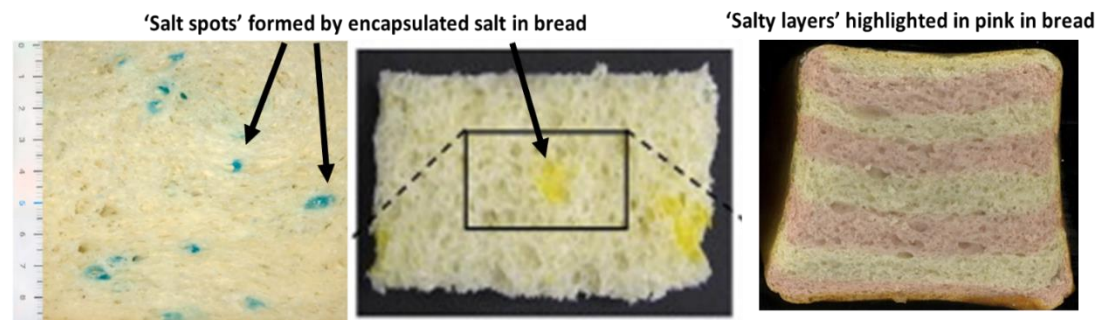


Figure 1.16. Diagrams to exemplify the different approaches to form an inhomogeneous distribution of salt within a product matrix (Monteiro et al., 2021, Noort et al., 2010a, Noort et al., 2012a). Images republished with permission conveyed through Copyright Clearance Center, Inc.].

on; cereals (Fan, 1991), sausages (Mosca et al., 2013), crackers (Vasques et al., 2020) and semi-solid gels (Li et al., 2020).

Creating an inhomogeneous distribution in solid matrices can be accomplished in low moisture products and when using materials where

a moisture barrier (such as fat) can be incorporated. This is because in high moisture products the salt can dissolve into water within the system, which could lead to the salt to a more homogenous distribution.

In bread, this approach is promising for the reduction of salt while maintaining saltiness perception. Noort et al. (2010a) established that up to 50 % salt reduction could be achieved by incorporating layers of dough that are high salt and low salt to cause contrasting salt intensities. Similarly, the use of encapsulated salt (salt enrobed with fat to maintain the structure of the salt crystals, forming 'salt' spots within the bread) could allow for 50% salt reduction in bread (Noort et al., 2012a) while maintaining saltiness perception. However, using encapsulated salt increases the fat content, which is a concern for the overall nutritional profile of the product. Therefore Guilloux et al. (2013) attempted to use coarse grain salt (800-3500 μm) to form 'salty' spots in pizza dough without the fat on the outside. This was unsuccessful as the crystalline salt structure could not be preserved and simply dissolved into the water within the dough. In contrast, a separate study found that the use of coarse grain salt (400-1400 μm) enhanced saltiness perception compared to standard salt grain size (160-700 μm), with a proposed salt reduction of up to 25 % that could be achieved while still maintaining taste quality when using coarse-grained salt in bread (Mueller et al., 2016). In sausages, it has been shown that using layers of sausages with different salt concentrations to form an inhomogeneous distribution of salt, provided a saltier taste and were most preferred compared to the

sausages that were layered using the same salt concentrations (Mosca et al., 2013). However this study was carried out by making homogenous sausages with different salt concentrations i.e. 2 %, 0.5 % and 3.5 % and then once the sausages were made and cooked they were then sliced and layered for tasting. When manufacturing sausages, this layered approach may not be easily achievable since sausages are typically made using a homogenous mixture of ground meat 'stuffed' into casings. Alternatively, the use of encapsulated salt within sausages may be more feasible since encapsulated salt can be added to the meat mixture and processed in the same way and the salt crystal structure should remain intact. In this way, Beck et al. (2021) found that salt encapsulated in carnauba wax could reduce the quantity of salt by 25 % in fresh sausages whilst maintaining saltiness intensity.

As mentioned in section 1.7, salt is required within products such as meat and bread to form the appropriate textural, flavour profiles and preserve the foods. Since the salt is separated from the rest of the food components, the disadvantage of this approach is that salt cannot carry out its functional role in these foods other than the salty taste that it provides. Therefore, this could impact shelf-life and other quality parameters discussed in section 1.7. This needs to be considered and evaluated before implementing these approaches to maintain food safety and quality. Furthermore, in some cases, the use of a large salt grain size (2000 μm) provided a much higher saltiness intensity than consumers expected. These breads were significantly less liked than the breads

made with standard salt grain sizes (Noort et al., 2012a). Therefore when implementing this approach, it is also essential to investigate the optimum encapsulated salt size to enhance saltiness and maintain consumer liking.

The reason behind the success of this approach is due to taste receptors ultimately responding to contrasts in stimulation. Thus this could be potentially an approach worth investigating in snack foods by creating a less-homogenous distribution of salt on the surface through creating 'pockets' for salt to deposit in, e.g. in ridges. The other reason for its success is that when salt remains unbound within the bread matrix due to encapsulation, the taste receptor cells can detect the salt taste more quickly as the salt can dissolve straight into the saliva without requiring the bound sodium to be released from the complex bread matrix first.

1.8.6 Inert fillers

Inert fillers are components in food that do not carry a specific active biological or chemical property. The most common inert filler used in the food industry is air. It is free and helps reduce fat, calories and sugar content of food products on the current market such as confectionary. Air has also been found to be successful for sodium reduction in liquid and semi-solid foods such as gels since air displaces the sodium into the liquid phase making it more concentrated within the same volume. Previously in soft agar gels, an inclusion of 40 % air and a reduction of 40 % salt (w/v) compared to a control had equivalent saltiness ratings

while samples with the same salt concentration to the control (w/v) but with the addition of 40 % air bubbles enhanced the saltiness rating (Goh et al., 2010). Results showed promise that salt (and sugar content) can be reduced in aqueous or gel-like products without affecting the taste profile (Goh et al., 2010). These findings were supported by a more recent study by Chiu et al. (2015), demonstrating that a sodium reduction of up to 80 % could be possible through the use of air as an inert filler in gelatine gels without impacting saltiness perception. By assessing the rate of diffusion of sodium from the gels, it was determined that the increased air inclusion enhances the diffusion rate due to the increase in surface area and thus optimising the perception of saltiness. Although this approach is successful, it is limited by the type of product it can be used in (limited to oil based and water-based sauces with gel properties). Furthermore, this approach would also impact the texture properties (making the product into more of a foam), macronutrient content and reduce calorie content which may not always be desirable to the food producer or consumer.

1.9 Aims and thesis structure

This chapter has highlighted the evident requirement for salt reduction strategies in various foods and summarised previous research completed in this area. More specifically, a number of snack foods have been brought in to be included in the new UK salt reduction targets set by the Food Standards Agency, including peanuts and popcorn. With

continuing salt reduction targets required to be met for other snacks like potato crisps, this is an area that requires more research, as many current and new products being launched do not meet the UK salt reduction targets or are unlikely to meet the 2024 future targets. Additionally, most previous studies assessing the success and potential of salt reduction strategies focus on meat, dairy and bakery products. Since most snack foods typically are seasoned with topically applied salt, this thesis focussed on the salt reduction of a topical application. To maintain a clean label which most manufacturers desire, the most efficient way to achieve significant sodium reduction in these foods is enhancing saltiness through the optimisation of salt dissolution. Therefore, this thesis focuses on the utilisation of this mechanism to achieve salt reduction in snack foods.

Alongside product strategies, we recognised in this chapter that consumer interactions with the product also determine the saltiness perception of a product. Therefore, this thesis also studied the influence of food oral processing on perception and liking. Chapter 3 assesses the salivary parameter impact on the salt taste sensitivity of consumers and the possible subsequent levels of sodium intake, and chapter 4 considers the influence of mouth behaviour type on individual perception of salt-reduced potato crisps and commercial competitors.

The main aims of this PhD were to explore the capabilities of salt reduction techniques while understanding the contribution that individual


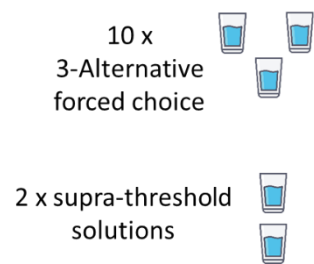
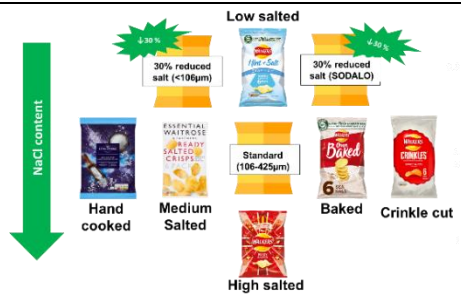
consumer differences in oral processing parameters have on saltiness perception and subsequent sodium intake by:

- Developing physicochemical sodium reduction design rules for salt particles by comparing the various properties, morphology, adhesion capabilities, sodium dissolution kinetics and temporal saltiness profiles of a range of salt particles with varying parameters altered by salt processing techniques (chapter 2).
- Exploring the relationships between oral physiology and salt taste sensitivity and how these could consequently affect sodium intake, using model solutions and assessing salt taste threshold and consumption habits (chapter 3).
- Validating the physicochemical design rules established in results chapter 2 through evaluation of the potential of model salts for sodium reduction in potato crisps using a dual sensory approach (chapter 4).
- Investigating the potential of other salt reduction strategies in potato crisps, such as direct salt removal from potato crisps and processing and texture alterations, on the saltiness perception and consumer acceptance (chapter 4).
- Exploring the impact of consumer mouth behaviour type on consumer product preference and perception of appropriate saltiness level (chapter 4).

The following chapters of this thesis will be presented in a publication format, with Table 1.5 on page 102 showing a thesis structure and summary.

Chapter 1: Introduction to thesis and literature review

Table 1.5. Thesis chapter structure and summary.

Chapter number	Chapter 2	Chapter 3	Chapter 4
Title	Physicochemical design rules for the formulation of novel salt particles with optimised saltiness	The relationship between salt taste sensitivity, saliva composition and self-reported sodium intake	Sensory perception and consumer acceptance of commercial and salt-reduced potato crisps formulated using salt reduction design rules
Paper status	https://doi.org/10.1016/j.foodchem.2021.129990 Date published: 20/10/2021	Under review by Food Quality and Preference (correction submitted December 2021)	https://doi.org/10.1016/j.foodres.2022.111022 Date published: May 2022
Samples used	<p>Salted peanuts</p> 	<p>Salt solutions</p> 	
Overarching aim of each chapter	To generate a series of design rules for novel salt particles and inform ingredient design for future product development for food, flavour and other industries.	To examine the relationships between individual salt taste perception, certain salivary parameters and salt intake measures and behaviours.	To explore sensory and consumer acceptance performance of salt reduced potato crisps within the current potato crisp market and validate previous physicochemical salt reduction design rules set out in chapter 1
Methods	<ul style="list-style-type: none"> • Physicochemical characterisation • Adhesion measurements • Dissolution kinetics using conductivity • Sensory evaluation – Time-intensity methodology 	<ul style="list-style-type: none"> • Salivary characterisation • Sensory evaluation – Detection and recognition threshold determination and determination of perceived saltiness intensity of supra-threshold concentrations • Dietary assessment 	<ul style="list-style-type: none"> • Sensory evaluation – Sensory Descriptive Analysis and consumer testing • Consumer clustering using overall liking scores • Characterising consumers by mouth behaviour typing tool • Cluster analysis
Key findings	<ul style="list-style-type: none"> • Physicochemical ‘design’ rules for sodium reduction were developed. • Transfer efficiency was driven by size, density and flow properties. • Loss from the product in packaging was driven by particle size. • Dissolution and/or saltiness were driven by size and hydrophobicity. • Findings informed the selection of salt crystals used in chapter 4 • To maximise potential perceived saltiness, salt particles should be designed with small particle size, low density and hydrophobicity and have a particle shape associated with optimal flow properties. 	<ul style="list-style-type: none"> • NaCl taste sensitivity is primarily dependent on salivary sodium concentration. • High NaCl taste sensitivity group consumed on average more salt. • Individuals perceiving NaCl as more intense ate fewer calories. • Trends observed between NaCl addition to food and perceived NaCl intensity. 	<ul style="list-style-type: none"> • 30% salt reduction achieved without impacting saltiness or consumer acceptance. • Salt reduction design rules were validated. • Direct salt removal of 14-15% resulted in compromised consumer acceptance. • Salt content drives liking while texture profiles were polarising and segmented consumers. • Baked and thick cut crisp increased oral residency time but this did not impact saltiness perception or liking. • Crinkled potato crisps did not enhance the saltiness through cross model interaction. • Mouth behaviour groups did not differ in their preferences across the product set.

Chapter 2

2. Physicochemical design rules for the formulation of novel salt particles with optimised saltiness

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2.1. Abstract

Novel sodium reduction strategies are urgently required by the food industry. We hypothesised that redesigning salt crystals (size, density, hydrophobicity and flow properties) will offer a new route to increase saltiness and therefore reduce sodium. Eight salts were compared with different physicochemical properties, the resultant particles were characterised and adhesion to product, loss in-pack, rate of dissolution and ultimately saltiness perception evaluated. Principle findings included that particle adhesion was driven by particle size ($r=-0.85$, $p=0.008$), bulk density ($r=-0.80$, $p=0.017$) and flow properties ($r=0.77$, $p=0.015$); loss in-pack was associated with particle size and hydrophobicity of the salt particle while dissolution and/or saltiness perception was driven by particle size and hydrophobicity of the salt particle. The findings offer a new set of design rules for future ingredient design for the food and flavour industries.

Keywords: Sodium reduction, time-intensity, sodium dissolution kinetics, particle adhesion, foam-mat processing

2.2. Highlights

- Unique salts with varying physicochemical properties were designed
- Salts were applied to a model system with a controlled surface area
- Transfer efficiency was driven by size, density and flow properties
- Loss from the product in packaging was driven by particle size
- Dissolution and/or saltiness were driven by size and hydrophobicity

2.3. Introduction

Over-consumption of sodium chloride (NaCl) salt can lead to an increased risk of high blood pressure, cardiovascular disease, stomach cancer and kidney-related disease (Adler et al., 2014). Average NaCl salt consumption exceeds the recommended levels of <5 g NaCl/day (World Health Organisation, 2018), with consumption of >8 g/day, >9 g/day and >12 g/day in the UK, USA and Asia (Public Health England, 2016). As 85 % of consumed sodium originates from NaCl in processed foods, there is a high demand for food manufacturers to produce sodium-reduced foods while maintaining flavour profile, appearance and consumer acceptability (Gibson et al., 2000). However, sodium is included for structure formation, taste and flavour enhancement, making reduction challenging due to its multifaceted benefits.

To perceive saltiness in foods, NaCl must first dissociate in saliva forming free sodium ions (Na^+), move close to oral taste receptor cells (TRC) and passively diffuse through epithelial amiloride-sensitive sodium channels (ENaC) on the TRC surface (Chandrashekar et al., 2006). This ultimately leads to the transmission of taste signals and saltiness perception (Delwiche and O'Mahony, 1996).

In dry snack foods, such as crisps and peanuts, a significant proportion of sodium, in the form of topically applied salt particles, is lost during processing, packaging, transport or storage due to poor adhesion of the

dense crystal structures or is consumed without being perceived as swallowing occurs before the salt is dissolved (Tian and Fisk, 2012, Yucel and Peterson, 2015b, Yucel and Peterson, 2015a). To optimise sodium perception, it is imperative to; maximise the fraction of Na⁺ that successfully adheres to the product and delivers to the oral cavity and optimise the rate at which the salt particle dissolves.

We, therefore, propose three key phases for the optimal design of salt particles: Phase I: Adhesion during application and before packaging: Poor adhesion results in unnecessary wastage and directly impacts the heterogeneity of product and sodium levels in-pack; Phase II: Adhesion during packaging and transport: Salt should remain on the product as salt particles not associated with the product are unlikely to be consumed, leading to elevated pack declared sodium levels; Phase III: Release during oral processing: Salt release should occur quickly and remain separate from the bolus to enable effective diffusion of free Na⁺ ions to TRCs.

Redesigning salt particles (particle size, morphology, surface properties and flow properties) has previously been proposed as a route to optimise adhesion, enhance dissolution rate and increase saltiness perception (Rama et al., 2013, Chindapan et al., 2018, Vinitha et al., 2020, Moncada et al., 2015, Yi et al., 2017). In addition to milling (Rama et al., 2013), the size and shape of salt particles can also be modified through controlled drying such as spray-drying to produce small NaCl enriched particles

(Chindapan et al., 2018) or the addition of sodium ferrocyanide during vacuum crystallisation of brine to produce dendritic salt with a high surface area (Buck and Barringer, 2007, Matz, 2012). Drying of a foamed brine also produces a product with a high surface area (foam-mat drying) (Rajkumar et al., 2007a, Teoh et al., 2016, Chokumnoyporn et al., 2016). However, despite studies showing sodium reduction is possible by increasing crystal surface area (Rama et al., 2013, Shen et al., 2013), this is often technically challenging in humid environments due to caking of highly hygroscopic finely milled salt.

Adhesion of salt particles is also key to producing well-seasoned products (Buck and Barringer, 2007). Changing the size or shape of salt particles has been shown to enhance adherence (Halim and Barringer, 2007, Buck and Barringer, 2007). However, adhesion must be reversible, enabling salt release and dissolution during oral processing (Rama et al., 2013, Quilaqueo et al., 2015). Smaller particles adhere more efficiently than larger particles (Halim and Barringer, 2007, Buck and Barringer, 2007), while flakes coated more efficiently than cubic salt particles due to an increased surface area (Miller and Barringer, 2002). Finding a salt crystal morphology that provides strong adhesion whilst achieving effective sodium delivery to the oral receptor is critical in searching for a reduced salt alternative for topical application (Rama et al., 2013, Shen et al., 2013).

Due to the multifaceted role of salt in food and the wide-ranging interacting factors that impact its efficacy (Phase I, II and III), it is unlikely that a single pronged approach will result in the successful development of novel salt particles. We, therefore, explored the potential of modifying a range of physicochemical properties simultaneously (particle size, density, hydrophobicity and flow properties) on adhesion to product, loss in-pack, rate of dissolution and saltiness perception. Ultimately the aim is to generate a series of design rules for novel salt particles and inform ingredient design for future product development for food, flavour and other industries.

2.4. Materials and methods

Eight diverse salt samples produced using a range of processing methods were evaluated. Their physicochemical properties, the efficiency of transfer to product and adhesion to product, the release of sodium ions during dissolution and subsequent saltiness perception was assessed.

2.4.1. Formulation of model salt particles

Regular salt (RS) (Sainsbury's, London, UK) was milled using a coffee grinder and mechanically sieved using nickel sieves (Fisher, Loughborough, UK) into three different fractions: <106, 106-425 and 425-600 μm . Samples are referred to as RS plus the sieve sizes used.

SODA-LO® Salt Microspheres Extra Fine salt (Tate and Lyle, London, UK) was formed by spray-drying a salt solution with maltodextrin and has a lower density/higher bulk porosity than RS. Dendritic salt has a modified surface area and was purchased from Madar Corporation Ltd, Hampshire, UK.

Foam-mat salt (FMS) has a lower density/higher bulk porosity than RS crystals. Three fractions varying in particle size, were prepared by blending commercial NaCl salt (Sainsbury's, London, UK) in ultrapure water with a hydrophobic egg albumen powder (MyProtein, Cheshire, UK) and methylcellulose (Special Ingredients, Chesterfield, UK) followed by grinding and sieving. The process is further outlined in 2.4.1.1.

2.4.1.1. Preparation of foam-mat salt particles

Method and formulation for FMS particles preparation was developed in preliminary experiments (data not published). A solution of 21.7 % NaCl, 5.4 % egg albumen powder, 0.36 % methylcellulose and 72.5 % ultrapure water was stirred with a magnetic stirrer at 1000 rpm with a constant temperature (5 °C for 15 hours). After mixing, the solution was foamed using a Kenwood Chef mixer (Kenwood Limited, Havant, UK) with a stationary bowl and whisk attachment at room temperature (17-18 °C) for 8 mins on speed setting 6 to form a stable gas-liquid foam. The resultant stable foam was spread over a baking sheet at a thickness of < 5 mm and dried in a fan convection oven at 60 °C until it reached a constant

weight. Once dry, the thin porous honeycomb structure was scraped from the tray and ground using a mortar and pestle and mechanically sieved using nickel sieves (Fisher, Loughborough, UK) to standardise the particle size into 3 fractions (<106, 106-425, 425-600 µm). These samples are referred to as FMS and mechanically sieved sizes.

2.4.2. Physicochemical characterisation of model salt particles

2.4.2.1. Morphological characterisation

Morphological observations of the salt particles were made using a JEOL 6060LV Scanning Electron Microscope (SEM) (JOEL Ltd, Tokyo, Japan) at 10 kV for isolated salt particles (Rama et al., 2013).

2.4.2.2. Moisture content and water activity

Moisture content (MC) and water activity (a_w) measurements were based on methodologies outlined in (Yu et al., 2012). The MC of samples was determined gravimetrically by oven drying at 105 °C for 24 hours (Memmert GmbH and Co. KG, Schwabach, Germany). MC was calculated using Equation 1.

$$\text{Equation 1: } MC (\%) = \frac{\text{Initial weight} - \text{dry weight}}{\text{dry weight}} \times 100$$

A_w was analysed using the AquaLab water activity meter (METER Group, Munich, Germany). For A_w measurements, samples were placed in a

standard A_w container with a lid and parafilm wrap to seal the container (samples filled just under half of the sample container, as per manufacturer's instructions). Samples were left to equilibrate in the sealed containers for 3-4 hours at 20 °C (room temperature) before analysis.

2.4.2.3. Bulk density and tapped density

The bulk density (ρ_b) of salt samples were obtained gravimetrically using a dry glass 10 mL graduated cylinder at 20°C (room temperature) and was calculated using the weight and corresponding volume according to Equation 2. The tapped density (ρ_t) was measured in the same way, but the measuring cylinder was tapped strenuously until no further change in volume took place (Basu and Athmaselvi, 2018).

$$\text{Equation 2: } \rho_b \text{ or } \rho_t \text{ (g/mL)} = \frac{\text{Mass of powder (g)}}{\text{Volume of powder (mL)}}$$

2.4.2.4. Carr's Compressibility Index (%)

Carr's Compressibility Index (CI%) of the salt particles was evaluated using the relationship between tapped and bulk densities of the samples (Basu and Athmaselvi, 2018) and expressed as a percentage (Equation 3).

$$\text{Equation 3: } CI \% = \frac{\rho_t - \rho_b}{\rho_t} \times 100$$

CI% indicates flowability of a powder. A free-flowing powder compacts readily, resulting in a similar bulk to tapped density. A powder that flows poorly has a greater bulk density to tapped density suggesting a greater number of inter-particle interactions. Fine rough particles or those with complex surfaces are known to flow more poorly, and larger, smoother particles flow more readily and have a higher CI%. In general, particles greater than 250 μm tend to be free-flowing, while those below 100 μm tend to be cohesive. A CI% of <10 represents excellent flow, 11-15% good flow, 16-20 Fair flow, 21-25% passable flow, 26-31 poor flow, 32-39 very poor flow, and >40 very, very poor flow (Carr, 1965). Whilst this is an empirical measure, it offers a rapid tool for powder flow characterisation.

2.4.2.5. Particle size

Particle size analysis was performed using a LS 13 320 Laser Diffraction Particle Size Analyser equipped with Tornado dry powder system (Beckman Coulter, Brea, California, USA). The Fraunhofer theory was used to determine the mean diameters of the particles, as explained in Soukoulis et al. (2013).

2.4.2.6. Colour

Salt samples were placed in small plastic cuvettes for analysis. Lightness (L^*), redness (a^*) and yellowness (b^*) were measured by a HunterLab

colorimeter, and the whiteness index was calculated using Equation 4 (Chokumnoyporn et al., 2016).

$$\text{Equation 4: Whiteness Index} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

2.4.2.7. Total sodium content

The following method was adapted from Ayed et al. (2021). Nitric Acid 68% for Trace Metal Analysis (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and ultra-pure water, (Millipore, Bedford, Massachusetts, USA) was used. All salt samples except FMS samples were prepared by dissolving 0.05 g salt into 10 mL of 2 % nitric acid and were then diluted by a factor of 100 using the same 2 % nitric acid solution and transferred to clean polypropylene inductively coupled plasma mass-spectrometry (ICP-MS) tubes (Sarstedt Inc, Newton, North Carolina, USA). RS, dendritic and SODA-LO® samples dissolved readily in the 2 % nitric acid matrix. Foam-mat samples required further digestion steps due to lower solubility and were prepared by adding 10 mL of 68 % nitric acid to 0.2 g of FMS in polypropylene digestion tubes (Anton Paar, Graz, Austria), and digested (teflon-coated graphite Block Digestor, Analysco Ltd, Oxford, UK) at 95 °C (2 hr) with polypropylene watch glasses (Anton Paar, Graz, Austria) placed on top to allow for refluxing. After cooling, samples were topped up to 50 mL with ultrapure water. Samples were mixed well, 1 mL of sample was removed from the top, diluted by 100 and transferred to ICP-MS tubes. Multi-elemental analysis of the diluted

solutions was undertaken by ICP-MS (Thermo Fisher Scientific, Bremen, Germany). Samples were introduced at a flow rate of 1.2 mL/min from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a perfluoroalkoxy (PFA) Microflow PFA-ST nebuliser (Thermo Fisher Scientific, Bremen, Germany). Sample processing was undertaken using Qtegra™ software (Thermo Fisher Scientific, Bremen, Germany) and external cross-calibration between pulse-counting and analogue detector modes were used when required. Internal standards, used to correct for instrumental drift, were introduced to the sample stream on a separate line (equal flow rate) via the ASXpress unit. Internal standards included combinations of Sc (10 µg/L), Ge (10 µg/L), Rh (5 µg/L), Re (5 µg/L) and Ir (5 µg/L). The matrices used for internal standards, calibration standards and sample diluents were 2% nitric acid (Fisher Scientific, Loughborough, UK) with 4% methanol (to enhance ionisation of some elements). Multi-element calibration solutions were prepared at different concentration levels of Ca, Mg, Na and K (0-30 mg/L) from a bespoke external multi-element calibration solution (SCP Science, Quebec, Canada).

Concentration was converted to sodium concentration using dilution factors. High purity sodium chloride (Sigma-Aldrich, St. Louis, Missouri, USA) was used as a reference for 100 % sodium chloride, percentage NaCl for each sample was calculated. Samples were measured in triplicate with blank samples to remove any contamination effects.

Coefficient of variation for analytical triplicates was 0.2-2.8 % indicating high precision.

2.4.3. Salt particles - peanut interactions

2.4.3.1. Particle adhesion to lightly oiled peanuts measurements

Salt adhesion properties were determined using a modified method from Buck and Barringer (2007) and Sumonsiri and Barringer (2011). Salt particles (2g) were added to unsalted pre-oiled peanuts (100 g). Pre-oiled peanuts were made by mixing 1 g sunflower oil (Sainsbury's, London, UK) per 100 g unsalted peanuts (KP Snacks Limited, Slough, UK). The weight of oiled peanuts was recorded as the weight before coating with salt for each sample (W_{t1}). Each salt sample (2g) and the appropriate amount of pre-oiled peanuts were weighed (W_{t2}) and mixed in a cylindrical plastic container by hand for 30 seconds using the same rotating motion to mimic a tumble drum used to coat snack foods. The coated salted peanuts were then placed in a 16 cm x 23 cm packaging pouch made from polyethene terephthalate, aluminium foil and polyethene (Fresherpack Ltd, Huddersfield, UK). The weight of the salted peanuts inside the packaging was recorded as the weight of the sample after mixing (W_{t3}). Transfer efficiency (TE) was then calculated by equation 5. For the packaging test, packaged peanuts (W_{t3}) were sealed and inverted 10 times. Salted peanuts were then poured out into a separate container, and the weight was recorded as the weight after the

packaging test (Wt_4). Adhesion after packaging test (Ad %) was then calculated using equation 6.

$$\text{Equation 5: } TE (\%) = \frac{Wt_3 - Wt_1}{Wt_2 - Wt_1} \times 100$$

$$\text{Equation 6: } Ad (\%) = \frac{Wt_4 - Wt_1}{Wt_2 - Wt_1} \times 100$$

Wt_1 : weight of oiled peanuts before coating with salt

Wt_2 : weight of salted peanuts after coating

Wt_3 : weight of the salted peanut sample after mixing once inserted into packaging

Wt_4 : weight of salted peanut sample after packaging test

2.4.3.2. Salt particle dissolution kinetics in water from lightly oiled peanuts

Salt dissolution was evaluated by measuring conductivity over time, modified from Rama et al. (2013). Samples ($2 \text{ g} \pm 0.2 \text{ g}$) were placed in a dissolution vessel (4.5cm diameter stainless-steel tea strainer, Arktek Group Limited, Sunderland, UK) and suspended in stirred RO water (500 mL, 20 °C, 200 rpm). Conductivity (micro siemens per cm^3) was recorded every 5 s for 200-300 s (SevenExcellence pH/Ion/Conductivity meter, 4-pole platinum conductivity probe (inLab 710, 0.01-500 micro

Siemens/cm³) (Mettler Toledo, Columbus, Ohio, USA). Conductivity was normalised to percentage conductivity over time (s). The area under the curve of dissolution graphs was determined using the trapezoidal rule, and labelled as AUC_{diss} , presented without units. Other dissolution parameters were extracted including; initial slope (determined by calculating the gradient of the curve between 0 and 20 seconds) and time to 25% (T25%), 50% (T50%), 75% (T50%) and 90 % (T90%) conductivity, in seconds.

2.4.4. Sensory evaluation of model salt particles

2.4.4.1. Sensory panel and samples

A sensory panel consisting of 12 screened and highly experienced assessors (3 men and 9 women, aged 48-72 years) assessed final product samples for saltiness intensity. All assessments took place in individual tasting booths designed to meet ISO 8589:2007 standards with red coloured lighting to minimise product appearance differences. Batches of salted peanut samples were prepared using 1.31-1.83 g of model salt (depending on NaCl content) added to 100g of oiled peanuts to reach a final concentration of 1.3g NaCl per 100g of oiled peanuts. Panellists were served 2 g (\pm 0.1 g) of salted peanut sample in small plastics pots. In total, seven of the eight salts underwent sensory assessment (dendritic salt was excluded as it could not be classified as food grade).

2.4.4.2. Time-intensity (TI)

TI methodology was carried out based on the ASTM E1909-13 (2017) standard. Before data collection, three 2-hour training sessions took place to familiarise the panellists on the methodology, assessment protocol, saltiness scale, reference samples and test samples. Panellists were instructed to record their perception of saltiness intensity over 90 seconds by moving a mouse on a linear scale. Data was captured using EyeQuestion software version 4.11.6. Panellists started their saltiness ratings once the sample was placed in the mouth from the pot by clicking start on the screen. Chewing rate and swallowing time were controlled to minimise variation caused by individual differences in their chewing behaviours. Panellists chewed at a rate of 70 beats per minute controlled by the sound of a metronome while simultaneously evaluating saltiness intensity using a continuous line scale, where the left end represented a saltiness intensity of 0 and the right end a saltiness intensity of 100. All panellists swallowed at 25 seconds and data collection finished at 90 seconds. Samples were assessed in duplicate by each panellist in a randomised order. Water and unsalted crackers were provided as a palate cleanser, with a 10-minute break between samples. Data from 10 panellists (3 men, 7 women, aged 48-72) were used from the total of the 12 panellists based on consistent performance and attendance.

2.4.4.3. Extracted TI parameters

A number of parameters were extracted from TI curves relating to saltiness intensity, rate and duration, including perceived maximum intensity of saltiness (I_{max}), area under the TI curve (AUC_{sensory}) and the maximum perceived saltiness over time to I_{max} (rate I_{max}). Extracted parameters are further detailed in Figure 2.3 (page 134) and Figure 2.4 (page 137).

2.4.5. Statistical analysis

Data analysis was performed using XLStat Sensory version 2020.1.2. Experiments were performed in triplicate, and mean values reported unless otherwise stated. Differences between samples for each variable were determined using analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test, $p < 0.05$. Average TI curves were constructed by calculating the mean intensity value at each time point across panellists and replicates. The extracted TI parameters were subjected to ANOVA (products, panellists and their interactions as fixed variables), followed by Tukey's HSD. Correlations between variables were determined using the Pearson product-moment correlation coefficient (see also supplementary materials). Data was subjected to Partial Least Squares Regression (PLS-R) to visualise relationships between variables.

2.5. Results and discussion

In this study, a range of salts of varying physicochemical properties and macroscopic flow behaviours were assessed to understand the key drivers behind salt adherence and transfer properties, dissolution kinetics and saltiness perception. Exploration of the morphology of the salt particles and their physicochemical properties will first be discussed, followed by the dissolution properties (the rate at which the salt particles dissolve in model saliva) and then ultimately the influence of these parameters on saltiness perception. Relationships, interactions and main drivers for each of the 3 key phases outlined are discussed considering these findings, concluding with proposed design rules for future product development.

2.5.1. Morphology of model salt particles evaluated by scanning electron microscopy

SEM images of salt particles are shown in Figure 2.1. Similarly to SEM images presented in Rama et al. (2013), very fine salt crystals (<106 µm) have an irregular shape due to the milling (Figure 2.1A), the larger RS samples are dense crystals with smooth topology (Figure 2.1B, 2.1C) that pack closely together due to their smooth flat surfaces (Figure 2.1C) and have no internal voids. SODA-LO® particles (Figure 2.1G) are smooth pseudospherical structures; these pack loosely and are often damaged or cracked showing internal voids and a higher surface area than RS.

The process patent for SODA-LO® (Shen et al., 2013) shows clumped aggregates of microspheres; this can be seen in Figure 2.1 where numerous smaller spheres can be found inside the larger SODA-LO® particles (Figure 2.1G x 500 magnification). This is similar to other spray-dried products such as fruit powders (Darniadi et al., 2018). The dendritic salt (Figure 2.1H) has an overall cubic shape with surface irregularities resulting in a rough surface topology with a layered appearance, with evidence of small internal voids and a slightly elevated surface area compared to RS. In this study and previous studies (Triyastuti, Kumoro, & Djaeni, 2017), foam-mat powders have a spikey/flake-like structure due to the surface bubbles on the films that are broken up during grinding resulting powder that packs loosely with no apparent clumping (Figure 2.1D-F). FMS samples appear to have a high surface area and many internal voids at both the x 100 and x 500 magnification.

