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An investigation of saltiness perception of lysine and calcium lactate and their application in developing reduced salt meat products

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Abstract

Excessive salt intake is associated with a growing risk of cardiovascular disease. In order to reduce salt levels in food, one of the popular strategies is to use other metallic salts to partially replace salt. However, this often causes a significant loss in saltiness, leads to additional tastes (i.e., bitter) and reduces shelf-life. According to pervious research, lysine and calcium lactate may hold the key to solve this problem, and hence, enable successful salt substitution. This experiment aimed to explore whether lysine and calcium lactate can be used as salt substitutes and their effect on the quality of low-sodium meat products.

Since umami taste has been used widely in sodium reduction by enhancing flavour perception, therefore, this thesis first aimed to gain a better understanding of the interaction of the five basic taste sensations (sweetness, sourness, saltiness, bitterness, umami), and especially the role of umami in complex taste systems. A trained sensory panel was used to rate the taste intensity of equi-intense aqueous solutions. The results concluded that umami did not enhance or suppress the perception of any other taste, whereas sweetness, saltiness, sourness and bitterness significantly suppressed the perception of umami. Therefore, the study changed focus to consider whether lysine and calcium lactate could contribute to salty taste. In aqueous solution, calcium lactate did not offer saltiness, but 1% lysine produced weak saltiness. Overall, 1% lysine with or without 0.75% calcium lactate would replace 50% salt (NaCl) in solution system without compromising saltiness perception. The effects of lysine and calcium lactate as substitutes were further tested in a real food matrix (low-salt meat products).

Physicochemical characteristics, sensory properties and microbiological analysis were used to evaluate their effectiveness in salt-reduced pork patties. The results concluded that lysine increased the yield and calcium lactate improved shelf-life of a salt-reduced pork patty. Calcium lactate and lysine could offer effective way to reduce salt by 50% without compromising shelf life and eating quality. Because lysine, as a basic reactive amino acid, may be involved in Maillard reaction and modify the flavour profile of meat products during heating processing, thereby affecting the salty taste. So, gas chromatography-mass spectrometry (GC-MS) was used to study the volatile flavour compounds in salt-reduced pork patties in a range of meat pH (5.5 to 6.5). Results showed that Maillard reaction-related volatile flavour compounds were very low in the low salt patties prepared with lysine and calcium lactate under normal meat pH conditions, and the modification to flavour profile of cooked pork patty was minimum. To sum up, the combination of lysine and calcium lactate could be used as a new salt substitute in meat products offering comparable eating quality and shelf life to full salt products.

Chapter 1 Research update of sodium reduction in meat products with special focus on taste and flavour

Abstract

NaCl is one of the most important ingredients in meat products, and it has multi-functions including developing texture, improving taste/flavour and extending the shelf life amongst others. However, there is an increasing demand for salt reduction in meat products due to the health concern. In this literature review, the taste and flavour aspects of salt reduced meat products were critically reviewed according to the available salt reduction strategies for meat products. Saltiness is mainly perceived through epithelial sodium channels (ENaCs) and paracellular pathways, while other basic tastes including sourness, sweetness, bitterness and umami significantly affect the perception of saltiness in salt reduced food products at different extents, which may shed some light on developing new ingredients used in meat products for salt reduction, such as lysine, calcium lactate, MSG etc. Salt is also associated with flavor development in meat products via interference with lipid oxidation and Maillard reactions, which implies the changed flavor profile may risk the consumers' acceptance for salt reduced products. Current salt reduction strategies include reduction by stealth, changing physical form/distribution of the salt crystals, employing processing technologies and using flavour enhancers. In conclusion, successful salt reduction in meat products should take a collaborative approach by combining processing technologies, ingredients with manipulation of taste perception to achieve a desirable product for consumers.

1.1 Introduction

Saltiness is one of five taste qualities in taste perception, and the prototypical stimulus is sodium chloride (NaCl) (Dötsch *et al.*, 2009). It is one of the most frequently used food preservatives for extending the shelf-life of meat products and has been used for thousands of years. Salt also affects the flavour and texture of meat products. In addition to the perceived saltiness, salt brings out the characteristic taste of meat products, enhances the flavour, and improves the water and fat binding properties of the meat product, resulting in a desirable gelatinous texture after cooking (Liem Miremadi and Keast, 2011).

Sodium, the cation within table salt, is responsible of many physiological functions of the human body like acid-base balance, functioning of cells, transmission of nerve impulses and maintenance of plasma volume, because it is the main determinant of the volume of extracellular fluid and the major cation in extracellular fluid (Logan, 2006). According to the recommendation of the World Health Organization (2020), the average sodium consumption should be approximately 2 g sodium per day (equivalent to about 5 g salt per day) for adults to maintain physiological functions. However, Ashford, Jones and Collins (2020) reported that the average salt intake for age 19 to 64 is estimated to be 9.2 g salt per day in men and 7.6 g salt per day in women in UK. A high sodium diet has been identified by the Global Burden of Disease as one of the two major dietary risk factors for disease along with high potassium diet. Epidemiology research showed that excessive intake of sodium led to a high risk of hypertension due to increase in blood pressure (Aaron and Sanders, 2013), while 49% of coronary heart disease and 62% of stroke are reported with association with high blood pressure (He

and MacGregor, 2010). If global salt consumption could fall to the recommended level, it was estimated that 2.5 million deaths could be avoided each year (WHO, 2020). In a typical western diet, natural foods only contribute to 10% to 12% of dietary sodium, while the main sources of dietary sodium intake are processed foods and foods eaten outside the home (Partearroyo *et al.,* 2019), among which 20% comes from meat products (Inguglia *et al.*, 2017). Naturally, salt is present in small quantities in fresh foods like meat, vegetables, and fruit, but salt levels would increase exponentially when foods are processed. For example, the fresh pork typically contains only about 0.18 g of salt per 100 g, but the salt content spikes to about 2.2 g per 100 g when it is processed into sausages, and even up to 2.7 g per 100g in cooked ham (Inguglia *et al.*, 2017). Therefore, reducing salt content in processed food products has attracted extensive attention in the past decades. To address the issues of high salt intake, Public Health England (2020) has set ideal salt content for various processed foods, for example, 2.59 g of salt per 100 g should be targeted for bacon by the end of 2024, a reduction of 0.29 g of salt per 100 g compared to 2019.

In past years, many literature reviews associated with salt reduction have been published with focus on the roles of salt in meat products, and/or the perception and sensory effects of salty taste along with evaluating the salt reduction strategies in food products. However, food is a complex system, and how tastants within the food matrix interact with each other and affect the efficiency in salt reduction was rarely addressed. Therefore, this work approached from this angle and summarized the theory understanding about taste/flavour perception of salt and its interaction with meat protein

and other tastes in order to provide theory exploration about the sodium reduction in meat products. The latest technologies for reducing the sodium content in processed meat products were also summarized and discussed to explore the novel salt substitutes for meat industry. The overall aim of this research project is to investigate the feasibility of lysine and calcium lactate as salt substitutes in developing salt reduced meat products. The effects of lysine and calcium lactate on saltiness perception within aqueous solutions were investigated first in order to elucidate their contribution to taste and taste interaction. Furthermore, lysine and calcium lactate were applied to a food matrix (pork patties) to assess their impacts on a broader range of properties; including processing properties, texture, colour, shelf life and flavour profile of final meat products. Finally, a recommendation was made to the food industry concerning the use of lysine and calcium lactate as novel salt substitutes in food products.

1.2 Salty Taste perception

The taste system is subserved by five taste qualities: sourness, sweetness, bitterness, umami and saltiness. Sourness is elicited by protons indicating acidic foods; sweetness is elicited by sugars indicating carbohydrates in foods; bitterness is often elicited by multiple bitter chemicals (such as propylthiouracil (PROP), quinine-hydrochloric acid (QHCl)) indicating the toxic compounds in foods; umami is elicited by glutamic acid and other amino acids indicating protein in foods; and saltness is elicited by sodium content of foods (Keast and Breslin, 2003). Compounds taken into the oral cavity are detected through taste receptor cells (TRCs) that are aggregated into taste buds (Ishimaru, 2009). Taste bud has onion-like shape and is typically composed of 50–100

TRCs (Delay, Roper and Kinnamon, 1986). Observations from electron microscopy have revealed that the TRCs in each taste bud can be classified into four morphological types: type I (dark), type II (light) and type III (intermediate) cells with elongated and spindle shape (Figure 1.1), and basal, a nonpolarized, presumably undifferentiated cell, sometimes termed type IV (Chaudhari and Roper, 2010). Type II cells sense taste stimuli and type III cells transmit taste signals to sensory afferent nerve fibers, type IV cells are located at the bottom of the taste buds and are considered as progenitor cells of other types of TRCs (Suzuki, 2007). In general, bitter, sweet and umami stimuli are detected by type II cells, sour stimuli are detected by type III cells, where salty stimuli are undefined yet (Roper and Chaudhari, 2017).

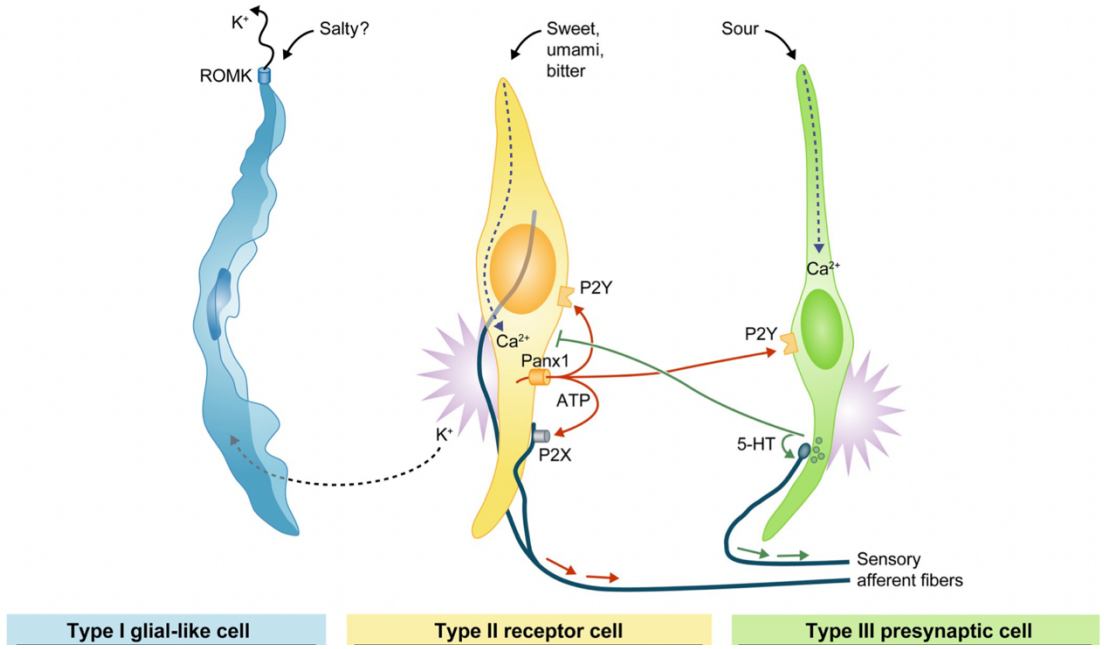

Figure 1.1 The three major classes of taste cells (Chaudhari and Roper, 2010). As it is unclear whether all Type IV in taste buds represent a common class of undifferentiated cells, no specific images are shown in this figure.

When food or drink enters the mouth, the chemicals in these foods will activate the taste receptors to produce chemical signals which are converted into electrical signals and then sent to the taste processing areas of the brain via the seventh, ninth and tenth cranial

afferent nerve fibres (Chandrashekar *et al.*, 2006). Three of the five basic taste qualities, sweet, bitter and umami, are detected by two families of G protein-coupled receptors (GPCRs), i.e., T1Rs and T2Rs, which contain seven transmembrane domains. Sweet and umami compounds are detected through different combinations of T1R family members, and the sweet and umami taste receptors are T1R1 + T1R2 and T1R1 + T1R3 heteromers respectively. Bitter compounds are detected by T2Rs, which contain 25 members in humans. In contrast, sour and salty compounds are detected through ion channels (Lindemann, 2001). Figure 1.2 shows the detail of a plethora of proteins, including ion channels, ligand-gated channels, enzymes and GPCRs, serve as receptors for sensory qualities such as salty, sour, sweet, umami and bitter taste.

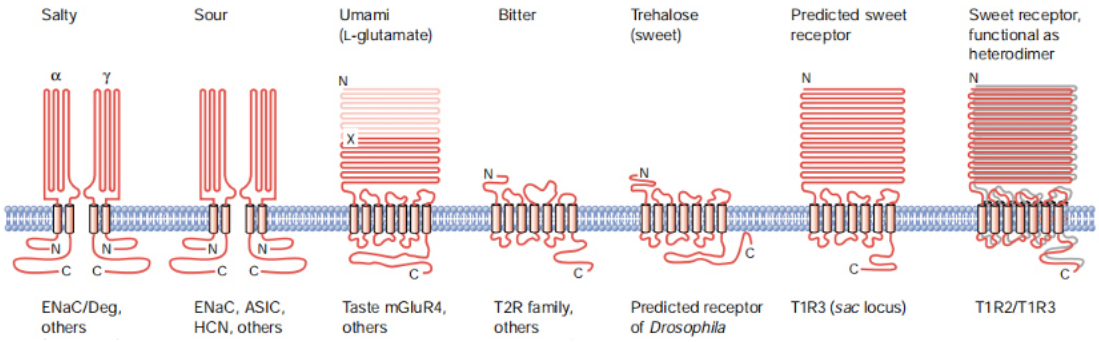

Figure 1.2 The known primary structure of taste receptors (Lindemann, 2001).

1.2.1 Salt perception and transduction

Saltiness perception guides the incorporation of NaCl into the human diet, alongside other required minerals, and enable NaCl to provide essential functions in ion and water homeostasis (Lindemann, 1996). Although salt taste can be elicited by many ionic species, sodium ion (Na^+) is predominantly responsible for the salt taste of most foods (Lindemann, 1997). Saltiness is a distinctive sensory quality primary linked to sodium or lithium containing compounds, and other cations like potassium and calcium may

also exhibit salty taste, but it is not their dominant taste quality (Vanderklaauw and Smith, 1995). Salty taste transduction is complicated, and epithelial sodium channel (ENaCs) and paracellular pathway are considered as the most known sodium pathways for the perception of salty taste.

1.2.1.1 The epithelial sodium channels (ENaCs)

The amiloride-sensitive Na^+ specific epithelial sodium channels (ENaCs) is considered as one of the most important receptors for saltiness perception. ENaCs allow primarily sodium (and lithium) dissolved in saliva to move in the taste receptor cell. In principle, Na^+ activates the ENaCs to produce electrical pulses which are then transmitted via the sensory neurons to the brain to form salty taste (Yamamoto and Ishimaru, 2013). At low sodium concentrations (detection threshold), the afferent signal may be too weak to produce a noticeable difference compared to a solution without sodium. As the sodium concentration increases, the intensity of the afferent signal will increase. When the sodium concentration is high enough (recognition threshold), it not only activates the taste receptors, but also produces electrical impulses which can be transmitted via sensory neurons to the brain where they are decoded and the quality of the taste can then be recognized (Keast and Roper, 2007). The ENaCs is a hetero-oligomer complexes containing four homologous subunits (α-, β-, γ- and δ- respectively) that act as salty receptors by providing a specific pathway for sodium currents to enter the taste cells in human (rodents do not contain δ-) (Stähler, 2008). The sodium current triggers action potential of the basolateral membrane of the taste cell, followed by synaptic events (Avenet and Lindemann, 1991). The location of the subunits in the human taste

system is important because it determines the transduction pathway of sodium ions. If the δ-subunit is located at the apical membrane, sodium ions will be transduced through ENaCs, whereas paracellular pathway will be mode of transduction if subunits are located at the tight junctions of the taste buds (Bigiani, 2020).

1.2.1.2 Paracellular pathway

Tight junctions were observed by electron micrographs at the apical end of the connecting cells in taste buds from several species (Chaudhari and Roper, 2010). Taste buds, like most epithelial cells, impede the penetration of water and many solutes through their cellular interstices. However, Na^+ had been proved to penetrate the paracellular pathway of taste buds to produce salty tastes (Chaudhari and Roper, 2010). Neurons responsive to salts are not simultaneously both anion and amiloride sensitive. Rehnberg *et al.* (1993) studied N-fibres and H-fibres in the hamster chorda tympani nerve which are responsive to sodium salts and found that amiloride-insensitive H fibres were found to be sensitive to anions, whereas responses of N fibres could be blocked by amiloride but were relatively anion insensitive). Anion-specific permeability of tight junctions surrounding taste cells may play a role in determining the overall stimulatory effectiveness of a sodium salt. Large or multivalent anions would not traverse this paracellular pathway as easily as small monovalent anions, and their salts would be less stimulatory (Elliott and Simon, 1990). Thus, sodium chloride is the saltiest compound compared to any other sodium salt.

1.2.2 The interaction between salt and other tastes

For individual taste stimuli, as the physical concentration increases the perceived intensity elicited by that compound also increases, but the rate of increase is not always directly proportional. For the concentration at relatively low levels (just above threshold), an accelerating relationship would exist; moderate concentration, linear relationship for tastant at moderate concentrations or decelerating relationship for tastant at high concentrations (Bartoshuk, 1975). When two compounds with different taste qualities are mixed, a number of interactions may occur, like enhancement or suppression. Saltiness may also influence other taste qualities independent of intensity or concentration in food matrices (Keast and Breslin, 2003). Interactions between tastes get more complex when three or more taste qualities interact within the food matrices. In general, the degree of suppression depended on the individual's unscripted function; perception of a sharp increase in taste with increasing concentration tended to lead to greater suppression (Bartoshuk, 1975).

1.2.2.1 Interaction between saltiness and sourness

Keast and Breslin (2003) summarized the interaction between four tastes (sourness, saltiness, bitterness, sweetness) in different taste intensity concentrations as shown in Figure 1.3. Saltiness and sourness affect each other symmetrically in the mixture, enhancing at low/medium intensity concentration range and inhibiting or having no effect at higher concentration range. Breslin (1996) indicated that NaCl suppressed the sourness of lactic and critic at strong suprathreshold, while a little enhancement at weak suprathreshold.

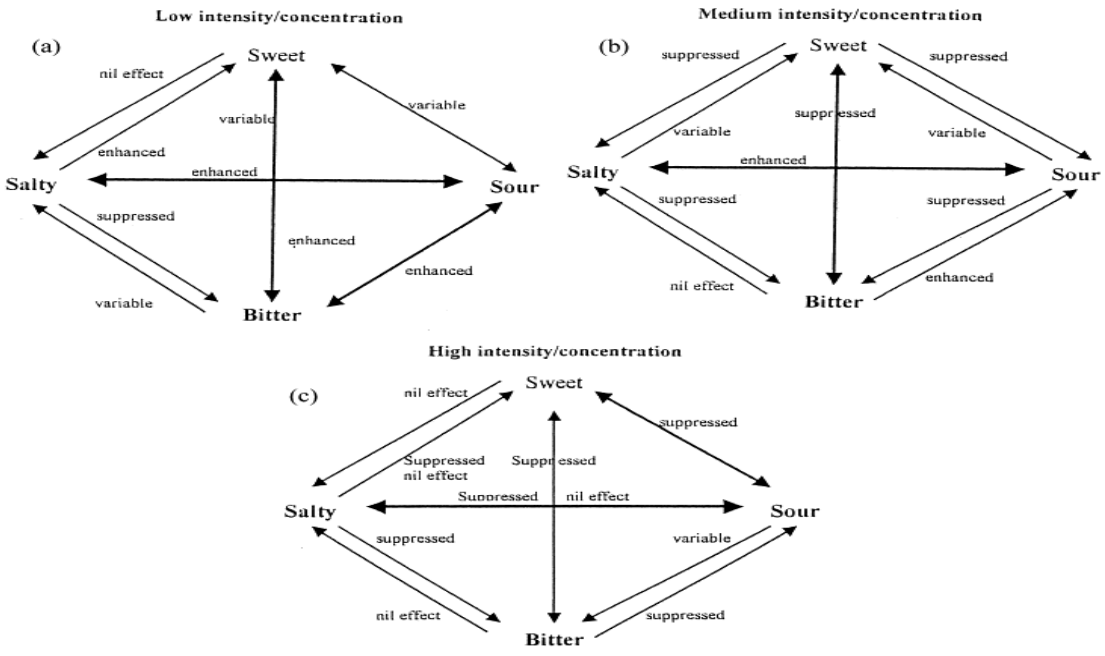

Figure 1.3 Schematic review of binary taste interactions (Keast and Breslin, 2003).