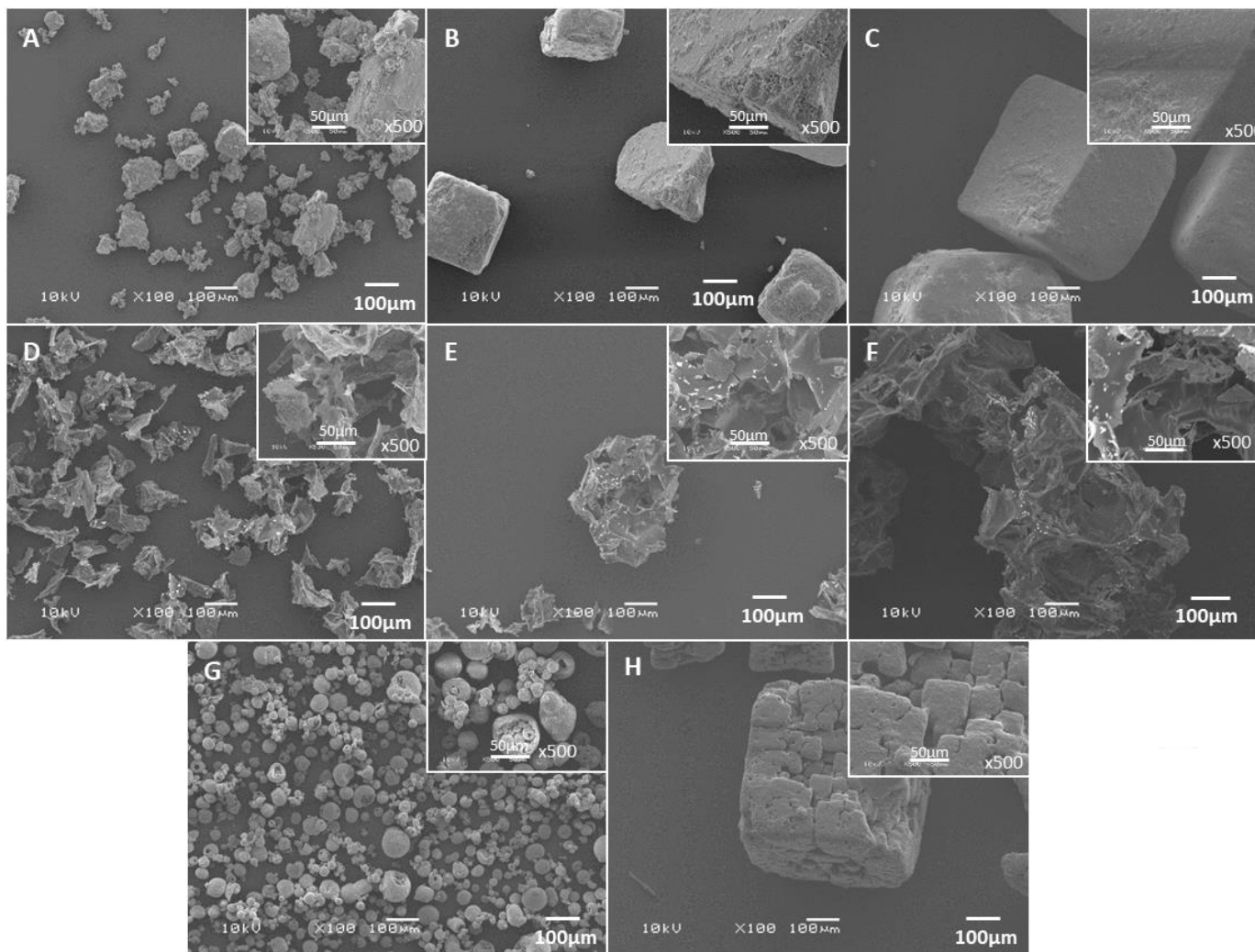


Figure 2.1. Scanning Electron Microscopy images of model salts: (A) RS <106µm, (B) RS 106-425µm, (C) RS 425-600µm, (D) FMS < 106 µm, (E) FMS 106-425µm, (F) FMS 425-600µm, (G) SODA-LO® and (H) dendritic at magnification x 100 for each of the main images and x 500 magnification images are displayed in the top right corner for each salt sample.

2.5.2. Moisture content and water activity of model salt particles

MC and a_w are presented in Table 2.1. FMS and SODA-LO® have a slightly higher MC and a_w . RS samples have a lower MC and a_w . MC and a_w are significantly higher ($p < 0.05$) in the samples that contain secondary materials, such as maltodextrin in the SODA-LO® sample and egg albumin and methylcellulose in FMS samples. According to the product specification, the MC of SODA-LO® is 0.7 % which is close to that measured in this study (0.75 ± 0.03 %). A higher MC in these particles is probably due to inefficient evaporation of water from the salt crystal during spray-drying and foam-mat drying (Vinitha et al., 2020) due to water binding with the proteins and sugars. A_w measures the amount of water available for biochemical reactions. It can be used as a predictor for the microbial stability of food. A_w values in the modified salts were between 0.35 and 0.44, while RS and dendritic salts were between 0.28 and 0.31 and were significantly lower ($p < 0.05$) than the modified salt samples, except FMS 106-425 μm (Table 2.1). An increase in MC and A_w may have consequences on the long term microbial stability of the salt powder as a product. However, as all the salt samples had an MC of < 1 % and an A_w of < 0.5 , this is unlikely to be an issue as typically, an a_w value of > 0.6 is quoted as being required for microbial growth (Barbosa-Cánovas et al., 2007) and these small differences in water content are unlikely to have a significant impact on the physical properties of the samples.

Table 2.1 Physicochemical properties, transfer efficiency and adhesion capabilities (mean \pm SD) of different types of salt crystal and modified salt particles. Values in the same row with different letters are significantly different (P <0.05).

	Salt Particles ¹							
	RS <106 μ m	RS 106-425 μ m	RS 425-600 μ m	Dendritic	SODA-LO®	FMS <106 μ m	FMS 106-425 μ m	FMS 425-600 μ m
Moisture content (%)	0.01 \pm 0.01 d	0.06 \pm 0.02 cd	0.04 \pm 0.02 d	0.06 \pm 0.03 cd	0.75 \pm 0.03 a	0.35 \pm 0.11 bc	0.48 \pm 0.35 ab	0.48 \pm 0.10 ab
Water activity	0.31 \pm 0.01 cd	0.30 \pm 0.01 cd	0.28 \pm 0.02 d	0.30 \pm 0.00 cd	0.41 \pm 0.02 ab	0.44 \pm 0.02 a	0.35 \pm 0.03 bc	0.39 \pm 0.04 ab
NaCl (%)	99.0 \pm 1.0 a	99.0 \pm 1.0 a	99.0 \pm 1.0 a	100 \pm 3.0 a	93.4 \pm 1.0 b	58.6 \pm 2.0 d	73.5 \pm 0.0 c	76.6 \pm 2.0 c
Particle Size diameter (μ m)	44.3 \pm 34	299.6 \pm 115.2	542.6 \pm 101.4	239.9 \pm 106.2	34.5 \pm 23.9	60.69 \pm 40.1	150 \pm 116.1	294.4 \pm 147
Bulk Density (g/ml)	0.69 \pm 0.02 c	1.23 \pm 0.03 a	1.30 \pm 0.02 a	1.02 \pm 0.01 b	0.39 \pm 0.01 d	0.13 \pm 0.01 g	0.26 \pm 0.01 f	0.34 \pm 0.01 e
Tapped density (g/ml)	1.00 \pm 0.08 c	1.42 \pm 0.05 a	1.39 \pm 0.01 a	1.18 \pm 0.01 b	0.52 \pm 0.02 d	0.23 \pm 0.05 e	0.43 \pm 0.02 d	0.45 \pm 0.02 d
CI (%)	30.8 \pm 5.7 ab	10.4 \pm 4.8 d	6.8 \pm 1.3 d	13.0 \pm 1.1 cd	25.1 \pm 2.3 bc	41.8 \pm 9.0 a	41.0 \pm 1.3 a	24.0 \pm 1.5 bc
L*	91.85 \pm 0.07 a	86.35 \pm 0.99 d	82.45 \pm 1.56 e	91.07 \pm 0.05 abc	91.28 \pm 0.28 ab	89.91 \pm 0.04 bc	89.28 \pm 0.20 c	85.40 \pm 0.33 d
a*	0.15 \pm 0.01 a	0.01 \pm 0.02 c	-0.05 \pm 0.01 cd	0.08 \pm 0.03 b	-0.08 \pm 0.01 d	-0.55 \pm 0.04 e	-1.20 \pm 0.03 f	-1.54 \pm 0.05 g
b*	0.57 \pm 0.03 d	0.17 \pm 0.05 e	0.03 \pm 0.10 e	0.10 \pm 0.02 e	2.50 \pm 0.06 c	8.24 \pm 0.09 b	9.70 \pm 0.15 a	9.55 \pm 0.28 a
Whiteness index	91.82 \pm 0.07 a	86.35 \pm 0.98 b	82.45 \pm 1.56 c	91.07 \pm 0.05 a	90.93 \pm 0.28 a	86.97 \pm 0.09 b	85.49 \pm 0.05 b	82.48 \pm 0.42 c
Transfer Efficiency (%)	97.7 \pm 1.3 a	93.6 \pm 4.1 bc	91.6 \pm 2.9 c	98.3 \pm 0.6 a	98.4 \pm 0.6 a	98.7 \pm 1.7 a	98.8 \pm 0.3 a	97.2 \pm 0.1 ab
Adhesion after packaging (%)	95.5 \pm 0.6 a	89.9 \pm 4.1 b	88.4 \pm 1.6 b	96.8 \pm 0.8 a	95.7 \pm 0.8 a	95.6 \pm 1.0 a	94.7 \pm 1.8 a	87.5 \pm 2.3 b

¹ Samples: FMS = foam-mat salt; RS = regular salt. Colour: L* = lightness level from 0 = black and 100 = white; a* = redness from red (+) to green (-); b* = yellowness from yellow (+) to blue (-). CI% = Compressibility index: <10 = excellent flow; 11-15% = good flow; 16-20 = fair flow; 21-25% = passable flow; 26-31 = poor flow; 32-39 = very poor flow; >40 = very very poor flow.

2.5.3. NaCl content of model salt particles

SODA-LO® and FMS samples had a significantly lower NaCl content when compared to RS and dendritic salt due to the inclusion of non-NaCl components used to create the three-dimensional structures of these salt particles (Table 2.1). The three FMS samples would theoretically have similar NaCl % as they were manufactured in the same batches before being separated by sieving. However, FMS <106 µm sample has a lower NaCl % than the other two FMS samples ($p < 0.05$). A likely explanation for this is that particles of methylcellulose and egg albumen, smaller than 106 µm in diameter, settle in this fraction, increasing the proportion of other materials. Due to the differences in NaCl levels across all samples, altered amounts of each salt sample was added to the oiled peanuts prepared for sensory evaluation to ensure equivalent NaCl content between samples. Dendritic salt is the purest of all the salts with 100 % NaCl as it does not contain any additional anti-clumping agents.

2.5.4. Colour of model salt particles

Whilst sodium concentration is key for saltiness perception, the appearance of salt particles is essential for consumer acceptability, as consumers expect typically salted snack products to be coated in white or slightly clear salt crystals. Colour properties are, therefore, outlined in Table 2.1. There is a strong negative correlation between whiteness and particle size ($r = -0.84$, $p = 0.009$) due to the increased compactness. The largest particle fractions (FMS and RS 425-600 µm) have significantly

lower whiteness indices ($p < 0.05$) compared to all other samples. SODA-LO®, dendritic and RS 106 μm have a significantly higher whiteness value than the other salts ($p < 0.05$). The a^* values for all samples are all very close to 0, showing no red or green contribution to colour. FMS samples all have values over 8 for b^* , meaning these samples have a slight yellow hue ($+b^*$) due to the addition of egg albumen powder (Katekhong and Charoenrein, 2017). In this study, colour differences were minimised during sensory assessment using red booth lights, however, increasing yellowness of samples could impact consumer acceptance.

2.5.5. Tapped density, bulk density and Carr's Compressibility

Index of model salt particles

Tapped density, bulk density and CI% are calculated for powders as indicators of ease of reconstitution, packaging, transportation, storage and processing (Marques et al., 2014). Tapped density and bulk density (Table 2.1) positively correlated to each other ($r = 0.99$, $p < 0.001$). Both are also highly negatively correlated with CI % ($r = -0.86$, $p = 0.006$ and $r = -0.91$, $p = 0.002$, respectively).

The size of RS directly impacted bulk density and tapped density, with larger particle fractions having higher densities (Table 2.1). This is expected as relatively larger particles flow more readily while more uniformly shaped pack more efficiently. This is also observed for CI %, where the larger particles have a CI % of $< 11\%$, indicating "excellent" or

“good” flow, the smallest RS (RS <106 μm) has a CI % of 30.8%, which indicates “poor” flow properties.

Dendritic salt had a bulk density, tapped density and CI% that in all cases is similar or sits between RS<106 μm and RS 106-425 μm . Given that dendritic salt has a mean particle size (239 μm) that also sits between these two fractions (44 μm and 299 μm), it can be assumed that it behaves similarly to RS particles.

SODA-LO® and FMS particles have lower bulk and tapped densities than all other samples. Whilst SODA-LO® (CI% of 25.1 %) has “passable” flow properties, which is similar to RS<106 μm (CI% 30.8 %), FMS has a high CI % indicating “very very poor” flow properties for FMS <106 μm and FMS 106-425 μm , and only “passable” flow for FMS 425-600 μm . This indicates a marked difference in powder properties and flow behaviour for the FMS samples compared to RS, dendritic and SODA-LO®. Differences are assumed to be due to the very low bulk density and complex surface geometry formed due to air incorporation during the drying process for SODA-LO® and the inherent structure of dendritic salt particles.

2.5.6. Adhesion and transfer efficiency of model salt particles

2.5.6.1. Transfer efficiency of model salt particles

Transfer efficiency and particle diameter are negatively correlated ($r=-0.85$, $p=0.008$). ANOVA results (Table 2.1) show that RS 106-425 μm

and RS 425-600 μm samples were significantly lower in transfer efficiency than all other samples, except FMS 425-600 μm , which was not significantly different to RS 106-425 μm . Our results show that reducing the particle size of regular table salt increased transfer efficiency during coating. This supports previous findings by Miller and Barringer (2002) and Sumonsiri and Barringer (2011). However, despite the change in particle size within the three FMS samples, the level of transfer efficiency is not significantly different, suggesting that both FMS processing and a reduction in particle size may increase transfer efficiency.

Adhesion is the main factor determining the coating efficiency of the peanuts and is mainly due to the viscous oil holding salt particles via liquid bridges formed through capillary forces (Takenaka et al., 2006). Miller and Barringer (2002) explained that finer particles have a smaller mass and therefore have improved adhesion initially as gravity has less effect than for larger masses. Whereas there is a more significant effect of gravity on larger particles, counteracting the total adhesion force, causing less coating. When comparing the same fraction sizes, e.g. RS 106-425 μm and FMS 106-425 μm , there was significantly higher transfer efficiency ($P < 0.05$) in the salt processed using foam-mat drying (Table 2.1). The same can be said for fraction size 425-600 μm but not the < 106 μm fraction, possibly due to the slightly smaller mean particle size of the FMS samples (Table 2.1). The improved transfer efficiency of FMS could therefore be, in part, also due to the reduction in density. A decrease in

density contributes to the rise in transfer efficiency with correlation values of -0.76 to -0.81 ($p < 0.05$). CI (%) is also significantly positively correlated to transfer efficiency ($r = 0.77$, $p = 0.015$). Whilst correlation cannot directly imply causality, our findings indicate that particles with poor free flowing properties are likely to have a higher transfer efficiency, suggesting that not only particle size and density, but also surface properties (i.e. how the particles mutually interact) may play a role in transfer adhesion.

2.5.6.2. Adhesion of model salt particles after packaging

Poor seasoning and salt adhesion efficiency can be problematic once products are packaged. Particles become unattached and drop to the bottom of the packaging resulting in loss of potential flavour. In this study, all peanut samples lost some surface salt within the packaging. Samples: RS 106-425 μm , RS 425-600 μm and FMS 425-600 μm , all had significantly lower ($P < 0.05$) adhesion after the packaging test compared to the other samples (Table 2.1). Similarly to transfer efficiency results, smaller particle sizes remain adhered to the peanuts with less particle loss. Whilst the global correlation between mean particle diameter and adhesion after packaging ($r = 0.60$) was weaker than transfer efficiency and was not significant ($p = 0.11$, Table 1), there was a significant impact of particle size on adhesion losses for RS ($P < 0.05$) and FMS ($P < 0.05$).

As mentioned previously in section 2.5.6.1, adhesion forces between particles and food surface are made up of capillary forces due to the presence of oil. Particles are lost from the surface when the external

influences are strong enough to split the capillary bridges. Differences in adherence can be interpreted via two different mechanisms. Firstly, friction between peanuts and packaging, and secondly, the impact of the peanut colliding with the bottom of the packet due to gravity and resulting in the loss of salt crystals from the peanut surface. In the first instance, larger salt crystals are more exposed to mutual contact than smaller particles, so the larger particles in this study are lost first. Larger particles are more likely to pack more closely. When coated in fat, they cling to each other, thereby further overcoming adhesion forces.

In the second instance, as the peanuts fall and impact the bottom, they 'shake' off some of the salt particles. This is due to the transfer of kinetic energy from peanuts to salt crystals. The kinetic energy of a salt particle is proportional to its mass and hence its volume. Therefore, larger particle sizes achieve greater kinetic energy than smaller particles with the same density. If this kinetic energy is greater than the adhesion energy, then particles detach from the peanut. The adhesion energy is assumed to be proportional to the contact surface area (Halsey and Levine, 1998). This contact surface area can be estimated as the surface area of one of faces of the salt particles. Therefore, the ratio between kinetic and adhesion energy is proportional to the ratio of salt particle volume and the area of its contacting surface. For larger particles (if their shape is similar to smaller particles), this ratio is greater than for smaller particles, so they detach more easily. Manufacturers aiming to reduce cost and reduce the loss of coating materials could therefore decrease

particle size. This study demonstrates a new method of assessing salt transfer and adhesion, taking into account forces incurred during packaging and transport.

2.5.7. Dissolution kinetics of model salt particle

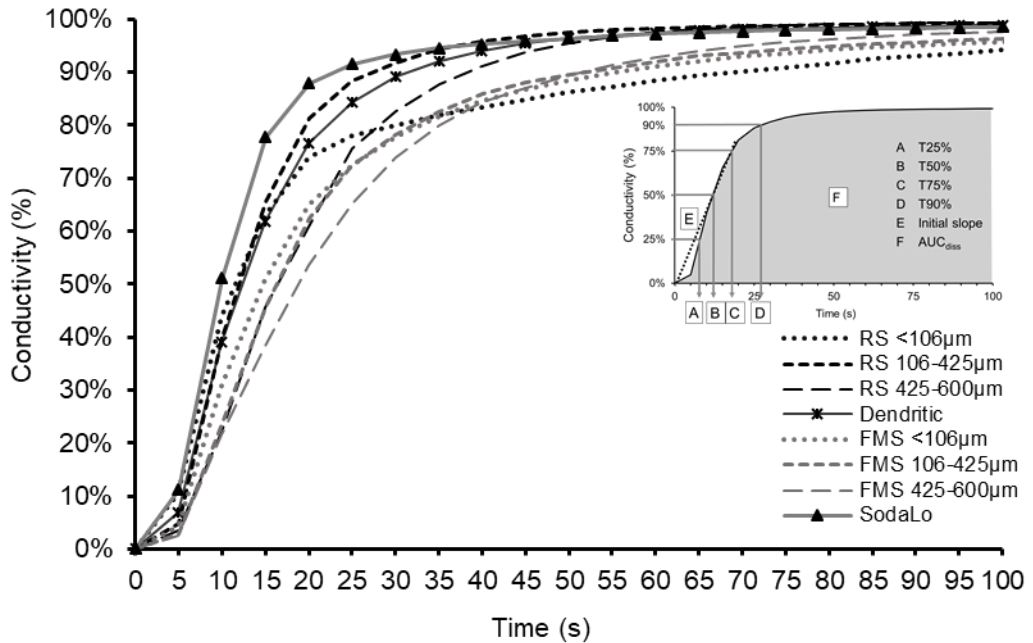


Figure 2.2. Dissolution curve presented as percentage conductivity of dissolution media (RO water) after immersion for each model salt sample when applied to oiled peanuts contained in a dissolution vessel at 0 seconds at a constant temperature of 20°C. Samples denoted as RS is regular table salt (NaCl), FMS is foam-mat salt. Fraction size is indicated for RS and FMS samples. Dendritic and SODA-LO® samples were commercially purchased.

Oral processing is a rapid event. In most cases, salt crystals cannot fully dissolve before a bolus is formed and swallowed (Tian and Fisk, 2012). This incomplete dissolution limits potential saltiness perception. To evaluate this, the salt particles were applied to a real food matrix, oiled peanuts, and dissolution of salt was observed by the change in conductivity of the dissolution media (RO water). Raw conductivity data was converted to a percentage of total conductivity to observe

comparative dissolution kinetics between samples over time. The dissolution graph in Figure 2.2 shows a slow increase in conductivity (%) in all samples until 5 seconds and then followed by a rapid increase in conductivity (%) between 5 s – 20 s. After 20 s, the increase in conductivity slows again.

Significant differences were found between the salts for all extracted dissolution parameters (Supplementary Material 2.1). SODA-LO® had a significantly higher ($p < 0.05$) initial dissolution slope (determined by calculating the gradient of the curve between 0 and 20 seconds) than all other samples (4.8 % increase per second), except for RS 106-425 μm (4.4 % increase per second). SODA-LO® took the shortest time to reach 25, 50, 75 and 90 % conductivity, while FMS 425-600 μm required the longest time to reach these same points (except for time to 90 % where $< 106 \mu\text{m}$ was slowest).

2.5.7.1. Relationship between salt properties and dissolution kinetics

Due to the complexity of the various interacting factors, the experimental results from the physicochemical characterisation of samples, *in vitro* dissolution data and sensory TI parameters underwent PLS-R to elucidate relationships between these variables (Figure 2.3). Sensory TI parameters and their relationships with dissolution parameters are introduced and discussed in the subsequent sections (section 2.5.8). In general, samples can be seen to be separated on the biplot by particle

size along the axis t1 and by NaCl content and processing type on axis t2.

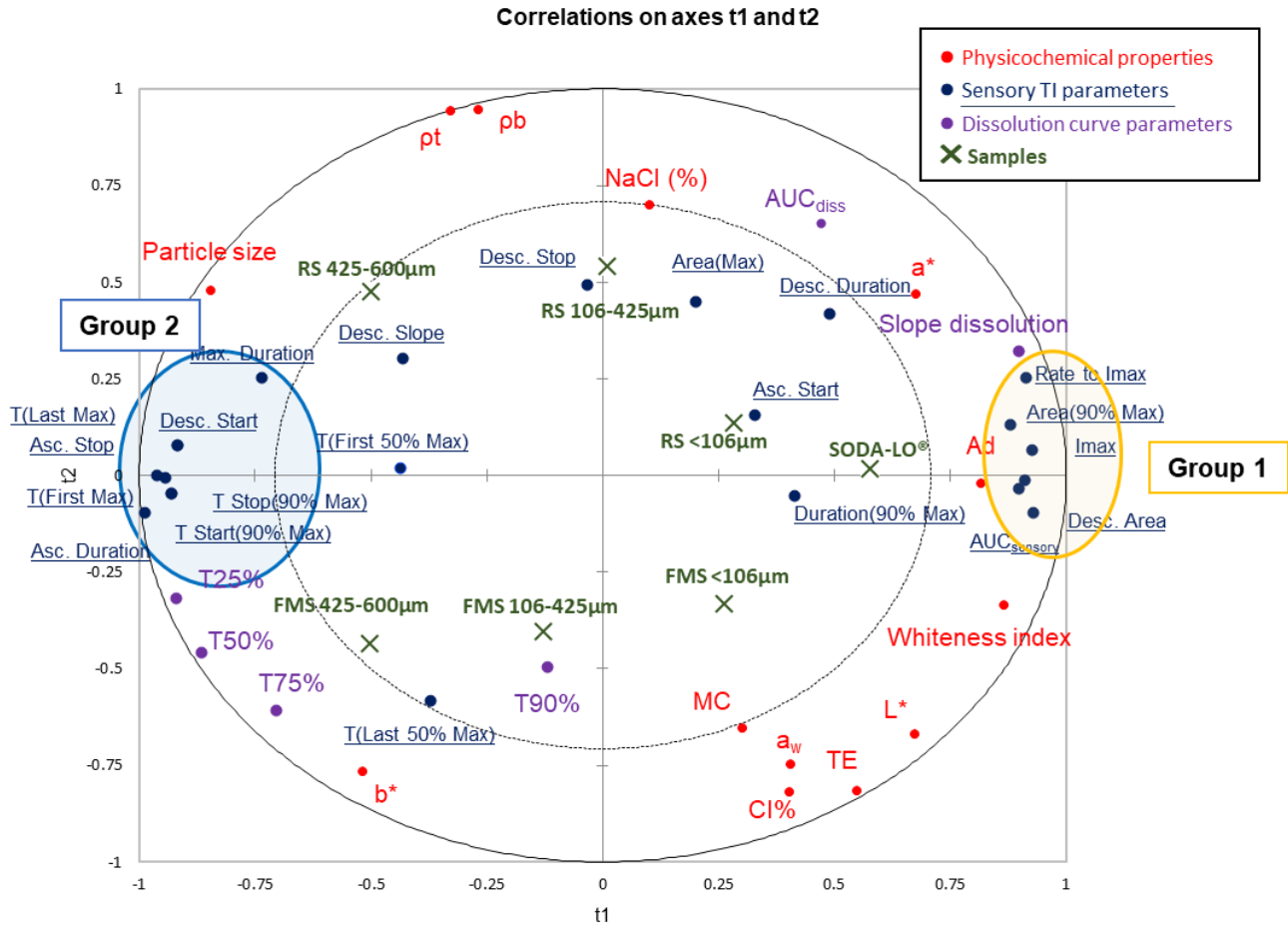


Figure 2.3. Partial Least Square Regression projections of model salt samples (found in green, where RS denotes regular salt and FMS denotes foam-mat salt). The physicochemical and adhesion properties are in red (X), *in vitro* dissolution parameters in purple (X) while sensory variables (Y) are projected in blue. Variables X: pt and pb: tapped density and bulk density respectively, T25%, T50%, T75%, T90%: time taken to reach 25%, 50%, 75% and 90% respectively, MC: moisture content percentage, a_w: water activity, CI%: Compressibility Index, TE: transfer efficiency, ad: adhesion percentage after packaging test, L*: lightness from 0=black to 100=white, a*, a* = red (+) to green (-) axis, b* = yellow(+) to blue (-) axis, AUC_{diss}: area under the curve for dissolution curve and slope is the gradient of increase between 0-20 s of dissolution curve. All Y variables are detailed in the materials and methods section.

Extracted parameters from the dissolution study are clearly separated on axis t1. T25%, T50% and T75% are closely correlated and are negatively presented on axis t1. T90% is less well resolved. This is partly due to the

dissolution kinetics of RS <106 μm sample, which follows a different dissolution profile, as shown in Figure 2.2. The dissolution for this sample slows more quickly than the other samples. This is proposed to be due to the strong adhesion forces between surface oil and the highly compact salt particles of small particle size.

Particle size is positively correlated with the time to reach 25% conductivity ($r=0.65$), and this was almost significant at $p=0.06$. The linear regression is weaker than expected due to the outlying trend of RS <106 μm mentioned previously. ANOVA results confirmed significant differences between different particle sizes for curve parameters (further information can be found in Supplementary material 2.1). Samples on the negative side of axis 1, RS and FMS 425-600 μm , can be described as dissolving more slowly due to their larger particle size and lower surface area.

FMS samples are presented closely to T25%, T50% and T75%, indicating that foam-mat drying creates particles that dissolve more slowly. This restricted dissolution in the FMS samples is proposed to be due to the hydrophobic egg albumen and methylcellulose encapsulating the salt and slowing the rate at which it can dissolve. Sodium ions can also chemically interact with negatively charged amino acids within the protein. This binding of free Na^+ will reduce sodium ion mobility and further slow release and dissolution (Mosca et al., 2015). This can be supported by solubility values (the degree that a compound can dissociate in water). Solubility values for egg albumen and

methylcellulose are 50 mg/ml and 20 mg/ml respectively (Sigma-Aldrich, 2020c, Sigma-Aldrich, 2020b) which are substantially lower than sodium chloride alone (358 mg/ml) (Sigma-Aldrich, 2020a). While this limits FMS use for topical applications, the particles inhibited NaCl release via protein binding may better suit other salt reduction strategies. One example may be an encapsulated salt in bread. It has been previously shown that an inhomogeneous distribution of salt or 'salty spots' within bread can compensate for a reduction in salt. This concept has been previously demonstrated using a fat enrobed salt offering significant sodium reduction potential in bread (Noort et al., 2012b). However, FMS contains protein rather than fat, which could offer a nutritional benefit. The approach warrants further exploration.

2.5.8. Sensory evaluation of model salt particles

Temporal saltiness perception was assessed by TI and average TI curves for each salt are shown in Figure 2.4. All curves show a similar curve profile; initial increase in saltiness to a peak, followed by a plateau and gradual decrease until saltiness is no longer perceived, although differences in peak, plateau and time can be observed between samples.

TI curve parameters were extracted and included for PLS-R, identified in blue (Y variables) on the PLS correlation circle (Figure 2.3). TI curve parameters; desc. duration, desc. slope, desc. stop, T (First 50% Max), T (Last 50% Max), asc. start, area(max) are all found in the inner circle of Figure 2.3, indicating that these parameters are not significantly

correlated with the X variables or the samples. These parameters showed no significant differences between samples and were not presented in Table 2; however, due to their importance in understanding the complex interactions of dissolution kinetics and saltiness perception, they are included in the PLS-R. Samples differed significantly in the following curve parameters; I_{max} , rate to I_{max} , T(Last Max), $AUC_{sensory}$, T Start (90% Max), T Stop(90% max) and Desc. area and are presented in Table 2.

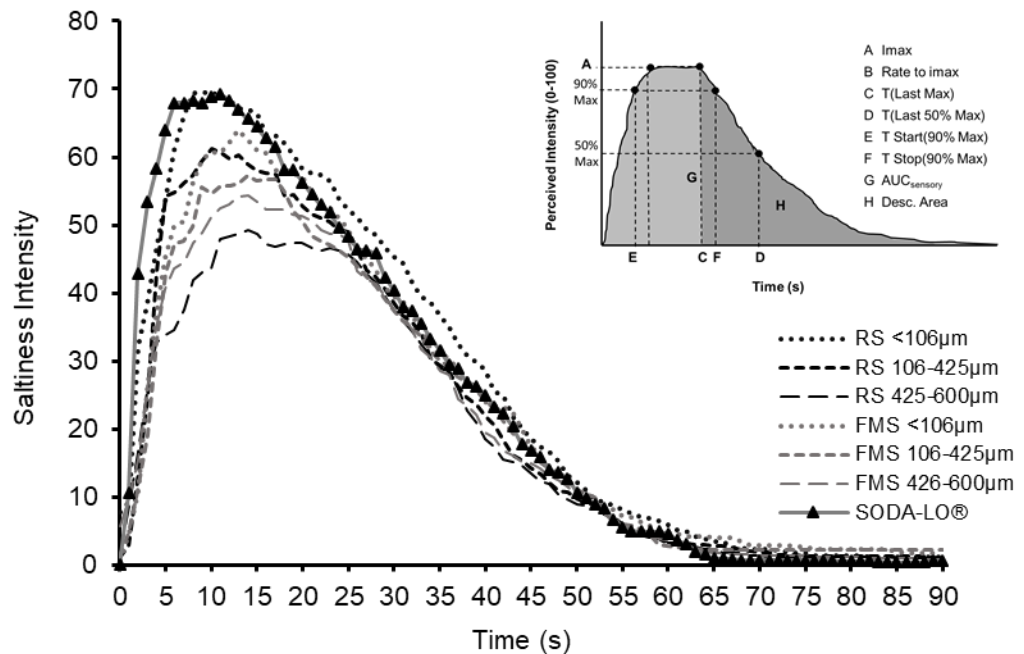


Figure 2.4. Time-intensity curves averaged across panellists and replicates for perceived saltiness intensity of model salts applied to slightly oiled peanuts with equivalent sodium chloride levels (1.3% in the final product).

Table 2.2. Parameters extracted from time-intensity (TI) curves for perceived saltiness (mean \pm standard error) which showed significant differences between samples. Values in the same row with different letters are significantly different ($P < 0.05$).

TI Curve parameters	Salt Particles						
	RS <106 μ m	RS 106-425 μ m	RS 425-600 μ m	SODA-LO®	FMS <106 μ m	FMS 106-425 μ m	FMS 425-600 μ m
I _{max}	77.1 \pm 2.6 A	66.9 \pm 3.1 ABC	56.4 \pm 3.4 C	75.6 \pm 3.2 A	67.5 \pm 3.0 AB	62.9 \pm 2.9 BC	59.3 \pm 3.1 BC
Rate I _{max}	11.5 \pm 1.8 AB	8.91 \pm 1.9 AB	7.34 \pm 1.8 AB	13.7 \pm 2.5 A	9.47 \pm 2.3 AB	6.62 \pm 2.0 B	5.96 \pm 0.67 B
T (Last Max)	16.8 \pm 1.6 AB	18.0 \pm 1.8 AB	20.2 \pm 1.9 A	14.6 \pm 1.9 B	18.0 \pm 1.6 AB	17.7 \pm 1.6 AB	19.4 \pm 1.6 A
AUC _{sensory}	2370 \pm 176 A	2010 \pm 188 ABC	1750 \pm 180 C	2250 \pm 190 AB	2110 \pm 167 ABC	1950 \pm 186 ABC	1870 \pm 156 BC
T Start (90% Max)	6.35 \pm 0.78 CD	8.55 \pm 1.1 ABC	10.4 \pm 1.2 A	5.25 \pm 1.2 D	7.65 \pm 0.93 BCD	9.20 \pm 1.1 AB	9.85 \pm 1.0 AB
T Stop (90% Max)	18.5 \pm 1.8 AB	19.5 \pm 2.0 AB	22.2 \pm 2.1 AB	17.2 \pm 1.9 B	19.6 \pm 1.8 AB	20.6 \pm 2.0 AB	21.0 \pm 1.7 AB
Desc. Area	1460 \pm 140 A	1140 \pm 140 ABC	883 \pm 120 C	1430 \pm 150 AB	1230 \pm 120 ABC	1150 \pm 130 ABC	1000 \pm 120 BC

TI parameters are separated by PLS-R along axis t1 (Figure 2.3) and are grouped into two clusters: Group 1: Rate to I_{max}, area (90% max), I_{max}, desc. area, and AUC_{sensory}; and Group 2: (T (first max) and T(last max), T Start(90% Max) and T Stop(90% max), Asc. Stop and max. duration). In general, group 1 is related to the intensity of saltiness and total saltiness. Group 2 is related to the temporal aspects of saltiness perception (correlation highest with slower time to maximum saltiness). These two groups are highly negatively correlated.

SODA-LO® followed by <106 µm samples of both RS and FMS are positively correlated with group 1 TI parameters (Figure 2.3) with the highest peak intensity of mean TI curves (Figure 2.4). ANOVA on extracted parameters from the TI curves confirmed that these salts have the highest values for Group 1 parameters (Table 2.2). In comparison, 425-600 µm samples of both RS and FMS resulted in the lowest mean TI curve peaks (Figure 2.4), saltiness intensity (Table 2.2) and are positioned further away from Group 1 parameters in the PLS-R biplot (Figure 2.3). This supports previous studies showing a reduction in particle size results in a higher I_{max} (Rama et al., 2013) and the hypothesis that SODA-LO® with its low density and hollow structure containing internal voids (Figure 2.1) would be likely to dissolve much more rapidly than larger particles or those that consist of a more dense crystalline structure (RS).

FMS samples have a less compact structure than RS particles and greater surface area, with more exposed voids (Figure 2.1A-F) and would be expected to hydrate more quickly than their size equivalents in RS particles. However, foam-mat samples of equivalent size to RS particles did not significantly increase in group 1 saltiness parameters. The act of processing is orthogonally presented on the PLS-R with separation on t_2 , corresponding with the previously presented dissolution data. It is assumed to result from a combination of the encapsulation or binding of Na^+ by proteins, as explained in 2.5.7.1, and hydrophobic interactions of salt particles with surface fat which restricted dissolution rates in saliva.

A direct comparison of the equally sized foam-mat particle (FMS < 106 μm) and SODA-LO® suggests that when comparing the two particles with similarly high levels of internal voids and low densities, that the hydrophobic proteins in the FMS samples are binding to the Na^+ and changing the hydrophobicity of the particle which ultimately restricts Na^+ release and dissolution (Supplementary material 2.1).

2.5.8.1. Relationship between dissolution kinetics and sensory perception

Dissolution and TI curve parameters showed significant correlations (Supplementary material 2.2). The initial slope gradient extracted from between 0 and 20 s of the dissolution curves is positively correlated with rate to I_{max} ($r=0.85$, $p=0.01$), which is a key marker of Group 1.

Furthermore, the initial dissolution slope is also positively correlated with area (90% Max) ($r = 0.75$, $p=0.062$), I_{max} ($r = 0.68$, $p=0.087$) and $AUC_{sensory}$ ($r = 0.59$, $p=0.12$), although it should be noted that these correlations were not found to be significant ($p>0.05$). Dissolution slope is significantly inversely correlated with the group 2 sensory parameters, T(first max) ($r= -0.77$, $p=0.026$), T Start(90% max) ($r=-0.77$, $p=0.026$) and T Stop(90% max) ($r=-0.74$, $p=0.036$). Overall, this suggests that the *in vitro* method can be used as a proxy for the group 1 and group 2 saltiness attributes.

It can, therefore, be shown that samples with a higher dissolution slope value will take less time to reach peak intensity in sensory trials., When aiming for salt reduction, this is a desirable insight since salt release is more efficient at the start of consumption meaning the product reaches a higher peak saltiness intensity compared to those with a slower dissolution slope where the peak intensity onset is later with a lower peak saltiness intensity (Figure 2.4). It is therefore thought that a higher dissolution slope provides an initial more intense salty 'hit', allowing salt to be removed without impacting saltiness perception. Whilst AUC_{diss} and T90% from the dissolution data did not show significant correlations with TI curve parameters ($p>0.05$); time to 25%, 75% and 50% conductivity are significantly correlated to sensory data (further detailed in bold in Supplementary material 2.2). T25% has higher correlation values and lower p-values than T50% and T75%, not unexpected given

consumption, last a relatively short time. After the initial Na⁺ release, other factors come into play, such as saliva flow, clearance, and taste adaptation; therefore, dissolution parameters extracted at relatively longer times do not strongly represent real-life consumption. We conclude that dissolution slope and time to 25% are the best predictors for predicting saltiness perception. In this study, it is also important to note that assessment of saltiness intensity was performed following a clearly defined protocol that standardised oral processing, thereby minimising variation in perception due to eating behaviour. This should be addressed in future studies with product consumers using free eating paradigms.

Other studies have also shown strong correlations between dissolution rates and TI curve parameters using artificial saliva as the *in vitro* dissolution media (Vella et al., 2012). Our current study used RO water as a dissolution media. It successfully predicted sensory outcomes suggesting RO water could be used as a simple alternative to the time and cost expense of artificial saliva if dissolution is the primary experimental aim.

It is noteworthy that whilst the *in vitro* sodium dissolution method used in this study was able to predict defined saltiness TI parameters, multiple factors relate to eating behaviour such as; oral processing and mouth behaviour, saliva composition and flow rates, bolus clearance rates, taste

adaption and chewing patterns should also be considered for investigation in future studies.

2.6. Conclusions

A range of model salt particles which varied in size, density, hydrophobicity and flow properties were used to explore the impact of particle design on adhesion to product, loss in-pack, rate of dissolution and saltiness perception, ultimately to generate a series of design rules that address each of the initial three phases proposed as potential routes to optimise saltiness perception.

Phase I: Adhesion during application and before packaging:

Key Finding: Transfer efficiency is driven by particle size ($r=-0.85$, $p=0.008$), bulk density ($r=-0.801$, $p<0.05$) and flow properties ($r=0.77$, $p=0.015$)

- Decreasing regular table salt particle size increased transfer efficiency during coating, likely due to increased interaction with surface fat on the product.
- Foam mat processing increased transfer efficiency indicating this is due to reduced bulk density.
- Flow properties were correlated with transfer efficiency suggesting particle-particle interactions also play a role.

Phase II: Adhesion during packaging and transport:

Key Finding: Loss from the product in packaging is driven by particle size ($p < 0.05$)

- Smaller particle sizes exhibited less loss due to enhanced adhesion energy between surface oil on the product and the smaller salt crystals.

Phase III: Release during oral processing:

Key Finding: Dissolution and/or saltiness are driven by particle size ($p < 0.05$) and hydrophobicity

- Smaller particles sizes were associated with faster sodium dissolution rates; however, this was compromised for highly dense small particles due to high levels of interaction with surface fats.
- Smaller particle sizes had a greater saltiness intensity (I_{max}) due to faster dissolution in saliva.
- Greater particle hydrophobicity resulted in slower sodium release.

In summary, to maximise potential perceived saltiness, salt particles should be designed with small particle size, low density and hydrophobicity and have a particle shape associated with optimal flow properties. Also, the *in vitro* sodium dissolution method used in this study was able to predict key parameters associated with *in vivo* saltiness time-

intensity. Future studies should investigate these design rules within a commercial product context and seek to validate the potential for sodium reduction whilst retaining consumer acceptability.

In addition to salt, these physicochemical design rules may apply to new product development and ingredient design of sugar, seasonings and other aligned pharmaceutical and oral care industries, where crystalline structures with controlled dissolution rates are essential for product efficacy.

Credit authorship contribution statement

Katherine Hurst contributed to the conceptualisation of the study, carried out the investigation, performed data analysis and wrote the initial draft. Charfedinne Ayed contributed to the conceptualisation of the study, and contributed to the data analysis, interpretation of results and writing of the manuscript. Ian Fisk acquired funding, supervised the project, helped conceptualise the idea and contributed to the writing of the manuscript. Louise Hewson provided insight and guidance into sensory implications and contributed to the writing of the manuscript. Ivan Derbenev helped to interpret the adhesion data and contributed to the writing of the manuscript. All authors reviewed, edited and approved the final draft of the manuscript.

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MS analysis and his assistance with the digestion process, and Tate & Lyle for supplying SODA-LO® Salt Microspheres.

Supplementary material (chapter 2)

Supplementary material 2.1. Dissolution curve parameters (mean \pm SD). T25%, T50%, T75% and T90% are all expressed in seconds (s). Values in the same column with different letters are significantly different ($p < 0.05$).

Dissolution curve parameters	Salt crystals				Modified salt			
	RS 106 μ m	RS 106-425 μ m	RS 425-600 μ m	Dendritic	SODA-LO®	FMS <106 μ m	FMS 106-425 μ m	FMS 425-600 μ m
Initial Slope (% per second)	4.0 \pm 0.0 BC	4.5 \pm 0.2 AB	3.3 \pm 0.3 DE	4.2 \pm 0.2 B	4.8 \pm 0.1 A	3.5 \pm 0.2 CD	3.4 \pm 0.3 DE	2.9 \pm 0.1 E
AUC _{diss}	7750 \pm 78 CD	8480 \pm 100 A	7980 \pm 170 BC	8340 \pm 120 AB	8620 \pm 30 A	7650 \pm 130 CD	7630 \pm 230 CD	7500 \pm 170 D
T25% (s)	7.3 \pm 0.4 CD	8.0 \pm 0.5 CD	10.7 \pm 1.2 AB	8.0 \pm 0.5 CD	6.7 \pm 0.3 D	8.8 \pm 0.3 BC	10.3 \pm 0.4 AB	11.00 \pm 1.00 A
T50% (s)	11.3 \pm 1.1 D	11.6 \pm 0.8 D	16.3 \pm 1.4 AB	12.2 \pm 1.4 CD	9.7 \pm 0.3 D	14.7 \pm 1.0 BC	16.0 \pm 0.0 AB	18.67 \pm 0.58 A
T75% (s)	20.5 \pm 0.7 CD	17.7 \pm 1.0 AB	24.8 \pm 1.8 BC	19.3 \pm 1.2 D	14.0 \pm 0.0 E	27.0 \pm 2.0 AB	27.0 \pm 1.4 AB	30.83 \pm 2.02 A
T90% (s)	68.5 \pm 2.8 A	26.5 \pm 2.5 CD	37.3 \pm 2.8 BC	31.0 \pm 3.0 CD	21.7 \pm 1.2 D	55.0 \pm 5.0 A	53.5 \pm 16.3 AB	51.67 \pm 2.89 AB

Supplementary material 2.2. Pearson correlations between sensory, dissolution measures and particle diameter. Values in bold are different from 0 with a significance level of $p=0.05$

Variables	Mean particle diameter	T25%	T75%	T50%	T90%	AUC dissolution	Slope dissolution
lmax	-0.83	-0.94	-0.70	-0.88	0.05	0.39	0.77
T(First Max)	0.76	0.91	0.77	0.87	0.18	-0.53	-0.79
Rate to lmax	-0.64	-0.94	-0.83	-0.92	-0.25	0.62	0.86
T(Last Max)	0.82	0.86	0.74	0.83	0.23	-0.53	-0.77
Max. Duration	0.75	0.62	0.55	0.62	0.25	-0.43	-0.60
AUCsensory	-0.87	-0.89	-0.58	-0.79	0.21	0.23	0.69
Area(Max)	-0.07	-0.39	-0.19	-0.33	0.37	-0.04	0.22
Area(90% Max)	-0.70	-0.87	-0.66	-0.81	0.04	0.36	0.72
T(First 50% Max)	0.38	0.55	0.51	0.47	0.31	-0.45	-0.37
T(Last 50% Max)	-0.01	0.57	0.58	0.55	0.39	-0.57	-0.55
T Start(90% Max)	0.83	0.93	0.72	0.86	0.08	-0.46	-0.81
T Stop(90% Max)	0.82	0.94	0.75	0.87	0.18	-0.53	-0.83
Duration(90% Max)	-0.34	-0.32	-0.12	-0.24	0.30	-0.10	0.21
Asc. Start	-0.27	-0.29	-0.15	-0.30	0.11	0.06	0.44
Asc. Stop	0.76	0.91	0.77	0.87	0.18	-0.53	-0.79
Asc. Duration	0.81	0.95	0.78	0.92	0.14	-0.53	-0.89
Desc. Start	0.82	0.86	0.74	0.83	0.23	-0.53	-0.77
Desc. Stop	0.21	-0.10	-0.19	-0.18	-0.22	0.27	0.27
Desc. Duration	-0.26	-0.57	-0.59	-0.64	-0.33	0.55	0.69
Desc. Area	-0.91	-0.88	-0.61	-0.81	0.13	0.28	0.71
Desc. Slope	0.55	0.40	0.15	0.27	-0.33	0.11	-0.12
Saltiness rating	-0.83	-0.88	-0.70	-0.84	0.02	0.38	0.70

Supplementary material 2.3. Pearson correlations between physicochemical properties and dissolution parameters. None of the values in are different from 0 with a significance level of $p=0.05$.

Variables	Mean particle diameter	T25%	T75%	T50%	T90%	AUC dissolution	Slope dissolution
Mean particle diameter	1.00	0.63	0.27	0.51	-0.30	0.02	-0.54
Moisture content (%)	-0.45	0.01	0.06	0.07	-0.14	-0.01	0.08
Water activity	-0.58	-0.02	0.23	0.12	0.12	-0.24	0.18
NaCl (%)	0.19	-0.46	-0.68	-0.55	-0.40	0.62	0.22
Bulk Density (g/ml)	0.70	-0.07	-0.39	-0.21	-0.47	0.51	0.05
Tapped density (g/ml)	0.62	-0.14	-0.43	-0.27	-0.39	0.49	0.07
Porosity (%)	-0.75	0.00	0.37	0.12	0.65	-0.58	0.00
Transfer efficiency (%)	-0.85	-0.31	0.04	-0.17	0.33	-0.21	0.21
Adhesion after packaging test (%)	-0.60	-0.67	-0.52	-0.67	-0.03	0.31	0.65
T25%	0.63	1.00	0.88	0.98	0.28	-0.66	-0.91
T75%	0.27	0.88	1.00	0.95	0.63	-0.91	-0.83
T50%	0.51	0.98	0.95	1.00	0.39	-0.76	-0.91
T90%	-0.30	0.28	0.63	0.39	1.00	-0.89	-0.46
AUC dissolution	0.02	-0.66	-0.91	-0.76	-0.89	1.00	0.73
Slope dissolution	-0.54	-0.91	-0.83	-0.91	-0.46	0.73	1.00
Lightness	-0.90	-0.33	0.09	-0.17	0.52	-0.35	0.29
a*	-0.28	-0.33	-0.24	-0.34	0.12	0.06	0.44
b*	0.06	0.45	0.49	0.50	0.15	-0.36	-0.53
Whiteness index	-0.84	-0.49	-0.13	-0.37	0.40	-0.15	0.49

Chapter 3

3. The relationship between salt taste sensitivity, saliva composition and self-reported sodium intake

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3.1. Abstract

Individual differences in salivary parameters can affect taste experiences, including the perception of saltiness and the quantity of salt consumed in foods. Few studies have considered the natural inter-individual variation of both unstimulated saliva (US) and stimulated saliva (SS) and its effects on sodium chloride salt (NaCl) taste sensitivity and dietary intake and behaviours. This study explored the relationship between key salivary parameters (flow rate, sodium, and protein concentrations), individual NaCl salt thresholds and NaCl consumption using 36 participants (29F, 7M, 19-47yrs). Detection and recognition thresholds were determined using ASTM E679 3-AFC method, and dietary intake and NaCl salt behaviours were measured using 72-hour food diaries and self-report questionnaires. Despite high intra and inter-individual variation in salivary parameters, flow rate and sodium concentration increased significantly during chewing, as expected, while protein concentration slightly decreased. While flow rate and sodium concentration show trends with NaCl taste threshold and sensitivity groups, salivary parameters did not predict the perceived saltiness intensity of supra-threshold concentrations. Negative trends were found between NaCl taste threshold and some NaCl intake measures, and when assessed using a food questionnaire approach, the high NaCl taste sensitivity group reportedly consumed significantly more NaCl than the low NaCl taste sensitivity group. Results suggest that 'salt-responsive'

individuals may actively seek a greater number of NaCl salt-containing foods, or high salt consumers may develop these behaviours due to their diet, leading to self-perpetuating negative dietary habits. Overall, there is evidence suggesting a probable relationship between NaCl taste function and food behaviours relating to NaCl consumption and dietary intake.

Keywords: saliva flow rate, saliva composition, taste threshold, salt perception, salt taste sensitivity, salt intake

3.2. Highlights

- Salivary parameters, NaCl taste sensitivity and intake was assessed in 36 subjects
- NaCl taste sensitivity is primarily dependent on salivary sodium concentration
- High NaCl taste sensitivity group consumed on average more salt
- Individuals perceiving NaCl as more intense ate fewer calories
- Trends observed between NaCl addition to food and perceived NaCl intensity

3.3. Introduction

Salt over-consumption and its impact on disease is an ongoing issue and a popular topic of research since over-consumption of sodium chloride salt (NaCl) is a risk factor for high blood pressure and cardiovascular diseases (World Health Organisation, 2021). Despite NaCl reduction efforts through food reformulation and behavioural interventions, NaCl intake remains high with averages of > 8 g/day, > 9 g/day and > 12 g/day in the UK, USA and Asia respectively (Public Health England, 2016). Therefore, more research is required at the consumer level to understand differences in food choices to guide salt reduction strategies to ultimately help achieve the World Health Organisation target of < 5 g/day of NaCl by 2025 (World Health Organisation, 2018).

Consumers are attracted to NaCl in food due to its unique taste, flavour-enhancing qualities, and functionality (e.g. reducing bitter notes) (Ley, 2008). However, individuals vary highly in NaCl salt consumption, preferences and salty food choices (Pilic et al., 2020, Hayes et al., 2010), as well as NaCl salt thresholds and perceived saltiness intensities (Yang et al., 2014, Zaidan et al., 2009, Yang et al., 2011). This is also reported for other basic tastes (Jayasinghe et al., 2017, Han et al., 2017). One link that could help unpick the multi-faceted issue of salt over-consumption is understanding individual differences in NaCl intake and taste sensitivity, and since saliva has a mechanistic role in taste and sensory perception

(Feron, 2019), all of these variables and their interrelationships require a deeper understanding.

Saliva is a complex bodily fluid composed of mainly water (99.5%), proteins, peptides and ions. It has multiple functions, from acting as a lubricant and a solvent for tastants during food oral processing to maintaining the health of taste receptor cells and supporting food clearance (Carpenter, 2012). It is continually secreted and removed from the mouth (via natural drainage and swallowing) at varying inter and intra-individual rates due to; circadian cycles, sex, age, hydration status, disease state and pharmacological agents (Dawes, 1972, Muñoz-González et al., 2018). During food oral processing, cues such as aroma, taste, and chewing stimulate the salivary glands, causing compositional changes and increased flow rate (Watanabe and Dawes, 1988, Dawes, 1996, Delvadia et al., 2012, Edgar, 1990). Salivary flow rate influences the perception of saltiness (Heinzerling et al., 2011), while several components of saliva have previously been implicated in determining salt taste thresholds and perception, including salivary sodium concentrations (Bartoshuk, 1974, Delwiche and O'Mahony, 1996), salivary proteins including endoprotease enzymes (Stolle et al., 2018, Stolle et al., 2017) and the abundance of tongue film (Feng et al., 2018) suggesting an essential interrelationship between saliva composition and salt perception.

A fundamental function of taste in mammals is to act as a gatekeeper, distinguishing nutrient-rich from toxic foods. However, sensitivity to basic tastes varies considerably across individuals. A proportion of this variation can be attributed to genetics; for example, the extensively studied sensitivity to the bitterness of 6-n-propylthiouracil (PROP) results from a single nucleotide polymorphism (SNP) on the TAS2R38 bitter receptor gene (Un-kyung et al., 2003). Intriguingly, individuals classified as PROP super-tasters also have elevated oral sensations to other tastes (Yang et al., 2014), including saltiness (Hayes et al., 2010). Additionally, considerations such as physiology, environmental factors, past exposure, and current diet also play a role in determining an individual's taste phenotype (Hayes et al., 2010). This variation in taste sensitivity, typically assessed either by supra-threshold intensities or taste thresholds, has been proposed to relate to variation in preference and, ultimately, food choice. Several studies have correlated sensitivity to the bitter compound PROP with liking and consumption of cruciferous vegetables, coffee and alcohol (Dinehart et al., 2006, Lanier et al., 2005, Tepper et al., 2009), whilst sweet liking, rather than sweet taste thresholds or supra-threshold intensities, has been suggested to predict sweet consumption (Garneau et al., 2018). Similarly, preference for NaCl may result from experience and familiarity with highly salty foods, but sensitivity to NaCl taste may also play a role. However, current findings on the association between NaCl intake and NaCl taste sensitivity

(assessed either by threshold levels or saltiness intensity of supra-threshold NaCl concentrations) are contradictory (Pangborn and Pecore, 1982, Martinelli et al., 2020, Veček et al., 2020). Understanding an individual's salt phenotype may enable informed dietary communication and intervention tailored to those most at risk of over-consumption of salt within the diet.

It is generally accepted that one single measure cannot incorporate the full view of taste function as it is genuinely multi-faceted (Webb et al., 2015). Several sensory measures can be used to assess taste function or, in other words, taste sensitivity, including but not limited to; threshold and supra-threshold measures. The detection threshold is the lowest tastant concentration discriminable from water, and the recognition threshold is the lowest concentration correctly identified as its taste quality. Supra-threshold measures are the perceived intensity ratings of supra-threshold NaCl concentrations, higher than the detection and recognition thresholds, and they may reflect the NaCl levels more commonly encountered in foods. While studies assessing the relationships between taste sensitivity (i.e. thresholds and supra-threshold intensities) and dietary intake often show no associations or contradictory results, hedonic ratings have been found to be a better indicator of dietary intake (Tan et al., 2021, Tan and Tucker, 2019). Nevertheless, Jayasinghe et al. (2017) showed negative correlations between sweet taste intensity with sweet food, total energy and

carbohydrate intake, while positive correlations have been found between thresholds and NaCl intake or consumption habits (Martinelli et al., 2020, Veček et al., 2020, Cattaneo et al., 2019).

In common with other tastants, for NaCl to be perceived it must first be released from the food matrix, before diffusion through the saliva to the taste receptor cells. Diffusion rate depends on the sodium ion concentration gradient between the extra- (i.e. saliva) and intracellular fluid of the taste receptor cells (Muñoz-González et al., 2018). Therefore saltiness perception is intricately linked with salivary sodium concentration (Delwiche and O'Mahony, 1996, Bartoshuk, 1974), and studies have suggested that salivary parameters could underlay the variation in sensory perception and even preference between individuals (Méjean et al., 2015, Feron, 2019, Gilles and Christian, 2018).

To understand individual variation in NaCl perception, deeper investigations into saliva composition changes between unstimulated saliva (at rest) and stimulated saliva (during consumption) and the relationship with threshold and supra-threshold perceptual measures are warranted. Additionally, this study examined the relationship between NaCl taste sensitivity and appetite for dietary NaCl intake using self-reported food diaries (over 72hrs), food frequency, and behaviour questionnaires.

3.4. Materials & Methods

3.4.1. Participants

Approval for this study was granted by the Faculty of Medicine and Health Sciences Research Committee, University of Nottingham, reference No. 381-1909. Thirty-six healthy participants (29 female, 7 male, 19-47 yrs) were recruited from students and staff of the University of Nottingham using email invitations. More details on demographic and anthropometric data can be found in Table 3.1. Participants self-reported to be in good health with no history of hypertension, cardiovascular diseases or renal diseases, not pregnant or lactating and were not taking medication (excluding oral contraceptives). Participants self-reported that they had no known sensory impairments in taste and smell. The study took place before the COVID-19 pandemic, and therefore prior to the increased prevalence of viral-induced sensory impairments.

3.4.2. Overview of study sessions

Over a total of 4 sessions, all participants provided three stimulated saliva (SS) and three unstimulated saliva (US) samples and performed a series of sensory tests at approximately the same time of day for each individual (between 9:15 am and 12:15 pm). Before each session, participants were required not to consume any food or drink (except for water) or brush their teeth within the 2 hours preceding the session.

Participants also completed a self-reported food diary (collected over a period of 72hrs), food frequency questionnaire and a salt behaviour questionnaire during the final session to assess regular NaCl salt intake.

Total number of participants	36
Age (mean yrs, range)	26, 19-47
Gender	
% Male	19 %
% Female	81 %
BMI (kg/m ²)	
% 16-25	82 %
% 26-44	14 %
Ethnicity	
% Hispanic	8 %
% Asian	25 %
% White	64 %
% Black	3 %

3.4.3. Saliva collection and measurements

3.4.3.1. Collection of unstimulated and stimulated saliva and determination of saliva flow rate

US and SS was collected by draining saliva into sterile pre-weighed 50 mL polypropylene graduated centrifuge tubes (Greiner Bio-One, Kremsmünster, Austria) with use of disposable funnels (VWR International, Pennsylvania, USA) for 10 (SS) or 15 (US) minutes. For the collection of US, participants were sat upright and asked to lean slightly forward to allow saliva to collect at the front of the mouth, allowing the saliva to fall into the collection vessel while making minimal facial or

mouth movements. For SS samples, participants were seated in the same position as for the collection of US and continually chewed a standardised size of parafilm (5 cm x 5 cm) (Bemis Company Inc, Wisconsin, USA), expectorating when they felt the need. All saliva samples were collected over ice. Salivary flow rate was determined by calculating the weight of saliva over the collection time, using the assumption 1 g of saliva is equal to 1 mL. Saliva samples were separated into 0.5 to 1 mL aliquots and immediately frozen at -80 °C until further analysis. For all following saliva analyses, aliquots of saliva were removed from the -80°C freezer and allowed to defrost (~ 5 mins).

3.4.3.2. Determination of salivary sodium concentration by Inductively Coupled Plasma Mass-Spectrometry (ICP-MS)

Saliva samples were measured in duplicate for each sample collected. Saliva aliquots were centrifuged at 5000 g for 15 minutes at a constant temperature of 4 °C to allow a protein-containing pellet to form at the bottom of the tube. Using a 2 % nitric acid solution, the supernatant was diluted to either a 1:20 or 1:40 dilution depending on the volume of saliva available for analysis (low volume of saliva required a 1:40 dilution which occurred in 15 % of total samples tested, dilution factors were altered accordingly). After dilution, samples were left overnight (at room temperature) to allow any precipitation to occur. Each sample was then pushed through a < 0.45 µm syringe filter (Merck Life Science, Dorset,

UK) into clean polypropylene ICP-MS tubes (Sarstedt Inc, Newton, North Carolina, USA). Samples were introduced at a flow rate of 1.2 mL/min from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a perfluoroalkoxy (PFA) Microflow PFA-ST nebuliser (Thermo Fisher Scientific, Bremen, Germany). Sample processing was undertaken using Qtegra™ software (Thermo Fisher Scientific, Bremen, Germany). For instrument calibration, internal standards included combinations of Scandium (Sc) (10 µg/L), Germanium (Gr) (10 µg/L), Rhodium (Rh) (5 µg/L), Rhenium (Re) (5 µg/L) and Iridium (Ir) (5 µg/L). The matrices used for internal standards, calibration standards and sample diluents were 2 % nitric acid (Fisher Scientific, Loughborough, UK). Multi-element calibration solutions were prepared at different concentration levels of Ca, Mg, Na and K (0-30 mg/L) from a bespoke external multi-element calibration solution (SCP Science, Quebec, Canada).

3.4.3.3. Total protein content of saliva

After samples had defrosted (~5 minutes) at room temperature (18-20 °C), saliva samples underwent gentle centrifugation (1500 x g for 15 minutes) to remove large cellular debris prior to protein. Total protein content (mg/mL) was determined by using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Massachusetts, USA). An aliquot of the supernatant (50 µL) was diluted in 200 µL of PBS buffer. Standard

calibration curves were obtained by using bovine serum albumin (BSA) in a dilution series of 9 concentrations between 0 and 2000 µg/mL. Prepared standard solution and diluted saliva samples (25 µL) were each placed in duplicates on a microplate wells. The working reagent (200 µL) was added to each sample and gently mixed (30 s) and incubated at 37 °C for 2 hours before cooling for 15 minutes at room temperature (18-20°C). A GENios plate reader (Tecan, Zürich, Switzerland) was used to measure the light absorbance of samples at a wave length of 580 nm.

3.4.4. Sensory Assessment

A range of NaCl salt solutions were prepared using Evian mineral water (Danone, Paris, France) and NaCl (Sigma-Aldrich, St. Louis, Missouri, USA) and stored at 4°C overnight before removing to allow to equilibrate to room temperature (22 ± 2 °C) prior to use. Sensory assessments took place in a standardised sensory environment following ISO 8589:2007 international standard. Compusense sensory software was used to collect participants' responses for all sensory measurements (Compusense Five 5.4, Ontario, Canada). Before sensory assessment, participants were only provided with necessary information on how to assess the samples, while no information was provided on the type of tastant used.

3.4.4.1. Detection and recognition salt threshold measurements

Best Estimate Threshold (BET) for both detection and recognition of NaCl salt solutions were determined following the ASTM standard E679-19 (ASTM International, 2011) for each participant. The concentrations ranged between 1-24 mM and were established using previous literature (ASTM International, 2011, Yang et al., 2014) and further modified by a preliminary pilot test. Participants were presented with a series of ten 3-Alternative Forced Choice (3-AFC) tests comprising two blank water samples and one NaCl salt solution increasing in concentration at a constant factor ($\sim x1.4$) each time. Triads were presented in ascending order of salt concentration. Each sample was 15 mL and presented in identical plastic 30 mL pots labelled with random 3-digit codes. Participants were told that 2 samples were the same for each triad and 1 differed and asked to indicate which out of the 3 samples was the odd sample out. Participants were then asked to state how that sample differed. Between each 3-AFC test, participants were instructed to cleanse their palate with unsalted crackers (Rakusens Limited, Leeds, UK) and Evian mineral water (Danone, Paris, France), then take a 90-second break before tasting the next set of samples. Each individual's BET for both detection and recognition were calculated based on Yang et al. (2014), Peng et al. (2012), where the detection threshold of each participant was calculated by the geometric mean of the last not correctly detected sample concentration, and the first correctly detected

concentration (when no other incorrect answers have been given after this). No individual correctly selected the lowest concentration. Therefore, all individuals' detection thresholds fell within the tested concentration range. Recognition threshold for each participant was determined by the geometric mean of the last not correctly recognised as salty and the first correctly recognised concentration as salty (when no other incorrect answers have been given after this). Four individuals incorrectly recorded the taste quality across the whole concentration range, thus, as in Giguère et al. (2016) if the subjects did not correctly recognise the tastant as salty within the testing range, the recognition threshold was calculated as follows "the geometric mean of the highest concentration tested and the next concentration that would have been used in the series if it had been continued".

3.4.4.2. Supra-threshold Intensity measurements

Prior to assessment, participants were trained in using the generalised labelled magnitude scale (gLMS) and subsequently asked to measure the intensity of saltiness sensation perceived of two supra-threshold concentrations of NaCl (0.056 M and 0.56 M) (Yang et al., 2014). Each individual assessed samples in the same order (0.056 M then 0.56 M) to minimise any carryover effect of the highest concentration.

3.4.5. Participant physiology, dietary intake and salt behaviours

Participants were asked to complete self-report food diaries and record everything they ate and drank over 3 consecutive days after the first study session. Consumption information was entered into the Nutritics Software version 5.0 (Nutritics, Dublin, Ireland), and for each participant, average daily calorie (kcal/day) and NaCl intake (mg/day) was extracted. NaCl per kcal was also determined from the extracted data. During the final session, all participants completed a food frequency questionnaire that included salt behaviour questions outlined in Supplementary material 3.1 and questions on frequency of consumption of 34 food groups of dietary NaCl sources outlined in Supplementary material 3.2 developed from Nguyen and Wismer (2019). Participants were asked how many times on average, they had consumed each food item in the previous week. The six frequency response options ranging from “never” to “3+ times a day” were converted to frequency of consumption per day for each participant. Standard average portion sizes (g) (Mills, 2002, British Dietetic Association, 2019, Winkler et al., 2012, Rippin et al., 2019), average NaCl contents (McCance and Widdowson, 2014) and frequency of consumption values (all found in Supplementary material 3.2) were used to calculate each participant’s estimated NaCl intake..

3.4.6. Statistical analysis

XL Stat software (Addinsoft, Paris, France) and GraphPad Software (San Diego, California USA) was used for all data analysis. A mixed-model ANOVA with interaction (saliva type as fixed effect and participant as a random effect) was adopted to determine differences between US and SS for each salivary parameter. Before analysis, Shapiro-Wilk normality tests were conducted, assessing the distribution of residuals based on the variation in participant means for each treatment. Coefficient of variation was used to measure intra-individual variation across biological replicates and inter-individual variation across the means of participants for each salivary parameter.

BET values for detection and recognition were \log_{10} transformed before further analysis due to concentrations being part of a logarithmically spaced series and supra-threshold intensity values being \log_{10} transformed due to the scale being logarithmically spaced. Variables were tested for normality using the Shapiro-wilko test. A number of variables used in the correlation analysis were not normally distributed, and therefore a non-parametric test was used. Spearman correlation coefficients were calculated between all measured variables. As in Low et al. (2016) *p*-values obtained by correlation analysis were not adjusted using Bonferroni or another equivalent method to account for multiple comparisons due to approaches being overly conservative (increasing

risk of type II error) and possibly masking outcomes (Perneger, 1998, Armstrong, 2014). Therefore, significance was accepted at $p < 0.01$ to reduce the chance of making a type 1 error due to many tests. Individuals were grouped into NaCl taste sensitivity groups using quartile analysis of individual detection threshold values. Individuals with detection thresholds of 1.59 mM (minimum value) up to and including 4.89 mM (lower quartile) were classified as highly sensitive to NaCl, between 4.89 mM and 14.01 mM (upper quartile) excluding these values were classified as having a medium sensitivity to NaCl and those with a detection threshold of 14.01 to 20.05 mM (maximum) were classified as having low taste sensitivity to NaCl. A one-way ANOVA with Fishers LSD post hoc test adjusting for heteroscedacity was used to determine differences between sensitivity groups for salivary parameters and continuous dietary intake values. Chi-square test and Fisher's exact test were used to determine differences between sensitivity groups for table salt behaviours and self-estimated intake (categorical data).