1.2.2.2 Interaction between saltiness and sweetness

Saltiness enhances sweetness at low intensity concentration range, but the effect can vary at the medium intensity concentration range, while salt can inhibit or has no effect on sweetness at high intensity concentration range. Whereas sweetness inhibits saltiness at medium concentration range and has no effect on saltiness at low/high intensity concentration range. Pangborn (1962) verified the taste interaction of sucrose and NaCl by highly trained subjects using single and paired sample presentation. The data indicated that sucrose reduced the apparent saltiness of NaCl samples at 0.12 - 3.24%. However, there was no obvious change in the sweetness of sucrose solution. The sweetness of 0.75, 2.25 and 6.75% sucrose solutions were enhanced with NaCl at low concentration but depressed by NaCl at higher concentration. At the same time, all levels of salt reduced the sweetness of 20.25% sucrose.

1.2.2.3 Interaction between saltiness and bitterness

Saltiness inhibited bitterness at all intensities or concentrations, while salt taste was less affected by bitterness in medium/high intensity concentration range, but different effects were observed in the low intensity concentration range. According to Breslin and Beauchamp (1995), NaCl could significantly suppress the bitterness of quinine hydrochloride (QHCl), about 41 ± 11% of the maximum bitterness sensation was suppressed. At the same time, the inhibitory effect of NaCl on bitterness was related to the concentration of bitter substances. The bitterness of low-concentration QHCl (10^{-4} M) would be inhibited by all concentrations of NaCl (0.1, 0.3, 0.5M), while the bitterness of high-concentration QHCl (10^{-3} M) inhibited only by 0.3 and 0.5 M NaCl. However, saltiness was less affected by bitterness, only the highest concentration of QHCl (10^{-3} M) could inhibit the saltiness of 0.1 M NaCl solution.

1.2.2.4 Interaction between saltiness and umami

Umami, as the last taste to be discovered, is the least studied among all tastes, while the understanding on the interaction between umami and other tastes in a mixture tastant is scarce. Woskow (1969) concluded that sodium salts of 5′-ribonucleotides (umami/savory quality) enhanced saltiness only at moderate concentrations, but Kemp and Beauchamp (1994) reported monosodium glutamate (MSG) could enhance saltiness only at or above supra-detection threshold concentration. Some of the contradictory findings in the literature may be due to differences in the levels, compounds and testing strategies applied in sensory testing. Although the controversy was reported about the enhancing effect of umami in saltiness, umami tastants are widely used as flavour enhancers in developing salt reduced food products, for example,

MSG was used to reduce NaCl in a Japanese soup (Sumash-Jiru) with a much stronger umami taste (Yamaguchi and Takahashi, 1984). More examples can be given here to support the point.

Generally speaking, reducing sodium in food would result in a loss of saltiness. Consequently, bitterness could increase due to the loss of sodium in bitterness inhibiting capacity, while perception of sweetness would decrease as well (Breslin and Beauchamp, 1997). This may also lead to a reduction in the perception of appetitive aromas associated with this taste, which would have a negative impact on food preferences.

1.3 Interaction between salt and flavour perception

Flavour is a single perception, but it is considered as part of a unitary whole which can combine the inputs from separate sensory systems: taste, smell and chemical stimuli (Keast, Dalto and Breslin, 2004). This central integration ensures that there is ample opportunity for interaction between the senses. Salt imparts more than just saltiness, and it also enhances the palatability of foods. When salt is added to food, it can improve the thickness perception, enhance sweetness, mask metallic or chemical off-flavours, refine the overall flavour, and increase flavour intensity (Gillette, 1985). The enhancement effect of salt on flavour perception can be partially explained by the sodium cation. Various sodium-containing ingredients such as MSG, sodium bicarbonate is known to reduce bitterness in foods and enhance other flavour attributes such as sweetness (Breslin and Beauchamp, 1995). Another reason for its enhancing effect is that salt can decrease the water activity (aw) of the food, which would

effectively increase the flavour concentration and improve the volatility of the flavour components (Hutton, 2002). The flavour of foods is considerably influenced by their constituents, like water-soluble small molecules, monosaccharides, disaccharides or salts (such as NaCl). These compounds bind considerable amounts of water to build hydration shells during solubilization, while the decreased availability of water molecules due to salt binding would result in flavour release (Rabe, Krings and Berger, 2003). As a result, the high volatility of flavour components would improve the aroma and flavour perception. Along with bitterness blocking and increasing volatility of flavour compounds, salt is also found to affect the flavour formation through two main pathways, i.e., Maillard reaction and lipid oxidation (Mariutti and Bragagnolo, 2017; Gokmen and Senyuva, 2007).

1.3.1 Maillard Reaction

Maillard reaction, also known as non-enzymatic browning reaction, is widely present in food production. It is a reaction between carbonyl compounds (reducing sugars) and amino compounds (amino acids and proteins), which typically happens at the temperature from around 140 to 165 °C (280 to 330 °F). At higher temperatures, caramelization (the browning of sugars, a distinct process) and subsequently pyrolysis (final breakdown leading to burning and development of acrid flavors) become more pronounced (Ames, 1992). Figure 1.4 shows the mechanism of the Maillard reaction, which is usually divided into three stages. The first stage of the reaction is the condensation reaction between reducing sugars and amino acids. In the second stage, sugars are degraded and accompanied by the release of amino compounds. The last

stage is closely related to the formation of flavour. In this stage, the amino compounds undergo dehydration, decomposition, cyclization, and polymerization. According to the chemical composition, a series of aromatic compounds can be formed including ketones, aldehydes, alcohols, furans, and their derivatives such as pyrrole, pyridine, pyrazine, thiophene, and sulfides. Even though the flavour of each compound is unique, in particular, sulphur-containing compounds are important for the flavour of meat (Van Boekel, 2006).

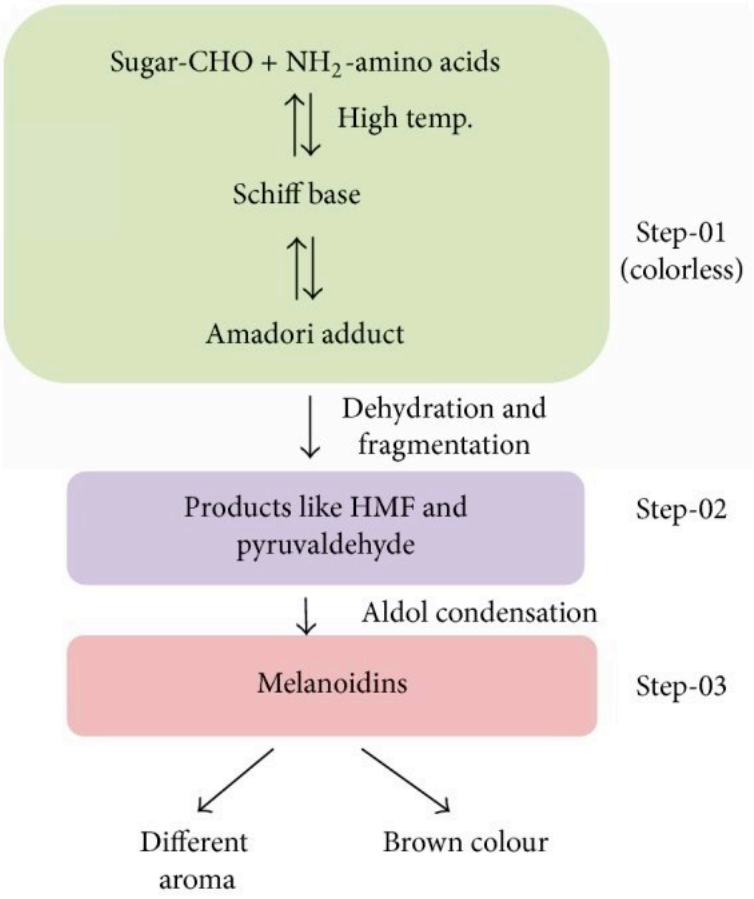

Figure 1.4 Mechanism of the Maillard reaction (Tamanna and Mahmood, 2015).

Most of the flavour compounds identified in cooked meat are the result of Maillard reaction. For example, the precursors formed from 1-deoxypine interact with the products of the Strecker reaction to produce many aromatic compounds including

furans, pyrazines, pyrroles, oxazoles, thiophenes, thiazoles and other heterocyclic compounds (Mottram, 1998). Thermal degradation of thiamin produces a number of sulfur compounds, such as thiols, sulphides and disulphide compounds, which offer meaty flavour or contribute to the flavour development of cooked meat (Grosch, 2001). Sulphur-containing amino acids, particularly cysteine, is one of the most important amino acids responsible of meaty flavour produced by Maillard reactions (Aaslyng and Meinert, 2017).

The impact of NaCl on the chemistry of Maillard reactions in meat was mentioned in some publications. Gokmen and Senyuva (2007) reported that the presence of NaCl in a reaction mixture of fructose and asparagine decreased the Schiff base formation, hence slowing down the formation of some Maillard compounds such as acrylamide. Reduction of NaCl may lead to an increase in proteolysis, the production of free amino acids and small molecule peptides associated with the Maillard reaction and Strecker degradation which can affect the flavour development of the meat, as their concentration usually exceeds the identification threshold (Luo *et al.*, 2021). So NaCl might influence Maillard reaction, either directly or indirectly, modifying the nature and number of volatile molecules formed.

1.3.2 Lipid oxidation

Lipids play an important role in the production of volatile flavour compounds. Meat flavour and palatability are influenced by fat content and types of fatty acids (Khan, Jo and Tariq, 2015). The degradation of unsaturated fatty acids would produce a variety of flavour compounds during the heating process which determines the flavour profile

of meat products (Sun *et al.*, 2022). Many of these flavour compounds have relatively high odour thresholds, but they can still have an impact on meat flavour because they are abundant (Mottram, 1992). Lipolysis leads to the production of large amounts of non-volatile compounds that are important for promoting meat flavour, while most endogenous enzymes are responsible for such reactions (Toldrá and Flores, 2000). It is mainly phospholipids that produce flavour compounds, while intramuscular triglycerides and structural phospholipids are the main contributors (Mottram and Edwards, 1983). Figure 1.5 shows the reaction mechanism of how lipids are oxidized to produce meat aroma. During the heating process, phospholipids and triglycerides are degraded, releasing short-chain fatty acids. At high temperatures, fatty acids are oxidized to produce hydroperoxides. Finally, hydroperoxides can be degraded to form alkoxy groups, and converted into volatile carbonyl compounds.

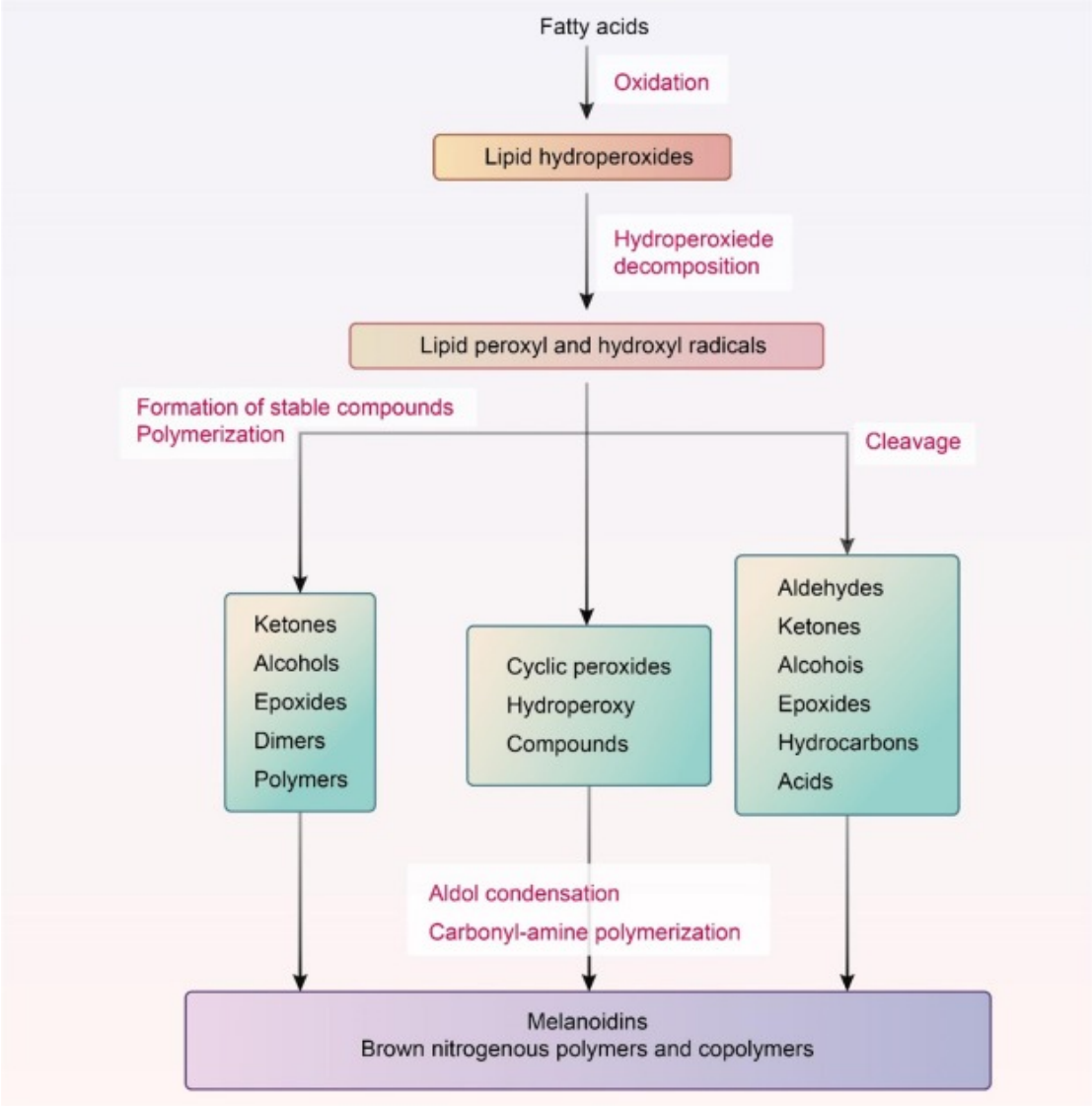

Figure 1.5 The mechanism of fat oxidation to produce meat flavour (Sun *et al.*, 2022).

It is generally assumed that salt accelerates lipid oxidation, which can cause undesirable changes in the colour and flavour of meat and meat products (Kanner, Harel and Jaffe, 1991). One of the most important volatile compounds produced by lipid oxidation is hexanal, which has a rancid flavour at excess level (Campagnol, Dos Santos and Rodriguez-Pollonio, 2017). Most of the studies suggested that salt acted as a prooxidant agent involved in the lipid oxidation of meat products. Purriños *et al.* (2012) confirmed the dry-cured pork shoulder "lacón" that were salted for longer period produced more volatile compounds from lipid oxidation, such as pentanal, heptanal and so on. Corral,

Salvador and Flores (2013) also indicated that volatile compounds from lipid oxidation like 1-pentanol, 2-octenol were significantly lower in salt-reduced fermented sausage than these in control sample. The mechanism of accelerated oxidation by NaCl may be attributed to its ability to disrupt cell membrane integrity, thereby facilitating access of oxidants to lipid substrates (Mariutti and Bragagnolo, 2017). Min, Cordray and Ahn (2010) studied the involvement of NaCl in a model system containing washed muscle residues and iron ions in cytosol and found that catalytic free iron ions were detected with an increased amount and they could penetrate the lipid phase to increase lipid peroxidation. Except grilled meat, meats with subcutaneous fat contain significantly high level of lipid-derived volatiles, whether cooked or uncooked. Because fatty acids can react with Maillard reaction compounds to form flavour compounds with a lower odour threshold and therefore it may have a greater impact on flavour (Aaslyng and Schäfer, 2008).

1.4 Salt as key ingredient in meat processing

Meat itself contains sodium but the amount is less than 100 mg Na per 100 g (Strazzullo and Leclercq, 2014). The main source of sodium in meat products is sodium chloride which is added during processing. As shown in Table 1.1, most meat products contain salt between 1.2 g/100 g to 4.3 g/100 g. Salt has a flavour enhancing effect in meat products and the perceived saltiness is mainly due to the perception of sodium ion. Both fat and salt together contribute to many of the sensory properties of processed meats (Miller and Barthoshuk, 1991).

Table 1.1 Sodium content in meat products (Pretorius and Schönfeldt, 2018).

Food	Sodium content (mg/100g)	Salt content (g/100g)
Gammon	711	1.78
Frankfurters	1074	2.69
Cooked Hams	1206	3.02
Pork sausages	1018	2.54
Hot dog	488	1.22
Bacon	1270	3.17
Cooked turkey breast	595	1.49
Salami	1695	4.24
Chicken nuggets	661	1.65

1.4.1 Formation of Meat Texture

One main function of salt in processed meat is to solubilise the functional myofibrillar proteins in meat, i.e., actin and myosin (Xiong, 1997), and increase their hydration and water holding capacity (WHC), ultimately result in an improved texture (e.g., tenderness) and high processing yield (Desmond and Vasilopoulos, 2019). The effect of NaCl on meat proteins is mainly attributed to Cl^-, probably because Cl^- are bound to the myofibril filaments more strongly than Na^+ and thus increase the negative charges of proteins (Petit *et al.*, 2019). This leads to repulsion between myofibrillar proteins, and further causes an electrostatic repulsive force between individual molecules, which results in a swelling of myofibrils (Offer and Trinick, 1983). The adsorption of Cl^- with positively charged groups of myosin results in a shift of the isoelectric point to lower pH, causing a weakening of the interaction between oppositely charged groups at a pH greater than the isoelectric point, as a result, WHC is increased (Puolanne and Halonen, 2010). Increasing the WHC of meat will reduce cooking loss and increase the tenderness and juiciness of meat products.

In addition, the extraction of myosin from myofibrils is important in processed meat (Desmond and Vasilopoulos, 2019). The salt-soluble myofibrillar protein forms a sticky exudate on the surface of the meat product, and this exudate will form a matrix of heat-coagulated protein and bind the meat pieces together after cooking (Desmond, 2006). In chopped or emulsified meat products (such as sausages), the salt-soluble proteins in the continuous phase form a protein film around fat globules, thereby retaining the fat during cooking (Monahan and Troy, 1997). NaCl is therefore essential for the texture of processed meat products. The addition of 1.5% to 2.5% (w/w) salt enables the protein to bind more water, thus increasing the tenderness and reducing fluid loss in heat-processed meat products (Doyle and Glass, 2010).

1.4.2 Salt as Preservative

Fresh food generally has water activity (aw) value between 0.95 and 0.99, while raw meat has aw 0.99 or higher. Hence meat is considered as a highly perishable food with risk of immediate growth of microorganisms (Lund *et al.*, 2000). In general, water activity at 0.85 and 0.90 are considered as the lowest levels which the eukaryotic and prokaryotic pathogens can grow respectively, while for most spoilage bacteria aw above 0.90 is required, and some may grow at 0.85 or even lower in extreme cases (Houtsma *et al.*, 1993). For example, *Staphylococcus aureus* can grow at high salt concentrations (10 - 20%) and low water activity (0.83 to 0.86) due to its great adaptive response to osmotic stress (Medveďová and Valík, 2012).

Salt has been used as a preservative in meat products including ham, sausages, salami, bacon and others (Hutton, 2002). It can inhibit the growth and survival of undesirable

421 microorganisms, prevent rapid spoilage and extend shelf life (Inguglia *et al.*, 2017).
422 Salt influences the growth of most microorganisms. It is generally accepted that 10%
423 salt inhibits the growth of most germs, whereas 5% salt can only inhibit anaerobes (Petit
424 *et al.*, 2019). Reducing the NaCl level below the level normally used without adding
425 any other preservative would shorten the shelf life of food products (Desmond and
426 Vasilopoulos, 2019). For example, Desmond (2006) reported that 40% of salt reduction
427 for frankfurters (from 2.5 to 1.5 % w/v) without any salt substitutes caused the natural
428 flora to grow more rapidly. Stringer and Pin (2005) also found that bacon at 2% (w/w)
429 salt content had vinegary off odour after 3 weeks storage, whereas it took only 2 weeks
430 to develop this off odour was perceived after 2 weeks if the salt level was reduced
431 to 1% (w/w).at same storage condition.
432 Salt works as a preservative mainly by lowering water activity (Albarracín *et al.*, 2011).
433 The addition of salt causes water within bacteria to flow out through their semi-
434 permeable membranes and triggers osmotic shock, leading to bacterial cell death or
435 serious injury. As a result, bacterial growth is significantly reduced (Davidson, Taylor
436 and Schmidt, 2012). In addition, salt may reduce the solubility of oxygen, interfere with
437 cellular enzymes or force cells to expend energy to remove sodium ions from the cell,
438 all of which can reduce growth rates (Shelef and Seiter, 2005).

439 **1.5 Strategies of sodium reduction in meat products**

440 Meat manufacturers and consumers have become more aware of the relationship
441 between sodium and chronic diseases such as high blood pressure, as a result, demand
442 for a variety of low-salt meat products has increased greatly in many countries. Food

processors are developing a wide range of low-salt products to meet consumer demand. Current approaches to reduce the sodium content of meat products include the following strategies.

1.5.1 Reduction of salt content by stealth

Stealth salt reduction means a gradual reduction of salt in processed foods over a long period of time (Dubow and Childs, 1998). This strategy has achieved a decent level of saltreductionwithin foods, but consumers perceived no significant sensory difference in products (Kilcast and Den Ridder, 2007). Studies on the perception of taste have shown that difference between the two concentrations of taste substances are often undetectable when their difference is less than approximately 10% (Henney, Taylor and Boon, 2010). This is now a common approach in the UK, and it has been successfully used all over the world for a variety of food products. For example, the sodium content of white bread, was reduced by 25% in six weeks, but consumers did not notice the difference in flavor (Girgis et al., 2003). In the UK, the sodium content of many processed foods has been reduced by 20-30% in three years, and it resulted in a reduction in NaCl intake of approximately 1 g/day for the UK population (He and MacGregor, 2009). For the food industry, this meant that sodium reduction goals can be achieved by gradually reducing the sodium content of their products over a period of years without losing consumers. However, the biggest limitation of this strategy is time consuming, and it may take years to reach the target. In addition, in practice, it is generally only possible to reduce salt by a limited amount without making the product unpalatable.

1.5.2 Changing the physical form or distribution of salt

1.5.2.1 Changing the size/shape of salt crystal

The size and shape of salt particles play important roles in food matrices. Dissolution of salt in the mouth is necessary to impart salt taste, but ordinary salt particles usually do not dissolve completely. As a consequence, the perceived saltiness is compromised. Desmond (2006) stated that the perception of saltiness in solid form is influenced by the structure of salt crystals. The dissolution rate of sodium chloride in the oral cavity depends on the exposed surface area and is a function of crystal size and shape (Kilcast and Den Ridder, 2007). It is estimated that between 70% and 95% of NaCl is retained in the food matrix without being dissolved by saliva, in other words, most NaCl crystals are swallowed without being perceived any salty taste (Quilaqueo et al., 2015). Therefore, a smaller crystal size and lower bulk density will result in a faster dissolution rate and quicker transportation of sodium to the saliva. Consequently, a stronger salt taste will be perceived (Henney, Taylor and Boon, 2010).

Optimization of salt crystals allows to reduce the salt content but maintain the same salty taste. Based on a time-intensity sensory technique, Rama et al. (2013) found that NaCl crystal sizes smaller than 106 μm could offer snacks the fastest and highest maximum salty intensity, as well as the highest total salty taste. Moncada et al. (2015) demonstrated that the use of micronized salt allowed the salt content to be reduced from 1.5% to 1.0% in beef burger without affecting its colour, yield, saltiness and juiciness. Gaudette, Pietrasik and Johnston (2019) found that the use of 3mm sized fat-coated salt

crystals in beef patty could achieve 30% sodium reduction but with a similar salty taste comparable to control samples.

Various forms of salt crystal (such as flake, granular) have been trialed to explore the feasibility of reducing salt content in meat products as well. In general, flake salt has better and faster solubility than granular salt, which offers better water binding capacity and increases protein solubility, thus improving product cooking yield (Tunieva and Gorbunova, 2017). Flake salts may be beneficial for products without any water addition during processing like dry cured products. Rios-Mera et al. (2021) showed that the fine flake NaCl crystals (0.55 mm) dissolved rapidly and were highly permeable in the dry cured pork. In addition, dendritic salt possesses the most beneficial characteristics of both crystal and flake salts. Dendritic crystals are branched or star-shaped and have the low density, high specific surface area and fast dissolution properties of fine-grained salts, especially macro porosity (Inguglia et al., 2017). Moncada et al. (2015) found that cheese crackers with 1% w/w 15 μm Cargill flake salt even had higher saltiness than with 2% w/w regular salt. However, this method is mainly used in the food seasoning industry and is only applicable to dry and solid foods (Rama et al., 2013).

1.5.2.2 Inhomogeneous salt distribution

Controlling the distribution of salt has been used for salt reduction in bakery products. Monteiro et al. (2021) indicated inhomogeneous distribution of salt agglomerates could reduce the salt content of bread by up to 30% without changing other quality attributes. Guilloux et al. (2015) found that uneven salt distribution could achieve 30% salt

reduction in pizza without altering its organoleptic properties. The taste enhancement in an inhomogeneous system is thought to be the result of discontinuous stimulation of taste receptors (Busch et al., 2013). Uneven distribution of salt would create a partial salt contrast, which prevents adapting and gradually decreasing in taste perception caused by continuous exposure of taste buds, especially in high doses of salt (Nakao et al., 2013). Xiong et al. (2020) reported that edible coating with salt uneven distribution could reduce the salt content by even up to 60% for beef frankfurter sausage without affecting its salty intensity. Mosca et al. (2013) demonstrated that sausage with uneven distribution of salt was saltier and more desirable than with even distribution of salt at a constant salt concentration. However, this strategy has limited application to reduce salt in meat products due to a high moisture content. Consequently, the dissolution of salt would minimize the contract in concentration within meat products.

1.5.3Alternative processing techniques

1.5.3.1 High pressure treatment

High Pressure Processing (HPP) is a non-insulated technique that uses pressure rather than heat to inactivate harmful pathogens and spoilage microorganisms (Rodrigues *et al.*, 2015). High hydrostatic pressures at 300 - 600 MPa at mild temperatures (<45 °C) are commonly used to treat foods for a few minutes, thus allowing most foods to be preserved with minimal impact on flavour, texture, appearance and nutritional value (Inguglia *et al.*, 2017). It is considered as a useful method to assist salt reduction in meat products as it can partially perform the functions of salt in meat products. When salt is reduced, the functional properties of protein molecules will be affected including

530 solubilisation of myofibrillar proteins, depolymerization of F-actin, dissociation of
531 actomyosin, aggregation of myofibrillar protein and alteration of enzymatic activity
532 within meat, but high-pressure treatment could perform these functions to facilitate the
533 formation of a gel network that retains water, and thus reduce the cooking losses of the
534 meat batter (Iwasaki *et al*., 2006). O'Flynn *et al.* (2014) reported that applying high-
535 pressure-treatment at 150 MPa on raw meat increased the yield of 20% salt reduced
536 breakfast sausages regardless of salt concentration. HPP can be used to partially replace
537 NaCl because it can help extract myofibrillar proteins from the muscle, which is one of
538 key functions of salt (Kim *et al.*, 2021). As a result, it helps to improve the cohesiveness,
539 stickiness and chewiness of meat products (Jimenez-colmenero *et al.*, 1998). Crehan,
540 Troy and Buckley (2000) found that hardness, cohesiveness, gumminess and chewiness
541 of 40% salt-reduced frankfurter sausages with HPP at 150 MPa were improved. High
542 pressure treated meat products have been shown to have an increased level of saltiness
543 intensity without increasing salt content. This increase in saltiness perception was
544 attributed to a weakening interaction between Na^+ and protein which resulted in more
545 sodium being released to the taste receptors on the tongue for a saltier taste (Clariana
546 *et al.*, 2011). Zhu *et al.* (2022) presented that 50% salt-reduced emulsified beef sausage
547 treated with HPP (200 - 400 MPa) had similar saltiness and juiciness compared to
548 sausage at regular salt content. Most importantly, HPP has been shown to successfully
549 inactivate harmful pathogens such as *E. coli, Salmonella* and *Listeria monocytogenes*
550 in a variety of meat products, thus ensuring food safety and shelf life (Cheftel and
551 Culioli, 1997). Myers et al. (2013) indicated that *L. monocytogenes* was inhibited in 25%

552 salt-reduced ham/turkey with 3 mins HPP (600 MPa). Luckose et al. (2015) also found
553 that 50% salt-reduced chicken nuggets with 600 MPa pressure treatment effectively
554 reduced all microbial counts to 10 CFU/g and remained low during the 60-day storage
555 so that shelf life was improved.
556 However, HPP require expensive initial investment, high operation and maintenance
557 costs, which can drive up the price of meat products (Kim *et al.*, 2021). In addition,
558 microorganisms vary in their sensitivity to high pressure, with Gram-negative bacteria
559 being the most sensitive and bacterial spores being the most resistant (Inguglia *et al.*,
560 2017). As a result, most high-pressure-treated foods require cold storage to maintain
561 their sensory qualities and may also require aseptic packaging conditions, which again
562 further increases the cost of food production.
563 **1.5.3.2 Ultrasound**
564 The ultrasound is considered an emerging technology with great potential for
565 application in food. In general, the range of sound used is divided into high-frequency
566 (>1 MHz) with low-intensity (<1 W cm^{-2}), and low-frequency (20–100 kHz) with high-
567 intensity (10–1000 W cm^{-2}), which is also known as power ultrasound (Alarcon-Rojo
568 *et al.*, 2015). Ultrasound is a form of vibrational energy produced by a transducer that
569 converts electrical energy into acoustic energy, which triggers a phenomenon known as
570 cavitation (Pinton *et al.*, 2021). Cavitation produces a large number of bubbles which
571 results in high local pressure and temperature when collapse (Boateng and Nasiru,
572 2019). This phenomenon also generates strong physical forces, such as shear, shock
573 waves and turbulence, which affect the functional properties of meat proteins and

574 increase the water retention capacity (Gómez-Salazar *et al.*, 2021). In addition, the
575 collapse of cavitation bubbles produces microjets that collide with the surface structure
576 of the myofibrils leading to the formation of micro fissures that alter the protein
577 structure and improve the additive diffusion, thus improving the texture of meat (Awad
578 *et al.*, 2012). Stadnik, Dolatowski and Baranowska (2008) found that beef (*m.*
579 *semimembranosus*) sonicated at 24 h after slaughter treated with ultrasound (45 kHz)
580 for 2 mins showed higher water holding capacity. Barretto *et al.* (2018) also presented
581 that applying ultrasound (20 kHz, 600 W cm^{-2}) for 10 mins on restructured cooked ham
582 with 50% salt reduction increased its hardness but without changing taste, texture and
583 global acceptance comparing with no salt reduction cooked ham. The use of ultrasound
584 during curing improves salt distribution in meat and enhances salt transfer during
585 processes such as meat curing (Ojha *et al.*, 2016), consequently a higher salt perception
586 can be achieved even at lower NaCl levels. Barretto *et al.* (2020) proved that low
587 sodium restructure cooked ham was subjected to power ultrasound treatment (20 kHz,
588 600 W cm^{-2}) for 10 mins, and the product exhibited better flavour, higher saltiness and
589 global acceptance. Leães *et al.* (2020) also indicated that ultrasound treatment (25 kHz,
590 175 W) for 20 min combined with basic electrolyzed water to replace salt would allow
591 to reduce up to 30% NaCl content of meat batters. As similar with high pressure
592 processing, ultrasound has also been proven the inactivation of microorganisms.
593 Inguglia et al. (2018) demonstrated that a reduction of $\log_{10}^{6}$ CFU ml^{-1} for *E. coli* K12
594 and $\log_{10}^{4}$ inactivation for *L. innocua* within a one-hour treatment were achieved with
595 a frequency ultrasound (20 kHz) in tryptic soy broth. Aguilar et al. (2021) also shown

596 that the ultrasound pulses (7.56 s wave pulse, 400 W) reduced the natural microflora,
597 *L. delbrueckii* and *L. monocytogenes* of a raw meat emulsion, even inactivation reached
598 up to 60% of the microbial population. The media particles present in the fluid are
599 compressed and thinned during ultrasound, leading to the formation of cavities or
600 bubbles. With successive cycles of ultrasound, they may become unstable and collapse,
601 leading to localized high temperatures and pressure release, which may disrupt the
602 cellular and functional components of the bacterial membrane and therefore microbial
603 inactivation (Zhou, Lee and Feng, 2012).
604 Ultrasound has been used commercially due to its high speed, reliability, low cost and
605 simplicity of application (Turantaş, Kılıç and Kılıç, 2015). However, similar to HPP,
606 spores and fungi are more resistant to inactivation by ultrasound, gradually decreasing
607 in yeasts, Gram-positive and Gram-negative cells (Inguglia *et al.*, 2017). Hence,
608 ultrasound parameters need to be optimized for each meat product that may result in a
609 difficult spread in manufacture.
610 **1.5.3.3 Pulsed Electric Field Processing**
611 Pulsed electric field (PEF) treatment is a non-thermal technology used primarily in food
612 processing to improve food quality and extend shelf life (Kim *et al.*, 2021). PEF
613 treatment is a brief application of high voltage pulses (1-100 μs) with electric field
614 strengths ranging from 0.1 to 80 kV/cm to food placed between two electrodes (Barba
615 *et al.,* 2019). This causes structural changes and rapid disruption (permanent or
616 temporary) of the cell membrane, resulting in the cell membrane to trigger an increase
617 in membrane permeability by enlarging existing pores or creating new pores, and then

allow membrane components exchange with the cellular environment and have a positive effect (Gómez *et al.*, 2019). The three most important parameters determined during PEF are electric field strength, processing temperature and energy delivery (Toepfl, Siemer and Heinz, 2014). Previous studies have reported that the pulsed electric fields affected the tenderness and other quality parameters of fresh meat and meat products. Bekhit *et al.* (2014) shown that PEF beef *Longissimus lumborum* muscles (0.27-0.56 kV/cm, 20 μs) had lower cooking loss and higher tenderness. The beneficial tendering effect of pulsed electric fields may associate with membrane damage which result in releasing of calcium, thereby activating calcium-dependent proteases, calpain and accelerating glycolysis; releasing of cathepsins from lysosomes, thereby accelerating protein hydrolysis (Warner *et al.*, 2017). PEF can also improve the shelf life of food because the formation of hydrophilic pores and the forced opening of protein channels in the membrane by PEF lead to enzyme inactivation and destruction by spoilage and pathogenic microorganisms (Buckow *et al.,* 2014). Limited research on the use of PEF to treat low-salt meat products. Bhat *et al.* (2020) found that PEF (0.52 kV/cm, 20 μs) treatment could reduce salt content in beef jerky by 40% without any negative effects on lipid oxidation, sensory quality and microbiological stability of the product. Treatment with PEF affects the diffusion, distribution and release of sodium from the meat matrix, thereby altering the interaction between protein and salt ions and influencing sodium release during mastication (Bhat *et al.,* 2019).

PEF has the advantage of low energy consumption, short processing time and continuous operation in food processing (Puértolas and Barba, 2016), but the initial

capital investments and cost is high (Jeyamkondan, Jayas and Holley, 1999). The electrolysis products of PEF can have a detrimental effect on food and the uneven treatment distribution in non-uniform by PEF can lead to the presence of air bubbles (Gómez *et al.*, 2019). In addition, the technique also fails to inactivate bacterial spores because the high electric field strength required for inactivation which usually means that the distance gap between the electrodes is very small (in millimeters) (Oziembłowski and Kopeć, 2005).

1.5.4 Use of flavour enhancer and salt substitutes

1.5.4.1 Flavour enhancers

Flavour enhancers are substances or ingredients that can alter or increase the overall intensity of the perceived taste or smell of a food by enhancing desirable flavour or inhibiting undesirable flavour, which has little or no flavour/aroma in itself (Campagnol, Dos Santos and Rodriguez-Pollonio, 2017). Among them, salt enhancers are substances or ingredients that are added to food preparations that already include salt, with the aim of amplifying or intensifying the taste of salt and make the salt flavor more pronounced (Henney, Taylor and Boon, 2010). They can significantly help and balance the salty taste of reduced salt products by activating taste receptors in the mouth and throat (Brandsma, 2006). There are many flavour enhancers and flavour masking agents include nucleotides, yeast extracts, glutamates and amino acids on the market and the number of products entering the market is increasing.

1.5.4.1.1 Monosodium glutamate

Compared to the other four basic taste (sweetness, sourness, bitterness and saltiness), umami has it unique function to rebalance the taste of low sodium products and increase their savoury perception. The most commonly used source of umami is monosodium glutamate. Yamaguchi and Takahashi (1984) demonstrated that MSG could be used to reduce NaCl in a Japanese soup (Sumash-Jiru), where MSG was used in combination with 5′-nucleotides, such as inosine-5′-monophosphate (IMP) and guanosine-5′-monophosphate (GMP), to achieve a much stronger umami taste. Dos *et al.* (2014) found that MSG, disodium inosinate, disodium guanylate could enhance flavour and maintain saltiness at 50% reduction of NaCl in fermented cooked sausages. Quadros *et al*. (2015) also proved that 0.3% added MSG could compensate the saltiness loss caused by 50% salt reduction in low-sodium fish burgers. However, MSG itself contains sodium, so using MSG would lead to more sodium added than salt alone in some cases (Pangborn and Braddock, 1989). Additionally, some literature mentioned that continuous intake of high levels of MSG may increase risk of neurological diseases, including Alzheimer's dementia and Parkinson's disease (Blaylock, 1999). Therefore, MSG concentrations in food must be controlled. An acceptable daily intake of MSG which established by European Food Safety Association is 30 mg /kg (Zanfirescu *et al.*, 2019). For example, the acceptable daily intake for a 70 kg adult is 2.1 g.

1.5.4.1.2 Yeast extract

Yeast autolysates are also commonly used in low salt preparations, they are practically used to mask the metallic flavour of potassium chloride (KCl), one of the popular salt replacers. Campagnol *et al.* (2011b) found that 2% yeast extract could be used to

683 develop 50% salt reduced fermented sausage, while the sensory quality defects caused
684 by KCl could be compensated by the yeast extract. They reported that yeast extract
685 could increase volatile compounds production during sausage fermentation such as 3-
686 methylbutanal which relevant to the aroma of cured meat product and may mask the
687 unpleasant taste of KCl. Vidal *et al.* (2020) also demonstrated that the addition of 5%
688 yeast extract significantly reduced the rancid aroma of mixtures containing NaCl, KCl
689 and calcium chloride ($CaCl_2$) in low sodium salted beef with 50% reduction of salt.
690 Yeast extracts are rich in compounds or precursors, such as amino acids, and most of
691 these volatile and non-volatile substances, as well as aroma-active compounds, are
692 released during the heating process, thus improving the flavour (Alim *et al.*, 2018).
693 According to Desmond (2006), yeast extracts can produce tasty products with low salt
694 content, but it has a particular meaty flavour which may not be acceptable for some
695 people.

696 **1.5.4.2 Salt substitutes**

697 An ideal strategy for maintaining or improving the quality of low-salt foods would be
698 replacing NaCl with a compound that produces a similar pure salty taste while
699 containing lower amounts of sodium or using alternative ingredients, which identified
700 as salt substitute (Liem Miremadi and Keast, 2011). The food industry currently uses
701 many salt substitutes to replicate some functions of salt. Common salt substitutes are
702 mineral salts such as KCl, $CaCl_2$ and magnesium sulphate, which have been used
703 widely as salt substitutes in many foods, while certain type of amino acids also attracted
704 lots of attention recently (Ruusunen and Puolanne, 2005; Kilcast and Den Ridder, 2007).

While both flavour enhancers and salt substitutes can enhance the taste of food, their mechanisms and purposes differ. From the definition, it can be seen that flavour/salt enhancers are additional ingredients which added in food, while salt substitutes are ingredients which replacing part of NaCl in food. Flavour enhancers focus on intensifying existing flavours, and salt enhancer specifically refers to the enhancement of saltiness, while salt substitutes aim to provide a salty taste while reducing sodium intake (Campagnol, Dos Santos and Rodriguez-Pollonio, 2017).

1.5.4.2.1 Potassium chloride

One of the most common mineral salts used to replace or reduce salt is KCl which has been widely used in meat products, because the two salts have similar chemical properties. Particularly KCl has beneficial effect on lowering blood pressure (Geleijnse *et al*., 2007). Paulsen *et al.* (2014) found that using KCl to replace NaCl from 20% to 40% did not change the meaty flavour, juiciness, hardness and cohesiveness in sodium reduced sausage. Wu *et al.* (2014) indicated that the replacement of 40% of salt in the dry-cured bacon by KCl did not affect the proteolysis, colour, hardness and juiciness, but the saltiness was reduced. When the concentration of KCl reached to 70%, the saltiness decreased significantly and the bitterness increased obviously, even though it is juicier. KCl has been shown to have the same antibacterial effect as sodium chloride against a wide range of pathogenic bacterial species, such as *Aeromonas hydrophila*, thus it could ensure the shelf life is not shortened in salt-reduced foods (Bidlas and Lambert, 2008). Terrell *et al.* (1983) proved that the microbial load of *Micrococcus, Moraxella* and *Lactobacillus* in ground pork containing 1.6% or 3.19% KCl were close

to that of ground pork containing 2.5% NaCl stored at 5°C for 10 days. Although KCl does have some salty taste, it may also result in some unpleasant aftertastes, such as bitter, metallic and astringent taste, which limit its application in food manufacturing (Reddy and Marth, 1991). The substitution of salt with KCl in most foods must be limited to 30%, as higher levels can produce bitter and metallic tastes (Doyle and Glass, 2010). A significant increase in bitterness and loss of saltiness were observed in foods treated with blends where the KCl is more than 50% (Desmond, 2006). That means KCl should be added with other salt substitutes or flavour enhancer in a salt-reduced meat product to cover unpleasure taste or maintain salty taste when the concentration of KCl is more than 30%. What is more, high potassium load is associated with impairments in people with type 1 diabetes, renal disease and adrenal insufficiency (Khaw and Barrett-Connor, 1984).