3.5. Results and discussion

3.5.1. Salivary parameters measured in unstimulated saliva (US) and stimulated saliva (SS)

To observe the effect of stimulation by chewing on three salivary parameters considered important for salt taste perception, mean values for flow rate, sodium concentration and total protein concentration for each participant was presented within box plots for each treatment (US and SS) (Figure 3.1). Despite high inter-and intra-individual variation (Table 3.2) the mixed model ANOVA identified that there was a significant increase in flow rate and sodium concentration upon chewing ($p < 0.0001$) (Figure 3.1a-b). In contrast, total protein concentration

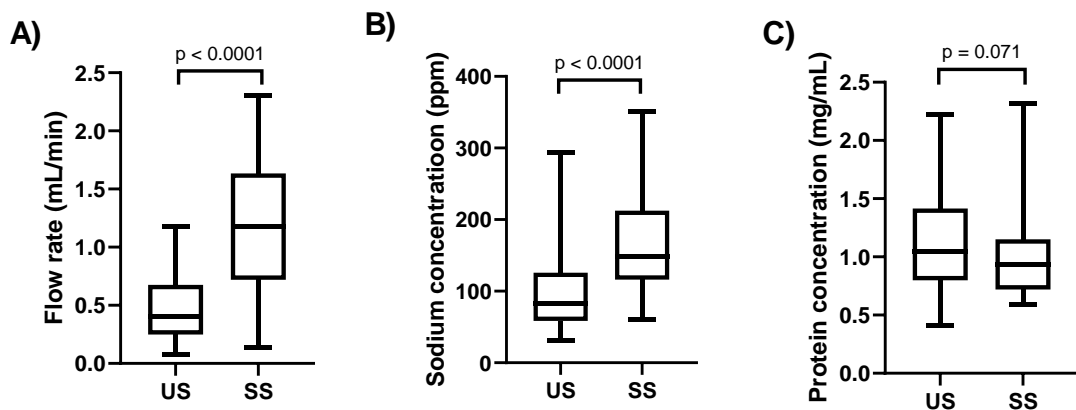


Figure 3.1. Boxplots displaying flow rate (A, n=36), sodium concentration (B, n=34, two individuals removed due to extreme data points) and protein concentration (C, n=36) for unstimulated (US) and stimulated saliva (SS). Values plotted were means of replicates collected per participant. Box represents median value, 25th and 75th percentile. Whiskers represent maximum and minimum values. Mixed model analysis with interaction (participant as a random factor) was used to find p -values for each salivary parameter.

decreased slightly upon chewing stimulation, approaching significance ($p = 0.07$) (Figure 3.1c). Salivary parameter levels are consistent with results previously reported for flow rate (Tukia-Kulmala and Tenovu, 1993, Ghezzi et al., 2000, Aframian et al., 2006, Carpenter, 2012, Gittings et al., 2015), sodium concentration (Rehak et al., 2000, Tomás et al., 2008) and total protein concentration (Jenzano et al., 1986, Lin and Chang, 1989, Neyraud et al., 2012).

Table 3.2. Inter- and Intra- individual variability of salivary parameters expressed as coefficient of variation (%) for both unstimulated (US) and stimulated saliva (SS).

	Flow Rate (n=36)		Sodium concentration (n=34)		Protein concentration (n=36)	
	US	SS	US	SS	US	SS
Inter-individual variation						
CV % of individual means	60 %	45 %	59 %	42 %	38 %	36 %
Intra-individual variation						
CV % of biological replicates of individuals (mean, range)	30 % (7-96 %)	22 % (3-56 %)	24 % (4-75 %)	21 % (6-44 %)	24 % (5-65 %)	22 % (1-65 %)

The observed increase in sodium concentration is typical when the flow rate is increased and can be explained by a reduction in the reabsorption rate of sodium ions at the striated and excretory ducts. The reabsorption of sodium and chloride ions during secretion ensures saliva is hypotonic, which is vital for taste perception; however, sodium can only be reabsorbed at a limited capacity from the primary saliva (Thaysen et al., 1954) and therefore sodium increases with flow rate. Spearman's correlation indicated significant positive relationships between

unstimulated and stimulated measures for each salivary parameter ($r > 0.57$, $p < 0.0001$), showing that individuals with typically higher levels of salivary parameters in unstimulated saliva had higher levels in their stimulated saliva (Supplementary material 3.3). The relationship between naturally occurring individual variation in salivary parameters and NaCl taste sensitivity was explored further by capturing perceptual measures within the same participant cohort.

3.5.2. NaCl taste thresholds and perceived intensities of supra-threshold NaCl concentrations

Figure 3.2 shows individual detection threshold values ranging from 1.6 to 20.1 mM with a group median of 6.9 mM, and recognition threshold values ranging from 2.3 to 28.4 mM with a group median of 14.07 mM. Feron (2019) determined mean detection and recognition threshold values from 19 publications as 9.5 mM and 15.2 mM, respectively. The group average values in this study were just slightly below these values, with threshold ranges within those noted by Feron (2019). The range of perceived intensity values of supra-threshold solutions are also found in Figure 3.2. The supra-threshold intensity values for the lower NaCl salt concentration (ST1) varied from being perceived in the 'weak' to 'moderate' strength region to the 'very strong' region on the gLMS. The supra-threshold intensity values for the highest NaCl concentration (ST2) varied from a region between 'moderate' and 'strong' up to the 'strongest

imaginable sensation'. The broad ranges for threshold values and supra-threshold intensity ratings support previous studies (Yang et al., 2014, Zaidan et al., 2009, Yang et al., 2011), highlighting extensive individual variation in NaCl thresholds and perception (indicating individual variations in NaCl taste sensitivity). It is accepted that those with a high

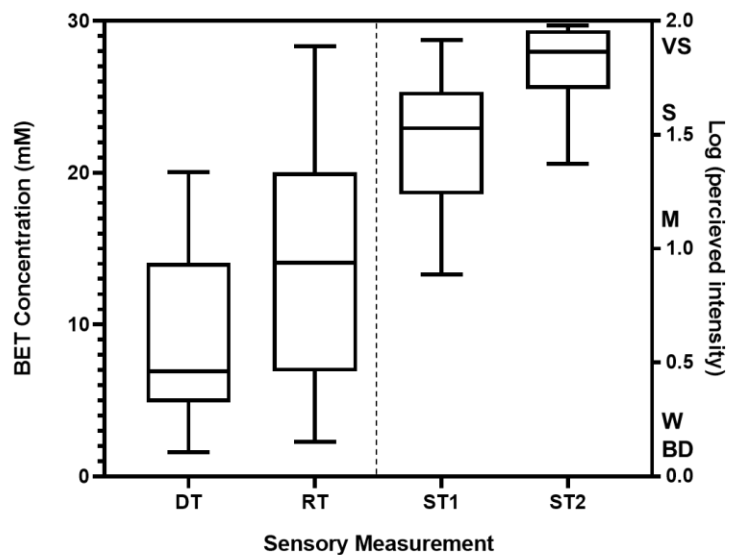


Figure 3.2. Boxplots displaying the Best Estimate Threshold (BET) concentrations for detection (DT) and recognition (RT) threshold (left) and log perceived intensity ratings of supra-threshold concentrations (ST1 = 0.056 M, ST2 = 0.56 M) (right) for 36 participants. Box represents median value, 25th and 75th percentile. Whiskers represent maximum and minimum values. On the right Y axis, BD, W, M, S and VS stand for; barely detectable, weak, moderate, strong, very strong respectively.

detection threshold are considered less sensitive to the stimuli in question since a higher concentration is required before detection. In contrast, those with a low detection threshold are more sensitive since they have the ability to detect lower concentrations.

3.5.2.1. Relationships between the sensory measurements of NaCl taste perception

Although not significant ($p > 0.01$), detection and recognition thresholds were positively correlated to each other ($r = 0.35$, $p = 0.04$), while the supra-threshold measures were more strongly positively correlated to each other ($r = 0.54$, $p = 0.001$). On the other hand, no correlations were found between thresholds and supra-threshold intensities ($r = -0.09$ to -0.23 , $p > 0.17$) (Supplementary material 3.4). In common with previous findings (Webb et al., 2015), NaCl taste threshold measures are not predictive of perceived intensities of supra-threshold concentrations. Therefore, they should be considered independent of each other whilst providing complementary insights into both detection and perception of a stimulus.

3.5.3. NaCl intake determined by food diaries, food frequency questionnaires and consumption behaviours of the cohort studied

Food diaries estimated the average NaCl intake to be 5.56 g/day (± 2.00 g/day), whilst the food frequency questionnaire estimated the average NaCl intake to be 4.45 g/day (± 1.74 g/day). The total participant cohort consumed more than the current World Health Organisation recommendation of 5 g NaCl per day when assessed using food diaries;

however, the average was less than the recommendation when determined using the food frequency questionnaire. In general, the group studied consumed less than the national average of 8.1 g NaCl/day (Public Health England, 2016). However, comparison across the two methods for measuring dietary NaCl intake did highlight inconsistencies between them. Under-reporting intake is a common problem of self-report questionnaires and food diaries, especially among food items with an adverse health image, e.g. highly salty items which may have influenced the self-reported dietary intake data in this study (Macdiarmid and Blundell, 2007). Therefore, individual NaCl intake values may be higher than those recorded using these self-report methods. NaCl intake per calorie calculated by dividing individuals self-reported daily NaCl intake by calorie intake obtained from food diaries ranged from 1.3 mg NaCl per kcal to 6.0 mg NaCl per kcal. The results of NaCl salt behaviour questions are reported in Supplementary material 3.1. Half of the participants declared that they always added NaCl salt during cooking, 17 % often, 14 % sometimes, and 19 % rarely. In contrast, the frequency of NaCl salt added during consumption rather than during cooking, was more divided between individuals with the highest proportion of participants (28 %) rarely adding NaCl salt during consumption, 19 % sometimes, 22 % often, and 19% always. The smallest proportion of participants (11 %) declared that they never added NaCl salt during consumption. Overall in this study, participants were more likely to

contribute to NaCl intake by adding NaCl salt during cooking over at the table. A similar finding was also found in a previous survey of 493 Australian adults. A greater number of respondents reported that they always add NaCl salt during cooking than at the table (Grimes et al., 2010). In developed nations, studies have shown that discretionary NaCl salt intake (added during cooking and at consumption) contributes to 9-15 % (James et al., 1987, Andersen et al., 2009) of the total NaCl salt intake, whereas processed foods contribute to the majority of salt intake (75 %) (Dyer et al., 1997). Despite most NaCl salt consumption being attributed to processed foods, more NaCl salt reduction strategies aimed at the population level are required to lower discretionary NaCl salt use. There is also evidence that the addition of table salt (NaCl) likely results in a higher liking for salty foods (Bertino et al., 1986), contributing to potentially further increasing NaCl salt intake, highlighting the importance of implementing ongoing NaCl salt reduction initiatives. In Supplementary material 2.1, it is observed that 47 % of the studied participants perceived themselves as consuming the recommended NaCl salt level (5 g/day), while 20 % considered themselves as consuming 'less' or 'much less' than this and 33 % 'more' than this amount. Overall the dietary NaCl intake estimated by self-reporting questionnaires and food diaries show that there is a considerable inter-individual variation in self-recorded intake and discretionary NaCl salt use.

3.5.4. Relationships between salivary parameters, sensory measures of NaCl salt taste and intake

3.5.4.1. Salivary parameters and NaCl salt sensory measures

Although no significant correlations were found between salivary parameters and NaCl taste sensory measures (Table 3.3), the positive trends between unstimulated salivary sodium concentration and thresholds ($r = 0.30-0.31$, $p = 0.08-0.09$), and between both types of salivary flow rate and thresholds ($r = 0.22-0.29$, $p = 0.08-0.19$) agree with previous literature which determined that sodium concentration and flow rate drive NaCl taste thresholds as a result of taste adaption (O'Mahony and Heintz, 1981, Delwiche and O'Mahony, 1996, McBurney and Pfaffmann, 1963), as did the trends shown in Figure 3.3. Our study confirms that even naturally occurring, inter-individual differences in salivary sodium concentration can impact NaCl taste thresholds, without altering salivary sodium levels through chewing (Delwiche and O'Mahony, 1996) or rinsing the mouth prior to tasting with concentrations of NaCl stimuli (O'Mahony and Heintz, 1981). Since there were no consistent trends between threshold measures and salivary protein concentrations ($r = -0.27-0.09$, $p = 0.11-0.95$) (Table 3.3), no further analysis took place to determine protein concentration differences across NaCl taste sensitivity groups. It is important to highlight that the method used in this study for estimating threshold values for each individual is

suggested not to be appropriate for use on an individual basis. Therefore correlations were used as an exploratory tool to identify potential relationships and should be considered alongside the results of the NaCl sensitivity groupings.

The trend between flow rate and NaCl taste thresholds (Table 3.3), or in other words, NaCl taste sensitivity (Figure 3.3), is explained by the associated increase in salivary sodium concentration with increased flow rate (Supplementary material 3.3). Since the supra-threshold concentrations (0.056 and 0.56 M) are much higher than typical salivary sodium concentrations (Rehak et al., 2000, Tomás et al., 2008), perhaps

Table 3.3. Correlation matrix^a of salivary parameters and NaCl salt taste measures.

Salivary parameters ^b		Sensory Measures			
		NaCl Salt Threshold		Perceived supra-threshold intensity	
		Detection	Recognition	ST1 Low NaCl	ST2 High NaCl
US flow rate	r	0.23	0.23	0.05	-0.02
	p	0.18	0.17	0.78	0.90
SS flow rate	r	0.29	0.22	0.23	0.08
	p	0.08	0.19	0.17	0.66
US Sodium concentration	r	0.31	0.30	0.06	0.15
	p	0.08	0.09	0.71	0.39
SS Sodium concentration	r	0.23	0.17	-0.02	0.05
	p	0.19	0.32	0.89	0.80
US Total protein concentration	r	0.01	0.09	0.04	-0.08
	p	0.95	0.59	0.84	0.65
SS Total protein concentration	r	-0.12	-0.27	-0.17	-0.20
	p	0.50	0.11	0.33	0.24

^a Determined by Spearman's correlation coefficient. Top number is the correlation coefficient (r), and the bottom number is p-value (p). No pairwise correlations were deemed significant ($p < 0.01$).

^b US: Unstimulated saliva, SS: Stimulated saliva

not surprisingly, supra-threshold intensity values showed no trends with salivary parameters (Table 3.3).

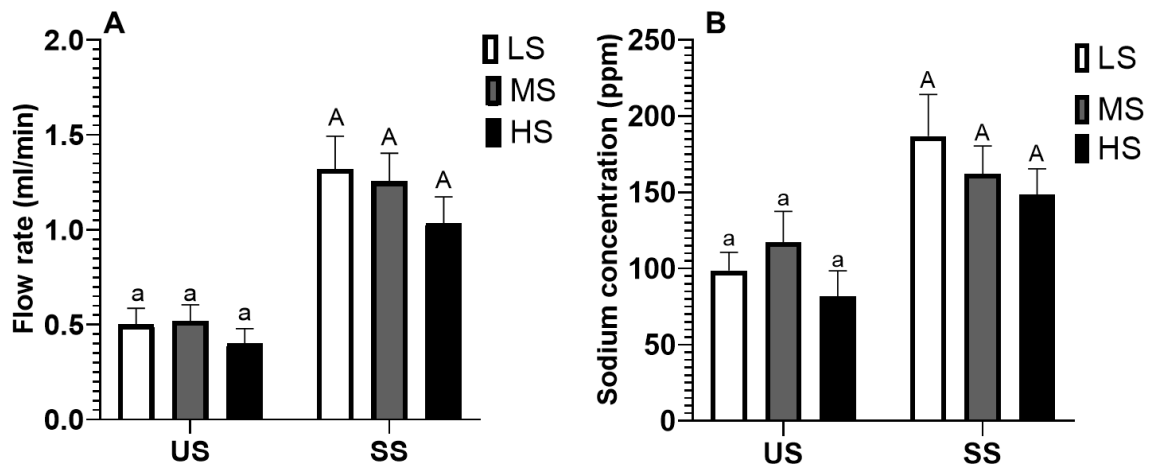


Figure 3.3. Salivary flow rate (A) and sodium concentration (B) of unstimulated saliva (US) and stimulated saliva (SS) of NaCl salt sensitivity groups. Groups were determined by quartile analysis of detection threshold data of 36 individuals where LS is low sensitivity, MS is medium sensitivity, and HS is high sensitivity to NaCl salt taste. Bars represent mean \pm standard error. Different letters represent significant differences determined by one-way ANOVA and Fishers LSD post hoc test ($p < 0.01$).

3.5.4.2. Salivary parameters and dietary intake

Although no significant correlations were determined ($p > 0.01$) between salivary parameters and dietary intake measures, slight trends were observed between unstimulated salivary protein concentration and total daily NaCl intake and NaCl intake per kcal ($r = -0.35-0.37$, $p = 0.03-0.04$) (Table 3.4). Previously, Méjean et al. (2015) also found that salivary total protein concentration did not significantly affect sodium intake or liking for salt. However there is some evidence that specific proteins such as

alpha-amylase (Ferry et al., 2006), endo proteases (Stolle et al., 2018, Stolle et al., 2017) and carbonic anhydrase VI (Lamy et al., 2021), and amount of pellicle film layer on the tongue (Dsamou et al., 2012, Feng et al., 2018) may impact saltiness perception, thus potentially NaCl intake.

Table 3.4. Correlation matrix^a of NaCl salt intake measures and salivary parameters measured for both unstimulated (US) and stimulated saliva (SS).

NaCl salt intake measures		Salivary Parameters					
		Flow rate		Sodium concentration		Total protein concentration	
		US	SS	US	SS	US	SS
Daily NaCl Intake (FD ^b)	r	-0.01	-0.16	-0.23	-0.23	-0.37	-0.14
	p	0.97	0.35	0.20	0.20	0.03	0.42
Daily NaCl Intake per kcal (FD ^b)	r	0.04	-0.19	-0.17	-0.10	-0.35	-0.08
	p	0.83	0.27	0.34	0.61	0.04	0.64
Daily NaCl Intake (FFQ ^c)	r	-0.18	-0.18	-0.13	-0.15	-0.19	0.04
	p	0.31	0.29	0.46	0.40	0.28	0.80
Daily Calorie intake (FD ^b)	r	-0.15	-0.04	-0.13	-0.21	0.09	0.06
	p	0.37	0.80	0.46	0.23	0.59	0.75
NaCl added during cooking	r	-0.11	-0.21	-0.18	0.08	-0.16	-0.11
	p	0.53	0.22	0.32	0.66	0.35	0.51
NaCl added to meal at table	r	-0.12	-0.29	-0.17	-0.21	-0.08	0.10
	p	0.49	0.09	0.33	0.23	0.64	0.57
Self-estimated NaCl intake	r	0.15	-0.11	-0.11	0.07	-0.23	-0.01
	p	0.38	0.51	0.53	0.68	0.18	0.95

^a Determined by Spearman correlation coefficient. Top number is the correlation coefficient (r), and the bottom number is *p*-value (*p*). No pairwise correlations were deemed significant using a significance level of $p = 0.01$.

^b Daily NaCl intake or NaCl intake per kcal as determined by a 3-day food diary (FD)

^c Daily NaCl intake as determined by a food frequency questionnaire (FFQ)

3.5.4.3. Dietary intake and NaCl salt sensory measures

Table 3.5 presents the correlations between dietary intake and NaCl taste measures. A negative trend (not significant, $p > 0.01$) was noted between detection threshold and NaCl intake determined by FFQ ($r = -0.39$, $p = 0.02$), and recognition threshold had a significant negative trend with NaCl intake determined by food diary ($r = -0.42$, $p = 0.01$). When testing differences in dietary intake between NaCl taste sensitivity groups, no significant differences were found apart from NaCl intake determined by FFQ (Figure 3.4). The high NaCl taste sensitivity group consumed a significantly higher amount of NaCl than the low NaCl taste sensitivity group ($p = 0.01$). On the contrary, there were no associations determined by Fisher's exact test between NaCl taste sensitivity grouping and the categorical data regarding responses to NaCl added during cooking, NaCl addition during eating and self-estimated NaCl intake. Supplementary Material 3.5 The significant difference in NaCl intake (determined by FFQ) found between high and low NaCl taste sensitivity groups and the trends that were identified by the correlations between NaCl intake and thresholds (

Table 3.5 3.5) suggest that individuals most sensitive to NaCl (lower detection threshold), or those who recognise salt more easily (lower recognition threshold), consume the highest NaCl in their diets. These results oppose previous findings which determined significant positive associations between NaCl taste thresholds and NaCl intake, behaviours or salty food consumption levels (Martinelli et al., 2020, Cattaneo et al.,

2019, Veček et al., 2020), which had previously suggested that low taste sensitivity to NaCl results in a higher intake of NaCl. In support of the current findings, Pangborn and Pecore (1982) also identified a negative association between recognition threshold of NaCl solutions and NaCl intake. The reason for this identified trend is unclear. One theory could be that those that are more salt-sensitive (i.e. recognise and detect NaCl at lower levels) may actively seek a more significant number of salt-containing foods due to their individual preferences, leading to self-perpetuating negative dietary habits.

Table 3.5. Correlation matrix^a of sensory NaCl salt measures and NaCl salt intake.

		Sensory Measures			
		NaCl Taste Threshold		Perceived supra-threshold intensity	
		Detection	Recognition	ST1 Low NaCl	ST2 High NaCl
NaCl intake measures					
Daily NaCl Intake (FD ^b)	r	-0.14	-0.42	-0.01	-0.18
	p	0.43	0.01	0.96	0.29
Daily NaCl Intake per kcal (FD ^b)	r	-0.22	-0.32	0.20	0.18
	p	0.20	0.06	0.23	0.30
Daily NaCl Intake (FFQ ^c)	r	-0.39	-0.08	0.08	0.12
	p	0.02	0.66	0.61	0.50
Daily Calorie intake (FD ^b)	r	-0.08	-0.19	-0.26	-0.52
	p	0.63	0.27	0.12	<0.001
NaCl added during cooking	r	0.07	0.08	-0.38	-0.02
	p	0.70	0.66	0.02	0.89
NaCl added to meal at table	r	-0.16	-0.10	-0.26	0.01
	p	0.35	0.55	0.13	0.95
Self-estimated NaCl intake	r	-0.02	-0.27	0.08	0.00
	p	0.90	0.11	0.64	0.98

^a Determined by Spearman correlation coefficient. Top number is the correlation coefficient (r), and the bottom number is p-value (p). Pairwise correlations deemed significant at $p < 0.01$ are in bold.

^b Daily NaCl intake or NaCl intake per kcal as determined by a 3-day food diary (FD)

^c Daily NaCl intake as determined by a food frequency questionnaire (FFQ)

As evidenced, findings are inconsistent and many more studies show a lack of associations between threshold and salt intake and consumption variables (Pilic et al., 2020, Pilic and Mavrommatis, 2018, Simpson et al., 2012, Lee et al., 2014). Similarly, most studies have determined that there are no significant associations between sweet taste thresholds and dietary intake (Low et al., 2016, Jayasinghe et al., 2017, Smith et al., 2016). The discrepancies in the direction of associations may be as a result of the specific population studied. In the current study, participants reported an average NaCl intake lower than the UK average of 8.1 g salt per day. Participants were recruited primarily from a food science and nutrition background, and as such, may differ in dietary habits from general population cohorts studied previously.

Calorie intake was significantly negatively correlated with perceived saltiness intensity ($r = -0.52, p < 0.001$), and there was a negative trend between the NaCl added during cooking and perceived saltiness intensity ($r = -0.38, p = 0.02$). These associations suggest that those who perceive supra-threshold levels of NaCl salt as more intense add less NaCl to their food during cooking and consume fewer calories. This is logical since if one perceives NaCl as typically more intense, they would require less NaCl added to food to provide a pleasant salty taste. Although some studies found no associations between saltiness intensity ratings and dietary intake (Pangborn and Pecore, 1982, Veček et al., 2020), Shepherd et al. (1984) found, similar to the current study, that lower table salt usage was associated with higher saltiness intensity

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ratings. It is important, however, to remember that other factors that contribute to saltiness perception and NaCl intake, including genetics, food preference and other salivary parameters (Pilic et al., 2020, Stolle et al., 2018, Stolle et al., 2017, Tan et al., 2021) have not been measured here and may also have had an influence on the outcomes in this study.

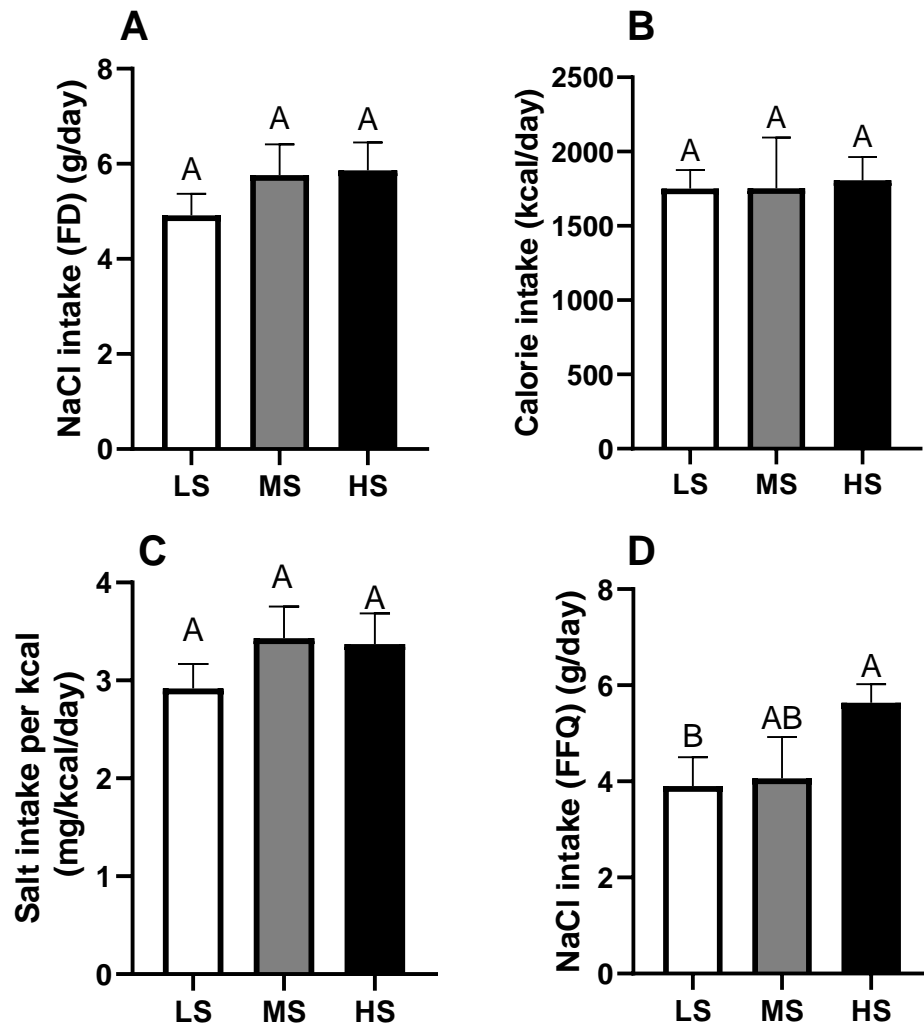


Figure 3.4. NaCl Intake calculated by food diaries (FD) (A), calorie intake (B), NaCl intake per kcal (C), and NaCl intake calculated using food frequency questionnaire (FFQ) (D) of each NaCl salt sensitivity group. Groups were determined by quartile analysis of detection threshold data of 36 individuals where LS is low sensitivity, MS is medium sensitivity, and HS is high sensitivity to NaCl salt taste. Bars represent the mean \pm standard error. Different letters represent significant differences determined by one-way ANOVA and Fisher's LSD post hoc test ($p < 0.01$).

3.5.5. Strengths and limitations

The current study explored the natural inter-individual variations in salivary sodium concentrations to determine relationships between salivary parameters and sensory perception within the context of each participant's physiological 'norm', rather than altering salivary sodium concentrations through chewing or through mouth rinses of NaCl solutions (Delwiche and O'Mahony, 1996, McBurney and Pfaffmann, 1963).

Dietary NaCl intake was estimated using self-report food diaries and questionnaires, which come with their limitations, such as over and under-reporting (and not accounting for individual differences in portion size). The gold standard NaCl intake assessment is 24h urinary excretion (McLean et al., 2017) which is onerous for the participant and unsuitable for the current study due to practical constraints. Self-reporting food diary and frequency questionnaires are widely used and considered suitable for estimating intake within the constraints highlighted with the additional benefit of capturing information on multiple nutrients and dietary behaviours. Inclusion of options for food/meal photo uploads may be considered to improve reporting accuracy in future studies.

Exploring consumers' relationship with salt and consumption patterns would benefit from a larger scale study group to confirm current findings with balanced gender groups to study any effects of gender. Furthermore, future studies should use an extensive range of

concentrations for threshold testing to capture even the highest thresholds, and the use of real food systems or model foods similar to salty food systems typically consumed in the population studied could be considered.

There was considerable variation within individuals across collection days for some salivary parameters, which is challenging to minimise due to the dynamic physiological changes through various factors. Nevertheless, this study has demonstrated several trends between salivary components, taste perception and dietary intake, expanding the analytical and psychophysical measures to include consumer behaviour and investigating their influence on dietary NaCl intake.

3.6. Conclusion

To conclude, relationships between NaCl taste perception measures and dietary intake remain uncertain. The trends found between salivary parameters and threshold measures confirm that inter-individual variations in salivary flow rate and sodium concentration contribute to the determination of NaCl taste thresholds. Conversely, supra-threshold intensity values showed no associations with salivary parameters. Other factors, therefore, determine the perceived intensity of saltiness in higher concentrations above the threshold level. In terms of relationships between NaCl taste measures and intake, results suggest that those with lower thresholds (i.e. more sensitive to NaCl taste) consume a higher amount of dietary NaCl, while those who perceived NaCl as more intense

typically added less NaCl to their foods. Therefore individuals considered more NaCl taste sensitive may actively seek foods with higher quantities of NaCl or a more significant number of salt-containing foods. These individuals presumably have a high preference towards salty taste, leading to self-perpetuating negative dietary habits. However, those who perceive NaCl as more intense require less NaCl added to foods to provide their preferred salty taste. Overall, this study has contributed to understanding the complex relationship between salt taste sensitivity and salivary composition, which may influence dietary salt behaviours, with larger studies required to fully elucidate relationships.

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Funding sources

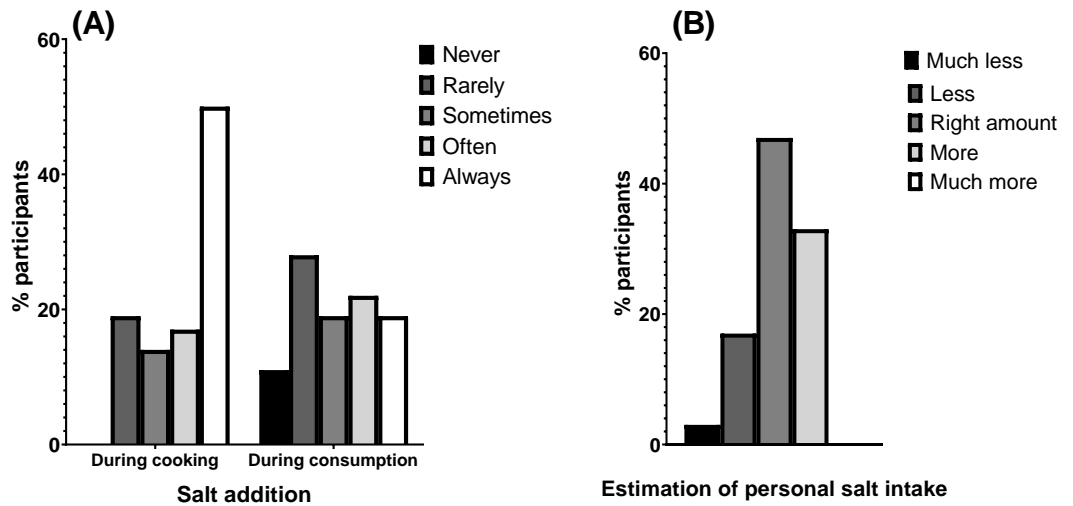
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Author contributions

Katherine Hurst: conceptualisation, formal analysis, investigation, writing – original draft. , Charfedinne Ayed: formal analysis, writing – review and editing and supervision. Ian Fisk: supervision, funding acquisition and writing – review and editing. Louise Hewson: writing – review and editing. Sophie Lester: investigation, writing – review and editing.

Supplementary material (chapter 3)

Supplementary material 3.1 Self-reported salt consumption behaviour of 36 participants as determined by questionnaire. Graph A presents the frequency of NaCl salt addition during cooking and during consumption of the meal. Graph B presents the results of the estimation of personal NaCl intake.



^A Percentage of participants who answered “Never”, “rarely”, “sometimes”, “often” or “always” to the questions “How often do you add table salt during cooking?” and “How often do you add table salt during the consumption of a meal?”.

^B Percentage of participants who answered “much less”, “less”, the “right amount”, “more” or “much more” to the question “How much salt do you think you consume compared to the recommended intake of less than 6g per day.”.

Supplementary material 3.2 Food frequency of consumption by food category.

Food Category	Frequency of consumption per day Mean (SD)	Portion size	Mean NaCl content in food category (g/100g)	Mean NaCl intake (g/day/person)	% NaCl consumed by category
White bread	0.20 (0.28)	45 ^a	1.02	0.09	0%
Brown bread	0.32 (0.38)	45 ^a	1.09	0.16	0%
Breakfast cereal	0.27 (0.35)	41 ^a	0.56	0.06	0%
Savoury Crackers and biscuits	0.10 (0.19)	11 ^a	0.68	0.01	0%
Sweet biscuits	0.36 (0.37)	19 ^a	0.84	0.06	0%
Sweet desserts e.g. Cakes and muffins	0.33 (0.15)	62 ^a	0.76	0.10	0%
Pizza	0.13 (0.14)	204 ^a	0.96	0.25	0%
Pasta/noodle dishes with sauces e.g. macaroni cheese, noodles	0.22 (0.23)	258 ^a	0.54	0.30	0%
Salted popcorn or peanuts	0.12 (0.22)	20 ^a	1.60	0.04	0%
Salted crisps	0.26 (0.24)	30 ^a	1.67	0.13	0%
Processed meat (salami, bacon, chorizo, pork sausage)	0.16 (0.24)	75 ^b	2.85	0.34	0%
Savoury pastry products such as pies and sausage rolls	0.13 (0.25)	178 ^a	0.55	0.13	0%
Fried chicken	0.12 (0.5)	102 ^a	1.21	0.15	0%
Gravy, made with stock or gravy powder	0.09 (0.16)	65 ^a	0.96	0.06	0%
Milk	1.16 (1.11)	200 ^b	0.17	0.40	0%
Cheese	0.50 (0.38)	30 ^b	1.56	0.24	0%
Yoghurt	0.46 (0.44)	125 ^b	0.16	0.09	0%

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Eggs	0.48 (0.47)	120 ^b	0.44	0.25	0%
Tinned fish	0.14 (0.25)	140 ^b	1.14	0.22	0%
Sea food e.g. fish not tinned	0.13 (0.20)	140 ^b	0.54	0.10	0%
Chips/french fries	0.16 (0.17)	136 ^a	0.30	0.07	0%
Canned vegetables	0.27 (0.28)	80 ^b	0.24	0.05	0%
Soup	0.17 (0.23)	246 ^a	0.60	0.26	0%
Condiments	0.4 (0.47)	21 ^a	1.28	0.11	0%
Ice cream	0.14 (0.17)	66 ^a	0.18	0.02	0%
Margerine/butter	0.40 (0.40)	10 ^a	1.37	0.06	0%
Chutneys/pickles	0.09 (0.16)	26 ^a	1.08	0.02	0%
Soy sauce	0.35 (0.45)	5a	13.75	0.24	0%
Salt	0.85 (0.75)	0.35d	98.35	0.29	0%
Mustard	0.11 (0.19)	10 ^a	5.71	0.06	0%
Peanut butter	0.26 (0.36)	10 ^c	0.88	0.02	0%
Savoury spreads such as bovril/marmite	0.04 (0.10)	10 ^c	10.84	0.04	0%
Chocolate/sweet	0.57 (0.46)	43 ^a	0.17	0.04	0%

^a Mean frequency of consumption for each food category of the study group. Mean was determined by using participants responses in a food frequency questionnaire where responses were coded as “never”=0, “1-3 times a week”=2/7, “4-6 times week”=5/7, “1 time a day”=1, “2 times a day”= 2 and “3 times a day”=3).