1.5.4.2.2 Lysine

Lysine is colourless crystal required for human growth as one of the nine essential amino acids in the human body that cannot be produced by the body and therefore must come from food (Blemings and Benevenga, 2007). It has a high nutritional value and is essential for protein synthesis for human metabolism (Wolfe, 2017). Foods rich in protein are generally good sources of lysine, such as meat, especially red meat (1.57 g/100 g) (Liu *et al.*, 2016). Lysine itself could reduce the level of triglycerides in blood to prevent cardiovascular and cerebrovascular disease (Flodin, 1997). According to Li *et al.* (2019) report that L-lysine increases the solubility of myosin at low ionic strength, suggesting that lysine has great potential for improving the quality of low-salt meat

749 products. Recently, lysine has been successfully added to salt-reduced meat products
750 as flavour enhancer to improve eating quality (Dos Santos Alves *et al.*, 2017; Zheng *et*
751 *al.*, 2017; Dos Santos Alves *et al.*, 2014; Campagnol *et al.*, 2012; Campagnol *et al.*,
752 2011a). Lysine is also as salt substitute and try to add in meat products, but Guo *et al.*,
753 (2020) demonstrated that increasing concentration of lysine increased yield, WHC and
754 global acceptance, improved mouthfeel, appearance of ham with 50% NaCl reduction,
755 but saltiness intensity could not achieve similar level with non-salt-reduced ham at
756 highest concentration (0.8%). Vidal *et al.*, (2020) also found that 50% NaCl reduced
757 salted meat with KCl and 3% lysine had enhanced flavour and overall acceptance, but
758 saltiness intensity still could not completely compensate saltiness intensity loss
759 comparing with non-salt-reduced meat, even at high concentration level (3%). This
760 could provide an idea that lysine can increase the salty taste intensity of salt-reduced
761 meat products, but it needs to be at a relatively high concentration range when the
762 consumers is not able to distinguish the difference in saltiness between salt-reduced
763 meat products and non-salt-reduced meat products. As for the mechanism of action of
764 lysine to produce salty taste is currently unknown, this need to be further explored. For
765 example, whether it stimulates ENaC channel resulting in the transduction of salty taste
766 signals in the brain, or alternatively whether it can enhance the overall taste of foods by
767 interacting with salt receptors in the taste buds to make the perception of salt stronger,
768 is not known. In addition to enhancing the eating quality of meat, lysine also contributes
769 to the absorption of calcium in the human body and decreases the amount of calcium

lost in the urine, which is used with calcium to prevent and treat osteoporosis (Fini *et al.*, 2001).

1.5.4.2.3 Calcium lactate

There is less literature on the use of calcium lactate as a salt replacer, however, it has following potential benefits which could be consider as an feasible salt substitute. Calcium lactate is a white or gray crystalline salt, the most common form is pentahydrate (Shelef, 1994). It can be used directly as food ingredients or food additives (E327), such as flavor enhancers, thickeners or others in the food industry when it is used as a monohydrate (World Health Organization, 2011). Calcium lactate is associated with saltiness because the salts of divalent metal cations are mainly perceived with saltiness and bitterness (Lawless *et al.*, 2003), but calcium lactate also has a considerable sour component (Kilcast and Den Ridder, 2007). It is interesting to note that insufficient intake of calcium would stimulate the salty appetite (Tordoff, 1996), which indicates that people with calcium deficiency prefer to eat more salt and lead to a vicious circle finally. The most prominent advantage of lactates as a salt substitute is that lactate anion can inhibit the growth of bacteria in meat products and antilisterial properties (Devlieghere et al., 2009), which can compensate for the drawbacks of most salt substitutes. Weaver and Shelef (1993) found that 2% calcium lactate could inhibit the growth of *Listeria monocytogenes* (*L. monocytogenes*) which was very common in the meat products. In addition, Lawrence *et al.*, (2003) also indicated that the beef longissimus (muscle) marinated with calcium lactate was more resistant to the growth of aerobic bacteria than marinated with calcium ascorbate or

792 calcium chloride. Calcium lactate also affects the colour, texture and flavour of meat
793 products. Yang *et al.* (2021) presented that 0.2–0.4% calcium lactate resulted in greater
794 redness, oxidative stability and increased hardness, gumminess, chewiness in cured
795 beef sausage. Irshad *et al.* (2016) also found a similar trend for redness, yellowness,
796 hardness in restructured buffalo meat loaves with calcium lactate added at 1-1.25% but
797 there was no change in sensory attributes. The sensory results were further confirmed
798 by Aggarwal, Ahlawat and Sharma (2009), and they demonstrated that calcium
799 enriched chicken meat roll with 1.5-2% calcium lactate had same flavour, colour,
800 tenderness, juiciness and overall acceptability as control. In addition, calcium is not
801 only an important mineral to support bone health, but also maintain the metabolism of
802 human (Adluri *et al.,* 2010). Lack of calcium in the diet will cause rickets, osteoporosis
803 and so on (Shaw, 2016). According to Lutz, Mazur and Litch (2014), adults were
804 recommended a daily intake of calcium at l000 mg/day, but the calcium content in the
805 meat is relatively poor, only about 10 mg/100 g (Okuskhanova *et al.*, 2016). Therefore,
806 it is useful to enrich the calcium level in meat products for people's health and help
807 people maintain a healthy appetite for salt.
808 In conclusion, although the literature has indicated that lysine could be used as a salt
809 substitute to improve the quality of reduced-salt meat products, the relationship
810 between the concentration of lysine and the perceive saltiness has not been explored. In
811 addition, there is scarce information about how calcium lactate interacts with saltiness
812 in aqueous or food model systems, although it can effectively extend the shelf life.

Therefore, the feasibility of using the combination of lysine and calcium lactate as salt substitutes is worth exploring.

1.5.5 Challenges in reducing salt

Developing low-salt meat products is not an easy task, so far there is no comparable salt substitute with all essential functions as salt. Quite often, several agents or salt substitute need combine with processing technologies to achieve successful salt reduction. One of the biggest barriers to salt substitution is the cost, as salt is one of the cheapest food ingredients. Sodium chloride plays multiple roles in meat products. A particular problem associated with low-salt meat products is that when salt is reduced, not only the perceived saltiness, but also the intensity of the characteristic flavour is reduced. Ideally, the quality characteristics of low-salt meat products must therefore be the same as those of the conventional meat products. What is more, maintaining microbiological stability and safety is an essential requirement for any salt reduction programme, and aspects related to process ability must also be considered.

1.6 Conclusions

Salty taste is an important sensory attribute of many foods and sodium chloride contributes to the characteristic flavour of many food types beyond just the salty taste. When salt intake is within recommended levels, it plays a very important physiological role in the body. However, higher concentrations of sodium-containing salt can pose a serious risk to human health. Reducing the dietary sodium intake for the public are facing lots of challenges. For meat industry, simply reducing the salt addition level in products would compromise the eating quality of products, particularly in saltiness and

overall acceptability. This review summarised the principles of saltiness perception in foods and discussed the mechanism, strength and weakness of different salt reduction strategies which were adapted by the meat industry, governments and manufacturers. Despite the progress made in the development of salt replacement ingredients and flavour enhancers, there are still factors associated with their negative sensory impact. Salt substitutes not only need to be effective in maintaining food safety, but also must meet consumer perceptions of low-salt meat products, such as taste, colour, flavour, texture and so on, all parameters that may become unacceptable if too much sodium is removed. There are evidence that combing lysine and calcium lactate can be effective strategy to improve the eating quality and maintain shelf life of salt-reduced foods. However, understanding for their perceived saltiness and shelf life in low salt foods is scarce. This needs to be fully validated by subsequent experiments.

Reference

Aaron, K. and Sanders, P., 2013. Role of Dietary Salt and Potassium Intake in Cardiovascular Health and Disease: A Review of the Evidence. *Mayo Clinic Proceedings*, 88(9), pp.987-995.

Aaslyng, M.D. and Schäfer, A., 2008. The effect of free fatty acids on the odour of pork investigated by sensory profiling and GC-O-MS. *European Food Research and Technology,* 226(5), pp.937-948.

Aaslyng, M.D. and Meinert, L., 2017. Meat flavour in pork and beef–From animal to meal. *Meat science*, 132, pp.112-117.

856 Adluri, R., Zhan, L., Bagchi, M., Maulik, N. and Maulik, G., 2010. Comparative effects
857 of a novel plant-based calcium supplement with two common calcium salts on
858 proliferation and mineralization in human osteoblast cells. *Molecular and Cellular*
859 *Biochemistry*, 340(1-2), pp.73-80.
860 Aggarwal, P., Ahlawat, S. S., and Sharma, D. P., 2009. Development of calcium
861 enriched chicken meat rolls. Indian J. Poultr. *Sci*, 44(2), 233-237.
862 Aguilar, C., Serna-Jiménez, J., Benitez, E., Valencia, V., Ochoa, O., and Sotelo, L. I.,
863 2021. Influence of high power ultrasound on natural microflora, pathogen and lactic
864 acid bacteria in a raw meat emulsion. *Ultrasonics Sonochemistry*, 72, 105415.
865 Alarcon-Rojo, A.D., Janacua, H., Rodriguez, J.C., Paniwnyk, L. and Mason, T.J., 2015.
866 Power ultrasound in meat processing. *Meat science*, 107, pp.86-93.
867 Albarracín, W., Sánchez, I.C., Grau, R. and Barat, J.M., 2011. Salt in food processing;
868 usage and reduction: a review. *International Journal of Food Science & Technology,*
869 46(7), pp.1329-1336.
870 Alim, A., Song, H., Liu, Y., Zou, T., Zhang, Y., and Zhang, S., 2018. Flavour-active
871 compounds in thermally treated yeast extracts. *Journal of the Science of Food and*
872 *Agriculture,* 98(10), 3774-3783.
873 Ames, J.M., 1992. The maillard reaction. In *Biochemistry of food proteins* (pp. 99-153).
874 Springer, Boston, MA.
875 Ashford, R., Jones, K. and Collins, D., 2020. National Diet and Nutrition Survey:
876 Assessment of salt intake from urinary sodium in adults (aged 19 to 64 years) in
877 England, 2018 to 2019. GOV. UK, pp.133-155.

878 Avenet, P. & Lindemann, B., 1991. Noninvasive recording of receptor cell action
879 potentials and sustained currents from single taste buds maintained in the tongue: the
880 response to mucosal NaCl and amiloride. *J. Membr. Biol.* 124, 33–41.
881 Awad, T.S., Moharram, H.A., Shaltout, O.E., Asker, D.Y.M.M. and Youssef, M.M.,
882 2012. Applications of ultrasound in analysis, processing and quality control of food: A
883 review. *Food research international*, *48*(2), pp.410-427.
884 Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., ... and
885 Vorobiev, E., 2015. Current applications and new opportunities for the use of pulsed
886 electric fields in food science and industry. Food Research International, 77, 773-798.
887 Bartoshuk, L.M., 1975. Taste mixtures: Is mixture suppression related to compression?.
888 *Physiology & behavior*, 14(5), pp.643-649.
889 Barretto, T.L., Pollonio, M.A.R., Telis-Romero, J. and da Silva Barretto, A.C., 2018.
890 Improving sensory acceptance and physicochemical properties by ultrasound
891 application to restructured cooked ham with salt (NaCl) reduction. *Meat Science*, 145,
892 pp.55-62.
893 Barretto, T.L., Bellucci, E.R.B., Barbosa, R.D., Pollonio, M.A.R., Romero, J.T. and da
894 Silva Barretto, A.C., 2020. Impact of ultrasound and potassium chloride on the
895 physicochemical and sensory properties in low sodium restructured cooked ham. *Meat*
896 *science*, 165, p.108130.
897 Bekhit, A. E. D. A., van de Ven, R., Fahri, F., and Hopkins, D. L., 2014. Effect of
898 pulsed electric field treatment on cold-boned muscles of different potential tenderness.
899 *Food and Bioprocess Technology*, 7(11), 3136-3146.

900 Bhat, Z.F., Morton, J.D., Mason, S.L. and Bekhit, A.E.D.A., 2019. Current and future
901 prospects for the use of pulsed electric field in the meat industry. *Critical Reviews in*
902 *Food Science and Nutrition,* 59(10), pp.1660-1674.
903 Bhat, Z.F., Morton, J.D., Mason, S.L. and Bekhit, A.E.D.A., 2020. The application of
904 pulsed electric field as a sodium reducing strategy for meat products. *Food Chemistry,*
905 306, p.125622.
906 Bidlas, E. and Lambert, R.J., 2008. Comparing the antimicrobial effectiveness of NaCl
907 and KCl with a view to salt/sodium replacement. *International Journal of Food*
908 *Microbiology*, 124(1), pp.98-102.
909 Bigiani, A., 2020. Does ENaC work as sodium taste receptor in humans?. *Nutrients*,
910 12(4), p.1195.
911 Blaylock, R. L., 1999. Food additive excitotoxins and degenerative brain disorders.
912 *Medical Sentinel*, 4(6), 212-215.
913 Blemings, K. and Norlin J. Benevenga, N., 2007. Unique Aspects of Lysine Nutrition
914 and Metabolism. *American Society for Nutrition*, 137(6), pp.1610S-1615S.
915 Boateng, E.F. and Nasiru, M.M., 2019. Applications of ultrasound in meat processing
916 technology: A review. *Food Sci. Technol,* 7, pp.11-15.
917 Brandsma, I., 2006. Reducing sodium: a European perspective. *Food technology*
918 *(Chicago)*, 60(3), pp.24-29.
919 Breslin, P.A.S. and Beauchamp, G.K., 1995. Suppression of bitterness by sodium:
920 variation among bitter taste stimuli. *Chemical senses*, 20(6), pp.609-623.

921 Breslin, P.A.S., 1996. Interactions among salty, sour and bitter compounds. *Trends in*
922 *Food Science and Technology,* 7(12), pp. 390–399.
923 Breslin, P.A.S. and Beauchamp, G.K., 1997. Salt enhances flavour by suppressing
924 bitterness. *Nature*, 387(6633), pp.563-563.
925 Brown, I.J., Tzoulaki, I., Candeias, V. and Elliott, P., 2009. Salt intakes around the
926 world: implications for public health. *International journal of epidemiology*, 38(3),
927 pp.791-813.
928 Buckow, R., Chandry, P.S., Ng, S.Y., McAuley, C.M. and Swanson, B.G., 2014.
929 Opportunities and challenges in pulsed electric field processing of dairy products.
930 *International Dairy Journal*, 34(2), pp.199-212.
931 Busch, J.L.H.C., Yong, F.Y.S. and Goh, S.M., 2013. Sodium reduction: Optimizing
932 product composition and structure towards increasing saltiness perception. *Trends in*
933 *Food Science & Technology*, 29(1), pp.21-34.
934 Campagnol, P.C.B., dos Santos, B.A., Morgano, M.A., Terra, N.N. and Pollonio,
935 M.A.R., 2011a. Application of lysine, taurine, disodium inosinate and disodium
936 guanylate in fermented cooked sausages with 50% replacement of NaCl by KCl. *Meat*
937 *science*, 87(3), pp.239-243.
938 Campagnol, P. C. B., dos Santos, B. A., Wagner, R., Terra, N. N., and Pollonio, M. A.
939 R., 2011b. The effect of yeast extract addition on quality of fermented sausages at low
940 NaCl content. *Meat science*, 87(3), 290-298.

941 Campagnol, P., dos Santos, B., Terra, N. and Pollonio, M., 2012. Lysine, disodium
942 guanylate and disodium inosinate as flavor enhancers in low-sodium fermented
943 sausages. *Meat Science*, 91(3), pp.334-338.
944 Campagnol, P.C.B., dos Santos, B.A. and Rodriguez-Pollonio, M.A., 2017. Strategies
945 to reduce the salt content in fermented meat products. *Strategies for Obtaining*
946 *Healthier Foods*, p.291.
947 Chandrashekar, J., Hoon, M.A., Ryba, N.J. and Zuker, C.S., 2006. The receptors and
948 cells for mammalian taste. *Nature*, 444(7117), pp.288-294.
949 Chaudhari, N. and Roper, S.D., 2010. Review series: The cell biology of taste. *The*
950 *Journal of cell biology*, 190(3), p.285.
951 Cheftel, J.C. and Culioli, J., 1997. Effects of high pressure on meat: a review. *Meat*
952 *science*, *46*(3), pp.211-236.
953 Clariana, M., Guerrero, L., Sárraga, C., Díaz, I., Valero, Á. and García-Regueiro, J.A.,
954 2011. Influence of high pressure application on the nutritional, sensory and
955 microbiological characteristics of sliced skin vacuum packed dry-cured ham. Effects
956 along the storage period. *Innovative Food Science & Emerging Technologies*, *12*(4),
957 pp.456-465.
958 Corral, S., Salvador, A. and Flores, M., 2013. Salt reduction in slow fermented sausages
959 affects the generation of aroma active compounds. *Meat science*, 93(3), pp.776-785.
960 Crehan, C. M., Troy, D. J., and Buckley, D. J., 2000. Effects of salt level and high
961 hydrostatic pressure processing on frankfurters formulated with 1.5 and 2.5% salt. *Meat*
962 *Science,* 55(1), 123-130.

963 Davidson, P.M., Taylor, T.M. and Schmidt, S.E., 2012. Chemical preservatives and
964 natural antimicrobial compounds. *Food microbiology: fundamentals and frontiers*,
965 pp.765-801.
966 Desmond, E., 2006. Reducing salt: A challenge for the meat industry. *Meat science*,
967 74(1), pp.188-196.
968 Desmond, E. and Vasilopoulos, C., 2019. Reducing salt in meat and poultry products.
969 In *Reducing salt in foods* (pp. 159-183). Woodhead Publishing.
970 Delay, R. J., Roper, S. D., & Kinnamon, J. C., 1986. Ultrastructure of mouse vallate
971 taste buds: II. Cell types and cell lineage. *Journal of Comparative Neurology*, 253(2),
972 242-252.
973 Devlieghere, F., Vermeiren, L., Bontenbal, E., Lamers, P.P. and Debevere, J., 2009.
974 Reducing salt intake from meat products by combined use of lactate and diacetate salts
975 without affecting microbial stability. *International journal of food science &*
976 *technology*, 44(2), pp.337-341.
977 Domínguez, R., Pateiro, M., Pérez-Santaescolástica, C., Munekata, P.E.S. and Lorenzo,
978 J.M., 2017. Salt reduction strategies in meat products made from whole pieces.
979 *Strategies for Obtaining Healthier Foods*; *Lorenzo, JM, Carballo, FJ, Eds,* pp.267-289.
980 Dos Santos, B., Campagnol, P., Morgano, M. & Pollonio, M., 2014. Monosodium
981 glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the
982 sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl
983 with KCl. *Meat Science*, 96(1), 509-513.

984 Dos Santos Alves, L.A.A., Lorenzo, J.M., Gonçalves, C.A.A., Dos Santos, B.A., Heck,
985 R.T., Cichoski, A.J. and Campagnol, P.C.B., 2017. Impact of lysine and liquid smoke
986 as flavor enhancers on the quality of low-fat Bologna-type sausages with 50%
987 replacement of NaCl by KCl. *Meat science*, 123, pp.50-56.
988 Dötsch, M., Busch, J., Batenburg, M., Liem, G., Tareilus, E., Mueller, R. and Meijer,
989 G., 2009. Strategies to Reduce Sodium Consumption: A Food Industry
990 Perspective. *Critical Reviews in Food Science and Nutrition*, 49(10), pp.841-851.
991 Doyle, M.E. and Glass, K.A., 2010. Sodium reduction and its effect on food safety,
992 food quality, and human health. *Comprehensive reviews in food science and food safety,*
993 9(1), pp.44-56.
994 Dubow, J.S. and Childs, N.M., 1998. New Coke, mixture perception, and the flavor
995 balance hypothesis. Journal of Business Research, 43(3), pp.147-155.
996 Elliott, E.J. and Simon, S.A., 1990. The anion in salt taste: a possible role for
997 paracellular pathways. Brain research, 535(1), pp.9-17.
998 Fini, M., Torricelli, P., Giavaresi, G., Carpi, A., Nicolini, A. and Giardino, R., 2001.
999 Effect of L-lysine and L-arginine on primary osteoblast cultures from normal and
1000 osteopenic rats. *Biomedicine & Pharmacotherapy*, 55(4), pp.213-220.
1001 Flodin, N.W., 1997. The metabolic roles, pharmacology, and toxicology of lysine.
1002 *Journal of the American College of Nutrition*, 16(1), pp.7-21.
1003 Gaudette, N.J., Pietrasik, Z. and Johnston, S.P., 2019. Application of taste contrast to
1004 enhance the saltiness of reduced sodium beef patties. *LWT*, 116, p.108585.