^b Average portion size from number of sources (Mills, 2002, McCance and Widdowson, 2014, Rippin et al., 2019, Winkler et al., 2012)

^c Salt content in food categories obtained from McCance and Widdowson's the Composition of Food tables (McCance and Widdowson, 2014)

Supplementary material 3.3. Correlation matrix^a of salivary parameters in unstimulated saliva (US) and stimulated saliva (SS).

Salivary parameter ^a	Type		Flow rate (mL/min)		Sodium concentration (ppm)		Total protein concentration (mg/ml)	
			US	SS	US	SS	US	SS
Flow rate (mL/min)	US	r	1					
		p	x					
	SS	r	0.68	1				
		p	<0.001	x				
Sodium Concentration (ppm)	US	r	0.17	0.11	1			
		p	0.35	0.52	x			
	SS	r	0.12	0.34	0.57	1		
		p	0.50	0.05	0.001	x		
Total protein concentration (mg/ml)	US	r	-0.39	-0.11	-0.08	-0.14	1	
		p	0.02	0.51	0.66	0.41	x	
	SS	r	-0.35	-0.24	-0.38	-0.30	0.69	1
		p	0.04	0.16	0.02	0.08	<0.001	x

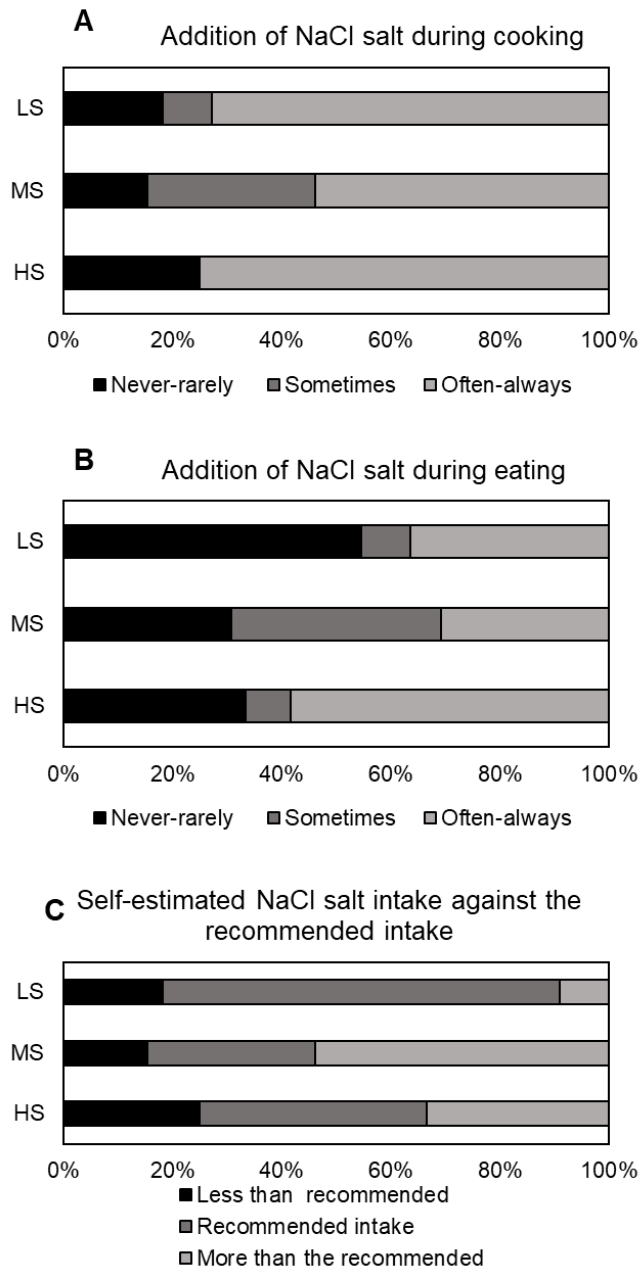
^a Determine by Spearman's correlation. Top number is the correlation coefficient (r), and the bottom number is p-value (p). Significant p-values ($p < 0.01$) are indicated in bold.

Supplementary material 3.4 Correlation matrix^a of sensory measures.

		Detection	Recognition	ST intensity (0.056 M)	ST intensity (0.56 M)
Detection	r	1			
	p	x			
Recognition	r	0.35	1		
	p	0.04	x		
ST intensity (0.056 M)	r	-0.15	-0.09	1	
	p	0.37	0.58	x	
ST intensity (0.56 M)	r	-0.23	-0.17	0.54	1
	p	0.17	0.33	0.001	x

^a Determined by spearman correlation, coefficients (r) and *p*-values are shown in relevant rows for each combination of sensory measures. In bold are correlations that are deemed significant (*p* = 0.01).

Supplementary material 3.5 Percentage of responses of each NaCl taste sensitivity group for three survey questions. Groups were determined by quartile analysis of detection threshold data of 36 individuals where LS is low sensitivity, MS is medium sensitivity, and HS is high sensitivity to NaCl salt taste. Chi-square and Fisher's exact test were performed on the responses for each question showing no associations ($p > 0.05$).



Chapter 4

4. Sensory perception and consumer acceptance of commercial and salt-reduced potato crisps formulated using salt reduction design rules

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4.1. Abstract

Successful salt (NaCl) reduction strategies are required to reduce the salt content of snacks while maintaining saltiness perception and consumer acceptance. Previous research suggests that particle physicochemical design rules (small particle size, low density, low hydrophobicity, optimised particle shape) can be leveraged to produce salt particles that enhance saltiness perception. This study aimed to validate these design rules by applying optimised model salts to unsalted potato crisps at a 30 % reduced salt content to produce prototype products. A selection of commercial products were also chosen to represent the salt content and crisp style of the broader market, with the aim to investigate the potential of other salt reduction strategies including; direct salt removal without compensation for loss of salt content and increasing time in mouth, while exploring the impact of consumer mouth behaviour type on consumer product preference. Nine products varying in salt content (6 standard, 1 crinkle-cut, 1 thick-cut batch-fried, 1 baked reconstituted potato) were subject to descriptive sensory analysis with a trained panel (n=11). A subset (seven products) were assessed for consumer acceptance (n=93). A salt reduction of 30 % was achieved while maintaining saltiness perception and consumer acceptance using model salts, while direct removal of salt without perceptual impact was only achievable by 15 %. To investigate key drivers of liking, consumers were segmented based on product liking and mouth behaviour. Results suggested that whilst salt content was the

primary driver, specific texture profiles were polarising. However, mouth behaviour had minimal influence on preference. These results validate previously described physicochemical design rules for developing novel salt particles for salt reduction and inform ingredient design for the food and flavour industries.

Keywords: salt reduction, saltiness perception, potato crisps, mouth behaviour, texture

4.2. Highlights

- Optimised model salt particles were selected based on physicochemical design rules
- Model salts applied to potato crisps were evaluated alongside commercial products
- Model salts allowed a 30% salt reduction without impacting saltiness or acceptance
- Only 15% salt could be directly removed without impact on perception and acceptance
- Salt content drives liking, and liking of texture profiles were polarising

4.3. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, contributing to one-third of global deaths in 2019 (World Health Organisation, 2021). CVD is a group of disorders associated with the heart and blood vessels, of which a diet high in sodium chloride salt (NaCl) is one of the major risk factors (World Health Organisation, 2021). In addition to the many preventable deaths caused each year, CVDs also place a substantial financial burden on the global economy through increased healthcare costs (Public Health England, 2019). It is recommended that adults consume no more than 5 g NaCl/day (World Health Organisation, 2018); however, average salt consumption still exceeds these levels in most countries, e.g. 8 g/day in the United Kingdom, 9 g/day in Australia and USA, and 14 g/day in China (Thout et al., 2019, Zhou et al., 2019).

Since the highest contributor to salt in our diet is processed foods (Gibson et al., 2000), most developed countries have continuing voluntary or mandatory salt reduction targets (Webster et al., 2011) to encourage food manufacturers to reduce salt in their products. Removal of NaCl salt is difficult due to its many functionalities, for example: imparting flavour, controlling yeast growth and fermentation rate, improving product texture, reducing spoilage through control of water activity, contributing to food preservation, and increasing water-binding capacity (Man, 2007). For savoury snacks specifically, it provides a salty

taste to an otherwise bland base, enhances flavour, provides a desirable product texture to expanded or reconstituted snacks, acts as a carrier for some seasonings, and may help to improve seasoning flowability (Man, 2007). Since salty taste is a key driver of consumer acceptance in many processed foods (Li et al., 2015), and direct removal is highly problematic due to its multifaceted role, significant research is required to enable food manufacturers to meet salt reduction targets without compromising consumer acceptance. Salt reduction strategies include: direct removal (partial removal of salt without compensatory strategy), salt substitution, leveraging cross-modal interactions for saltiness enhancement and modification of the product or salt particle to increase the availability of sodium and dissolution rate in saliva (Kilcast and Angus, 2007, Busch et al., 2013, Kuo and Lee, 2014). Salt reduction strategies should depend on the functional property of salt in that particular product. The use of direct removal without an attempt to mitigate the loss of salt content can be achieved up to 20 % in certain applications, including bread and dairy products (Mueller et al., 2016, Levings et al., 2014, Drake et al., 2011, La Croix et al., 2015, Jaenke et al., 2017), with even up to 40 % reduction being suggested as possible (Torrice et al., 2019). This is possible since partial reduction in salt concentration in a product can go unnoticed by the consumer. However, direct removal risks consumers rejecting the low salt product and ultimately choosing an alternative product that provides the desired saltiness. In addition, consumers may compensate for the loss of saltiness by adding their own salt (Zandstra et al., 2016). In order to combat this issue and reduce the risk of consumer rejection of any

new salt-reduced formulations by direct removal, a common approach is to use reduction by stealth, where gradual reduction over time can result in considerable reductions over an extended time period (Kilcast and Angus, 2007). Previously, in cereals and bread, a 33 % and 25 % reduction in NaCl salt content over time was achieved, respectively (Kilcast and Angus, 2007). Although successful, the approach required 7 years to ensure that the reduction remained unnoticed by consumers and still requires a concerted agreement within the specific product industry; to avoid the risks associated with the direct salt removal method, as mentioned. Potassium chloride (KCl) has been used to reduce salt by replacing NaCl salt up to 30 % in some products, including potato crisps (Kongstad and Giacalone, 2020, Torrico et al., 2019, Mueller et al., 2016). However, undesirable metallic and bitter flavour notes associated with increasing KCl content limit this approach (Sinopoli and Lawless, 2012). In potato crisps, it was also found that consumers perceive the use of KCl as a replacement of NaCl salt as less healthy and may therefore reject this change in reformulation (Kongstad and Giacalone, 2020). Another way to mitigate the loss of salt content during reduction is to increase sodium availability and dissolution either by altering product matrix composition (Lawrence et al., 2012a, Yucel and Peterson, 2015b, Yucel and Peterson, 2015a, Kuo and Lee, 2014) or modifying salt particles' properties (Rama et al., 2013, Hurst et al., 2021). These approaches are successful as they increase the dissolution speed of sodium from the food matrix to the saliva and the taste receptor cells, increasing the saltiness intensity perceived. Recently, physicochemical

design rules for salt particles for salt reduction were proposed, which included: small particle size, low density, low hydrophobicity and optimised particle shape (Hurst et al., 2021). Salt particles with reduced particle size (<106 µm) and specifically designed spray-dried salt (commercially available as SODA-LO® Salt Microspheres) adopted these design rules and enhanced saltiness perception compared to standard salt particles (106-425 µm) (Hurst et al., 2021).

Food texture impacts food oral processing, which can, in turn, influence the perception of saltiness and the acceptance of products (Lawrence et al., 2012b, Jeltema et al., 2015). Modifying food texture to regulate food oral processing is one way that could optimise sodium release in the mouth and enhance saltiness perception. Previously, Tian and Fisk (2012) highlighted that a high proportion of sodium in snacks is consumed without being perceived under regular eating patterns. Therefore, it is hypothesised that increasing the time of the product in the mouth will allow an increased proportion of sodium to be released into saliva and be detected by taste receptor cells. Differences in oral physiology (e.g. saliva flow rate and composition) and individual chewing behaviour can also influence sensory perceptions (Lawrence et al., 2012b, Chen, 2009). One way to assess typical chewing behaviour is the JBMB Mouth Behaviour Typing tool®, which classifies individuals into four groups (chewers, crunchers, smooshers and suckers) depending on individual preferences for the way one manipulates their foods (Jeltema et al., 2015). Individual mouth behaviour type has been suggested to

influence food choice and preferences (Jeltema et al., 2015), and although limited research has been conducted on the influence of mouth behaviour type on saltiness liking and perception, sodium release and saltiness has been shown to be affected by chewing behaviour (Lawrence et al., 2012b). Texture may also impact saltiness perception through cross-modal interactions, with some studies suggesting that a rough texture can enhance saltiness perception (Biggs et al., 2016, Pflaum et al., 2013, van Rompay and Groothedde, 2019).

Effective salt reduction strategies that allow reduction while maintaining saltiness and consumer acceptance are urgently required, thus a number of salt reduction strategies were investigated. This study firstly aimed to validate physicochemical design rules established previously for salt particles to maximise potential saltiness (small particle size, low density, low hydrophobicity and optimised particle shape) (Hurst et al., 2021). Therefore, prototypes produced using optimised model salts (<106 µm table salt and a spray-dried salt, SODA-LO®) with 30 % NaCl salt reduction were assessed within a broader product set of commercially available products and compared to a standard prototype (1.2 g NaCl / 100g, 106-425 µm salt particle size), using a dual sensory approach. Furthermore, with limited research on salt reduction of potato crisps in literature, this study also investigated: the impact of direct salt removal without any compensatory technique, the effect of changing oral residency time and breakdown speed by altering textural profiles and the impact of ridged texture compared to smooth on consumer acceptance

and sensory perception. Additionally, the influence of mouth behaviour on individual perception of potato crisps is explored as variation in oral manipulation and processing is thought to influence product liking. Thus, this study considers the influence of mouth behaviour type on individual perception of salt-reduced potato crisps and commercial competitors.

4.4. Materials and methods

4.4.1. Potato crisp product set

Nine ready salted potato crisp products were used within the study; 3 prototype products were produced using model salt particles topically applied to unsalted potato crisps (Walkers Salt & Shake, PepsiCo, Leicester, UK), and 6 were commercially available products (Table 4.1).

Table 4.1. Total potato crisp product set. All products outlined were used within the descriptive sensory analysis (n=9) and for the consumer study, crinkled and low salt commercial products were excluded as they were no longer available (n=7).

Prototype products				
Prototype reference	Salt particle description	Aimed NaCl content (g/100g)	NaCl content determined by sodium analysis (g/100g) ²	Product description
P1 (STD)	S1: Table salt 106-425 µm (99 % NaCl)	1.2	1.18 ± 0.05	Fried sliced potato, Standard NaCl level
P2	M1: Table salt <106 µm (99 % NaCl)	0.84	0.84 ± 0.01	Fried sliced potato, 30 % reduced NaCl
P3	M2: SODA-LO® Salt Microspheres Extra Fine salt (93.4 % NaCl)	0.84	0.80 ± 0.03	Fried sliced potato, 30 % reduced NaCl
Commercial products				
Product reference	Commercial Product name	Declared Back of Pack NaCl content (g/100g)	NaCl content determined by sodium analysis (g/100g)	Product description
Low salt	Walkers Hint of Salt	0.9	0.53 ± 0.07	Fried sliced potato, low NaCl
Medium salt	Waitrose Essential Ready Salted	1.15	1.23 ± 0.03	Fried sliced potato, medium NaCl
High salt	Walkers Ready Salted	1.4	1.40 ± 0.10	Fried sliced potato, high NaCl
Baked	Walkers Oven Baked	1.18	1.20 ± 0.10	Baked reconstituted potato flakes, medium NaCl
Crinkled	Walkers Crinkles	1.2	1.12 ± 0.10	Fried crinkle cut potato, medium NaCl
Hand-cooked	ASDA Extra Special Hand cooked crisps	1.2	1.64 ± 0.08	Thicker cut, batch fried potato, medium NaCl

² Measured salt content presented as mean of 4 replicates ± standard deviation

4.4.1.1. Potato crisp prototypes

Three potato crisp prototypes were produced (Table 4.1). Table salt (99 % NaCl) (Sainsbury's, London, UK), nickel sieves (Fisher, Loughborough, UK) and a coffee grinder (De'Longhi, Treviso, Italy) were used to produce standard salt particles (S1) at a diameter of 106-425 μm , and model salt 1 particles (M1) at a diameter $<106 \mu\text{m}$. Model salt 2 (M2) was provided by Tate and Lyle and is commercially available as SODA-LO® Salt Microspheres Extra Fine salt (93.4 % NaCl). SODA-LO® is formed by spray-drying a NaCl solution with maltodextrin and has been shown to have a lower density/higher bulk porosity than S1 (Hurst et al., 2021). S1, M1 and M2 were applied to unsalted potato crisps following the method outlined in Rama et al. (2013) using amounts indicated in Table 4.1 to form the potato crisp prototypes coded as P1 (STD), P2 and P3, respectively. Unsalted potato crisps (100 g) were seasoned by adding the model salt particles to the crisps and tumbling for at least 2 mins in clear polyethylene 300 mm x 250 mm vacuum flat bags (Nisbets, Bristol, UK). Bags were flushed with food-grade nitrogen gas and sealed using a tabletop vacuum packaging machine (Audion Elektro, Derby, UK) to maintain freshness before testing. The weight of the model salt particles (M1 and M2) added to the unsalted potato crisps corresponded to an aimed reduction in the NaCl content of 30 % compared to the standard salt level in P1 (1.2 g NaCl / 100g). A standard NaCl content of 1.2 g/100g was chosen based on the mode NaCl content in a survey of

30 ready salted fried potato crisp products commercially available in the UK, conducted by the authors (data not shown).

4.4.1.2. Commercial potato crisp products

Details of commercial products are outlined in Table 4.1, including NaCl content extracted from the composition tables on the back of pack and NaCl content measured using sodium analysis (method outlined in 4.4.1.3). To encompass a range of NaCl contents found in the current UK snack market, 3 potato crisp products were included in the product set with low salt (0.9 g/100g), medium salt (1.15 g/100g) and high salt (1.4 g/100g), and are referred to as these descriptors throughout the text. Three additional products were selected with a medium salt content level (1.18-1.20 g/100g) but differing in cut (crinkle-cut), slice thickness and frying method (thick cut, hand-cooked/batch-fried) and process (baked, reconstituted potato product); these products are referred to as crinkled, hand-cooked and baked in the text and figures. All commercial products were stored within their original packaging at room temperature, away from sunlight, before analysis. The low, medium and high salt potato crisps and all three prototype products (Table 4.1) are cut straight and were fried using a standard continuous frying method, as was the crinkled potato crisp. All of these products had a crisp thickness of 1.3-1.5 mm. The baked product is made using reconstituted potato flakes to form a dough that is sheeted and oven-baked, providing a product low in fat due to the absence of frying and had a thickness of 1.8 mm. The hand-

cooked product is cut thicker than the other products (2 mm) and produced using a 'kettle' or batch frying process.

4.4.1.3. Determination of salt content of products using flame photometry

Analysis of salt content was carried out by One Scientific, Bristol, United Kingdom (UKAS accredited in Food and Food Products). Representative test portions (4 x 50 g each) were sampled from 10 individual crisp packets or 2-3 large crisp packets (depending on pack weight) and incinerated to remove organic material. From the incinerated material, 1-2 g (per replicate) was dissolved in water and diluted to a known volume. Using flame photometry, sodium content of known standards was determined to generate a calibration curve to enable sodium content of product samples to be calculated and converted to salt weight per 100 g of product. Results are presented as mean \pm standard deviation of 4 replicates for each product (Table 4.1).

4.4.2. Descriptive sensory analysis of product set

4.4.2.1. Panellists

The sensory profiling panel was comprised of 11 panellists (3 Males, 8 Females, 38-68 years old). Panellists were employees of Sensory Dimensions Ltd, Nottingham, UK, and had previously been recruited to the companies trained panel based on their sensory acuity,

discriminating ability, motivation and availability. Panellists were highly experienced in sensory descriptive analysis techniques.

4.4.2.2. Sensory assessment protocol

Due to ongoing COVID-19 restrictions at the time of data collection, all training and feedback sessions took place in panellists' homes using a video calling platform that was easily accessible for all panellists. Panellists took part in five 2-hour training sessions in which panellists familiarised themselves with all nine samples, generated descriptive attributes, and developed the lexicon across appearance, aroma, flavour, texture, and aftertaste (Supplementary material 4.1). To understand the sensory characteristics of the whole product set, data across all modalities were collected from the trained panel to avoid 'dumping' effects (Abdi, 2002). Three saltiness attributes were included; initial saltiness, overall saltiness during eating and saltiness aftertaste, to provide temporal information on the saltiness profile of the samples. Between sessions 4 and 5, panellists undertook a practice rating assessment to assess panel performance and provide feedback and additional training before the final data collection.

Samples (25 g) were provided to panellists in a 16 oz clear pot with a lid (R&R Packaging, Cramlington, UK) labelled with random 3-digit codes. Samples were stored and served at room temperature. The crisp size was standardised across panellists, i.e. each panellist received similar sizes and amounts of each sample, and small fragments were discarded.

A 100-point unstructured line scale was used to rate each attribute, and data was collected using RedJade Sensory Solution Software, 2021 version (RedJade, Martinez, CA, USA). All panellists rated each sample in duplicate over 3 different sessions, tasting 6 samples in each session. The order of samples was randomised across the whole panel. Panellists were instructed to take a 5-minute break between each sample evaluation and cleansed their palate with unsalted crackers and tap water from panellists homes to reduce carry-over effects. Panellists were instructed to perform evaluations in the same place each time with no distractions.

4.4.3. Consumer acceptance of product set

Quantitative consumer acceptance testing was performed on a reduced product set (Table 4.1) due to 2 products (low salt and crinkled potato crisps) being delisted during the study.

4.4.3.1. Consumers

93 consumers (45 Males, 48 Females) were recruited from the Sensory Dimensions Ltd consumer volunteer database via a screening questionnaire. Participants were recruited using the inclusion criteria; 18-65 years old with no food-related allergies, in good health with no salt-related health issues (such as hypertension, cardiovascular disease, kidney disease), no taste impairments, must be a regular crisp user (at least once a month) and must accept ready salted potato crisps.

Information on demographics' (gender, age, and ethnicity) and frequency of snack product usage were captured using a self-report questionnaire.

4.4.3.2. Consumer assessment protocol

Consumers were invited to central location test facilities in the UK, and sample assessment took place within sensory booths (ISO 8589:2007). Samples were labelled using random 3-digit codes, and consumers assessed the 7 samples within one session following a sequential monadic presentation with a randomised order across consumers. Consumers were provided with 10 g of each sample to assess overall liking, saltiness liking and texture liking using 9-point hedonic category scales where 1 represented “dislike extremely” and 9 represented “like extremely”. For saltiness, they were provided with a 5-point Just-About-Right (JAR) scale where 1 represented “not at all salty enough”, 3 represented “Just-about-Right” and 5 represented “far too salty”. At the end of the final tasting session, consumers completed an online questionnaire about the preferred way they like to manipulate different types of foods in their mouth (JBMB Typing Tool®) in order to classify their mouth behaviour preferences (Jeltema et al., 2015). Based on their responses, consumers were grouped into four different mouth behaviour types: smooshers, crunchers, suckers, and chewers.

4.4.4. Ethical statement

All products were commercially purchased or produced from commercially available food-grade materials in food-grade environments using no novel ingredients. Participants provided their informed consent before taking part in the study. Testing procedures followed sensory, marketing and consumer research protocols including; ISO 11136, IFST Professional Sensory Science Group and Market Research Society guidelines (Market Research Society, 2022, IFST Sensory Science Group, 2020, British Standards Institute, 2017).

4.4.5. Data analysis

For all data analysis, an α risk of 0.05 was set as the level of significance and, unless otherwise stated, was conducted using XLSTAT (19.01, Addinsoft, New York, USA).

4.4.5.1. Descriptive sensory analysis

To determine if differences existed between products for each attribute assessed by panellists, a two-way fixed model ANOVA (product, panellist) with interaction was performed, followed by Tukey's HSD post-hoc test. Panel performance was evaluated using SenPAQ (version 5.01, Qi Statistics, Berkshire, UK).

4.4.5.2. Consumer acceptance analysis

To determine if differences existed between products for overall liking and specific attribute liking, a two-way mixed model ANOVA (product, consumer), with the consumer as a random effect, was performed, followed by Tukey's HSD post-hoc test. Agglomerative hierarchical cluster analysis using a dissimilarity matrix with Euclidean distance and Ward's method in the agglomeration was applied to classify consumers by patterns in overall liking. ANOVA was performed on the resulting clusters to determine if significant differences in liking existed between products within each cluster group.

Overall liking scores and saltiness JAR scores were used for penalty analysis following the steps outlined using the sensory analysis tool on XLSTAT (19.01, Addinsoft, New York, USA) and the procedure in Schraidt (2009). This analysis was performed for the whole consumer group (n=93) and each liking cluster group and mouth behaviour group. Chi-square and Fisher's exact tests were used to explore the differences in demographic, product usage and behavioural characteristics among cluster membership and mouth behaviour types.

An internal preference map was constructed using a PCA bi-plot of the multivariate space of the products, with mean overall liking scores of cluster groups and sensory attributes as supplementary variables to visually display and explore drivers of liking and disliking for each cluster.

4.5. Results

4.5.1. Salt content of the product set

Salt content was assessed to check the accuracy of the declared salt content found on the back of pack (BoP) for commercial products and to ensure prototype samples were within the desired salt content range to exhibit a 30 % salt reduction between standard salt level (P1, STD) and reduced salt prototypes (P2, P3). Measured NaCl content was within range for BoP reported content for most products (Table 4.1), with the exception of the low salt product (BoP: 0.9 g/100g, measured: 0.53 g/100g) and the hand-cooked product (BoP: 1.2 g/100g, measured: 1.64 g/100g). Prototype products ultimately met the aimed salt levels to achieve two 30 % salt reduced prototypes (P2 and P3) compared to the standard prototype (P1). The declared BoP and aimed salt content values are referred to through the text of this paper for ease, where the measured salt content is similar to BoP. Any implications of out of specification products are discussed where appropriate in the text.

4.5.2. Overall descriptive sensory profiles of the product set

The evaluation of panel performance indicated that one panellist performed poorly relative to the rest of the panel in discrimination ability, repeatability, and consistency and was removed from the data set. The mean scores for all 32 attributes measured across the product set are presented in spider plots in Figure 4.1a-b. ANOVA results indicated that

for all attributes, significant differences were observed ($p < 0.0001$). For clarity, post-hoc groupings are not included in Figure 4.1 spider plots. This information can be found in Supplementary material 4.2-4.5 along with the mean scores and standard deviation of each attribute. Figure 4.1a-b confirms that the three prototype products (P1 STD, P2, P3) have similar sensory profiles, with P3 being significantly higher in some appearance attributes (amount of speckles and amount of dark edges) and texture attributes (hardness and thickness of cut) ($p < 0.05$). Figure 4.1a-b also indicates that the hand-cooked and baked products have the most contrasting sensory profiles from the product set, as expected, due to different cooking processes and crisp thickness. The remaining products exhibit similar sensory profiles across attributes with the most noticeable differences in flavour intensity attributes (initial and during eating) and the three saltiness attributes (initial, during eating and aftertaste). Figure 4.1a-b show that the hand-cooked product had significantly higher values in: all aroma and appearance attributes, most texture and mouthfeel attributes (greasiness, hardness, crunchiness) and some flavour and aftertaste attributes (earthy and oil) (Supplementary material 4.2-4.5). The baked product, on the other hand, was significantly lower in all aroma attributes, most appearance attributes and all oil associated attributes (flavour, texture, mouthfeel) ($p < 0.05$) (Supplementary material 4.2-4.5). Baked also had significantly higher sweetness attributes and attributes associated with reconstituted potato than other products ($p < 0.05$).

Chapter 4: Sensory perception and consumer acceptance of commercial and salt-reduced potato crisps formulated using salt reduction design rules

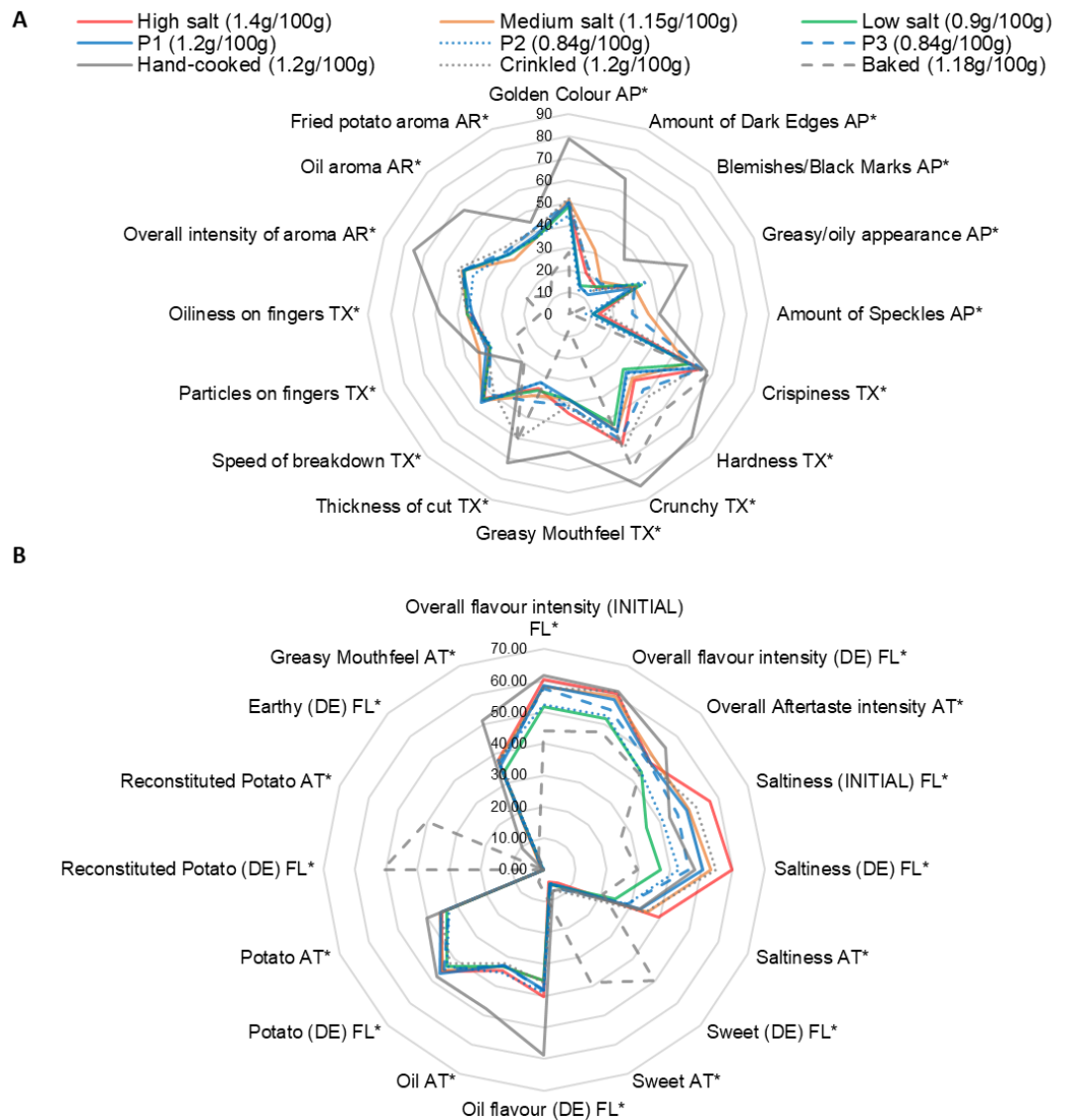


Figure 4.1. Spider plots of mean attribute ratings of 9 potato crisp products³ assessed by 10 panellists. Graph A presents aroma (AR), appearance (AP) and texture (TX) attributes. Graph B presents flavour (FL) and aftertaste (AT) attributes. Asterisks detail that a significant difference exists between products for that attribute ($p < 0.05$).

³ Potato crisp products include prototypes (P1, P2, P3) with the rest being commercial products detailed in the text. Prototype 1 (P1) was made using 1.2 g of 106-425 μm NaCl salt per 100g of unsalted potato crisps. P2 and P3 are 30 % reduced NaCl salt products when compared to P1 and were made using <106 μm NaCl salt or SODA-LO® salt microspheres respectively. DE stands for during eating.

4.5.2.1. Saltiness profiles of the product set

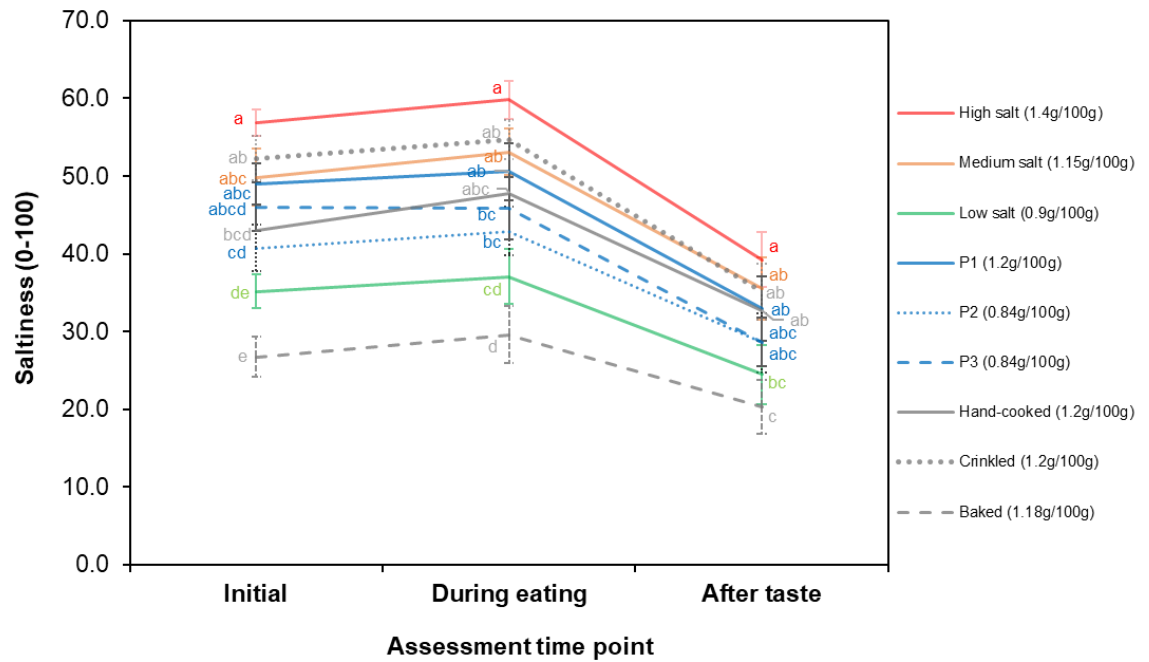


Figure 4.2. Saltiness intensity of 9 potato crisp products⁴ assessed by panellists (n=10) at 3 different assessment points via sensory descriptive analysis. Different letters within each assessment time point show significant differences ($p < 0.05$). Error bars represent standard error.

For each saltiness attribute (initial, during eating and aftertaste), significant differences were identified between the product set ($p < 0.0001$) (Figure 4.1b). Figure 4.2 displays the mean scores and post-hoc groupings for each saltiness rating across the product set. Most products slightly increase in saltiness from the initial rating to the rating during eating; saltiness intensity then decreases when assessed as an aftertaste (Figure 4.2). Products were ranked predominantly in the same

⁴ Potato crisp products include prototypes (P1, P2, P3) with the rest being commercial products detailed in the materials and methods section. Prototype 1 (P1) was made using 1.2 g of 106-425 μm NaCl salt per 100g of unsalted potato crisps. P2 and P3 are 30 % reduced NaCl salt products when compared to P1 and were made using <106 μm NaCl salt or SODA-LO® salt microspheres respectively.

order of saltiness intensity for each saltiness attribute, with the high salt product (1.4 g NaCl / 100g) being the saltiest and the least salty being the baked product (1.18 g NaCl / 100g). No significant differences were observed between prototypes for all three saltiness attributes measured ($p > 0.05$). In addition, when comparing the salt-reduced prototypes (P2, P3) to a commercially relevant competitor at a medium salt level (1.15 g NaCl / 100g), there were no significant differences between products for each saltiness attribute ($p > 0.05$) (Figure 4.2). Moreover, there was no significant difference in saltiness during eating between P3 and the high salt product (1.4 g NaCl / 100g) or between P2, P3 and the high salt product for saltiness aftertaste. However, there was no significant difference between P2, P3 and the low salt product (0.9 g NaCl / 100g).

Crinkled and hand-cooked products (1.2 g NaCl / 100g) were not significantly different to the standard prototype product (P1: 1.2 g NaCl / 100g) nor the medium potato crisp product (1.15 g NaCl / 100g) ($p > 0.05$). The baked product (1.18 g NaCl / 100g) compared to the rest of the product set was significantly different across all saltiness attributes, except for the low salt product (0.9 g NaCl / 100g) (Figure 4.2). No significant differences were found between the high salt (1.4 g NaCl / 100g) and medium salt potato crisps (1.15 g NaCl / 100g and P1 1.2 g NaCl / 100g) for all three saltiness attributes. However, there was a significant difference between the low salt and high salt product ($p < 0.05$) for each saltiness attribute.

Table 4.2. Demographic, mouth behaviour type and potato crisp consumption habits for the total consumer group and each cluster.

	Frequency response (%)			
	Total consumers (n=93)	Cluster 1 n=21	Cluster 2 n=43	Cluster 3 n=29
Product liking characteristics		Baked product dislikers	Liked Medium-salt product least	Hand-cooked product dislikers
Gender				
Female	52 %	38 %	56 %	55 %
Male	48%	62 %	44 %	45 %
Age				
18-24	14 %	10 %	12 %	21 %
25-34	12 %	5 %	14 %	14 %
35-44	17 %	5 %	16 %	28 %
45-54	27 %	38 %	26 %	21 %
55-64	30 %	43 %	33 %	17 %
Mouth behaviour				
Chewer	22 %	14 %	19 %	31 %
Cruncher	35 %	29 %	40 %	34 %
Smoocher	10 %	14 %	5 %	14 %
Sucker	23 %	24 %	26 %	17 %
Unclassified	11 %	19 %	12 %	3 %
Consumption habits (any flavour)				
Less than once a week	7 %	5 %	12 %	3 %
Once a week	10 %	19 %	7 %	7 %
2-4 times a week	57 %	52 %	51 %	69 %
Once a day or more	25 %	24 %	30 %	21 %
Consumption habits (ready salted)				
Less than once a week	21 %	33 %	21 %	14 %
Once a week	29 %	14 %	30 %	38 %
2-4 times a week	44 %	43 %	42 %	48 %
Once a day or more	4 %	10 %	7 %	0 %
Normal commercial crisp consumption				
Walkers ready salted	83 %	95 %	67 %	97 %
Walkers Hint of Salt	14 %	10 %	7 %	28 %
Supermarket own-brand ready salted crisps	43 %	48 %	33 %	55 %
Walkers oven baked sea salt	44 %	33 %	40 %	59 %
Kettle original sea salt	45 %	67 %	44 %	31 %
Pringles original salted	70 %	57 %	72 %	76 %
Smiths original crinkle cut salted	11 %	14 %	7 %	14 %
McCoy's Ridge Cut salted	49 %	71 %	35 %	5 %
Originally Smiths Walkers salt and shake	17 %	24 %	14 %	17 %

Tyrells Lightly salted	26 %	33 %	30 %	14 %
Walkers crinkles simply salted	30 %	14 %	28 %	45 %
Popchips sea salted	6 %	5 %	12 %	0 %
Pom Bear Original	29 %	19 %	26 %	41 %

■ High salt (1.4g/100g) ■ Medium salt (1.15g/100g) ■ P1 (1.2g/100g)
■ P2 (0.84g/100g) ■ P3 (0.84g/100g) ■ Hand-cooked (1.2g/100g)
■ Baked (1.18g/100g)

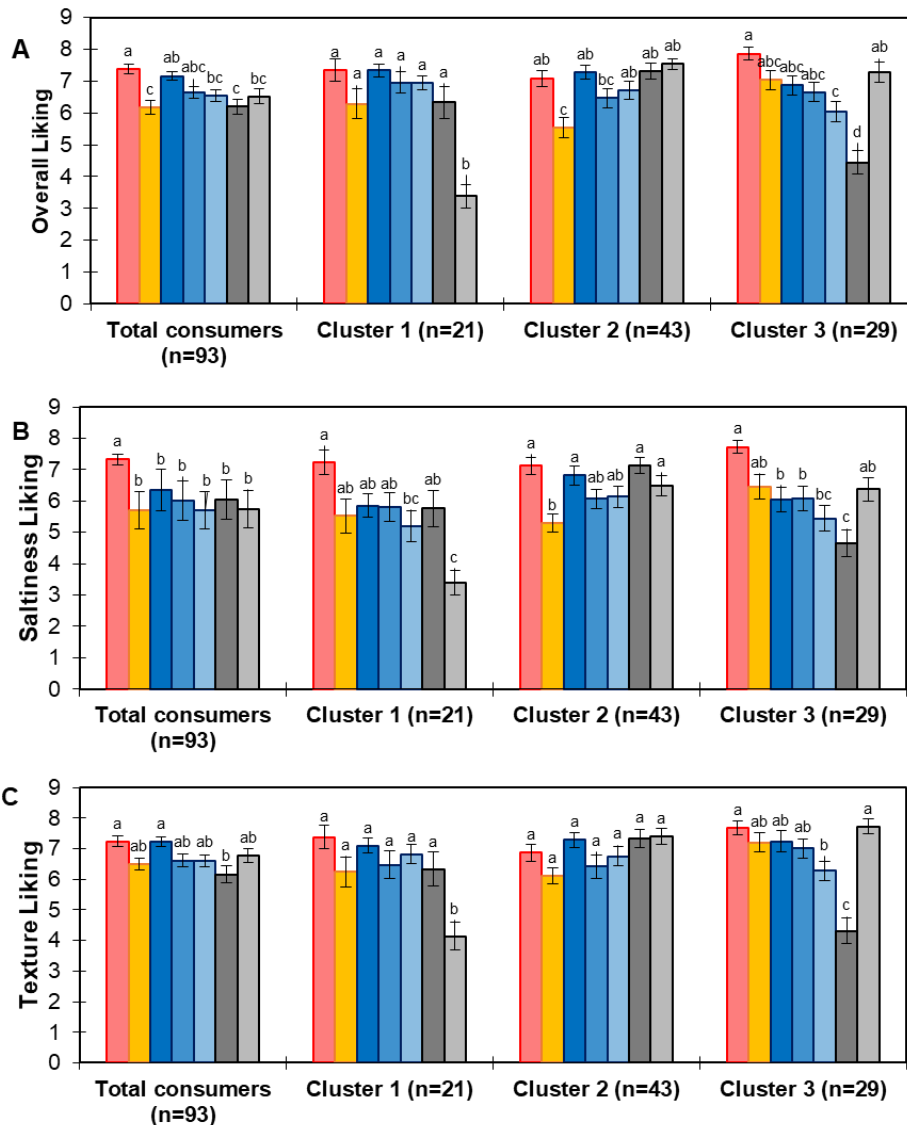


Figure 4.3. Liking scores for each liking attribute per product⁵ calculated for total consumer group (n=93) and for each cluster group. Different letters within cluster group or total consumer group determine a significant difference ($p < 0.05$). Error bars represent standard error.

⁵ Potato crisp products include prototypes (P1, P2, P3) with the rest being commercial products detailed in the materials and methods section of the text. Prototype 1 (P1) was made using 1.2 g of 106-425 μm NaCl salt per 100g of unsalted potato crisps. P2 and P3 are 30 % reduced NaCl salt products when compared to P1 and were made using <106 μm NaCl salt or SODA-LO[®] salt microspheres respectively.

4.5.3. Consumer acceptance of the product set

4.5.3.1. Consumer panel general demographics and crisp consumption habits

Information on demographics, mouth behaviour type and product usage characteristics can be found in Table 4.2 for the total consumer group. The majority of the consumers identified as white British (90 %), and there was an even spread of consumers across age ranges (18-24 years: 14 %, 25-34 years: 12 %, 35-44 years: 17 %, 45-54 years: 27 % and 55-65 years: 30 %). The consumer group tested were frequent crisp consumers; all consumed crisps at least once a month, with 82 % of consumers stating that they ate crisps more than two times a week.

4.5.3.2. Attribute liking ratings, saltiness just-about-right scores and penalty analysis of the total consumer group

Figure 4.3a-c describes the mean attribute liking scores across the whole consumer group (n=93) for each product, along with post-hoc groupings. ANOVA revealed significant differences between the product set across all liking attributes ($p < 0.0001$), yet, there was no significant differences in overall liking, saltiness liking and texture liking between the standard prototype (1.2g NaCl / 100g) and either of the model salt prototypes with 30 % less NaCl (P2, P3: 0.84 g NaCl / 100g) (Figure 4.3a-c). The high salt product (1.4g NaCl / 100g salt) had the highest overall liking ($7.4 \pm$

1.5), which was significantly higher than all the products except P1 (STD) and P2 (Figure 3a). The hand-cooked product was, on average, the least liked (6.2 ± 2.3), significantly less so than the high salt product and P1 (STD) (Figure 3a). Similar to overall liking scores, the high salt product had the highest mean saltiness liking score (7.4 ± 1.5), which was significantly higher than all other products ($p < 0.05$). No significant differences were observed between all three prototype products and the remaining products ($p > 0.05$) (Figure 3b). Figure 4.3c shows the most liked products for texture were the high salt product and P1 (STD), whose values were significantly higher than the hand-cooked ($p < 0.05$) but not significantly different to all other products, including the salt-reduced prototypes (P2, P3) ($p > 0.05$).

Figure 4.4 shows the Just-About-Right (JAR) responses for each product and the penalty score, which is the reduction in mean liking between JAR respondents and non-JAR respondents, calculated by penalty analysis. All products showed unbalanced JAR profiles with few consumers responding 'too salty' (4-9 %). With the exception of the high salt product (1.4g NaCl /100g), other products had a high percentage of 'not salty enough' responses (48-61 %). The product with the highest salt level had the greatest proportion of JAR responses (67 %), whilst the baked product had the lowest (31 %). The results from the penalty analysis indicated that all products not being salty enough or too salty is associated with a significant drop in overall liking of between 1.17 and 2.16 on a 9-point liking scale (table in Figure 4.4). The prototypes had

similar JAR profiles. Although there was a slightly lower percentage of consumers who rated P1 as not salty enough (48 %) compared to P2 and P3 (55 % and 57 %, respectively), these were similar to the medium salt product (59 %). When observing the penalty score (table in Figure 4.4), P3 decreased by (1.50) while P1 (STD) and P2 only decreased by 1.17 and 1.37, respectively.

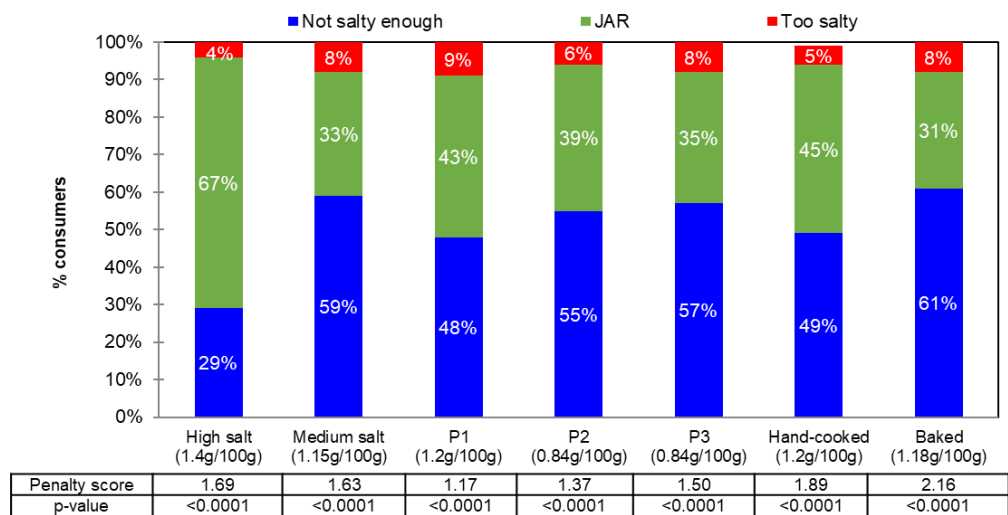


Figure 4.4. Just-about-right (JAR) responses⁶ for each product⁷ of the whole consumer base (n=93) shown as a percentage. The table underneath the graph details the penalty score⁸ and p-value⁹ from the penalty analysis.

⁶ Responses “not quite salty enough” and “not at all salty enough” were grouped into the “not salty enough” category and responses “too salty” and “far too salty” were grouped into the “too salty” category

⁷ Potato crisp products include prototypes (P1, P2, P3) with the rest being commercial products detailed in the text. Prototype 1 (P1) was made using 1.2 g of 106-425 µm NaCl salt per 100g of unsalted potato crisps. P2 and P3 are 30 % reduced NaCl salt products when compared to P1 and were made using <106 µm NaCl salt or SODA-LO® salt microspheres respectively.

⁸ Decrease in overall liking between respondents who scored products as JAR and those who scored samples at the other two levels (not salty enough and too salty).

⁹ p-value determined by the comparison test of the overall liking mean for JAR respondents and those who scored samples at the other two levels (not salty enough and too salty).

4.5.3.3. Consumer cluster groups based on overall liking patterns

To identify groups of consumers differing in overall liking patterns, a segmentation of the consumer group was performed by Agglomerative Hierarchical Clustering, which identified three groups (Supplementary material 4.6). ANOVA revealed a significant interaction effect between the product and cluster group for all three liking attributes ($p < 0.001$), indicating that attribute liking varied depending on the consumer cluster. Figure 4.3a-c show mean attribute liking scores across each cluster group. Significant differences were found between the product set for overall, saltiness and texture liking within each cluster group ($p < 0.001$) (Figure 4.3a-c). Of these differences observed, no significant differences between the three prototype products were found within the three cluster groups for all three liking attributes ($p > 0.05$). Cluster 1 (23 %) disliked the baked product (Figure 4.3a-c), with 100 % of cluster 1 also rating baked as 'not salty enough' (Supplementary material 4.7). Cluster 2 (46 %) liked the commercial medium salt product the least, and cluster 3 (31 %) disliked hand-cooked (Figure 4.3a-c), which also had the highest number of consumers (71 %) rating it as 'not salty enough' (Supplementary material 4.7). The high salt product was liked highly across all three cluster groups (Figure 4.3a-c).

Table 4.2 presents the demographic, mouth behaviour type and product usage characteristics for each cluster group. Chi-square and fisher test results (not presented) confirmed that clusters were independent of

mouth behaviour type, gender and crisp consumption frequency. However, cluster 1 had fewer consumers in the age category 35-44 years while cluster 3 had fewer consumers in the age range of 55-64 years. Unsurprisingly, cluster 1 (lower liking for the baked) has a lower percentage of consumers (33 %) who stated that they normally consume a baked variety of crisps than the other two clusters (40-52 %). Additionally, cluster 3 (lower liking for hand-cooked) had the lowest proportion of members who stated that they usually consume hand-cooked crisp varieties (Table 4.2).

4.5.4. Internal preference mapping

Internal preference mapping (Figure 4.5) was used to visualise overall preferences of the consumer clusters against the product sensory profiles. Two dimensions accounted for 78.62 % of the total variance (42.99 % and 35.63 % for F1 and F2, respectively), with both axes separating the three cluster groups within the multivariate space. F1 separated consumer cluster 1 (23 %) and cluster 2 (46 %) as F1 was strongly negatively correlated with cluster 1 overall liking (-0.757) and strongly positively correlated with cluster 2 (0.828). F2 was strongly positively correlated with cluster 3 (31 %) (0.926), separating this cluster from the other two. Overall liking of cluster 1 was significantly positively correlated with initial flavour (0.806), initial saltiness intensity (0.863), saltiness during eating (0.819), potato flavour (0.944), greasy mouthfeel (0.759), oiliness on fingers (0.815), saltiness aftertaste (0.904) and breakdown speed (0.772) while being significantly negatively correlated

with sweetness (-0.960), reconstituted potato flavour (-0.952). Overall liking of cluster 2 was significantly positively correlated to crispiness (0.886). Cluster 3 overall liking was significantly negatively correlated with oil aroma (-0.767), golden appearance (-0.767), dark edges (-0.797), and earthy flavour (-0.658).

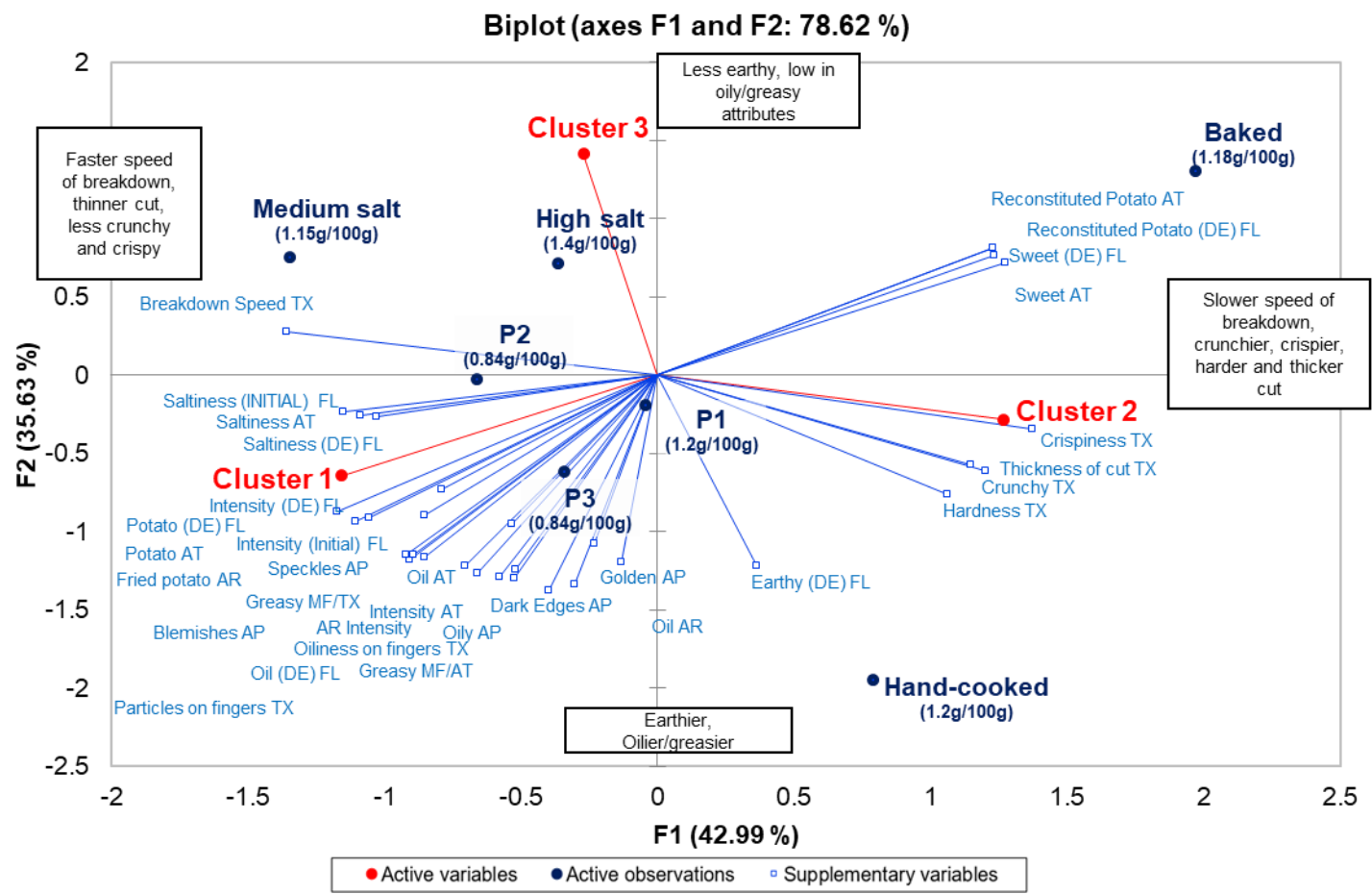


Figure 4.5. Internal preference map of mean overall liking data for each cluster with mean sensory attribute scores as supplementary variables. Each cluster is represented in red, the active observations in dark blue are the products and the supplementary variables in light blue are the sensory attributes.

4.5.5. Mouth behaviour typing using JBMB typing tool®

Using the JBMB typing tool® (Jeltema et al., 2015), 83 consumers were grouped according to mouth behaviour type (Table 4.2). Ten consumers could not be classified due to technical issues completing the surveys and were excluded from the subsequent analysis based on mouth behaviour type. After removing those unclassified, the most predominant mouth behaviour type was the crunchers (33 %), followed by suckers (24 %), chewers (20 %) and smooshers (11 %). Demographic and product usage characteristics of each mouth behaviour group is displayed in Supplementary material 4.8. Chi-square and Fisher exact tests (results not presented) determined that mouth behaviour type was independent of gender, age and frequency of crisps consumption (any flavour and ready salted). ANOVA revealed no interaction effect between product and mouth behaviour group for overall, saltiness and texture liking attributes ($p = 0.41 - 0.89$), suggesting that liking was not influenced by mouth behaviour type (Supplementary material 4.9). There was, however, a main effect of mouth behaviour type on texture liking ($p = 0.01$). The subsequent post-hoc test identified that chewers and crunchers gave significantly higher scores for texture liking than suckers ($p < 0.05$) (Supplementary material 4.10).

4.6. Discussion

This study aimed to validate the potential for NaCl salt reduction by leveraging physicochemical design rules set out to create optimised

model salt particles (Hurst et al., 2021). The previous study recommended a series of design suggestions for salt particles: small particle size, low density, and low hydrophobicity with a particle shape associated with optimal flow properties, which are applicable to the model salts selected for this current study. The model salts selected, reduced particle size (<106 µm) and a spray-dried salt with maltodextrin (commercially available as SODA-LO®), were previously shown to enhance sodium dissolution, saltiness perception, transfer efficiency and adhesion when applied to peanuts. SODA-LO® and <106 µm table salt have similar mean particle sizes; however, the morphology of SODA-LO® shows a higher surface area due to internal voids which enhances dissolution rate. Prototype products made using these salts at a 30 % salt reduced level were assessed and compared to a broader product set, including commercially available products, using a dual sensory and consumer assessment approach. Since this research uses mainly topically applied products, our findings may also apply to other topically applied snacks such as peanuts and popcorn.

4.6.1. Descriptive sensory analysis and saltiness profiles of the product set

Application of model salt particles to potato crisps that followed physicochemical design rules (Hurst et al., 2021) enabled a 30 % reduction in NaCl content since no significant difference in saltiness intensity was found across 3-time points: initial, during eating and aftertaste ($p > 0.05$) when compared to the standard prototype (P1: 1.2g

NaCl / 100g) and a commercially available medium salt product (1.15g NaCl / 100g). Additionally, P3 was not significantly different in initial saltiness nor saltiness aftertaste compared to the highest salt content product (1.4 g NaCl / 100g, $p > 0.05$), suggesting that the model salt (SODA-LO®) used to prepare the salt-reduced prototype (P3) could be successful in reducing NaCl content, even further, up to 40 %. Nevertheless, saltiness during eating was significantly different between P3 and the high salt product. No significant differences between prototype products were seen in most of the other attributes assessed (Supplementary material 4.2-4.5). The observed differences (some appearance attributes, hardness, and cut thickness) are probably due to the batch to batch variation of the unsalted crisps commercially purchased, despite randomisation of batch codes when generating the crisp prototypes. These slight differences are unlikely to impact saltiness perception. Thus, any changes in saltiness perception across the 3 prototypes can be attributed to the design of salt particles. Overall, we have validated that the physicochemical design rules (Hurst et al., 2021) can reduce NaCl by 30 % without significantly affecting saltiness perception.

Consistent with other research (Mueller et al., 2016, Levings et al., 2014, Drake et al., 2011, La Croix et al., 2015, Jaenke et al., 2017), our results suggest that limited salt reduction could be possible by direct NaCl removal without perceivable impact. Results indicated no significant difference in saltiness between the standard prototype (1.2 g NaCl /

100g) and the high salt level product (1.4 g NaCl / 100g), a reduction of approximately 15 % based on measured NaCl content (Table 4.1). These products were used to determine potential level of salt reduction since the base potato crisp was supplied by the same manufacturer (see section 4.4.1).

Previous studies have suggested that a rough texture, i.e. ridges or crinkles compared to a smooth texture, could enhance saltiness perception (Biggs et al., 2016, Pflaum et al., 2013, van Rompay and Groothedde, 2019). However, in the current study, no significant difference was seen in saltiness intensity between standard flat potato crisp and the crinkled potato crisp at similar NaCl contents (Table 4.1), supporting evidence from Kongstad and Giacalone (2020) that differences in the cut of potato do not influence the perception of saltiness.

The baked product is the only product in the set that contained NaCl salt in both the matrix and outside the product. The addition of salt within the potato base dough contributes to the desired flavour and texture of the final product during baking. Despite the baked product having the same NaCl salt content as medium salt level products (Table 4.1), it was significantly the least salty, suggesting that a proportion of salt contained in the baked product goes unperceived as a result of being 'trapped' within the matrix, thus, reducing its availability to interact with the taste receptor cells. Limited research has been published on the impact of salt removal and salt reduction strategies within the matrix of baked and

reconstructed snacks, e.g. thermomechanically extruded products. It would be a valuable area for further investigation, especially since expanded snacks often require much higher NaCl contents to develop the desired texture (van der Sman and Broeze, 2013).

Previously, Tian and Fisk (2012) highlighted that a proportion of sodium is consumed without being perceived under regular eating patterns of potato crisps. A thick cut hand-cooked product and a reconstituted product were included in the study to test whether texture and oral breakdown speed increase the proportion of perceived sodium. Despite the baked and hand-cooked products reducing the breakdown speed (hypothesised to optimise the release of sodium for detection by the taste receptor cells), their saltiness profiles were not enhanced significantly ($p > 0.05$). Since salt was topically applied, any available salt will dissolve in saliva within the first 10-20 seconds (Hurst et al., 2021) and therefore, break down speed or the subsequent length of time in mouth did not affect perception as any available NaCl would probably be dissolved rapidly at the beginning of consumption. However, chewing behaviour is more likely to affect perception when salt is incorporated into the food matrix and has a slower release of sodium ions from the matrix (Lawrence et al., 2012b), so this could be an exciting area to investigate to optimise salt release in the mouth. Surprisingly, the hand-cooked product did not have the highest perceived saltiness even though Table 4.1 shows that it has the highest measured NaCl content (1.64 g/100g), despite declared BoP content being 1.2 g/100g. This could be attributed

to a larger particle size (not measured in this study) or the higher level of greasiness suppressing saltiness perception (Supplementary material 4.3), due to oil acting as a barrier for tastant delivery to taste receptor cells (Metcalf and Vickers, 2002). On the other hand, this disparity may also be due to the high variability in NaCl content between product batches.

4.6.2. Consumer acceptance test of the product set

4.6.2.1. Attribute liking ratings, saltiness just-about-right scores and penalty analysis of the total consumer group

Results indicated that consumers liked the high salt product (1.4 g NaCl / 100g) the most. This product had an overall liking score that was significantly higher than P3, hand-cooked, baked and medium salt products (Figure 3a). Similarly, the high salt product had significantly higher saltiness liking than all other products, with the highest percentage of saltiness JAR responses (67 %) (Figure 4.4). In contrast, all other products have a much greater percentage of consumers reporting that the product is 'not salty enough' (Figure 4.4). The results from the penalty analysis in Figure 4.4 also revealed that liking was reduced significantly across all products when the product was not considered salty enough or too salty by the consumer. Although all products were at least 'slightly liked' (Figure 4.3), our findings highlight the difficulty in reducing salt whilst retaining consumer acceptance, given the common preference

towards higher salted potato crisps. Undoubtedly, food manufacturers cannot increase the salt content of products to obtain more balanced JAR profiles; however, these results highlight the importance of saltiness as a driver of liking.

Consistent with the saltiness profile results (Figure 4.2), there were no significant differences between the three prototype products (Figure 4.3a-c) for all three liking attributes. Therefore, salt content can be reduced by up to 30 % while maintaining saltiness liking and overall liking when using salt particles that adopt physicochemical design rules. Furthermore, saltiness ratings (as discussed in section 4.6.1) suggested that a 15 % (based on measured salt content) salt reduction via direct salt removal could be possible without significantly affecting saltiness perception. Despite differences in JAR profiles between high salt product (1.4 g/100g) and P1 (1.2 g/100g), overall liking scores confirm that this level of reduction through direct salt removal may be possible without impacting consumer acceptance since there was no significant difference between these two products. The higher percentage of consumers scoring the high salt product as JAR compared to P1 could be due to consumers' familiarity with this product since it is the most commonly consumed brand in the UK.

4.6.2.2. Consumer cluster groups based on overall liking patterns

Consumer segmentation groups were driven by preference for texture profiles which were altered by processing methods (e.g. batch fried product and reconstituted baked product) (Figure 4.3 and Figure 4.5). Cluster 1 (23 %) was characterised by disliking the baked product; cluster 2 (46 %) was characterised by liking the medium salt commercial product the least; cluster 3 (31 %) was characterised by disliking the thick-cut, hand-cooked product. Despite this, the most liked product across all cluster groups remained the highest salt product, further evidencing that most consumers seek a high salt level in their snacks. More research is required into altering saltiness preferences, for example, the role of early exposure to salt, to avoid damaging health impacts due to high NaCl salt consumption (DeSimone et al., 2013).

Drivers of liking are typically determined to understand consumer preferences towards products and the desirable sensory attributes required for high consumer acceptance. Drivers of liking were determined for each cluster group using the internal preference map in Figure 4.5 and correlations found between cluster liking and sensory attributes (presented in section 4.5.4). Cluster 1 (23 %) prefer products with a high potato flavour intensity, high saltiness, greasy mouthfeel and quick speed of breakdown, while cluster 2 (46 %) drivers of liking include crispiness and slow breakdown speed. Drivers of liking for cluster 3 (31 %) include low oil aroma, low earthiness and light in colour. The attributes

identified as drivers of liking for each cluster group reflect each clusters' most preferred products. Surprisingly, mouth behaviour type was not associated with cluster groups despite the differences in cluster groups being driven by texture, which is the primary determinant of mouth behaviour type (Jeltema et al., 2015).

4.6.2.3. Mouth behaviour typing using JBMB typing tool®

The JBMB typing tool® was used to characterise consumers by their preferred mouth behaviours. The proportion of consumers characterised as suckers in this study (24 %) was surprisingly higher than previously reported (8 %) (Jeltema et al., 2015). Nevertheless, differences in the spread of mouth behaviour groups have been reported between countries, with the proportion in this study being closer to that found in a UK cohort (15 %) (Licker, 2019). The relatively high proportion of suckers in this study is also surprising since the inclusion criteria was frequent crisp users (consuming crisps at least once a fortnight). It might be expected that a group of high-frequency crisp consumers would have a relatively lower proportion of suckers since suckers prefer foods that they can suck on until they dissolve like hard candies, whereas crisps may appeal more to crunchers as crisps are typically crispy and crunchy products. Therefore, the prevalence of suckers in our study suggests that mouth behaviour preference does not ultimately define an individual's consumption habits or that despite a hard and crunchy texture, individuals can still use their preferred mouth behaviours to manipulate the texture of the product. Although it was found that the mouth behaviour

group did not impact overall and saltiness liking of the different products, there was a main effect of mouth behaviour group on texture liking (Supplementary material 4.9). Suckers texture liking ratings were significantly lower across the whole product set compared to chewers and crunchers ($p < 0.05$). This is expected since crunchers are characterised by their preference towards food textures that they can crunch like crunchy granola, crispy vegetables, and crunchy cookies and chewers have a preference towards food that they can chew on (Jeltema et al., 2015).

4.7. Limitations of study

This study provides preliminary data investigating the effect of mouth behaviour type on consumer's crisp preferences; however, to fully explore the impact of mouth behaviour on perception and preference, a larger consumer group and a wider range of product textures should be considered. Increasing the number of consumers would also enable more robust segmentation to explore key differences between cluster group characteristics. Consumer choice is influenced by factors outside of a product's inherent sensory characteristics, including branding, packaging design, and nutritional information. For example, in a previous study when consumers were informed of reduced salt content of potato crisps on the packaging, consumers preferred the reference product over the salt reduced crisps; however, when tested blind, this was not the case. Therefore, there is a strong labelling effect on potato crisp product

liking and saltiness perception (Kongstad and Giacalone, 2020). Products within this study were assessed unbranded to enable the study's primary aim of determining the capability of the model salts to retain the perception of saltiness whilst reducing salt content. Nevertheless, the impact of these extrinsic product factors on preference and purchasing intent should not be disregarded when adopting salt reduction strategies by food manufacturers.