1005 Geleijnse, J. M., Witteman, J., Stijnen, T., Kloos, M. W., Hofman, A., and Grobbee, D.
1006 E., 2007. Sodium and potassium intake and risk of cardiovascular events and all-cause
1007 mortality: the Rotterdam Study. *European journal of epidemiology*, 22(11), 763-770.
1008 Girgis, S., Neal, B., Prescott, J., Prendergast, J., Dumbrell, S., Turner, C. and
1009 Woodward, M., 2003. A one-quarter reduction in the salt content of bread can be made
1010 without detection. *European journal of clinical nutrition*, *57*(4), pp.616-620.
1011 Gómez, B., Munekata, P.E., Gavahian, M., Barba, F.J., Martí-Quijal, F.J., Bolumar, T.,
1012 Campagnol, P.C.B., Tomasevic, I. and Lorenzo, J.M., 2019. Application of pulsed
1013 electric fields in meat and fish processing industries: An overview. *Food research*
1014 *international,* 123, pp.95-105.
1015 Gómez-Salazar, J.A., Galván-Navarro, A., Lorenzo, J.M. and Sosa-Morales, M.E.,
1016 2021. Ultrasound effect on salt reduction in meat products: a review. *Current opinion*
1017 *in food science*, *38*, pp.71-78.
1018 Grosch, W., 2001. Evaluation of the key odorants of foods by dilution experiments,
1019 aroma models and omission. *Chemical senses*, 26(5), 533-545.
1020 Guilloux, M., Prost, C., Courcoux, P., Le Bail, A. and Lethuaut, L., 2015. How
1021 inhomogeneous salt distribution can affect the sensory properties of salt-reduced multi-
1022 component food: contribution of A mixture experimental design approach applied to
1023 pizza. *Journal of sensory studies*, 30(6), pp.484-498.
1024 Guo, X., Tao, S., Pan, J., Lin, X., Ji, C., Liang, H., Dong, X. and Li, S., 2020. Effects
1025 of L-Lysine on the physiochemical properties and sensory characteristics of salt-
1026 reduced reconstructed ham. *Meat science*, 166, p.108133.

He, F.J. and MacGregor, G.A., 2010. Reducing population salt intake worldwide: from evidence to implementation. *Progress in cardiovascular diseases*, 52(5), pp.363-382.

Henney, J.E., Taylor, C.L. and Boon, C.S., 2010. Taste and flavor roles of sodium in foods: A unique challenge to reducing sodium intake. *Strategies to Reduce Sodium Intake in The United States; National Academies Press: Washington, DC, USA.*

Ho, C. T., Oh, Y. C., and Bae-Lee, M. (1994). The flavour of pork. Flavor of meat and meat products, 38-51.

Houtsma, P. C., De Wit, J. C., & Rombouts, F. M. (1993). Minimum inhibitory concentration (MIC) of sodium lactate for pathogens and spoilage organisms occurring in meat products. International Journal of Food Microbiology, 20(4), 247-257.

Hutton, T. 2002. Sodium: Technological functions of salt in the manufacturing of food and drink products. *British Food Journal*, 104(2):126-152.

Inguglia, E., Zhang, Z., Tiwari, B., Kerry, J. and Burgess, C., 2017. Salt reduction strategies in processed meat products – A review. *Trends in Food Science & Technology*, 59, pp.70-78.

Inguglia, E. S., Tiwari, B. K., Kerry, J. P., and Burgess, C. M., 2018. Effects of high intensity ultrasound on the inactivation profiles of Escherichia coli K12 and Listeria innocua with salt and salt replacers. *Ultrasonics Sonochemistry*, 48, 492-498.

Irshad, A., Sharma, B., Ahmed, S., Talukder, S., Malav, O. and Kumar, A. (2016). Effect of incorporation of calcium lactate on physico-chemical, textural, and sensory properties of restructured buffalo meat loaves. *Veterinary World*, 9(2), pp.151-159.

1048 Ishimaru, Y., 2009. Molecular mechanisms of taste transduction in vertebrates.
1049 *Odontology*, 97(1), pp.1-7.
1050 Iwasaki, T., Noshiroya, K., Saitoh, N., Okano, K., & Yamamoto, K., 2006. Studies of
1051 the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils
1052 and pork meat patty. *Food chemistry*, 95(3), 474-483.
1053 Jeyamkondan, S., Jayas, D.S. and Holley, R.A., 1999. Pulsed electric field processing
1054 of foods: a review. *Journal of food protection*, 62(9), pp.1088-1096.
1055 Jimenez-colmenero, F., Fernandez, P., Carballo, J. and Fernandez-martin, F., 1998.
1056 High-pressure-cooked low-fat pork and chicken batters as affected by salt levels and
1057 cooking temperature. *Journal of Food Science*, *63*(4), pp.656-659.
1058 Kanner, J., Harel, S., and Jaffe, R., 1991. Lipid peroxidation of muscle food as affected
1059 by sodium chloride. *Journal of Agricultural and Food Chemistry*, *39*(6), 1017-1021.
1060 Keast, R.S. and Breslin, P.A., 2003. An overview of binary taste–taste interactions.
1061 *Food quality and preference*, 14(2), pp.111-124.
1062 Keast, R.S., Dalton, P. and Breslin, P.A., 2004. Flavor interactions at the sensory level.
1063 *Flavor perception*, 228.
1064 Keast, R.S. and Roper, J., 2007. A complex relationship among chemical concentration,
1065 detection threshold, and suprathreshold intensity of bitter compounds. *Chemical senses*,
1066 32(3), pp.245-253.
1067 Kemp, S. E., Beauchamp, G. K., 1994. Flavor Modification by Sodium Chloride and
1068 Monosodium Glutamate. *Journal of Food Science.* 59:682-6.

1069 Khan, M.I., Jo, C. and Tariq, M.R., 2015. Meat flavor precursors and factors
1070 influencing flavor precursors—A systematic review. *Meat science*, 110, pp.278-284.
1071 Khaw, K.T. and Barrett-Connor, E., 1984. Dietary potassium and blood pressure in a
1072 population. *The American journal of clinical nutrition*, 39(6), pp.963-968.
1073 Kilcast, D. and Den Ridder, C., 2007. Sensory issues in reducing salt in food products.
1074 In *Reducing salt in foods* (pp. 201-220). Woodhead publishing.
1075 Kim, T.K., Yong, H.I., Jung, S., Kim, H.W. and Choi, Y.S., 2021. Technologies for the
1076 production of meat products with a low sodium chloride content and improved quality
1077 characteristics—A Review. *Foods*, 10(5), p.957.
1078 Lawless, H., Rapacki, F., Horne, J. and Hayes, A., 2003. The taste of calcium and
1079 magnesium salts and anionic modifications. *Food Quality and Preference*, 14(4),
1080 pp.319-325.
1081 Lawrence, T., Dikeman, M., Hunt, M., Kastner, C. and Johnson, D., 2003. Effects of
1082 calcium salts on beef longissimus quality. *Meat Science*, 64(3), pp.299-308.
1083 Leães, Y. S. V., Pinton, M. B., de Aguiar Rosa, C. T., Robalo, S. S., Wagner, R., de
1084 Menezes, C. R., and Cichoski, A. J., 2020. Ultrasound and basic electrolyzed water: A
1085 green approach to reduce the technological defects caused by NaCl reduction in meat
1086 emulsions. *Ultrasonics Sonochemistry*, 61, 104830.
1087 Li, S., Li, L., Zhu, X., Ning, C., Cai, K. and Zhou, C., 2019. Conformational and charge
1088 changes induced by L-arginine and L-lysine increase the solubility of chicken myosin.
1089 *Food Hydrocolloids*, 89, pp.330-336.

Liem, D., Miremadi, F. and Keast, R., 2011. Reducing Sodium in Foods: The Effect on Flavor. *Nutrients*, 3(6), pp.694-711.

Lin, W., Finger, T. E., Rossier, B. C. & Kinnamon, S. C., 1999. Epithelial Na+ channel subunits in rat taste cells: localization and regulation by aldosterone. *J. Comp. Neurol.* 405, 406–420.

Lindemann, B., 1996. Taste reception. Physiol. Rev. 76, 719–766.

Lindemann, B., 1997. Sodium taste. *Curr. Opin. Nephrol. Hypertension* 6, 425–429.

Lindemann, B., 2001. Receptors and transduction in taste. *Nature*, 413(6852), 219-225.

Liu, X., Zheng, L., Zhang, R., Liu, G., Xiao, S., Qiao, X., Wu, Y. and Gong, Z., 2016. Toxicological evaluation of advanced glycation end product Nε-(carboxymethyl)lysine: Acute and subacute oral toxicity studies. *Regulatory Toxicology and Pharmacology*, 77, pp.65-74.

Logan, A., 2006. Dietary Sodium Intake and Its Relation to Human Health: A Summary of the Evidence. *Journal of the American College of Nutrition*, 25(3), pp.165-169.

Luckose, F., Pandey, M.C., Chauhan, O.P., Sultana, K. and Abhishek, V., 2015. Effect of high pressure processing on the quality characteristics and shelf life of low-sodium re-structured chicken nuggets. *Journal of Food & Nutrition Research,* 54(4).

Lund, B., Baird-Parker, A.C., Baird-Parker, T.C., Gould, G.W. and Gould, G.W. eds., 2000. *Microbiological safety and quality of food* (Vol. 1). Springer Science & Business Media.

Luo, J., Nasiru, M.M., Zhuang, H., Zhou, G. and Zhang, J., 2021. Effects of partial NaCl substitution with high-temperature ripening on proteolysis and volatile

compounds during process of Chinese dry-cured lamb ham. *Food Research International,* 140, p.110001.

Lutz, C., Mazur, E. and Litch, N., 2014. Dietary Reference Intakes for Individuals. *Nutrition and diet therapy*, pp. 557-562.

Mariutti, L.R. and Bragagnolo, N., 2017. Influence of salt on lipid oxidation in meat and seafood products: A review. *Food Research International*, *94*, pp.90-100.

Medveďová, A., & Valík, Ľ., 2012. Staphylococcus aureus: Characterisation and quantitative growth description in milk and artisanal raw milk cheese production. Structure and function of food engineering, 71-102.

Miller Jr, I.J., 1991. Taste perception, taste bud distribution and spatial relationships. *Smell and taste in health and disease*, pp.205-233.

Min, B., Cordray, J.C. and Ahn, D.U., 2010. Effect of NaCl, myoglobin, Fe (II), and Fe (III) on lipid oxidation of raw and cooked chicken breast and beef loin. *Journal of agricultural and food chemistry*, 58(1), pp.600-605.

Monahan, F. J., & Troy, D. J., 1997. Overcoming sensory problems in low fat and low salt products. In *Production and processing of healthy meat*, poultry and fish products (pp. 257-281). Springer, Boston, MA.

Moncada, M., Astete, C., Sabliov, C., Olson, D., Boeneke, C., and Aryana, K. J., 2015. Nano spray-dried sodium chloride and its effects on the microbiological and sensory characteristics of surface-salted cheese crackers. *Journal of dairy science*, 98(9), 5946-5954.

1133 Monteiro, A.R.G., Nakagawa, A., Pimentel, T.C. and Sousa, I., 2021. Increasing
1134 saltiness perception and keeping quality properties of low salt bread using
1135 inhomogeneous salt distribution achieved with salt agglomerated by waxy starch. *LWT,*
1136 146, p.111451.
1137 Moody, W. G., 1983. Beef flavor-a review. *Food Technol.*, 37, 227-238.
1138 Mottram, D.S. and Edwards, R.A., 1983. The role of triglycerides and phospholipids in
1139 the aroma of cooked beef. Journal of the Science of Food and Agriculture, 34(5),
1140 pp.517-522.
1141 Mosca, A. C., Bult, J. H., & Stieger, M., 2013. Effect of spatial distribution of tastants
1142 on taste intensity, fluctuation of taste intensity and consumer preference of (semi-) solid
1143 food products. *Food Quality and Preference*, 28(1), 182-187.
1144 Mottram, D., 1994. Meat flavour. *Understanding natural flavors*, (pp. 140-163).
1145 Springer, Boston, MA.
1146 Mottram, D.S., 1998. Flavour formation in meat and meat products: a review. *Food*
1147 *chemistry*, 62(4), pp.415-424.
1148 Mottram, D.S. and Nobrega, I.C., 2002. Formation of sulfur aroma compounds in
1149 reaction mixtures containing cysteine and three different forms of ribose. *Journal of*
1150 *Agricultural and Food Chemistry*, 50(14), pp.4080-4086.
1151 Myers, K., Montoya, D., Cannon, J., Dickson, J. and Sebranek, J., 2013. The effect of
1152 high hydrostatic pressure, sodium nitrite and salt concentration on the growth of Listeria
1153 monocytogenes on RTE ham and turkey. *Meat Science*, 93(2), pp.263-268.

Nakao, S., Ishihara, S., Nakauma, M. and Funami, T., 2013. Inhomogeneous spatial distribution of aroma compounds in food gels for enhancement of perceived aroma intensity and muscle activity during oral processing. *Journal of Texture Studies*, 44(4), pp.289-300.

Offer, G. and Trinick, J., 1983. On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat science*, 8(4), pp.245-281.

O'Flynn, C. C., Cruz-Romero, M. C., Troy, D., Mullen, A. M., and Kerry, J. P., 2014. The application of high-pressure treatment in the reduction of salt levels in reduced-phosphate breakfast sausages. *Meat science,* 96(3), 1266-1274.

Ojha, K.S., Keenan, D.F., Bright, A., Kerry, J.P. and Tiwari, B.K., 2016. Ultrasound assisted diffusion of sodium salt replacer and effect on physicochemical properties of pork meat. *International journal of food science & technology*, *51*(1), pp.37-45.

Okuskhanova, E.; Assenova, B.; Rebezov, M.; Yessimbekov, Z.; Kulushtayeva, B.; Zinina, O.; Stuart, M., 2016. Mineral composition of deer meat pate. *Pak. J. Nutr.*, 15, 217–222

Olsen, N.V., Grunert, K.G. and Sonne, A.M., 2010. Consumer acceptance of high-pressure processing and pulsed-electric field: a review. Trends in Food Science & Technology, 21(9), pp.464-472.

Oziembłowski, M., and Kopeć, W., 2005. Pulsed electric fields (PEF) as an unconventional method of food preservation. *Pol. J. Food Nutr. Sci,* 14(55), 31-35.

Pangborn, R.M., 1962, Taste interrelationships. *Journal of Food Science,* 27(5), pp. 495–500.

1176 Pangborn, R. M., and Braddock, K. S., 1989. Ad libitum preferences for salt in chicken
1177 broth. *Food Quality and Preference*, 1(2), 47-52.
1178 Partearroyo, T., Samaniego-Vaesken, M.D.L., Ruiz, E., Aranceta-Bartrina, J., Gil, Á.,
1179 González-Gross, M., Ortega, R.M., Serra-Majem, L. and Varela-Moreiras, G., 2019.
1180 Sodium intake from foods exceeds recommended limits in the Spanish population: The
1181 ANIBES study. *Nutrients,* 11(10), p.2451.
1182 Paulsen, M. T., Nys, A., Kvarberg, R., and Hersleth, M., 2014. Effects of NaCl
1183 substitution on the sensory properties of sausages: Temporal aspects. *Meat Science*,
1184 98(2), 164-170.
1185 Petit, G., Jury, V., de Lamballerie, M., Duranton, F., Pottier, L. and Martin, J.L., 2019.
1186 Salt intake from processed meat products: Benefits, risks and evolving practices.
1187 *Comprehensive Reviews in Food Science and Food Safety*, 18(5), pp.1453-1473.
1188 Pinton, M.B., dos Santos, B.A., Lorenzo, J.M., Cichoski, A.J., Boeira, C.P. and
1189 Campagnol, P.C.B., 2021. Green technologies as a strategy to reduce NaCl and
1190 phosphate in meat products: An overview. *Current Opinion in Food Science*, *40*, pp.1-
1191 5.
1192 Pretorius, B. and Schönfeldt, H.C., 2018. The contribution of processed pork meat
1193 products to total salt intake in the diet. *Food chemistry*, *238*, pp.139-145.
1194 Public Health England. *Salt Reduction Targets for 2024*; Public Health England:
1195 London, UK, 2020

1196 Puértolas, E., and Barba, F. J., 2016. Electrotechnologies applied to valorization of by-
1197 products from food industry: Main findings, energy and economic cost of their
1198 industrialization. *Food and Bioproducts Processing,* 100, 172-184.
1199 Puolanne, E. and Halonen, M., 2010. Theoretical aspects of water-holding in meat.
1200 *Meat science,* 86(1), pp.151-165.
1201 Purriños, L., Franco, D., Carballo, J. and Lorenzo, J.M., 2012. Influence of the salting
1202 time on volatile compounds during the manufacture of dry-cured pork shoulder "lacón".
1203 *Meat Science*, 92(4), pp.627-634.
1204 Quadros, D. A., Rocha, I. F. O., Ferreira, S. M. R., and Bolini, H. M. A., 2015. Low-
1205 sodium fish burgers: Sensory profile and drivers of liking. *LWT - Food Science and*
1206 *Technology*, 63, 236–242.
1207 Quilaqueo, M., Duizer, L., & Aguilera, J. M., 2015. The morphology of salt crystals
1208 affects the perception of saltiness. *Food Research International,* 76, 675-681.
1209 Rabe, S., Krings, U. and Berger, R.G., 2003. Initial dynamic flavour release from
1210 sodium chloride solutions. *European Food Research and Technology*, 218(1), pp.32-
1211 39.
1212 Rama, R., Chiu, N., Carvalho Da Silva, M., Hewson, L., Hort, J., & Fisk, I. D., 2013.
1213 Impact of salt crystal size on in-mouth delivery of sodium and saltiness perception from
1214 snack foods. *Journal of Texture Studies,* 44(5), 338-345.
1215 Reddy, K.A. and Marth, E.H., 1991. Reducing the sodium content of foods: A review.
1216 *Journal of food protection*, 54(2), pp.138-150.

1217 Rehnberg, B.G., MacKinnon, B.I., Hettinger, T.P. and Frank, M.E., 1993. Anion
1218 modulation of taste responses in sodium-sensitive neurons of the hamster chorda
1219 tympani nerve. *The Journal of general physiology*, 101(3), pp.453-465.
1220 Resconi, V.C., Escudero, A. and Campo, M.M., 2013. The development of aromas in
1221 ruminant meat. *Molecules*, 18(6), pp.6748-6781.
1222 Rios-Mera, J.D., Selani, M.M., Patinho, I., Saldaña, E. and Contreras-Castillo, C.J.,
1223 2021. Modification of NaCl structure as a sodium reduction strategy in meat products:
1224 An overview. *Meat Science*, 174, p.108417.
1225 Rodrigues, F.M., Rosenthal, A., Tiburski, J.H. and Cruz, A.G.D., 2015. Alternatives to
1226 reduce sodium in processed foods and the potential of high pressure technology. *Food*
1227 *Science and Technology*, *36*, pp.01-08.
1228 Roper, S. and Chaudhari, N., 2017. Taste buds: cells, signals and synapses. *Nature*
1229 *Reviews Neuroscience*, 18(8), pp.485-497.
1230 Ruusunen, M. and Puolanne, E., 2005. Reducing sodium intake from meat
1231 products. *Meat Science*, 70(3), pp.531-541.
1232 Shaw, N., 2016. Prevention and treatment of nutritional rickets. *The Journal of Steroid*
1233 *Biochemistry and Molecular Biology*, 164, pp.145-147.
1234 Shelef, L., 1994. Antimicrobial Effects of Lactates: A Review. *Journal of Food*
1235 *Protection*, 57(5), pp.445-450.
1236 Shelef, L.A. and Seiter, J., 2005. Indirect and miscellaneous antimicrobials. *Food*
1237 *science and technology-new York-marcel dekker-,* 145, p.573.

1238 Stadnik, J., Dolatowski, Z. J., and Baranowska, H. M., 2008. Effect of ultrasound
1239 treatment on water holding properties and microstructure of beef (m. semimembranosus)
1240 during ageing. *LWT-Food Science and Technology*, 41(10), 2151-2158.
1241 Stähler, F., Riedel, K., Demgensky, S., Neumann, K., Dunkel, A., Täubert, A., ... &
1242 Meyerhof, W., 2008. A role of the epithelial sodium channel in human salt taste
1243 transduction?. *Chemosensory Perception*, 1(1), 78-90.
1244 Strazzullo P and Leclercq C., 2014. Sodium. *Advance in Nutrition*. 5(2), 188-90.
1245 Stringer, S. C. and Pin, C., 2005. Microbial risks associated with salt reduction in
1246 certain foods and alternative options for preservation. *Institute of Food Research:*
1247 *Norwich.* https://www. food. gov.
1248 uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/acm740a. pdf
1249 Sun, A., Wu, W., Soladoye, O.P., Aluko, R.E., Bak, K.H., Fu, Y. and Zhang, Y., 2022.
1250 Maillard reaction of food-derived peptides as a potential route to generate meat flavor
1251 compounds: A review. *Food Research International*, *151*, p.110823.
1252 Suzuki, T., 2007. Cellular mechanisms in taste buds. *The Bulletin of Tokyo Dental*
1253 *College,* 48(4), pp.151-161.
1254 Tamanna, N. and Mahmood, N., 2015. Food processing and Maillard reaction products:
1255 Effect on human health and Nutrition. *International Journal of Food Science*, 2015, pp.
1256 1–6.
1257 Terrell, R. N., Quintanilla, M., Vanderzant, C., and Gardner, F. A., 1983. Effects of
1258 reduction or replacement of sodium chloride on growth of Micrococcus, Moraxella and
1259 Lactobacillus inoculated ground pork. *Journal of Food Science*, 48(1), 122-124.

Toepfl, S., Siemer, C. and Heinz, V., 2014. Effect of high-intensity electric field pulses on solid foods. *In Emerging technologies for food processing* (pp. 147-154). Academic Press.

Toldrá, F. and Flores, M., 2000. The use of muscle enzymes as predictors of pork meat quality. *Food Chemistry*, 69(4), pp.387-395.

Tordoff, M. (1996). The importance of calcium in the control of salt intake. *Neuroscience & Biobehavioral Reviews*, 20(1), pp.89-99.

Tunieva, E.K. and Gorbunova, N.A., 2017. Alternative methods of technological processing to reduce salt in meat products. *Theory and practice of meat processing*, *2*(1), pp.47-56.

Turantaş, F., Kılıç, G. B., and Kılıç, B., 2015. Ultrasound in the meat industry: General applications and decontamination efficiency. International journal of food microbiology, 198, 59-69.

Van Boekel, M. A. J. S., 2006. Formation of flavour compounds in the Maillard reaction. *Biotechnology advances*, 24(2), 230-233.

Vanderklaauw, N. J., & Smith, D. V., 1995. Taste quality profiles for 15 organic and inorganic salts. Physiology & Behavior, 58(2), 295-306.

Vidal, V.A., Santana, J.B., Paglarini, C.S., da Silva, M.A., Freitas, M.Q., Esmerino, E.A., Cruz, A.G. and Pollonio, M.A., 2020. Adding lysine and yeast extract improves sensory properties of low sodium salted meat. *Meat Science*, 159, p.107911.

Warner, R. D., McDonnell, C. K., Bekhit, A. E. D., Claus, J., Vaskoska, R., Sikes, A., ... and Ha, M., 2017. Systematic review of emerging and innovative technologies for meat tenderisation. *Meat science*, 132, 72-89.

Weaver, R.A. and Shelef, L.A., 1993. Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage. *Journal of Food Safety*, 13(2), pp.133-146.

Werkhoff, P., Bruening, J., Emberger, R., Guentert, M., Koepsel, M., Kuhn, W., & Surburg, H., 1990. Isolation and characterization of volatile sulfur-containing meat flavor components in model systems. *Journal of Agricultural and Food Chemistry,* 38(3), 777-791.

Wolfe, R.R., 2017. Branched-chain amino acids and muscle protein synthesis in humans: myth or reality?. *Journal of the International Society of Sports Nutrition*, 14(1), p.30.

World Health Organization, 2011. Codex Alimentarius: general standard for food additives. *Codex Alimentarius: general standard for food additives*.

World Health Organisation, 2020. Salt Reduction, Fact Sheets. Retrieved 3 November 2020 from https://www.who.int/news-room/fact-sheets/detail/salt-reduction

Woskow, M. H. Selectivity in flavor modification by 50-ribonucleotides. *Food Technology*. 1969,23:32-7.

Wu, H., Zhang, Y., Long, M., Tang, J., Yu, X., Wang, J., and Zhang, J., 2014. Proteolysis and sensory properties of dry-cured bacon as affected by the partial substitution of sodium chloride with potassium chloride. Meat science, 96(3), 1325-1331.

Xiong, Y.L., 2017. Structure-function relationships of muscle proteins. In *Food proteins and their applications* (pp. 341-392). CRC Press.

Xiong, Y., Deng, B., Warner, R.D. and Fang, Z., 2020. Reducing salt content in beef frankfurter by edible coating to achieve inhomogeneous salt distribution. International *Journal of Food Science & Technology*, 55(8), pp.2911-2919.

Yamaguchi, S. and Takahashi, C., 1984. Interactions of monosodium glutamate and sodium chloride on saltiness and palatability of a clear soup. Journal of Food Science, 49(1), pp.82-85.

Yamamoto, K. and Ishimaru, Y., 2013. Oral and extra-oral taste perception. *Seminars in Cell & Developmental Biology*, 24(3), pp.240-246.

Yang, X., Sebranek, J.G., Luo, X., Zhang, W., Zhang, M., Xu, B., Zhang, Y. and Liang, R., 2021. Effects of calcium salts on the physicochemical quality of cured beef sausages during manufacturing and storage: A potential calcium application for sausages with alginate casings. *Foods*, 10(11), p.2783.

Zanfirescu, A., Ungurianu, A., Tsatsakis, A.M., Nițulescu, G.M., Kouretas, D., Veskoukis, A., Tsoukalas, D., Engin, A.B., Aschner, M. and Margină, D., 2019. A review of the alleged health hazards of monosodium glutamate. *Comprehensive reviews in food science and food safety*, 18(4), pp.1111-1134.

Zheng, Y., Xu, P., Li, S., Zhu, X., Chen, C. G., & Zhou, C. L., 2017. Effects of L-lysine/L-arginine on the physicochemical properties and quality of sodium-reduced and phosphate-free pork sausage. *International Journal of Nutrition and Food Sciences*, 6(1), 12-18.

1324 Zhou, B., Lee, H. and Feng, H., 2012. Microbial decontamination of food by power
1325 ultrasound. *In Microbial decontamination in the food industry* (pp. 300-321).
1326 Woodhead Publishing.
1327 Zhu, Y., Yan, Y., Yu, Z., Wu, T. and Bennett, L.E., 2022. Effects of high pressure
1328 processing on microbial, textural and sensory properties of low-salt emulsified beef
1329 sausage. *Food Control*, 133, p.108596.

Chapter 2. Interactions of umami with the four other basic tastes in equi-intense aqueous solutions

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Abstract

Previous research has shown that the addition of equi-intense concentrations of taste compounds leads to mixture suppression, with sweetness being the least suppressed taste while being the strongest suppressor of the other taste stimuli. However, perceived intensity of umami (savoury) within complex mixtures is less defined. Since maintaining savoury taste of foods at reduced salt levels is a growing need, this study aims to investigate the role of umami in complex taste systems. Initially the concentrations of single tastants were adjusted until a trained sensory panel rated them as equi-intense using general labelled magnitude scale (gLMS). In order to evaluate the impact of umami taste on other tastes, and vice versa, three sample sets were prepared as binary and quinary systems. The first two sets utilised monosodium glutamate (MSG) as the umami tastant; one set without balancing the sodium level in MSG (sodium unbalanced) and another set accounting for it by the addition of sodium at an equivalent molarity to all but the umami single tastant solution (sodium balanced). The third set used monopotassium L-glutamate monohydrate (MPG) as the source of umami to overcome the confounding influence of sodium. All samples were rated by trained sensory panellists. The results of the three studies conclude that umami taste does not enhance or suppress the perception of any other taste in binary aqueous taste systems

($p > 0.05$); whereas sweet, salty, sour and bitter significantly suppress the perception of umami in both binary and quinary systems ($p < 0.05$).

2.1 Introduction

Cross-modal interactions between two or more sensory modalities, have been investigated as a strategy for the reduction of salt and sugar (Ponzo et al., 2021). For example, odour-taste interactions have been explored for the reduction of sugar (Velazquez et al., 2020) and the reduction of salt (Thomas-Danguin, Guichard & Salles, 2019; Emorine et al., 2021). Mojet et al. (2004) described how taste-taste interactions influenced taste in various real foods, and found that tastants evoking salty, sweet, bitter or umami could alter the perception of one or more other taste qualities in the product which they had been added to. Such taste-taste interactions can be useful in salt reduction strategies. For example, where potassium chloride (KCl) is used to replace sodium chloride (NaCl) it can increase bitterness in the final product; however, Abu et al. (2018) found that adding sweetness (via trehalose or sucrose) to a KCl/NaCl mixture effectively reduced bitterness without changing saltiness. Therefore taste-taste interactions are of relevance to the food scientist, with applications in salt and sugar reduction continuing to be a growing interest.

Psychophysical functions are used to study and express relationships between a stimulus and a response, or perceived sensation, such as taste. For individual taste stimuli, as the physical concentration increases the perceived intensity elicited by that compound also increases, but the rate of increase is not always directly proportional. It is dependent on both the specific tastant and whether the concentration is at relatively

low levels (just above threshold, accelerating relationship), moderate levels (linear relationship) or high levels (decelerating relationship) (Bartoshuk, 1975; McBride, 1987).

Such stimulus response relationships are subsequently modified in tastant mixtures. In a previous review, Keast and Breslin (2002a) concluded that perception of binary taste mixtures is dependent on the position of the taste stimulus on the psychophysical curve. Whether the concentration is within the linear or decelerating (plateau) phase of the curve, helps predict whether a particular tastant would cause enhancement or suppression within a tastant mixture. In an earlier paper, McBride (1993) noted that the binary mixing of two different tastants produces three senses: an overall total intensity and a sensation from each of the two components; he suggested that the total intensity would be determined only by the strength of the stronger components.

In the case of more complex ternary and quaternary taste combinations, Bartoshuk (1975) found that tastants suppressed each other. The extent of suppression was dependent upon the function of the individual tastant; tastes where perception increased sharply with increasing concentration tended to cause greater suppression. Similarly on studying a tertiary taste mixture's intensity of sucrose, fructose, and citric acid, McBride and Finlay (1990) found that the total perceived strength of the mixture was determined by the perceptual intensity of the individual stronger components, and the sweetness and sourness of the mixture tended to suppress each other. Taking a modelling approach to understand the psychophysics of taste interaction, Schifferstein

and Frijters (1993) concluded that a summation model (addition of individual component intensities) was sufficiently able to predict total taste intensity of a mixture. Since many foods are formulated with tastants at moderate and not extreme levels, it is likely that the influence of taste stimuli in the linear phase of the psychophysical curve might be the most relevant. The approach taken by Green et al. (2010) focused on taste mixtures combined at perceptually equi-intense moderate (not extreme) concentrations. They tested taste interactions in the four taste mixtures (salt, sweet, bitter and sour) using equi-intense concentrations of sodium chloride, sucrose, quinine sulfate and citric acid. Moreover, four tastes qualities in binary, ternary and quaternary mixtures were also investigated. They concluded that suppression between stimuli in binary mixtures could predict taste perception in more complex combinations. For example, the sweet taste of sucrose tended to be the least suppressed quality, whereas it was a potent suppressor to all other tastes.

Umami tastants are widely used as flavour enhancers in food products, and especially in developing salt-reduced foods. In practice such enhancement may result from complex ingredients, such as yeast extracts, that comprise both amino acids (especially glutamate) and 5′- nucleotides. However, literature often focuses on the understanding of simpler systems. A review paper by Maluly et al (2017) recommended that monosodium glutamate (MSG) could be used to reduce NaCl in a broad range of foods. In specific applications, Yamaguchi and Takahashi (1984) demonstrated that MSG could be used to reduce NaCl in a Japanese soup (Sumash-Jiru). Where MSG is used in combination with 5′-nucleotides, such as inosine-5′-monophosphate (IMP) and

guanosine-5′-monophosphate (GMP), a much stronger umami taste can be achieved. Yamaguchi and Kimizuka (1979) found that the perceived umami intensity was affected by the ratio of IMP to MSG, and more recently Yamaguchi summarized that maximum taste intensity could be achieved with a 70:30 ratio of IMP to MSG (Yamaguchi, 1998). In using a combination of umami tastants, Dos et al. (2014) found that MSG, disodium inosinate, disodium guanylate could enhance flavour and maintain saltiness at 50% reduced NaCl when added into fermented cooked sausages.

However, there is limited understanding about how MSG performs in mixture of tastants, and how it interacts with other tastants, especially at equi-intense levels. Indeed, some of the findings in the literature appear contradictory which is perhaps due to the differences in levels, compounds, and test strategies applied in the sensory test. The early study by Woskow (1969), investigated the effects of umami on other tastes, but not vice versa. The study used a series of 50:50 combination of disodium 5′-inosinate and disodium 5′-guanylate from low to moderate levels (0.1mM to 0.5mM), while MSG was not included. This umami combination was found to enhance sweetness and saltiness but suppress sourness and bitterness. Reporting on work from their laboratory in 1979, Yamaguchi (1998) noted that MSG slightly enhanced saltiness from NaCl, but only at high MSG concentrations, and found that NaCl had no substantial influence on the perception of umami, while all other tastes did suppress umami. Kemp and Beauchamp (1994) demonstrated that at threshold levels, MSG had no influence on sweet, salt, sour and bitter, while at supra-threshold concentrations it suppressed sweet and bitter tastes and enhanced salt perception.

1439 Table 2.1: Summary of previous studies investigating the influence of umami taste in combined tastant aqueous mixtures.

Reference	Umami Tastant: Compound, Concentration and Recorded Intensity	Additional Tastants: Compounds, Concentration and Recorded Intensity	Sensory scale/ sensory test employed	Panelist type	Effect of Umami on Other Tastes*
Woskow (1969)	A 50:50 mixture of disodium 5'-inosinate and disodium 5'-guanylate: (0.1, 0.2, 0.3, 0.4, 0.5mM)	Quinine sulfate: 0.007 mM Citric acid: 0.005M NaCl: 0.09M Sucrose: 0.16M	Paired comparison: participants chose which one of the two was more bitter/sour/salty/sweet	11 volunteers (no information on their ability to discriminate, detect and recognize the different tastes)	Sweet ↑ (at 0.2mM and 0.4 mM of 5'Nucl) Salty ↑ (only at 0.5 mM) Sour ↓ (at concentrations ≥ 0.2mM of 5'Nucl) Bitter ↓ (at concentrations ≥ 0.2mM of 5'Nucl)

Kemp & Beauchamp (1994)	MSG: 0, 0.32mM (below detection threshold), 0.98mM (ca. detection threshold), 0.032M (moderately intense) and 0.059M (above level commonly found in foods)	Sucrose: 0.05M Citric acid: 0.0013M Quinine Sulfate: 0.025mM NaCl: 0.025M Tastes were all easily detected and were of moderate strength.	Ranking procedure. Sip and spit, rinsing after each test. They were allowed to re-taste as often as necessary.	15 trained panelists (screened for their ability to detect, recognize discriminate tastes)	Sweet ↓ Salty ↑ Sour = Bitter ↓ All changes found were at supra-detection threshold concentrations (0.032M and 0.059M).
Keast & Breslin (2002b)	MSG: 0.02M NaAMP: 0.02M	Pseudoephedrine: 0.01mM Ranitidine: 0.004M Acetaminophen: 0.05M Quinine: 0.0001M Urea: 1.2M (all scored moderate on gLMS)	General labelled magnitude scale (gLMS). Sip and spit, rinsing with water at least 4 times	14 trained panelists	Bitter ↓
Lioe *et al.*, 2005	MSG: 0.004M	NaCl: 0.08M	Ranking test. Taste and swallow	10 trained panelists	Salty ↑

*↑ enhancement, ↓ suppression, = no effect.

** Data reported as %, converted to Molarity assuming %w/v

The findings of Kemp and Beauchamp (1994) for bitterness suppression corroborates the work of Woskow (1969), which is perhaps unsurprising as the levels of bitter tastant, quinine sulfate, were relatively similar (0.007 and 0.025 mM respectively) in the two studies and the perceived intensity of MSG at the medium level was similar to the recorded umami intensity of the two ribonucleotides in the earlier study. However, for saltiness, Woskow (1969) concluded that ribonucleotides enhanced salty taste at moderate concentration (≥ 0.2mM), whereas Kemp and Beauchamp (1994) reported the enhancement of umami taste on salty taste only happened at high concentration of MSG (0.032mM and 0.059mM), as also concluded by Yamaguchi (1998). In relation to sweet taste, the conflicting result is likely to be due to the difference in sucrose levels used between the two studies. Sweetness was enhanced when the sucrose levels was 5% (w/v) or 0.16 M (Woskow, 1969), whereas it was suppressed when the level was three times lower at 0.05 M (Kemp & Beauchamp, 1994).

Bitterness suppression was later confirmed by Keast and Breslin (2002b), concluding that when using either MSG or adenosine monophosphate sodium salt (NaAMP), the bitter taste of any of five different bitter tastants was suppressed. However, according to the research by Fuke and Ueda (1996), NaAMP does not evoke umami taste alone, hence, inferring that taste suppression may not require the suppressing tastant to be perceived. Bitter and umami tastes are mediated via G-protein-coupled receptors, T1Rs and T2Rs which are found in type II taste receptor cells (Bachmanov & Beauchamp, 2007). Kim et al. (2015) established that the suppression of bitter taste by umami could occur at a cellular level, by investigating umami-bitter taste interactions with a cell-

based assay using hTAS2R16-expressing cells. They tested the effect of five umami peptides (Glu-Asp, Glu-Glu, Glu-Ser, Asp-Glu-Ser, and Glu-Gly-Ser) on the bitter tastant salicin and found that the glutamayl peptides inhibited the salicin-induced intracellular Ca2+ response. Specifically, the Glu-Glu peptide suppressed salicin-induced activation of hTAS2R16 to a greater extent compared with the probenecid, a specific antagonist of hTAS2R16.

Previous studies have considered taste-taste interactions within ternary and quaternary mixtures (Bartoshuk, 1975; Breslin & Beauchamp, 1997; Green et al., 2010). Breslin and Beauchamp (1997) investigated the interaction between sweet, salt and bitter, and found that bitter (urea) and sweet (sucrose) suppressed each other when mixed together. However, when salt (sodium acetate) was added the bitterness substantially decreased and the sweetness increased. While these papers focused on complex tastant mixtures, umami tastants were not included, and there are few studies exploring the specific interaction between umami and saltiness along with other basic tastes i.e., sweet, bitter and sour. Therefore, the aim of this study is to explore the effect of umami on the perception of other taste stimuli and vice versa. Progressing understanding from previous literature, this study specifically hypothesised that in an equi-intense aqueous solution umami would neither enhance saltiness/sweetness/bitterness, nor be suppressed by other tastes, anticipating therefore by the summation model that the overall savoury sensation would be increased by adding umami compounds.

2.2 Materials and Methods

2.2.1 Panelists

A total of 12 trained sensory panelists (11 females and 1 male, age 35 to 65) participated in all experiments. They were also screened for their detection, discrimination and description ability. All panelists were healthy and had no taste or olfactory defects or disorders. They were all employed as sensory panelists and provided consent through their employment to taste foods and for their data to be used.

2.2.2 Stimulus

The taste stimuli used (indicated in Table 1) were aqueous solutions of sucrose (granulated sugar, Co-op Food, Manchester, UK) for the taste quality sweet (S), sodium chloride (table salt, Co-op Food, Manchester, UK) for salty (N), citric acid (Sigma-Aldrich, Gillingham, UK) for sour (C), quinine hemisulfate salt monohydrate (Sigma-Aldrich, Gillingham, UK) for bitter (Q), monosodium glutamate MSG and monopotassium L-glutamate monohydrate (MPG) (Ajinomoto, Paris, France) for the taste quality umami (U). Each tastant solution was prepared in mineral water (Harrogate Spa, UK) a day before the panel session and kept in the fridge (4 °C) overnight. All tastant solutions were taken out of the fridge prior to the test to equilibrate to ambient temperature, then 15 mL of the sample was poured into 20 mL transparent polystyrene cups labeled with three-digit random codes and were served to the panel at ambient temperature (22 ± 2 °C).

2.2.3 Training

Prior to the data collection, all panelists participated in training on the use of the general labelled magnitude scale (gLMS). Compared to labelled magnitude scale (LMS) first developed by Green et al. (1993), the top of gLMS is defined as “strongest imaginable

of any sensation", which is more suitable for this experiment where intensity across modalities is compared (Bartoshuk et al., 2004). The descriptors of the magnitude estimates were "barely detectable", "weak", "moderate", "strong", "very strong" and "strongest imaginable of any sensation" (anchored values on gLMS scale 0.14, 0.76, 1.12, 1.52, 1.70, 1.98; exponentiated values 1.38, 5.01, 15.9, 31.6, 50.1 and 95 respectively) (Bartoshuk et al., 2004).

During the training period, panelists were asked to rate the taste intensity of the five basic taste stimuli respectively. The concentration of each stimulus used in this experiment was finalized when each stimulus was perceived as equi-intense (within the range from 'strong' to 'very strong' sensation on gLMS) by the panel. The training for finalizing the choice of concentration for stimuli was completed in three days.

2.2.4 Tastants preparation

Each of the three experiments described below in detail, contained a total of 10 tastants, including five single tastant solutions and five tastant mixtures (four binary, one quinary). All 12 panelists took part in all three experiments. After the training session, the first set of solutions (Experiment 1) using MSG as the source of umami with sodium unbalanced (UB) was scored by the panel, which were followed by solutions using MSG as the source for umami with sodium balanced (B) (Experiment 2). Finally, the panel was required to taste the third set of solutions (Experiment 3) which were prepared using MPG as the source for umami. For the three experiments, scoring for the samples were completed within two days.