4.8. Conclusions

This study was designed to evaluate the application of model NaCl salt particles, which adopt the salt reduction design rules in Hurst et al. (2021), to a common salty snack product: potato crisps. Potato crisp prototypes produced using model salts (<106 µm table salt and a spray-dried salt, SODA-LO®) with 30 % salt reduction were assessed within a broader product set of commercially available products to understand sensory and consumer acceptance performance within the market context. Overall, this study successfully demonstrated a route to 30 % salt reduction in a topical application, without loss of perceived saltiness intensity or impact on consumer liking, therefore validating the previously proposed physicochemical design rules for the formulation of model salt particles. Broader results suggest that salt reduction through direct salt removal (without compensating for reduction) may be successful, but only by 15 %, providing further evidence that specifically designed salt particles for salt reduction are required. Despite previous suggestions

that roughness may enhance saltiness perception, crinkled potato crisps did not enhance the saltiness through cross model interactions. This study also investigated the key drivers of liking and segmented consumers based on their overall liking patterns and mouth behaviour type. While varying textural profiles, caused by different processing techniques segmented consumers by overall liking, mouth behaviour groups did not differ in their preferences across the product set. Despite variations in oral breakdown speed across some products, saltiness perception was not enhanced by the increased time in mouth for salt dissolution. The presented findings will be of interest to both the food industry and academic researchers. Specifically, it will be relevant to those actively looking to reduce salt in foods containing topically applied seasonings and salt without compromising the perception of saltiness and consumer liking.

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Author contributions

KH: conceptualization, methodology, formal analysis, investigation, writing – original draft, visualisation. LH: conceptualization, methodology, writing – review & editing, supervision. IF: conceptualization, writing – review & editing, supervision, funding acquisition.

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4.9. Supplementary material (chapter 4)

Supplementary material 4.1. Attributes and definitions used in descriptive analysis of 9 crisp products.

Sensory attributes	Definition
<i>Aroma</i>	
Overall intensity	The overall intensity of aroma.
Oil	The intensity of aroma associated with fresh hot cooking oil.
Potato	The intensity of aroma associated with fried potato.
<i>Appearance</i>	
Golden colour	The intensity of golden colour
Amount of dark edges	Amount of dark edges present, including burnt edges and skins.
Amount of Blemishes/black marks	Amount of blemishes/black marks present.
Greasy/oily	Visible greasiness or oiliness on the surface of the crisps.
Amount of Speckles	Amount of speckles of potato skin present over the whole sample.
<i>Texture</i>	
Crispiness	The high-pitched sound produced when the product is bitten with the back teeth on the initial first bite.
Hardness	The force required to bite through the crisp. The harder the product the more force is required to bite through the sample.
Crunchiness	The amount of low-pitched noise and force with which the sample breaks or fractures during chewing with molars.
Greasiness	Greasy mouthfeel assessed during eating.
Thickness of cut	How thick the product feels whilst biting in the mouth from thin to thick.
Speed of breakdown	The amount of time for the food product to breakdown from the first chew to the swallow point from slow to fast.
Particles on fingers	The quantity of particles left on the fingers after touching the product.
Oiliness on fingers	The oiliness of the fingers after touching the product
<i>Flavour</i>	
Initial Overall Flavour	The overall intensity of flavour (initial impact).
Overall Flavour during eating (DE)	The overall intensity of flavour assessed during eating
Saltiness (intial)	The intensity of the basic taste associated with Sodium Chloride upon initial impression.

Chapter 4: Sensory perception and consumer acceptance of commercial and salt-reduced potato crisps formulated using salt reduction design rules

Saltiness during eating (DE)	The intensity of the basic taste associated with Sodium Chloride overall during eating.
Sweetness	The intensity of the basic taste associated with sugar.
Oil	The intensity of the flavour associated with hot cooking oil.
Standard Potato	The intensity of flavour associated with a potato that has been fried in oil.
Reconstituted potato	The intensity of aftertaste associated with a potato that has been reconstituted, e.g. instant mashed potato or pringles.
Earthy	Intensity of flavour associated with the blemishes/skins which provides an earthy flavour note.
<i>Aftertaste</i>	
Overall aftertaste intensity	The intensity of overall aftertaste.
Sweetness	The intensity of aftertaste associated with the basic taste sugar.
Saltiness	The intensity of aftertaste associated with the basic taste Sodium Chloride.
Oil	The intensity of aftertaste associated with cooked oil.
Standard potato	The intensity of aftertaste associated with a potato that has been fried in oil.
Reconstituted potato	The intensity of aftertaste associated with a potato that has been reconstituted, e.g. instant mashed potato or pringles.
Greasy mouthfeel	The oily film coating the mouth after swallowing.

Supplementary material 4.2. Sensory descriptive analysis mean panel data (\pm standard deviation) and post-hoc test groupings for aroma and appearance attributes of 9 products. Different letters within a column show significant differences ($p < 0.05$).

Product code	Aroma			Appearance				
	Overall intensity	Oil	Fried potato	Golden colour	Amount of dark edges	Blemishes/black marks	Greasy/oily	Amount of speckles
P1 (STD)	51.4 \pm 10.5b	38.1 \pm 12.5b	38.4 \pm 12.0b	38.4 \pm 10.2bc	13.6 \pm 8.9de	12.2 \pm 7.9cd	29.9 \pm 13.1b	10.9 \pm 8.4c
P2	46.6 \pm 10.1b	38.4 \pm 10.7b	37.4 \pm 8.6b	37.4 \pm 12.8c	11.5 \pm 8.9e	14.8 \pm 7.1bc	38.2 \pm 12.1b	7.8 \pm 9.6cd
P3	52.0 \pm 15.6b	39.7 \pm 14.0b	41.6 \pm 12.7b	41.6 \pm 8.0bc	22.7 \pm 8.6c	19.8 \pm 8.8bc	31.7 \pm 11.5b	28.4 \pm 14.8b
High salt	50.9 \pm 12.2b	38.4 \pm 10.2b	37.6 \pm 10.8b	37.6 \pm 12.8bc	20.4 \pm 11.4cd	17.2 \pm 7.8bc	32.7 \pm 8.4b	13.5 \pm 10.4c
Medium salt	52.0 \pm 11.7b	34.8 \pm 11.3b	37.3 \pm 10.9b	37.2 \pm 8.9b	31.1 \pm 15.0b	20.6 \pm 13.0b	31.9 \pm 11.7b	35.6 \pm 21.1ab
Low salt	51.1 \pm 12.7b	37.8 \pm 10.4b	37.4 \pm 11.5b	37.4 \pm 10.1bc	13.7 \pm 9.0de	17.5 \pm 9.9bc	35.2 \pm 9.9b	10.9 \pm 10.1c
Hand-cooked	75.3 \pm 10.5a	66.5 \pm 18.3a	44.7 \pm 13.3a	44.7 \pm 8.3a	65.8 \pm 17.7a	35.1 \pm 15.9a	57.4 \pm 14.3a	40.6 \pm 16.2a
Crinkled	53.8 \pm 10.6b	41.8 \pm 12.4b	40.9 \pm 12.2b	40.9 \pm 13.7b	22.1 \pm 11.1c	14.9 \pm 9.4bc	32.8 \pm 14.0b	16.2 \pm 12.3c
Baked	20.9 \pm 9.2c	11.2 \pm 12.9c	19.6 \pm 13.0c	19.6 \pm 12.5d	0.9 \pm 2.2f	0.2 \pm 0.5d	7.4 \pm 7.2c	1.0 \pm 2.7d

Supplementary material 4.3. Sensory descriptive analysis mean panel data (\pm standard deviation) and post-hoc test groupings for texture attributes for 9 products. Different letters within each column show significant differences ($p < 0.05$).

Product code	Crispiness	Hardness	Crunchiness	Greasiness	Thickness of cut	Speed of Breakdown	Particles on fingers	Oiliness on fingers
P1 (STD)	62.9 \pm 14.1abc	36.8 \pm 19.3e	57.3 \pm 13.9cd	38.2 \pm 14.5b	33.3 \pm 12.6d	55.8 \pm 15.9a	40.0 \pm 17.9a	43.5 \pm 19.4b
P2	62.6 \pm 13.6abc	37.3 \pm 16.7e	56.9 \pm 13.7cd	42.3 \pm 16.7b	33.2 \pm 12.3d	54.5 \pm 15.3a	37.6 \pm 18.8a	44.9 \pm 16.7b
P3	64.9 \pm 15.5abc	47.5 \pm 19.7cd	60.9 \pm 15.5cd	41.0 \pm 11.2b	42.6 \pm 12c	50.6 \pm 14.9a	38.3 \pm 18.3a	45.2 \pm 15b
High salt	64.1 \pm 10abc	41.9 \pm 20.7de	62.9 \pm 14.8c	44.4 \pm 15b	35.9 \pm 14.4cd	53.7 \pm 13.9a	38.5 \pm 17.9a	43.9 \pm 15.9b
Medium salt	57.5 \pm 14.2c	40.6 \pm 20.3de	56.4 \pm 16.6cd	38.2 \pm 14.4b	39.4 \pm 15.3cd	55.0 \pm 14.7a	43.6 \pm 19.5a	45.6 \pm 19.7b
Low salt	58.6 \pm 13.1bc	34.9 \pm 20.6e	53.8 \pm 16.1d	38.2 \pm 11.5b	36.8 \pm 13.6cd	54.2 \pm 15.1a	38.0 \pm 17.3a	45.5 \pm 18.7b
Hand-cooked	67.2 \pm 16.2ab	78.1 \pm 17.5a	83.7 \pm 7.2a	61.9 \pm 19.7a	72.1 \pm 15.1a	30.2 \pm 15.7b	43.8 \pm 16.1a	57.8 \pm 18.2a
Crinkled	62.7 \pm 11.4abc	51.6 \pm 15c	64.8 \pm 12.2cd	40.9 \pm 13.4b	60.4 \pm 16.7b	48.2 \pm 12a	39.8 \pm 21a	45.5 \pm 20b
Baked	68.7 \pm 10.2a	60.7 \pm 18.6b	74.9 \pm 12.4b	7.1 \pm 6.5c	61.9 \pm 14.9b	27.8 \pm 10.5b	26.0 \pm 17.6b	12.8 \pm 12.4c

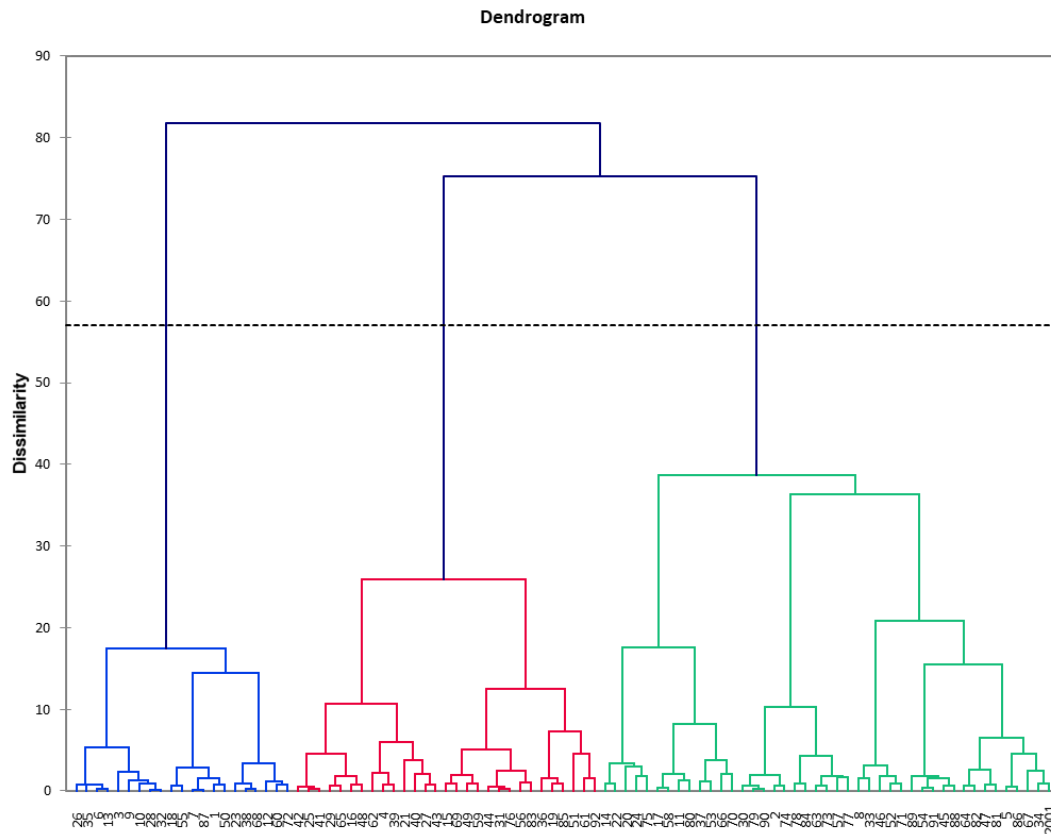
Supplementary material 4.4 Sensory descriptive analysis mean panel data (\pm standard deviation) and post-hoc test groupings for flavour attributes for 9 products. Different letters within each column show significant differences ($P < 0.05$).

Product code	Initial Flavour Intensity	Saltiness flavour (Initial)	Overall flavour intensity (DE)	Saltiness flavour (DE)	Sweet flavour (DE)	Oil flavour (DE)	Potato flavour (DE)	Reconstituted potato flavour (DE)	Earthy flavour (DE)
P1 (STD)	58.3 \pm 12.3ab	49 \pm 11.8abc	58.5 \pm 12.7a	50.6 \pm 16.3ab	7.4 \pm 7.4b	38 \pm 14.9b	46.5 \pm 12.5a	0 \pm 0b	0.5 \pm 1b
P2	52.4 \pm 13.9bc	40.8 \pm 13.4cd	52.9 \pm 13.8ab	42.9 \pm 14.1bc	8.5 \pm 7.6b	39 \pm 14.2b	43.4 \pm 13a	0 \pm 0b	0.3 \pm 0.7b
P3	57.4 \pm 13.5ab	46.1 \pm 14.1abcd	55.1 \pm 13.7ab	45.9 \pm 17.9bc	9.2 \pm 9.5b	38.4 \pm 10.6b	44.9 \pm 12a	0 \pm 0b	0.8 \pm 2.1b
Hlgh salt	60.4 \pm 9.4ab	56.9 \pm 10.1a	60.6 \pm 13.5a	59.8 \pm 14.5a	6.1 \pm 7.4b	40.2 \pm 13.3b	45.7 \pm 10.1a	0 \pm 0b	0.8 \pm 1.9b
Medium salt	58.3 \pm 11.1ab	49.9 \pm 16.5abc	59.5 \pm 10.9a	53.1 \pm 13.5ab	7.9 \pm 7.9b	35.2 \pm 12.3b	45.1 \pm 13.3a	0 \pm 0b	1.2 \pm 3.1b
Low salt	51.7 \pm 11.4bc	35.2 \pm 9.9de	51.8 \pm 13ab	37.1 \pm 15.7cd	7.5 \pm 7.5b	35.2 \pm 10.1b	43.3 \pm 12.8a	0 \pm 0b	0.4 \pm 1b
Hand-cooked	61.6 \pm 11.1a	43.1 \pm 7.4bcd	61.3 \pm 12.9a	47.8 \pm 10.7abc	8.7 \pm 11b	58.8 \pm 16.8a	47.8 \pm 12.2a	0 \pm 0b	9.4 \pm 11.2a
Crinkled	57.9 \pm 10ab	52.3 \pm 12.6ab	61.1 \pm 9.8a	54.7 \pm 11.3ab	10.4 \pm 8.8b	35.8 \pm 13.2b	42.2 \pm 11.4a	0 \pm 0b	0.6 \pm 1.7b
Baked	44.2 \pm 17.3c	26.7 \pm 11.5e	47.5 \pm 15.4b	29.6 \pm 16.4d	49.8 \pm 24.2a	7 \pm 5.2c	0 \pm 0b	51.1 \pm 27.5a	0.1 \pm 0.2b

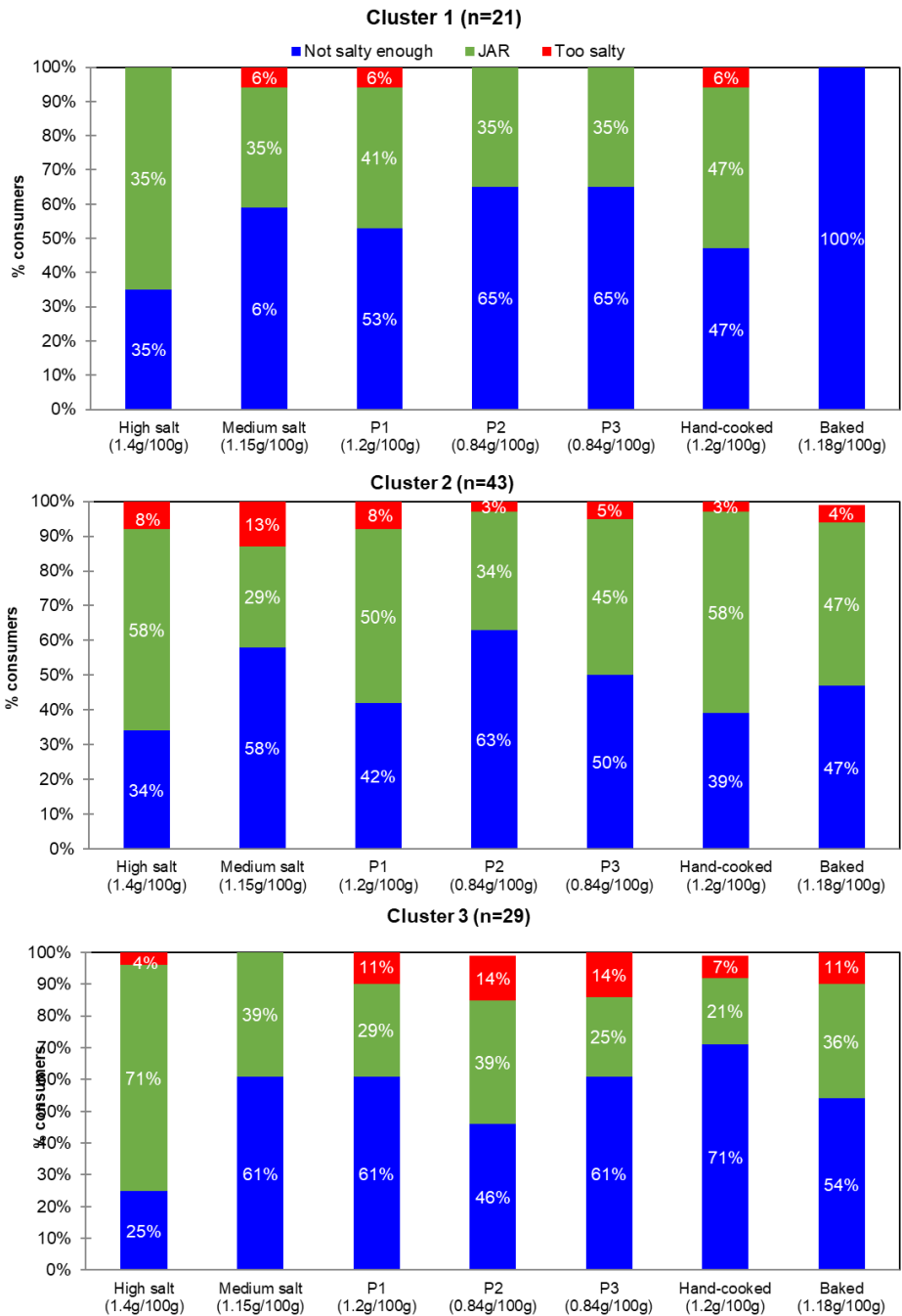
Supplementary material 4.5 Sensory descriptive analysis mean panel data (\pm standard deviation) and post-hoc test groupings for after taste attributes for 9 product. Different letters within each column show significant differences.

Product code	Overall Aftertaste Intensity	Sweet aftertaste	Saltiness aftertaste	Oil aftertaste	Potato aftertaste	Reconstituted Potato after taste	Greasy mouthfeel
P1 (STD)	48.1 \pm 16.9ab	5 \pm 5.9b	33 \pm 18.6ab	32.9 \pm 18.1b	35.5 \pm 13.2ab	0 \pm 0b	35.7 \pm 19.7b
P2	43.6 \pm 16b	4.7 \pm 5.6b	28.6 \pm 17.1abc	35.4 \pm 18.5b	32.5 \pm 16.1b	0 \pm 0b	37.7 \pm 20.6b
P3	47.2 \pm 12.5ab	5.7 \pm 7.2b	28.7 \pm 13.9abc	32.9 \pm 12.2b	32.6 \pm 12.9b	0 \pm 0b	36.7 \pm 14.5b
High salt	47.8 \pm 14ab	4.1 \pm 6.5b	39.3 \pm 16.8a	34.5 \pm 16.3b	34.9 \pm 12.9ab	0 \pm 0b	37.6 \pm 18.6b
Medium salt	49.5 \pm 14.9ab	5.2 \pm 6.9b	35.6 \pm 17.9ab	33.4 \pm 14.1b	35.8 \pm 12.7ab	0 \pm 0b	37.4 \pm 14b
Low salt	43.9 \pm 16.6b	4.8 \pm 6.2b	24.5 \pm 17.1bc	33.2 \pm 15.4b	33.3 \pm 13.7b	0 \pm 0b	33.3 \pm 18.7b
Hand-cooked	54.5 \pm 15.9a	7.4 \pm 9.4b	32.7 \pm 15.7ab	47.9 \pm 17.7a	40.2 \pm 13.6a	0 \pm 0b	51.2 \pm 19.5a
Crinkled	47.4 \pm 14.7ab	6.9 \pm 8.2b	35.1 \pm 16.1ab	32.1 \pm 10.6b	35.5 \pm 12.5ab	0 \pm 0b	37.2 \pm 16b
Baked	42.9 \pm 15.2b	38.9 \pm 15.1a	20.3 \pm 15.4c	4.1 \pm 3.9c	0 \pm 0c	40.1 \pm 30.3a	4.5 \pm 6.8c

Supplementary material 4.6. Dendrogram of agglomerative hierarchical clustering of consumers (n=93).



Supplementary material 4.7. Just-about-right (JAR) responses¹⁰ for each product¹¹ for each cluster group shown as a percentage.



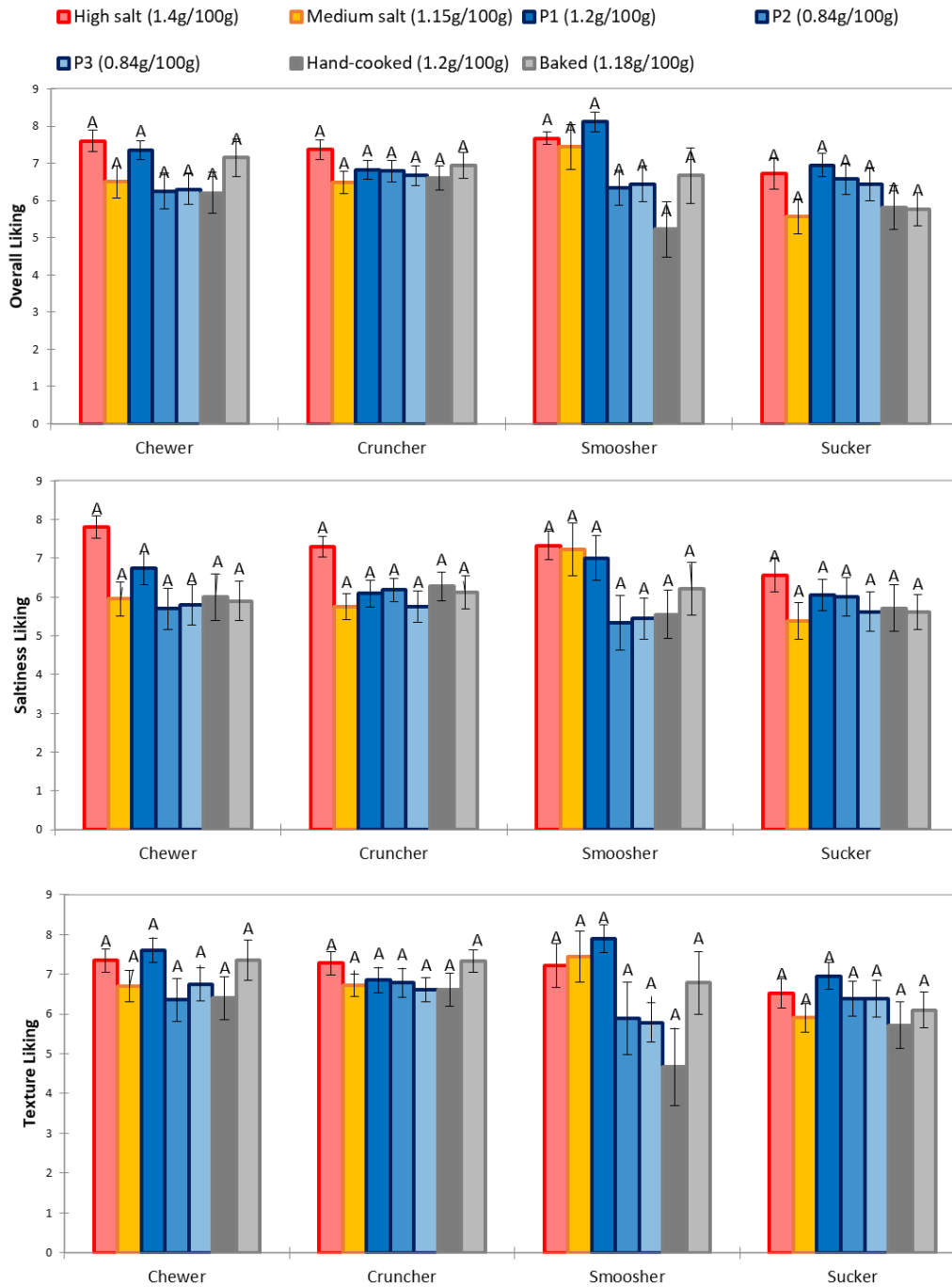
¹⁰ Responses “not quite salty enough” and “not at all salty enough” were grouped into the “not salty enough” category and responses “too salty” and “far too salty” were grouped into the “too salty” category

¹¹ Potato crisp products include prototypes (P1, P2, P3) with the rest being commercial products detailed in the text. P1 was made using 1.2g of 106-425 µm NaCl salt per 100g of unsalted potato crisps. P2 and P3 are 30% reduced NaCl salt products compared to P1 made using <106 µm NaCl salt or SODA-LO® salt microspheres respectively.

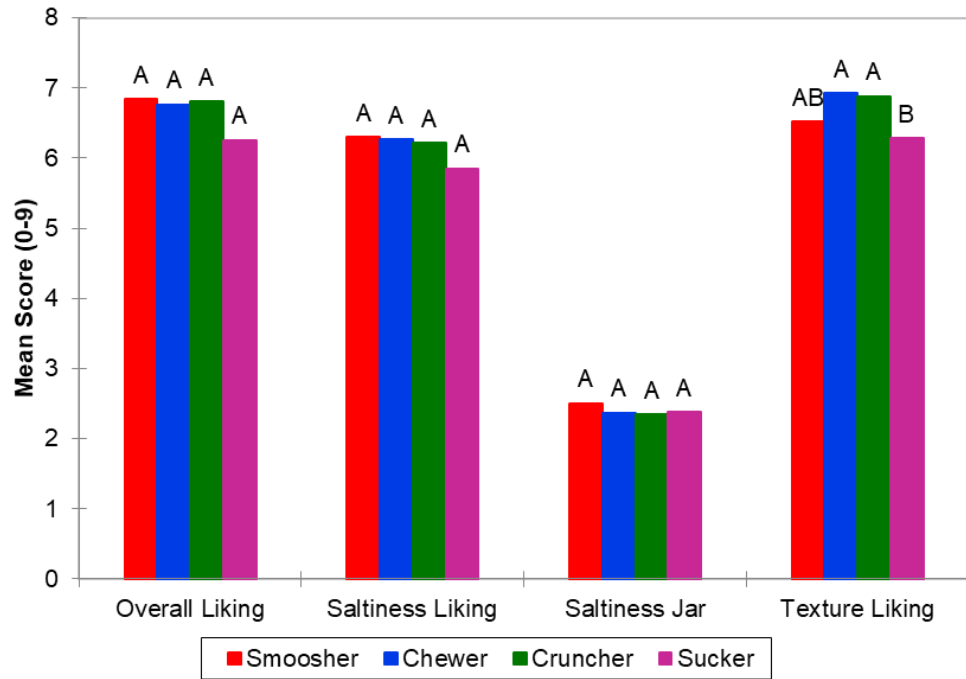
Supplementary material 4.8. Demographic, behavioural characteristics and potato crisp consumption habits for each mouth behaviour group

	Frequency response (%)			
	Chewer (n=20)	Cruncher (n=33)	Smoosher (n=9)	Sucker (n=21)
Gender				
Female	45%	52%	56%	52%
Male	55%	48%	44%	48%
Age				
18-24	20%	21%	0%	5%
25-34	10%	9%	11%	19%
35-44	25%	24%	11%	5%
45-54	25%	24%	44%	29%
55-64	20%	21%	33%	43%
Consumption habits (any flavour)				
Less than once a week	5%	12%	0%	10%
Once a week	10%	0%	22%	5%
2-4 times a week	50%	58%	56%	71%
Once a day or more	35%	30%	22%	14%
Consumption habits (ready salted)				
Less than once a week	5%	6%	0%	5%
Once a week	25%	24%	44%	38%
2-4 times a week	55%	42%	33%	43%
Once a day or more	5%	3%	11%	5%
Normal commercial crisp consumption				
Walkers ready salted	95%	76%	89%	76%
Walkers Hint of Salt	10%	18%	22%	10%
Supermarket own brand ready salted crisps	45%	36%	67%	48%
Walkers oven baked sea salt	40%	52%	33%	43%
Kettle original sea salt	45%	45%	44%	43%
Pringles original salted	65%	73%	56%	86%
Smiths original crinkle cut salted	5%	9%	11%	19%
Hula Hoops original salted	65%	64%	56%	71%
Chipstix ready salted	15%	3%	11%	14%
McCoys Ridge Cut salted	55%	52%	56%	48%
Originally Smiths Walkers salt and shake	20%	18%	22%	14%
Tyrells Lightly salted	20%	30%	22%	29%
Walkers crinkles simply salted	30%	30%	22%	33%
Popchips sea salted	20%	3%	0%	5%
Pom Bear Original	30%	30%	11%	29%
Walkers French fries ready salted	55%	36%	33%	43%
Hula hoops puft salted	20%	30%	22%	14%

Supplementary material 4.9. Mean liking scores of each product per mouth behaviour group for each liking attribute. Error bars represent standard error. Different letters indicate a significant difference between product ($p < 0.05$).



Supplementary material 4.10. Mean liking scores for each liking attribute for each mouth behaviour phenotype group. Different letters within each category determine a significant difference ($p < 0.05$)



Chapter 5

5. General discussion, conclusions and future work

In order to meet ever-evolving salt reduction targets in the global food industry, salt reduction solutions are urgently required across product categories. Snack foods are a growth market and employ salt for its characteristic salty taste that drives consumer liking and therefore repeated purchasing of the product. Furthermore, the impact of food oral processing parameters (e.g. saliva volume, saliva composition and chewing behaviours) on saltiness perception and acceptance of products is still not fully understood. Since salt preference and perception show a link to dietary salt intake, this is an important area to investigate to expand knowledge and support strategies to meet the challenge of reducing salt in the diet whilst retaining consumer acceptance of products.

Therefore, the main aims of this thesis were to explore the capabilities of salt reduction strategies, including the exploration of physicochemical properties to determine the particle design rules for optimised saltiness, while understanding the contribution that individual consumer differences in oral processing parameters have on saltiness perception and subsequent salt intake. Ultimately, this thesis aimed to provide

supporting strategies to reduce salt intake, through salt particle redesign and application to snack foods and through understanding the consumer to enable targeted salt reduction approaches.

This chapter will discuss the key findings from the aims set out in chapter 1 (page 98), review any limitations of the work and summarise key opportunities for further investigation to build on the findings presented within the project studies (section 5.6).

5.1. Developing physicochemical design rules for salt particles for salt reduction

By comparing physicochemical properties, morphology, adhesion capabilities, sodium dissolution kinetics and temporal saltiness profiles of a range of salt particles which varied by salt processing techniques, physicochemical design rules were developed to facilitate further particle design for salt reduction (chapter 2). Previous evidence suggested that to optimise saltiness perception, the dissolution kinetics of sodium should be increased (i.e. faster release rate increased the delivery of sodium to taste buds, increasing saltiness perception) (Tian and Fisk, 2012). Previous research exploring salt particles with altered morphology and particle size resulting in increased surface area, provided an increase in dissolution rate and enhanced perceived saltiness (Rama et al., 2013, Chindapan et al., 2018, Moncada et al., 2015). Within this thesis, this knowledge was expanded and extended by assessing a range of model salts varying in key physicochemical parameters to fully understand salt

particle properties' influence on potential saltiness and to consequently enable the development of specifically designed salt particles that optimise saltiness. Salt particles used in this thesis varied in size, density, hydrophobicity and flow properties to explore the impact of salt particle design in three key areas hypothesised to optimise saltiness of the final product (salted peanuts) (Figure 5.1). The three key areas ranged across the complete product and consumption cycle:

1. Adhesion during application and before packaging.
2. Adhesion during packaging and transport.
3. Release during food oral processing.

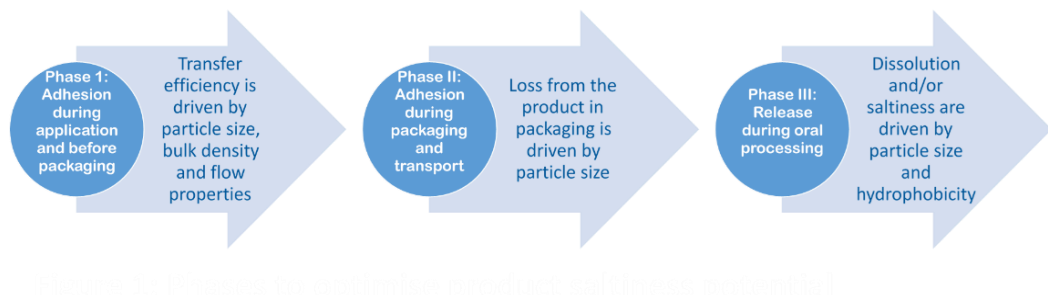


Figure 5.1 Proposed key phases for the optimal design of salt particles and summary of findings for each phase from chapter 2.