2.2.4.1 Experiment 1:MSG as the source of umami with sodium unbalanced (UB)

Based on the training results to determine equi-intensity, the single stimulus was selected at concentrations with the mean panel scores being between strong and very strong on the gLMS. The concentration of each tastant was kept constant in each binary and quinary tastant mixture as seen in Table 2.2.

2.2.4.2 Experiment 2: MSG as the source for umami with sodium balanced (B)

NaCl contains 39.34% (w/w) sodium whereas MSG contains 13.6% (w/w) sodium. Therefore, the experiment was designed to ensure that sodium levels were balanced in all samples. To achieve this, 0.015 M NaCl was added to all single tastants except MSG (Table 2.2). Based on the training results to determine equi-intensity, the single stimulus was selected at concentrations with the mean panel scores being between strong and very strong on the gLMS. The concentration of each tastant was kept constant in each binary and quinary tastant mixture as seen in Table 2.2.

2.2.4.3 Experiment 3: MPG as the source for umami

In order to remove the possible influence of sodium in glutamate when evaluating saltiness and umami, the source for the taste quality of umami was changed to MPG. The concentration of each tastant was also adjusted to achieve a slightly lower equi-intensity on the gLMS between the descriptors moderate and strong, which allows a liner relationship between stimuli and response on the psychophysical curve as the one achieved in experiments 1 and 2 (Table 2.2).

Table 2.2 Concentration of tastants used in binary and quinary mixture sets

Sample*	Experiment 1: Concentration used in MSG (sodium unbalanced) set MSG (UB)	Experiment 2: Concentration used in MSG (sodium balanced) set MSG (B)	Experiment 3: Concentration used in MPG set
S	S 0.19 M	S 0.19 M + N 0.015M	S 0.10 M
N	N 0.08 M	N 0.08 M + N 0.015M	N 0.05 M
C	C 0.005 M	C 0.005 M + N 0.015M	C 0.004 M
Q	Q 0.025 mM	Q 0.025mM + N 0.015M	Q 0.02 mM
U	U 0.015 M	U 0.015M	U 0.01 M
U+S	S 0.19M, U 0.015M	S 0.19M, U 0.015M	S 0.10M, U 0.01M
U+N	N 0.08M, U 0.015M	N 0.08M, U 0.015M	N 0.05M, U 0.01M
U+C	C 0.005 M, U 0.015M	C 0.005 M, U 0.015M	C 0.004 M, U 0.01M
U+Q	Q 0.025mM, U 0.015M	Q 0.025mM, U 0.015M	Q 0.02mM, U 0.01M
U+S+N+C+Q	S 0.19M, N 0.08M, C 0.005 M, Q 0.025mM, U 0.015M	S 0.19M, N 0.08M, C 0.005 M, Q 0.025mM, U 0.015M	S 0.10M, N 0.05M, C 0.004 M, Q 0.02mM, U 0.01M

*S = sucrose; N = sodium chloride; C = citric acid; Q = quinine hemisulfate salt monohydrate; U = monosodium glutamate (MSG) or potassium L-glutamate monohydrate (MPG)

2.2.5 Sensory evaluation

The experiments were conducted within a standard sensory environment using individual sensory booths, artificial daylight and controlled room temperature (22 ± 2 ℃). All samples were blind-coded and presented monadically. During tasting sessions, panelists were instructed to sip and hold the stimulus in their mouths for five seconds before swallowing and rating six attributes for each sample as follows: overall taste intensity, sweet, salty, sour, bitter and umami intensity. Between samples, the panel was instructed to cleanse their palate with plain crackers and water (filtered tap water at room temperature) to return the mouth back to a neutral state; an automatic reminder appeared during the countdown of ninety seconds between each stimulus after evaluating consecutive taste samples. Within each experiment scoring sessions included 10 samples and 2 replicates scored across two days. Sample presentation order was balanced across panelists; they each received different sample orders between each other, between replicates and between experiments. Data were captured using the sensory software Compusense® (cloud version, Guelph, Ontario).

2.2.6 Data analysis

Data from each of the three experiments was analysed separately. Log data from each panelist from the gLMS were captured by Compusense®. Data were exponentiated. Two-way analysis of variance (ANOVA) was carried out using Senpaq (QI Statistics, Reading, UK) where panelists were treated as random effects and samples as fixed effects, main effects were tested against the assessor by sample interaction. Multiple

pairwise comparisons were carried out using Tukey's HSD at a significance level of 0.05.

2.3 Results and discussion

The mean scores of perceived taste intensity for all single tastes and taste mixtures are given in Figures 2.1 to 2.3 (further statistical details given in supplementary Table 4 to 6). The aim was to have all single tastants rated "strong to very strong" on the gLMS (1.52 to 1.70 on the log scale, or 31.6 to 50.1 exponentiated values) in both the sodium unbalanced and balanced sets. Although panelists were extensively trained on each single tastant, saltiness and sourness were rated slightly lower than "strong". However, the mean ratings (exponentiated data) only fell below this descriptor by a maximum of 0.4 units, therefore it is suggested that this would not have greatly influenced the results. For samples using MPG as source of umami taste, all single tastants were rated as "moderate to strong" on the gLMS (1.21 to 1.52 on the log scale, or 15.85 to 31.62 as exponentiated values), while the concentration of tastants used was slightly lower in comparison to the MSG set samples.

2.3.1 Intensity of umami

The ratings of perceived intensity of umami in the different experiments are presented in Figure 2.1. It is clear from this figure that the perception of umami was significantly suppressed by all other tastes in both the binary and quinary mixtures. In all experiment sets, all the taste mixtures containing MSG were significantly ($p < 0.05$) lower in perceived umami intensity compared to MSG alone (U). The umami sensation was reduced from just above "strong" to "moderate" or "weak" in virtually all cases. The

main exceptions were where the binary mixture was with sodium chloride (U+ N), this led to a lower reduction in umami, leading to “moderate” sensation rather than “weak”. The intensity of umami in the quinary taste systems (U+S+N+C+Q) was the lowest for all experiment sets.

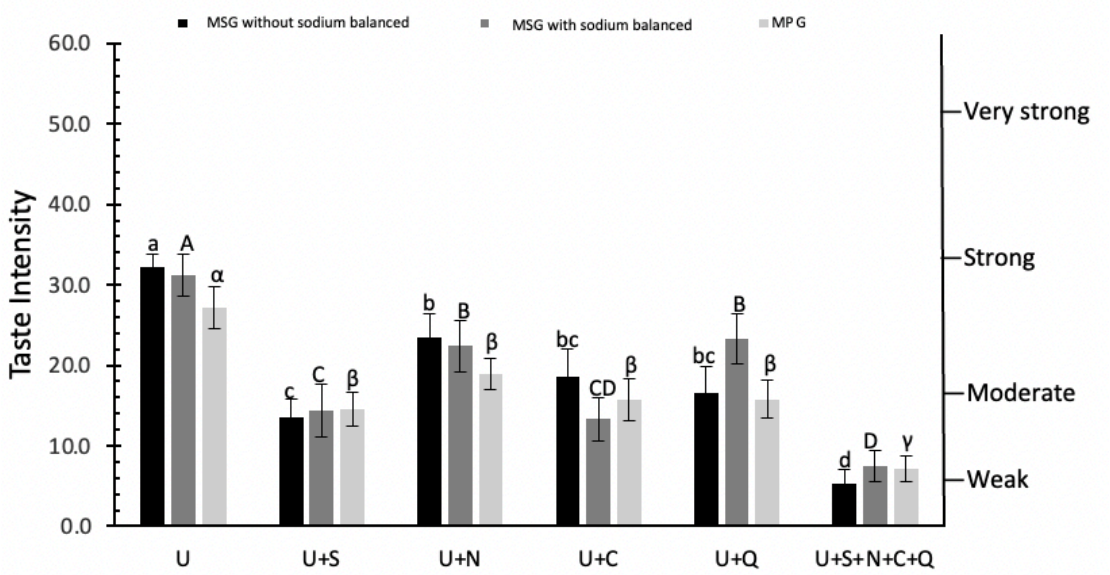

Figure 2.1. Ratings of perceived intensity (exponentiated values) of umami in the sodium unbalanced and balanced sets and using MPG as source of umami taste set. S = sucrose; N = sodium chloride; C = citric acid; Q = quinine hemisulfate salt monohydrate; U = monosodium glutamate (MSG) or potassium L-glutamate monohydrate (MPG). Within each sample set statistically significant differences between samples for the primary taste quality are indicated by different letters above the bar ($p < 0.05$). Lower case letters use for Experiment 1:MSG without salt balanced, upper case letters use for Experiment 2: MGS with salt balanced, and Greek letters use for Experiment 3: MPG.

2.3.2 Intensity of other tastes

The ratings of perceived intensity of sweetness, saltiness, sourness and bitterness can be seen in Figure 2.2. The umami taste did not enhance or suppress the perceived intensity of any other taste in the binary taste systems ($p > 0.05$) (further statistical details given in supplementary Table 4 to 6). This is an unusual phenomenon as all other taste modalities will suppress each other when added together (Green et al., 2010), and yet the addition of MSG as an umami tastant has neither suppressed, nor enhanced,

perception of the other four tastes. Kemp and Beauchamp (1994) concluded that MSG at medium concentration (0.032M) suppressed sweet and bitter tastes and at higher concentrations (0.059M) enhanced salty taste. The MSG levels used by Kemp and Beauchamp (1994) are higher than the 0.015M used in the current study which may have partly led to the different findings. However, the main reason is likely to be the different concentration of the other tastants. The present study used 0.19 M sucrose and 0.005 M citric acid for equi-intense perception of "strong to very strong".

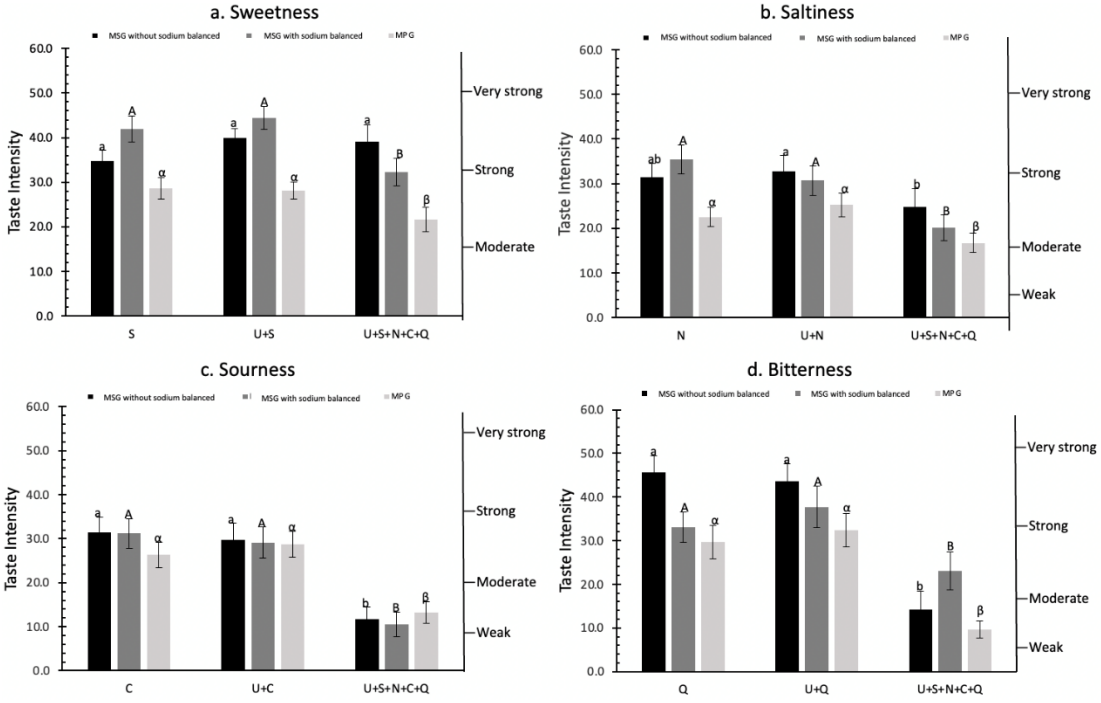

Figure 2.2. Ratings of perceived intensity (exponentiated values) of sweetness (a), saltiness (b), sourness (c), and bitterness (d) in the sodium unbalanced and balanced sets and using MPG as source of umami taste set. S = sucrose; N = sodium chloride; C = citric acid; Q = quinine hemisulfate salt monohydrate; U = monosodium glutamate (MSG) or potassium L-glutamate monohydrate (MPG). Within each sample set statistically significant differences between samples for the primary taste quality are indicated by different letters above the bar ($p < 0.05$). Lower case letters use for Experiment 1:MSG without salt balanced, upper case letters use for Experiment 2: MGS with salt balanced, and Greek letters use for Experiment 3: MPG.

2.3.3 Overall taste intensity

The ratings of perceived intensity of overall taste in the different experiments are presented in Figure 2.3. Results indicated that the total taste intensity of binary mixtures

was very similar to the total overall taste intensity of single tastants ($p > 0.05$), except for quinine hemisulfate with umami mixture (U+Q) in the sodium balanced set and sodium chloride with umami mixture (U+N) in MPG set, where the binary mixture was significantly higher in overall taste intensity ($P<0.05$). The total taste intensity of the quinary solution had a higher mean rating than all binary mixtures. In particular, it had a significantly higher rating compared to the binary mixture with citric acid (U+C) in both MSG sessions, and the binary mixture with sodium chloride (U+N) in sodium balanced set and MPG set ($p < 0.05$). The perception of all five tastes were all significantly and substantially lower in the quinary mixtures than as single tastants ($p < 0.05$) in the sodium balanced set and MPG set. In the sodium unbalanced set, sour, bitter and umami tastes were similarly significantly lower in the quinary mixtures than as single tastants ($p < 0.05$).

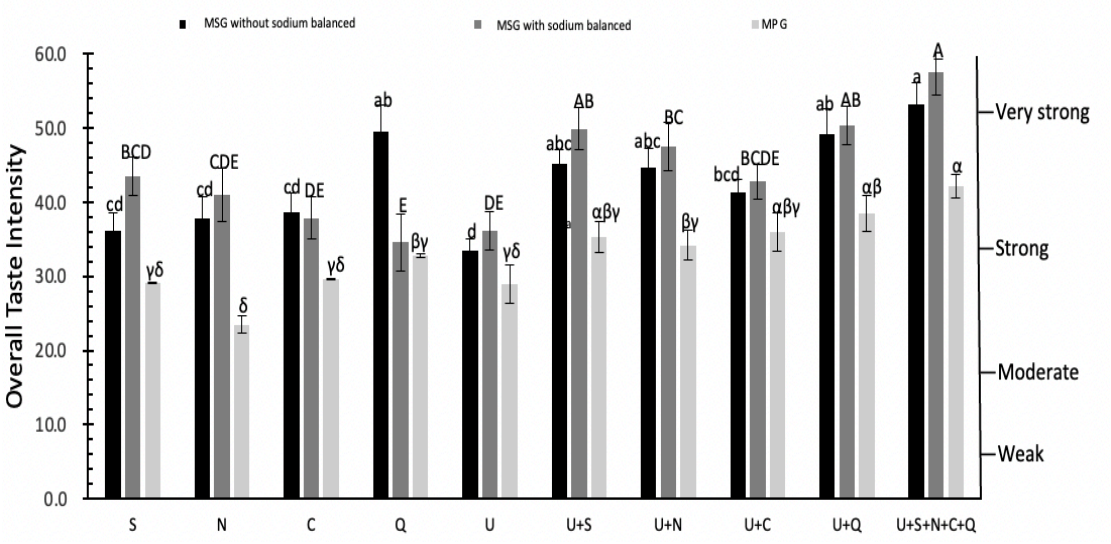

Figure 2.3. Ratings of perceived intensity (exponentiated values) of overall taste in the sodium unbalanced and balanced sets and using MPG as source of umami taste set. S = sucrose; N = sodium chloride; C = citric acid; Q = quinine hemisulfate salt monohydrate; U = monosodium glutamate (MSG) or potassium L-glutamate monohydrate (MPG). Within each sample set statistically significant differences between samples for the primary taste quality are indicated by different letters above the bar ($p < 0.05$). Lower case letters use for Experiment 1:MSG without salt balanced, upper case letters use for Experiment 2: MGS with salt balanced, and Greek letters use for Experiment 3: MPG.

The binary mixture with quinine hemisulfate (U+Q) had a significantly higher overall taste intensity than the sample of quinine hemisulfate alone (Q) only in sodium balanced set ($p < 0.05$), but not in sodium unbalanced set and MPG set. This could possibly be due to the inclusion of 0.015mM NaCl in quinine solution in the sodium balanced set. Keast and Breslin (2002a) reported that NaCl has suppression effect on the bitterness perception at low, medium and high intensity level. Therefore, 0.015M salt addition would lead to a lower intensity of bitterness for quinine solution in sodium balanced set (Experiment 2), while it is not the case in sodium unbalanced set (Experiment 1) and MPG set (Experiment 3). As the total overall intensity is determined by the dominant taste (bitterness), as a result, a low overall taste intensity in quinine hemisulfate alone solution (Q) was expected compared with that in quinine hemisulfate with umami mixture (U+Q) in sodium balanced set. The binary mixture of MPG and NaCl (U+N)

had a significantly higher overall taste intensity than the sample of NaCl (N) alone ($p < 0.05$). This indicates that umami may enhance the total intensity of a salt solution without enhancing the specific taste modality (saltiness) in the MPG mixture. The binary mixtures of U+N in the MSG sample set had a similar trend, but the differences were not significant ($p > 0.05$). These differences may be associated with the difference in concentrations used in the MSG and MPG sets (0.08M or 0.095M vs 0.05M). Finally, the total taste intensity of the quinary solution was the strongest, with all single tastants having a significantly and substantially lower overall taste intensity than the quinary mixtures except quinine hemisulfate ($p < 0.05$).

2.3.4 Taste interaction

The testing of the balanced sodium sample set allowed for an unbiased investigation of the influence of glutamate and the perception of all other tastes, and of the effect of sodium on glutamate, without the sodium within the MSG as a confounding factor. In conclusion, the results from both the sodium unbalanced and balanced trials were the same, increasing the confidence in the overall finding that umami from glutamate does not enhance or suppress other tastes when all tastes are presented at strong (but not excessive) intensity levels. The findings in this MPG set again confirmed that all other tastes suppressed umami ($p < 0.05$), whereby all binary mixtures had significantly lower umami intensity than MPG alone ($p < 0.05$), and the quinary mixture was significantly and very substantially lower in umami taste ($p < 0.05$). The results agree with the first two studies that the umami taste did not enhance or suppress the perceived intensity of

any other taste in the binary taste systems ($p > 0.05$), all other tastes could suppress the perception of umami taste in binary and quinary mixture ($p < 0.05$).

2.4 Discussion

The purpose of this work is to understand the interaction between umami and the other four tastes. However, it is unavoidable to have the impact of different cations involved when selecting glutamate, the predominant taste compound of umami. Therefore, different approaches were considered to make the results conclusive, including raising the Na^+ concentration when MSG was used, and using K^+ to remove the potential effect of Na^+ on saltiness and umami. However, their impact on the saltiness and umami taste is negligible. At low sodium concentrations, the afferent signal may be too weak and not able to produce a noticeable difference from a similar solution without sodium. As the concentration of sodium increases the afferent signal strength will increase and reach a level where an individual will be able to discriminate a sodium solution from water but remain unable to identify the taste quality. This is known as the detection threshold and is often used as a measure of individual sensitivity to sodium (Keast and Breslin, 2002a). Keast and Roper (2007) found that 0.015 M NaCl solution just reached recognition threshold, so the additional concentration of sodium chloride (0.015 M) added to achieve sodium balance has very little effect on the taste intensity. In addition, due to similar chemical properties, the same concentration of sodium ions and potassium ions has little difference in human umami and salty taste perception. Therefore, the effect on taste intensity could be ignore.

Figure 2.4 summarizes the overall findings which were common to all three studies presented in this paper, illustrating the associations between umami and the other four basic tastes. As seen in this figure the addition of umami taste did not enhance or suppress any other taste, however, the addition of sweet, salty, sour and bitter do significantly suppresses the umami taste.