Therefore, this thesis uniquely considers the physicochemical parameters of salt particles through the entire product cycle (including manufacture, transport, and consumption), which may influence the consumer's saltiness perception and have not previously been measured simultaneously in this context. This thesis extends knowledge by assessing additional stages of the potential loss of saltiness at

manufacturing and during packaging and transport. The rationale for each phase measurement, key findings and implications of each of these three stages when applied to oiled peanuts are outlined and discussed within this section. Peanuts were an ideal application for this initial study since they have a relatively uniform shape and surface across individual nuts and are easily accessible.

5.1.1. Phase 1: Adhesion during application and before packaging

Poor adhesion results in wasted material, impacting the heterogeneity of the product and sodium levels in the pack. Studies exploring the adhesion of salt to snack foods are limited within published literature. Of those published, the focus has been on the salt particle size and potato crisps rather than any other particle properties or products (Halim and Barringer, 2007, Buck and Barringer, 2007).

5.1.1.1. Key findings within phase 1

- Within this phase, it was found that transfer efficiency of the salt particles onto the surface of the peanut during salt-peanut mixing was driven by particle size, bulk density and flow properties of the particles.
- Decreasing regular table salt particle size increased transfer efficiency during coating, likely due to increased interaction with surface fat on the product.

- Foam-mat processing increased transfer efficiency indicating this was due to reduced bulk density.
- Flow properties were correlated with transfer efficiency suggesting particle-particle interactions also play a role.

5.1.2. Phase 2: Adhesion during packaging and transport

Like phase 1, ideally, all salt should remain on the product during packaging and transport, as salt particles that fall off the product are unlikely to be consumed and perceived by the consumer. Since salt may be lost from the product into the packaging due to poor adhesion, optimising salt adhesion to product is one approach (in line with others) that manufacturers could use to meet salt targets as they do not have to add additional salt to maintain perceived saltiness intensity whilst accounting for salt losses.

5.1.2.1. Key finding from phase 2

- Loss of salt from the product in packaging was driven by particle size as smaller particle sizes exhibited less loss due to enhanced adhesion energy between surface oil on the product and the smaller salt crystals.

5.1.3. Phase 3: Release during oral processing

In order to optimise saltiness perception, salt release should occur quickly, and dissociated ions should remain separate from the bolus to

enable effective diffusion of free Na⁺ ions to TRCs (Kuo and Lee, 2014). Salt release was therefore assessed using a modified method outlined in Rama et al. (2013), shown in Figure 1.13 in chapter 1, while perception was tracked using the time-intensity method.

5.1.3.1. Key findings from phase 3

- Salt dissolution and/or saltiness perception were driven by particle size and hydrophobicity.
- Smaller particles sizes were generally associated with faster sodium dissolution rates; however, this was compromised for highly dense small particles due to high levels of interaction with surface fats.
- Smaller particle sizes had a greater saltiness intensity (I_{max}) due to faster dissolution in saliva.
- Greater particle hydrophobicity resulted in slower sodium release.

5.1.4. Summary of design rules and other subsequent findings

In summary, studies detailed in chapter 2 concluded that to maximise potential perceived saltiness, salt particles should be designed with the following properties: small particle size, low density and hydrophobicity, and a particle shape associated with the optimal flow and dissolution properties. The validity of these design rules developed in chapter 2 was subsequently validated using a dual sensory and consumer evaluation approach in chapter 4 (discussed in section 5.3).

While the salt particles which exhibited optimised adhesion and saltiness perception were SODA-LO® and <106 µm table salt, foam-mat salt particle inhibited the release of salt from its matrix and saltiness perception was suppressed. Its inhibition of salt release is due to the hydrophobic egg albumen and methylcellulose encapsulating the salt and slowing the rate at which it can dissolve. Sodium ions can also chemically interact with negatively charged amino acids within the protein. This binding of free Na⁺ will reduce sodium ion mobility and further slow release and dissolution (Mosca et al., 2015). Only one study previously assessed the physicochemical and sensorial properties of a foam-mat salt (Chokumnoyporn et al., 2016). This study used methyl cellulose as the foaming agent and was applied to peanuts alongside soy sauce flavourings. The authors found that the foam-mat processing of salt altered the particle's physicochemical and morphological properties, and therefore this approach was used to provide a diverse range of properties for salt particles to be compared. Interestingly, foam-mat processing has been used extensively in the drying of other products such as fruits and vegetables and has shown to provide a spikey/flake-like structure due to the surface bubbles on the films; typically, it is thought that foam-mat processing increases surface area of the dried particle (Triyastuti et al., 2017). This is another reason why foam-mat drying was chosen to produce salt particles as the increased surface area increases the diffusion rate of sodium into saliva for perception. However, in chapter 2, the foam-mat salt was formulated using egg albumin and methyl cellulose as a foaming agent and stabiliser,

respectively, using proportions found in literature (Rajkumar et al., 2007b) and preliminary experiments to assess the stability of the formulation. The initial approach was to use methyl cellulose solely; however, preliminary experiments indicated that producing a stable foam with a high salt content without the methyl cellulose salting-out was unachievable, and therefore egg albumin was included as a foaming agent. Since the amino acid composition of egg albumen is known to be composed of 50 % hydrophobic amino acids (Guha et al., 2019), it is hypothesised that this property suppressed the ability of sodium to dissolve in the mouth. However, when forming stable foams using proteins, a mixture of hydrophobic and hydrophilic groups is required (Hardy and Jideani, 2017); therefore, it may be doubtful that a completely hydrophilic compound could be used as a foaming agent in foam-mat processing. It is suggested from this study that when designing salt particles through various drying/processing methods, which could include; foam-mat drying, drum drying, film drying onto products (Rama, 2016), spray drying, and fluid bed drying, a hydrophilic co-polymer should be used as a higher hydrophobicity would tend to repel or fail to mix with water. Since saliva is made up mainly of water (~99%), a hydrophilic compound is therefore recommended.

The study reported in chapter 2 also showed that the *in vitro* sodium dissolution method could predict defined saltiness TI parameters and be used by other researchers as a screening tool of product or salt particle prototypes before using more costly (in both time and budget) sensory

approaches. However, the *in vitro* approach should not replace sensory or consumer panels for the final assessment of the saltiness or acceptance of products as it does not mimic real-life consumption environments, cognitive factors or human physiological factors, such as oral processing and mouth behaviour, saliva composition, salivary flow rates, bolus clearance rates, taste adaption and chewing patterns. In separate studies, the impact of some of these physiological factors (salivary flow rate and composition or chewing patterns) on the saltiness perception of model salt solutions (chapter 3) and the saltiness and acceptance of potato crisp snack products (chapter 4) were also investigated.

5.2. Exploring the relationships between oral physiology and salt taste sensitivity and consequence on sodium intake

5.2.1. Salivary parameters and salt taste sensitivity

It is well known that individuals vary highly in their salt taste sensitivity and perception (Yang et al., 2014, Zaidan et al., 2009, Yang et al., 2011). However, the reasons behind this variation are not fully understood. Therefore, the research reported in chapter 3 investigated the influence of key salivary parameters on salt taste threshold (also termed salt taste sensitivity) and perceived saltiness intensity of above threshold concentrations (measured on gLMS scales). The findings showed that

salivary parameters can affect perception of salt and taste sensitivity.

Findings are summarised below:

- Trends were seen between salivary sodium concentration and salt taste sensitivity and between salivary flow rate and salt taste sensitivity.
- Supra-threshold intensity values showed no associations with salivary parameters measured.
- Measured total protein concentration was not associated with salt taste sensitivity or supra-threshold intensity.

Although there is previous research showing that salivary sodium concentration determines salt taste threshold, many of these studies used artificial methods to modify the saliva composition in the mouth. For example, in Delwiche and O'Mahony (1996), a chewing action was used to raise the sodium concentration in saliva to determine that sodium concentration drove salt taste sensitivity in individuals, and only a small number of subjects were used in the study. Meanwhile, O'Mahony and Heintz (1981) altered the natural sodium environment in the oral cavity by using mouth rinses of salt solutions. These studies showed that artificially increasing salivary sodium concentration increased individuals salt taste threshold. Chapter 3 builds on this knowledge and confirms that even small inter-individual differences in salivary concentration impacts salt taste sensitivity. The same could be said for flow rate, however, it is unclear whether the relationships elucidated between flow rate and taste

threshold are due to flow rate or a relationship between flow rate and sodium concentration driving the threshold level.

Looking across chapters, these factors, therefore could influence the saltiness intensities of samples perceived by participants in studies detailed in chapters 2 and 4. Although the impact of oral physiology on saltiness intensity of samples assessed by consumers and panellists was not investigated, some variation between sensory panellists (panel performance monitoring, data not shown) and between consumers JAR scores could be attributed to their individual salt sensitivities, driven by their salivary parameters (salivary flow rate, sodium concentration, salivary enzyme activities) or indeed, by other influences such as age, gender, ethnicity or taster status (Martin and Neyraud, 2021, Ogawa et al., 2017, Yang et al., 2014, Hayes et al., 2010, Williams et al., 2016). The salivary parameters were not investigated in the studies detailed in chapters 2 and 4 due to logistical challenges of collecting large numbers of saliva samples, not to mention the COVID-19 related restrictions in place at the time of data capture, which meant this was outside the scope of this project. However, this remains a valuable area for future investigations.

5.2.2. Salt taste sensitivity/perception and salt intake

Although some salt is required for normal physiological function of the body (around 500 mg/day according to the American Heart Association), overconsumption, attributed to individual consumer choices, increases

the risk of various diseases and is prevalent in developed countries (American Heart Association, 2021, He et al., 2010). Our innate physiological need drives a proportion of sodium intake. However, this natural appetite for salt that humans have can be enhanced by individual preferences modified by individual experiences (Beauchamp and Engelman, 1991). It is also thought that dietary consumption and therefore health outcomes may be influenced by individual sensory perception.

For salt intake, there is some evidence to suggest that taste sensitivity to salt or perceived intensity of salt is linked to salt intake (Pangborn and Pecore, 1982, Martinelli et al., 2020, Cattaneo et al., 2019, Veček et al., 2020). In chapter 3, results add to the understanding of this relationship. Key findings are outlined and discussed below:

- Individuals with lower detection thresholds (i.e. more sensitive to salt taste) consume a higher amount of dietary salt. It may have been hypothesised that individuals most sensitive to salt (lower threshold) would consume less salt in their diet due to enhanced perception of stimulus strength. However, this would not appear to be the case, possibly as preference rather than sensitivity per se is a better predictor of intake. One suggestion is that individuals considered more salt taste sensitive may actively seek foods with higher quantities of salt or a more significant number of salt-containing foods. These individuals presumably have a high

preference towards salty taste, leading to self-perpetuating negative dietary habits.

- Individuals who perceived supra-threshold salt solutions as more intense (more sensitive to higher than threshold salt concentrations) typically added less salt to their foods, indicating that these individuals require less salt added to foods to provide their preferred saltiness intensity. This would seem a reasonable response since if an individual perceives the same salt solution as less intense they are more likely to add more salt to their foods to provide an acceptable and familiar salt taste in their foods.

However, evidence is conflicting in this area with variable directions of impact, with many studies showing no significant relationship between sensory measures and intake (Pilic et al., 2020, Pilic and Mavrommatis, 2018, Simpson et al., 2012, Lee et al., 2014).

It is thought that preference drives intake more than salt taste sensitivity or the intensity in which salt is perceived (Tan et al., 2021). Therefore, salt reduction approaches could aim to alter these individual salt preferences to reduce individual's sodium intake. A proven technique successful in altering salt taste preferences to lower salt intake is reduction by stealth, whereby over repeated exposures, individuals slowly become more familiar with a lower salt level in food products (Methven et al., 2012b, Mattes, 1997). However, this is limited up to a point in foods due to technical limitations, food safety, or consumer

rejection. Reduction by stealth may be better suited to commodity products such as bread, where the reduction would more likely go unnoticed due to the salt present being 'hidden' since bread doesn't taste necessarily salty, evidenced by a successful 25 % reduction of salt in bread without affecting consumer acceptance using a series of 5 % salt reductions over 6 weeks (Girgis et al., 2003). Whereas for potato crisps, these snack products are selected based on the sensory appeal, with salt being a primary driver of liking (Meullenet et al., 2002). In bread products, salt taste is less likely to drive liking and purchase intent (Jervis et al., 2016). Moreover, in snack foods, reduction by stealth may have initially been implemented by manufacturers, potentially since the FSA set the first salt targets in 2005. It is likely these products have reached their limit for stealth reduction, since salty snacks an appealing level of saltiness is required to meet consumer hedonic expectations. Therefore, alternative solutions are required for this product category, with specifically designed salt particles aiming to maximise potential saltiness intensity an exciting strategy for snack foods.

5.3. Validating the physicochemical design rules for salt particles

In order to validate the selected physicochemical parameter design rules established and reported in chapter 2, chapter 4 evaluated the sensory profile and consumer acceptance of salt-reduced potato crisps and commercially available potato crisp products, using a dual sensory

approach. The optimal model salts from chapter 2, a reduced particle size (<106 µm) and a spray-dried salt with maltodextrin (commercially available as SODA-LO®), were chosen as they enhanced sodium dissolution assessed by an *in vitro* methodology, saltiness perception assessed through TI methodology, transfer efficiency and adhesion when applied to slightly oiled peanuts.

Overall, chapter 4 successfully demonstrated a method of salt reduction of up to 30 % in potato crisps through the design and application of novel salt particles without impacting the sensory profile of the products. The three prototypes (salt reduced vs standard salt content) showed no significant difference in the three saltiness attributes assessed by the trained panellists, including initial saltiness, overall and saltiness aftertaste. This study was expanded to assess consumer perception and acceptance of the reduced salt products. Results also confirmed that salt could be reduced by 30 % using the optimised salts, spray-dried product (SODA-LO®) and micronised salt (<106 µm) using grinding without significantly impacting consumer liking, saltiness liking or ideal saltiness levels for the products (JAR scores).

Furthermore, in chapter 1 it was suggested that there might be difficulty reducing salt content in salty snacks due to saltiness being the main driver of consumer liking (Meullenet et al., 2002). In chapter 4 this was confirmed since penalty analysis revealed overall liking was reduced significantly across all products when the product was not considered salty enough by the consumer. Additionally, the common preference of

the consumers was towards the higher salted potato crisps, with each consumer segmentation group liking the highest salt product the most, further evidencing that most consumers seek a high salt level in their snacks. Therefore, with this in mind, it is necessary to incorporate compensatory strategies for the loss of salt content to maintain the saltiness taste and consumer liking, since the simple removal of salt will likely reduce consumer liking (more discussion on direct salt removal in section 5.4.1).

The development and validation of physicochemical design rules for salt particles can not only help inform ingredient designs for sodium reduction, but could also be transferable to other types of flavour particles, to enhance or modulate the release of sugar or flavour compounds; and this potential opportunity warrants further investigation.

It should also be noted that peanuts were initially chosen as the base product for the studies in chapter 2 to reduce the variation in the coverage of salt applied to the surface within the samples, since there is less variation in the surface area of individual peanuts than potato crisps. However, to expand the product range able to be used in chapter 4, the most commonly consumed salty snack, potato crisps, were used to validate the design rules while assessing the potential of other salt reduction strategies and modifying oral residency times (discussed in the next section 5.4).

5.4. Investigating the potential of other salt reduction strategies

This thesis mainly focussed on the design of salt particles to optimise saltiness as a salt reduction strategy. Alongside this primary aim, other strategies were explored such as; direct salt removal, salt-texture cross-modal interactions and increasing oral residency time, all of which will be discussed in this section.

5.4.1. Direct salt removal

In chapter 1, it was discussed that it was possible to reduce salt to a limited extent via direct salt removal without the use of compensatory strategies like inhomogeneous salt distribution, cross-modal interactions or altering salt particle design. This is achievable since a specific level of reduction goes unnoticed by the consumer and has been successfully applied in a number of products previously, such as; bread, cheese and soup but with limited literature on snack foods (Jaenke et al., 2017). Therefore, our findings from chapter 4 contribute to bridging this knowledge gap in the literature.

When assessing direct salt removal as an approach for salt reduction it is most appropriate to compare the three products with varying salt contents manufactured using the same flat potato crisp base from Walkers, PepsiCo. These products were the low salt (0.9 g NaCl / 100g), P1 (STD) (1.2 g NaCl / 100g) and the high salt (1.4 g NaCl / 100g)

products. When comparing the high salt product and the P1 (STD) product in terms of objective saltiness assessed by the trained panel, there was no significant difference between the two products for all three saltiness attributes assessed (initial, during eating and aftertaste). Therefore, it is suggested that salt may be reduced by 14-15 % (measured-BoP salt content) in potato crisps without impacting saltiness perception. When comparing P1 (STD) to the low salt product, with a possible salt reduction level of 25 % (when considering declared BoP salt content), the saltiness perception at all three time-points was significantly different between these two products. Therefore, a level of salt reduction at 25 % is not achievable without impacting sensory perception. It should be noted, however, that the measured salt content shown in Table 4.1 would suggest that the salt content of the low salt product was much lower than that declared on the BoP (measured: 0.53 g / 100g, BoP: 0.9g/100g), making an actual reduction level of ~55 %. The discrepancy between BoP and measured salt content could be due to variation between batches or even natural variation between crisp packets, making determining the difference in salt content between these two products unclear.

Overall consumer acceptance scores further confirm that a 14-15 % reduction may be possible through direct salt removal without impacting consumer overall liking. The reduction range (14-15%) noted is due to a small difference between the salt content aimed for in P1 (STD) and its measured salt content. Since the actual salt content measured in the

STD prototype was 1.18 g/100g and the high salt product was measured at 1.4 g/100g, 15 % was declared as the level of salt reduction possible in chapter 4 when using the direct salt removal approach without impacting saltiness perception and consumer overall liking. Although overall liking was not significantly affected by the 15 % reduction in salt, saltiness liking decreased, but there was no evidence that this impacted on the overall acceptance score of the products, therefore this reduction would be accepted by the consumer. Reducing the salt content of the high salt product used in studies reported in chapter 4 by this amount would bring this product in line with the new salt targets set out for 2024 (Table 1.1). However, it may become increasingly difficult for manufacturers to reduce the salt content of crisps even further below these levels without compensatory strategies, as consumers will start to notice the change in salt content while further removal of salt to the bulk seasoning will give rise to processing and flowability issues.

Limited direct reduction of NaCl is achievable without impacting sensory properties and consumer acceptance in this product category. This supports the urgent need for alternative approaches to assist the snack food industry in meeting government targets.

5.4.2. Salt-texture cross-modal interactions

Cross-modal interactions have the potential to enhance saltiness in food products due to textural and aroma effects (see sections in 1.8.3). Saltiness enhancement through cross-modal salt-aroma interactions has

been extensively researched, with some congruent salt odours successfully enhancing saltiness in liquid and model cheese matrices (Batenburg and van der Velden, 2011, Syarifuddin et al., 2016, Lee et al., 2015). However, saltiness enhancement through texture-salt cross-modal interactions has been less well documented. Previously, saltiness was successfully enhanced when using cross-modal tactile touch sensations when Biggs et al. (2016) found that biscuits were perceived as saltier when served from a rough plate rather than smooth, and van Rompay and Groothedde (2019) concluded that the saltiness perception of salty potato crisps is enhanced when consumed from a bowl with a rough texture compared to smooth. However, these studies were based on the tactile touch of the serving object. When assessing the impact of rough texture in the mouth on saltiness perception, it was found that bread crumb texture affected the saltiness intensity of the bread crumb (Pflaum et al., 2013). Therefore, it was hypothesised that texture-induced tactile gustatory interactions in the mouth or brain could alter the perception of saltiness of the potato crisps. The ridged or crinkled texture of a potato crisp may also introduce an inhomogeneous distribution of salt (also shown to enhance saltiness perception, section 1.8.5) since the salt may deposit in the inverted ridges, which create 'pockets' for the salt to stick in. To investigate this, in chapter 4, potato crisps with a ridged texture and smooth straight cut potato crisps with similar salt contents were selected, assessing the impact of potato crisp texture and potentially an inhomogeneous distribution of salt on saltiness perception. Results showed that saltiness perception was not impacted based on the

texture of the potato crisp (ridged vs smooth). Similarly, Kongstad and Giacalone (2020) found that results did not align with expectations, where texture (wavy vs smooth) did not affect consumer acceptability or shift JAR results. Therefore potato crisps with a ridged shape would need salt reduction strategies applied in the same way as flat cut crisps, and their tactile texture does not advantage the product in providing an enhancement of saltiness. Salt particles designed exploiting the physicochemical design rules could also be of benefit applied to crinkle cut crisps, not only flat cut crisps, and other bland snack product bases (e.g. popcorn, extruded snacks, peanuts) to allow for salt reduction in these products too.

5.4.3. Increasing oral breakdown time to optimise salt release in mouth

Another strategy that could be leveraged for salt reduction is to increase the length of time the bolus or food product is in the mouth. This may optimise the proportion of sodium that can be released from the bolus and transported to the taste receptor cells for perception. Therefore, in chapter 4, two products differing from the standard flat cut potato crisp were incorporated into the product set. These were a reconstituted baked crisp and a thick-cut batch fried crisp product. Due to their differing textures caused by their processing and compositional differences, it was hypothesised that they would prolong the oral residency in the mouth compared to standard flat cut fried potato crisps to allow for optimised sodium release from the bolus. The trained sensory panel quantified the

hand-cooked and reconstituted baked products as being significantly harder in texture than the other products in the set (chapter 4, Supplementary material 4.3). Therefore, it would be expected that these products would increase the time in the mouth during food oral processing due to the greater level of oral processing required to break down the hard product structure and incorporate saliva. This expectation was confirmed by the decrease in the speed of breakdown for the two products compared to the others, i.e. slower speed of breakdown results in a longer time in the mouth (chapter 4, Supplementary material 4.3).

Even though the batch-fried thick-cut product significantly increased oral residency time compared to all other products in the set, saltiness was not significantly impacted at each time point assessed. However, it is difficult to directly compare the effect of oral residency time on the saltiness of these two harder products to other products since the process alterations and textural differences may also result in changes in salt adherence to the product surface and binding within the product matrix, affecting the release of salt from the bolus. For example, the baked potato crisp made from reconstituted potato flakes with 1.18g of salt /100g was found to be the least salty out of all of the product set despite a topically applied salted crisp being lower in salt (BoP: 0.9 g/100g). Therefore, it can be determined that a proportion of salt contained in the baked product goes unperceived as a result of being 'trapped' within the matrix, therefore reducing its availability to interact with the taste receptor cells. Furthermore, despite the hand-cooked

product having the highest measured salt content (table 4.1, 1.64 g/100g) even though BoP declared 1.2 g/100g, saltiness perception was not significantly higher than other products at the mid-salt range (1.18-1.2g/100g BoP). This could be attributed to a larger particle size (which was not measured; however, this type of crisp is known for using larger sea salt crystals than regular crisps or the higher level of greasiness quantified by the trained panel, suppressing saltiness perception. The hand-cooked had significantly higher values in attributes associated with oil than the other products in the set. Since oil acts as a barrier for tastant delivery to taste receptor cells (Metcalf and Vickers, 2002), this is a plausible reason for the relatively low saltiness intensity compared to the salt content value. On the other hand, this disparity may also be due to the high variability in NaCl content between product batches, or samples may not have been well homogenised.

5.5. Exploring the impact of consumer mouth behaviour type on preference and perception

There is some evidence to suggest that chewing activity impacts saltiness perception (Tournier et al., 2014, Lawrence et al., 2012b). Since the JBMB™ typing questionnaire (outlined in section 1.5.2) allowed for the segmentation of large number of consumers, this approach was used to classify consumers into mouth behaviour groups reported in chapter 4. It could be hypothesised that those who hold the product in the mouth for a longer length of time, e.g. presumably suckers and smoothers, may

have an enhanced saltiness sensation as perhaps more of the salt would be released from the bolus with these mouth behaviour types. Although it was found that mouth behaviour group did not impact liking of the different products, there was a main effect of mouth behaviour group on texture liking but no effect on saltiness liking or saltiness just-about-right scores. Suckers rated their texture liking of potato crisp product set as significantly lower than that of the chewers and crunchers. This is understandable since crunchers are characterised by their preference towards food textures that they can crunch like crunchy granola, crispy vegetables, and crunchy cookies and chewers have a preference towards food that they can chew on (Jeltema et al., 2015). Since the products in the set were probably difficult to suck on due to their hard, crunchy and crispy nature, this is reflected in sucker's texture liking score. With these preferences in mind, the food industry tailors products to the different consumer segments, ensuring a sufficient range of product textures to suit specific mouth behaviour preferences. For example, a sucker or smoosher may prefer an extruded puffed snack or one that melts more easily in the mouth compared to those in our product set. Therefore an extended product set incorporating a wider range of processing techniques and textures would be interesting in this context for further work.

Since the primary aim of studies reported in chapter 4 was to validate the use of physicochemical design rules and determine the salt reduction achievable without impacting saltiness perception and consumer

acceptance, a large number of consumers were required. The validated JBMB™ questionnaire (Jeltema et al., 2015) was used, which was easy to deploy to a relatively high number of consumers, simple for consumers to complete and able to rapidly segment a large number of consumers using the classification outputs. This approach should be considered in other research papers due to its rapid, easy to use design.

The study presented in chapter 3 identifies that mastication significantly increases salivary flow rate and sodium concentration. Therefore the extent to which an individual chews their food may alter the amount of saliva incorporated into the food (sufficient hydration of bolus required for sodium release). This could therefore be linked to individual mouth behaviour type. For example, crunching or chewing a particular food such as a potato crisp or extruded snack over sucking or smooshing, may secrete more saliva into the mouth, hydrating the food bolus efficiently, allowing for an optimised environment for the release of sodium for saltiness perception. Alternatively, smooshing or sucking the same product would mean that the individual would hold the food and the bolus in the mouth, increasing sodium contact to the taste receptor cell. There are, therefore, many unanswered questions in this area worth exploring.

5.6. Limitations and further work

Limitations and further work are primarily covered within each chapter more general considerations are outlined below:

- Eight model salt particles were evaluated in the study documented in chapter 2. Expanding this particle set to include a wider range of physicochemical properties, morphologies and co-polymers may provide additional insight into the optimisation of salt particles for perception. Furthermore, one salt (dendritic) was only used in the *in vitro* analysis due to it not being food-grade. Its use in the sensory TI trial would have been beneficial to help correlate TI parameters with *in vitro* dissolution curve parameters and to compare its saltiness intensity profile with the other model salts. Since we were not able to get certification that the dendritic salt used in chapter 2 was food grade, we were unable to include it in the sensory evaluation, and it remains unknown whether this model salt could have provided a similar saltiness profile to the other optimised model salts.
- Following the physicochemical design rules, particles with a very small particle size optimise potential salt perception; however, we recognise that smaller particle sizes habitually lead to concerns within a factory scale manufacture due to caking effects caused by changes in humidity or compaction of ingredients. This may limit the application of very small particle-sized salts within this setting.

The studies reported in this thesis have expanded on current knowledge but also opened up questions and opportunities for further investigation, which could help advance understanding of the contributions to variation in saltiness perception and intake and extend salt reduction strategies for snack foods. Future work could explore the following:

- *Extend the application of physicochemical design rules to particulates other than salt.*
 - Since the use of physicochemical design rules demonstrated a successful route to sodium reduction, these design rules warrant further investigation for other applications, such as sugar for sugar reduction and food flavourings to optimise the release of volatiles from particulates. Like salt, the food industry is continually working towards sugar reduction across a broad range of products, including confectionery, where sugar crystals are often topically applied to products. Sweetness perception may be optimised using these design rules for particulates as an increase in dissolution to the saliva may provide a higher sweetness intensity. Additionally, optimising the flavour perception of flavourings in the form of powder helps give a higher impact of flavour using the same weight of product, benefiting it over other flavourings on the market. These design rules could help achieve this upon further investigation when redesigning flavour particles for the topical application of snack foods.
- *Determine optimal conditions to minimise caking potential of salt particles*
 - Future studies should investigate the caking potential of salt particulates when adopting physicochemical design rules, determine optimum storage conditions, and look for solutions to reduce particulates' caking effect if required. This may

include incorporating varying sizes of particles to vary the particle size distribution to reduce the chance of caking (Modugno et al., 2015). This would provide insight into how the material should be transported and stored within the factory environment.

- *Expand investigation of potential applications of foam-mat salt*
 - Studies presented within this thesis include one of the first to develop and evaluate the performance of a foam-mat salt. Chapter 2 demonstrated that foam-mat salt suppressed saltiness intensity and dissolution rate due to the hydrophobicity of the protein in the egg albumin and the binding of the sodium to the protein. There remain opportunities to explore using more hydrophilic foaming agents to optimise the dissolution of the foam-mat structure, which could be used as an alternative salt particle for sodium reduction.
 - Since an inhomogeneous distribution of salt or 'salty spots' within some products can compensate for salt reduction (Noort et al., 2010b, Noort et al., 2012a, Monteiro et al., 2021), future research should certainly further test whether using the foam-mat structures evaluated in this thesis remain intact when incorporated into a low moisture food such as crackers. This could provide 'salty spots' within the product matrix. If successful, this inhomogeneous distribution of salt within the product would be advantageous over other encapsulation

techniques (section 1.8.5) since it would contribute to the protein content of the food without the need for fat (usually used for salt encapsulation) which negatively impacts the nutritional profile of the product.

- *Extend knowledge of the impact of salivary parameters on saltiness perception during consumption of real-life snack food systems rather than model solutions*
 - Chapter 3 contributes to the knowledge that salivary sodium concentration and flow rate determine salt taste threshold. Future research should be conducted on more realistic food products rather than model solutions. For example, one interesting research question derived from these studies: does the inter-individual variation in salivary flow rate and sodium concentration alter the saltiness perception of different snack foods? It would also be worth investigating whether changes in saltiness perception due to individual differences in salivary parameters are affected to different extents depending on salt location (within the matrix or outside the matrix) in order to help guide product reformulations to enhance salt release from specific product types, leading to a level of salt reduction in the product. For example, additional ingredients that trigger salivation could be used if a product requires a high degree of hydration to release salt for perception.
 - It is important to mention that saliva is a very complex biological fluid, and a limited number of salivary parameters

were measured due to time and a high number of samples to process. Evidence in the literature suggests that alpha-amylase, protease enzymes, and carbonic anhydrase VI may impact salt taste perception (section 1.5.1.4). It is worth more investigation into the effects of these salivary components on taste perception, not only for salt taste but also other taste modalities, as it is not fully understood, especially in the perception of real-life foods rather than model solutions.

- *Broaden investigations to fully elucidate relationships between salt taste perception and salt intake*
 - Chapter 3 contributed to understanding of the relationships between salivary parameters, salt taste perception and salt intake. More extensive large-scale studies are required to fully elucidate relationships between salt taste perception and salt intake. These studies should consider the following:
 - Balanced gender groups to study any effects of gender.
 - Use an extensive range of concentrations for threshold testing to capture even the highest thresholds.
 - Use of real-food systems or model foods similar to salty food systems typically consumed in the population studied could be considered (mentioned above).
 - More extensive evaluation of salt intake assessment combined with 24h urinary excretion (McLean et al., 2017).
 - Inclusion of options for food/meal photo uploads may be considered to improve reporting accuracy in future studies.

- *Investigate potential approaches to enhance salt release from within snack product matrices*
 - Chapter 4 identified that saltiness is suppressed when salt is incorporated into the reconstituted crisp as it becomes 'locked' into the product matrix, reducing its availability to TRCs. Future investigations are necessary to elucidate approaches that increase the release of sodium from the product matrix by reducing the matrix interactions with sodium, either in baked snack products or in extruded products where salt is also incorporated into the product matrix for functionality. For example, possible work could alter the surface area of the reconstituted/extruded product matrix by creating a more porous structure since previous research showed that aeration creating a porous structure of gels increased the release of tastants (Chiu et al., 2015). Preliminary studies not presented in this thesis on two commercial products (a highly porous potato snack vs a non-porous potato snack with a similar shape) also showed that the increase in the number of pores and therefore the surface area of the solid food matrix increased the rate of release when assessed using the *in vitro* methodology used in chapter 2.
- *Further explore the impact of oral breakdown time on saltiness perception*
 - To further elicit any impact of oral breakdown time on saltiness perception, future studies should develop crisp

textures/prototypes, using extrusion or baking to alter the breakdown speed required for swallowing and subsequently assess the sodium release from the products in mouth and differences in saltiness perception, and whether the changes in product structures or textures affect mouth/chewing behaviours. If a higher proportion of sodium can be released from within the product matrix, then more sodium is available for perception, enhancing saltiness perception and allowing some potential reduction in salt content.

- *Investigate the use of real-time techniques to quantify mouth behaviour and assess the impact on salt release from snack foods*
 - Chapter 4 provides insight into the effect of mouth behaviour group on consumer liking and perception of potato crisps. To further this research, studies should examine real time mouth/chewing behaviour during snack oral processing using quantitative techniques such as electromyography or through filming the chewing action of consumers coupled with the collection of in mouth sodium release data and sensory perception measures to elucidate associations with chewing behaviours and sodium release kinetics, and therefore saltiness perception. This approach would provide a more detailed insight into the impact various chewing parameters (e.g. chewing force, number of chews, chew length) has on the sodium release potential of specific foods such as snack product. This has previously been researched using

lipoprotein matrices (Lawrence et al., 2012b); however, since the product matrix of potato-based snacks is very different, this would provide useful information for researchers and the snack food industry. This research could allow the industry to target specific product sensory profiles towards specific consumer segments. It would also provide insight into the type of chewing actions that offer the optimum release of sodium to the saliva, which researchers could use to provide product structures that can manipulate consumers into using this kind of chewing action/mouth behaviour.

5.7. Final concluding remarks

The findings from this thesis broaden the knowledge on the potential use of salt reduction strategies in snack foods such as potato crisps. The novel development of salt reduction design rules provides a successful route to 30 % salt reduction when topically applied to potato crisps, without significant impact on sensory perception or consumer acceptance. At the same time, only 15 % of salt can be directly removed without using compensatory strategies like optimised salt particles. These findings will be useful for the food industry in developing optimised salt particles through various drying and processing techniques and may also be applicable to other flavourings such as sugar and powdered seasonings. This thesis also uncovers insight and the potential for future work around the impact of food oral processing on saltiness perception

and product liking. These findings suggest that individual differences in salivary parameters affect salt sensitivity, opening up questions on the influence of these oral processing parameters on the perception of saltiness in snack foods to help develop snack formulations for salt reduction. Although our findings suggest that consumer mouth behaviour type does not impact saltiness perception or liking of products, having certain mouth behaviour preferences reduced texture liking of crisps. Overall, the results outlined in this thesis can be utilised to benefit the formulation of new snack products with reduced salt content contributing to sodium reduction in the diet.

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