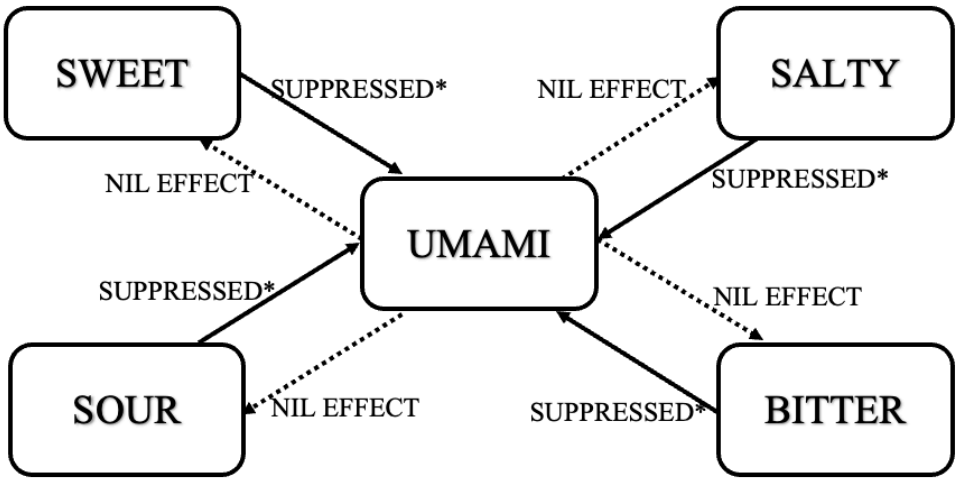

Figure 2.4 Binary interactions of taste qualities at equi-intense concentrations. Asterisks indicate statistically significant suppression of the primary taste quality ($p < 0.05$). Figure in line with schematic review of binary taste interactions by Keast and Breslin (2002a).

Keast and Breslin (2002a) have shown that the concentration of taste stimuli, and the position on the concentration-intensity psychophysical curve could predict the interactions of tastes in taste mixtures. In the current study however, no matter whether it was in the "moderate" perceived intensity region or in "strong" perceived intensity region, the umami taste did not enhance or suppress the perceived intensity of any other taste in the binary taste systems; where sweet, salty, sour and bitter all significantly suppressed the perception of umami intensity in the binary and quinary taste systems. Previous research has tended to agree that umami enhances salt perception in aqueous solutions (Woskow, 1969; Kemp & Beauchamp, 1994) and in foods (Dermiki et al.,

2013; Kremer et al., 2013; Khetra et al., 2019), and in recent years food manufacturers have been keen to use umami to enhance salty taste. However, the experimental results from this study conclude that umami taste did not affect the salty taste when presented at moderate or strong equi-intensities.

The disagreement between the current study and previous findings may be explained by the following factors: First, the levels of tastants used varies between studies. Compared to studies that previously used MSG, the 0.015M used in this study was lower than the levels found in the Kemp and Beauchamp study (1994) to enhance salty taste (0.032 and 0.059M MSG), and the level of sodium chloride used in the previous study was much lower (0.025M compared to 0.08M in the present study).

In addition, test procedure differences, i.e. a taste and spit procedure vs a taste and swallow procedure, are also responsible for the conflict. Running and Hayes (2017) have previously concluded that taste ratings resulting from model solutions that had been spat out are lower than ratings for swallowed samples on a gLMS scale. Taken together these arguments might infer that umami may enhance salty perception where salty taste is lower. Kawasaki et al. (2016) give an insight into the time over which the different tastes are perceived, for example saltiness and sourness tend to be perceived as dominant before swallowing, whereas umami was dominant after swallowing. This finding highlights the effect of the test methodology on the perceived intensity of taste. The sip and spit method was used by Kemp and Beauchamp (1994), while Keast and Breslin (2002b) did not include swallowing. But solutions were swallowed in the present study. Therefore, it is difficult to compare the results of studies where the tests

were not conducted in the same way. Kawasaki et al. (2016) also investigated the duration of impact of taste attributes of umami (MSG), salty (sodium chloride), sour (lactic acid) and their binary mixtures using temporal dominance of sensations methodology. They found that the presence of MSG increased the duration of NaCl saltiness but suppressed the sourness of lactic acid. On the other hand, the duration of umami taste of MSG was suppressed in the presence of NaCl but was not affected by lactic acid. This means that MSG could increase the duration of salty taste from NaCl rather than enhance the peak intensity. This might imply that where previous studies have reported an enhancement of salty taste, it could have been that the taste duration was extended rather than an increase in maximum intensity. However, our study was specifically set up to test maximum intensity following the sample remaining in the mouth for 5s, and so would not have captured an increase in duration that the Kawasaki study concluded.

A second explanation for such discrepancies might be that umami is a less recognised taste in Western countries and consumers may perhaps confuse it with salty perception, despite it being one of the five basic tastes (Cecchini et al., 2019). Although the panelists in this study were trained to recognise and score umami taste, they were UK assessors and as such they would not be habituated to umami taste throughout their lives, which might have affected their scoring. Certainly, in previous studies where functional magnetic resonance imaging (fMRI) was employed, it was confirmed that there was only a slight difference between the positions of the activation regions between umami and salty taste, which led to the conclusion that the basic perception

system of umami taste was very similar to the basic perception system of salty taste (Nakamura et al., 2011). Furthermore, Onuma, Maruyama, and Sakai (2018) had reported that the NaCl solutions with MSG increased responses in the frontal operculum but did not affect the hemodynamic salivary by functional near-infrared spectroscopy (fNIRS) data. This means that the umami induced saltiness enhancement effects occur in the central gustatory processing in the brain. Additionally, this might partly explain why umami, in the MPG model, was found to enhance the total taste intensity of the salt solution, without enhancing the specific taste modality (saltiness).

The type of panelist used in different studies should also be considered. Trained sensory panelists, such as the assessors in this study, “dissect” a product into its component attributes for rating, whereas consumers “synthesise” the information from the foods they are tasting (Ares & Varela, 2017). Compared with untrained consumers, trained panelists are more sensitive to taste discrimination, and they are significantly more aware of the flavour in the mixture and the intensity of suppression (McBride & Finlay, 1989; Prescott, Ripandelli & Wakeling, 2001), although their hedonic perception of the product may not fully represent the wide and varied perceptions from untrained consumers (Ares & Varela, 2017). So, one might expect a consumer would synthesise congruent taste information in a way that a trained panelist might not, leading more readily to the conclusion that a salt reduced food that is higher in umami might have an overall similar salty perception as the two tastes are congruent. However, the previous studies which concluded that umami enhanced salty taste perception were all carried out with trained panelists (Woskow, 1969; Kemp & Beauchamp, 1994; Keast & Breslin,

2002b), as employed in the current study; so, the differences in perception between trained panellists and consumers, does not lead to a satisfactory explanation of conflicting results.

When Green et al. (2010) studied binary, ternary and quaternary mixtures, they found that the overall perceived intensity of the mixtures was best predicted by perceptual additivity, the sum of the tastes perceived within the mixture (Green et al., 2010). In fact, their study concluded the sum of the unmixed taste intensities to be much higher than the sum of the taste intensities in the mixture, or the overall taste intensity ratings, thus ruling out stimulus additivity (Keast & Breslin, 2002a). In the current study, it was consistent that the overall taste intensity was lower than both the sum of the unmixed taste intensities and the sum of the taste intensities in binary system and quinary mixture. However, it was relatively easy to distinguish each taste in the binary system but much more difficult to distinguish each taste in the quinary mixture system, which may lead to a great reduction in intensity compared to a single tastant.

One limitation of this work was that when the source of umami was changed from MSG to MPG, the concentration level did not remain in the same taste intensity level. It means the relationship between the five basic tastes is only valid at certain taste intensity level and for certain umami compound, i.e., from moderate to strong when MPG was used as the source of umami; from strong to very strong when MSG was used as the source of umami. Even if the results presented same trend (suppression), the impact of concentration range on perception was uncertain. However, it provides a

prediction for the relationship of the five basic tastes when MSG is used as the source of umami at other concentration levels in the future.

In fact, taste interactions in a real food matrix are more complicated compared to aqueous solutions. This can explain why for example, MSG is added in variety of food products (e.g., soup, potato chips, sausage) to replace NaCl as well as to enhance flavour (Yamaguchi & Takahashi, 1984; Dos et al., 2014; Maluly et al., 2017). However, increasing saltiness perception using MSG in the aqueous model system of the current study was not observed. The discrepancy could be explained due to the complexity of food matrices which affects the perception. In a real food there are cross-modal interactions between two or more sensory modalities such as taste-flavour or flavour-texture interactions. Additionally, ingredients used in food products are often added at much higher concentrations than in the aqueous model systems to achieve the required taste intensity, considering that the texture can reduce intensity. In general, meat products have a high sodium content, and the salt content is around 2% (Inguglia et al., 2017), where only 0.29% or 0.55% salt was used in this study. Other research used higher MSG levels, 0.38% MSG was added to the sumashi-jiru (soup) to maintain the salty taste, and 0.3% MSG added to the sausage to compensate the saltiness loss caused by 50% salt reduction in low-sodium fish burgers (Quadros et al., 2015). In contrast, only 0.19% or 0.25% MSG was used in this study. Therefore, the conclusions reached by investigating aqueous model solution may not be applicable to food systems directly, however they offer the basis for the design of further experiments in real foods.

The present study employed a trained sensory panel to investigate taste interactions, with limited variability in taste sensitivities. Prescott et al. (2001) concluded that perception of tastes and interaction between tastes in binary mixture are affected by the 6-n-propylthiouracil (PROP) taster status, i.e., supertaster, medium taster and non-taster. However, the taste sensitivity is determined by many factors, such as genetic differences in taste receptors, including Tas2R38 gene that is predominantly responsible for PROP/PTC (phenylthiocarbamide) tasting (Hayes et al, 2008), and single nucleotide polymorphisms (SNPs) for epithelial sodium channel (ENaC) (Chamoun et al, 2021). For example, SNPs for the T1R receptors influence perception of sweet and umami taste. Therefore, to truly understand the influence of umami taste in taste mixtures for all consumers, a study considering taste sensitivities to basics tastes (each from more than one tastant) alongside genotyping would be needed in a large population cohort in the future.

2.3 Conclusions

MSG with umami taste has been popularly used as salty taste enhancer for developing salt reduction strategies. However, the exact role of MSG/umami was not sufficiently explored. The aim of this study was to investigate taste interactions in mixtures containing umami in the form of MSG and MPG. The addition of umami taste did not enhance or suppress any other taste in equi-intense aqueous solutions which indicated that umami is dissimilar to other tastants. However, the addition of sweet, salty, sour and bitter do significantly suppresses the umami taste. The findings of this study are significant because they fill the gap that existed in the literature considering the effect

of umami taste in taste mixture interactions and have an impact on our understanding of the underlying mechanisms of taste interactions that can be applied in food reformulation. Although umami was not found to enhance salty perception, as hypothesised, neither did it suppress it; hence when used together sodium chloride plus glutamate tastants maintained salty perception in addition to savoury taste perception, irrespective of the glutamate salt used. Overall, there is little evidence on the effect of umami on other taste stimuli, and the findings of the current study are difficult to compare directly with the limited information currently available in the literature. The reasons for this are the different sensory tests used (ranking vs gLMS), the different methodology (sip and spit vs swallowing), the different concentrations of tastants and the difference in perception of similar concentrations by the different groups studied. Although there are studies using umami as a flavour enhancer, real food systems are more complicated than aqueous systems. Further investigation is needed to determine whether these findings in aqueous solutions apply to real food systems where more complex and cross-modal interactions take place.

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References

Ares, G. & Varela, P. (2017). Trained vs. consumer panels for analytical testing: Fueling a long lasting debate in the field. *Food Quality and Preference*, 61, 79-86.

1875 Abu, N.B., Harries, D., Voet, H. and Niv, M.Y., (2018). The taste of KCl–What a
1876 difference a sugar makes. *Food chemistry*, 255, 165-173.
1877 Bachmanov, A.A & Beauchamp, G.K. (2007). Taste Receptor Genes. *Annual Review*
1878 *of Nutrition*, 27, 389–414.
1879 Bartoshuk, L. M. (1975). Taste mixtures: Is mixture suppression related to compression?
1880 *Physiology and Behavior*, 14(5), 643-649.
1881 Bartoshuk, L.M., Duffy, V.B., Green, B.G., Hoffman, H.J., Ko, C.W., Lucchina, L.A.,
1882 Marks, L.E., Snyder, D.J. and Weiffenbach, J.M. (2004). Valid across-group
1883 comparisons with labeled scales: the gLMS versus magnitude matching. *Physiology &*
1884 *behavior*, *82*(1), 109-114.
1885 Breslin, P. A. S. & Beauchamp, G. K. (1997). Salt enhances flavour by suppressing
1886 bitterness. *Nature*, 387, 563-563.
1887 Cecchini, M., Knaapila, A., Hoffmann, E., Boschi, F., Hummel, T. & Iannilli, E. (2019).
1888 A cross-cultural survey of umami familiarity in European countries. *Food Quality and*
1889 *Preference*, 74, 172-178.
1890 Chamoun, E., Liu A.S. Duizer, L.M., Feng, Z., Darlington, G., Duncan, A.M., Haines,
1891 J., Ma, D.W.L. (2021). Single nucleotide polymorphisms in sweet, fat, umami, salt,
1892 bitter and sour taste receptor genes are associated with gustatory function and taste
1893 preferences in young adults. *Nutrition Research*, 85, 40-46.
1894 Dermiki, M., Mounayar, R., Suwankanit, C., Scott, J., Kennedy, O., Mottram, D.,
1895 Gosney, M., Blumenthal, H. and Methven, L. (2013). Maximising umami taste in meat
1896 using natural ingredients: effects on chemistry, sensory perception and hedonic liking

in young and old consumers. *Journal of the Science of Food and Agriculture*, 93(13), 3312-3321.

Dos Santos, B., Campagnol, P., Morgano, M. & Pollonio, M. (2014). Monosodium glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl with KCl. *Meat Science,* 96(1), 509-513.

Emorine, M., Septier, C., Martin, C., Cordelle, S., Sémon, E., Thomas-Danguin, T. and Salles, C. (2021). Salt and aroma compound distributions influence flavour release and temporal perception while eating hot-served flans. *Molecules*, 26(5), 1300-1317.

Fuke, S. and Ueda, Y. (1996). Interactions between umami and other flavor characteristics. *Trends in Food Science & Technology*, 7(12), 407-411.

Green, B.G., Shaffer, G.S. and Gilmore, M.M. (1993). Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties. *Chemical senses*, 18(6), 683-702.

Green, B. G., Lim, J., Osterhoff, F., Blacher, K., & Nachtigal, D. (2010). Taste mixture interactions: Suppression, additivity, and the predominance of sweetness. *Physiology & Behavior*,101, 731-737.

Hayes, J.E., Bartoshuk, L.M., Kidd, J.R., Duffy, V.B. (2008). Supertasting and PROP bitterness depends on more than the TAS2R38 gene. *Chemical Senses*, 33 (3), 255-265.

Inguglia, E.S., Zhang, Z., Tiwari, B.K., Kerry, J.P. and Burgess, C.M., (2017). Salt reduction strategies in processed meat products–A review. *Trends in Food Science & Technology*, 59, 70-78.

1919 Kawasaki, H., Sekizaki, Y., Hirota, M., Sekine-Hayakawa, Y. & Nonaka, M. (2016).
1920 Analysis of binary taste-taste interactions of MSG, lactic acid, and NaCl by temporal
1921 dominance of sensations. *Food Quality and Preference*, 52, 1-10.
1922 Keast, R. S. J. & Breslin, P. A. S. (2002a). An overview of binary taste-taste interactions.
1923 *Food Quality and Preference,* 14, 111-124.
1924 Keast, R. S. J. & Breslin, P. A. S. (2002b). Modifying the bitterness of selected oral
1925 pharmaceuticals with cation and anion series of salts. *Pharmaceutical Research*, 19,
1926 1019-1026.
1927 Keast, R.S. and Roper, J., 2007. A complex relationship among chemical concentration,
1928 detection threshold, and suprathreshold intensity of bitter compounds. *Chemical*
1929 *senses*, *32*(3), pp.245-253.
1930 Kemp, S. E. & Beauchamp, G. K. (1994). Flavor Modification by Sodium Chloride and
1931 Monosodium Glutamate. *Journal of Food Science*, 59, 682-686.
1932 Khetra, Y., Kanawjia, S., Puri, R., Kumar, R. & Meena, G. (2019). Using taste-induced
1933 saltiness enhancement for reducing sodium in Cheddar cheese: Effect on physico-
1934 chemical and sensorial attributes. *International Dairy Journal*, 91, 165-171.
1935 Kim, M., Son, H., Kim, Y., Misaka, T. & Rhyu, M. (2015). Umami–bitter interactions:
1936 The suppression of bitterness by umami peptides via human bitter taste receptor.
1937 *Biochemical and Biophysical Research Communications*, 456(2), 586-590.
1938 Kremer, S., Shimojo, R., Holthuysen, N., Köster, E. & Mojet, J. (2013). Consumer
1939 acceptance of salt-reduced “soy sauce” foods over rapidly repeated exposure. *Food*
1940 *Quality and Preference*, 27(2), 179-190.

1941 Maluly, H.D., Arisseto-Bragotto, A.P. and Reyes, F.G., (2017). Monosodium glutamate
1942 as a tool to reduce sodium in foodstuffs: Technological and safety aspects. *Food science*
1943 *& nutrition*, 5(6), 1039-1048.
1944 McBride, R.L., (1987). Taste psychophysics and the Beidler equation. *Chemical Senses*,
1945 12(2), 323-332.
1946 McBride, R.L. and Finlay, D.C., (1989). Perception of taste mixtures by experienced
1947 and novice assessors 1. *Journal of Sensory Studies*, 3(4), 237-248.
1948 McBride, R.L. and Finlay, D.C., (1990). Perceptual integration of tertiary taste mixtures.
1949 *Perception & psychophysics*, 48(4), 326-330.
1950 McBride, R.L., (1993). Integration psychophysics: The use of functional measurement
1951 in the study of mixtures. *Chemical senses,* 18(2), 83-92.
1952 Mojet, J., Heidema, J. and Christ-Hazelhof, E., (2004). Effect of concentration on taste–
1953 taste interactions in foods for elderly and young subjects. *Chemical senses*, 29(8), 671-
1954 681.
1955 Nakamura, Y., Goto, T., Tokumori, K., Yoshiura, T., Kobayashi, K., Nakamura, Y.,
1956 Honda, H., Ninomiya, Y. & Yoshiura, K. (2011). Localization of brain activation by
1957 umami taste in humans. *Brain Research*, 1406, 18-29.
1958 Onuma, T., Maruyama, H. and Sakai, N. (2018). Enhancement of Saltiness Perception
1959 by Monosodium Glutamate Taste and Soy Sauce Odor: A Near-Infrared Spectroscopy
1960 Study. *Chemical Senses*, 43(3), 151-167.

Prescott, J., Ripandelli, N. and Wakeling, I., (2001). Binary taste mixture interactions in prop non-tasters, medium-tasters and super-tasters. *Chemical senses*, 26(8), 993-1003.

Ponzo, V., Pellegrini, M., Costelli, P., Vázquez-Araújo, L., Gayoso, L., D'Eusebio, C., Ghigo, E. and Bo, S., (2021). Strategies for reducing salt and sugar intakes in individuals at increased cardiometabolic risk. *Nutrients*, 13(1), 279-296.

Quadros, D. A., Rocha, I. F. O., Ferreira, S. M. R., & Bolini, H. M. A. (2015). Low-sodium fish burgers: Sensory profile and drivers of liking. *LWT - Food Science and Technology*, 63, 236–242.

Running, C. & Hayes, J. (2017). Sip and spit or sip and swallow: Choice of method differentially alters taste intensity estimates across stimuli. *Physiology & Behavior*, 181, 95-99.

Schifferstein, H.N. and Frijters, J.E., (1993). Perceptual integration in heterogeneous taste percepts. *Journal of Experimental Psychology: Human Perception and Performance*, 19(3), 661-675.

Thomas-Danguin, T., Guichard, E. and Salles, C., (2019). Cross-modal interactions as a strategy to enhance salty taste and to maintain liking of low-salt food: A review. *Food & function*, 10(9), 5269-5281.

Velázquez, A.L., Vidal, L., Varela, P. and Ares, G., (2020). Cross-modal interactions as a strategy for sugar reduction in products targeted at children: Case study with vanilla milk desserts. *Food Research International*, 130, 108920.

1982 Woskow, M. H. (1969). Selectivity in flavor modification by 5'-ribonucleotides. *Food*
1983 *Technology*, 23, 32-37.
1984 Yamaguchi, S. & Kimizuka, A. (1979). Psychometric studies on the taste of
1985 monosodium glutamate. *Advances in biochemistry and physiology*, 35-54.
1986 Yamaguchi, S., & Takahashi, C. (1984). Interactions of monosodium glutamate and
1987 sodium chloride on saltiness and palatability of clear soup. *Journal of Food Science,*
1988 49(1), 82– 85.
1989 Yamaguchi, S. (1998). Basic properties of umami and its effect on food flavour. *Food*
1990 *Reviews International*, 14(2&3), 139-176.

Chapter 3. Effect of lysine and calcium lactate on saltiness perception in an aqueous solution

Abstract

In order develop low-sodium foods, different types of metallic salts have been used to replace salt. However, they often lead to a significant loss in saltiness if used alone, or introduce substantial off-notes, such as bitterness. This study aimed to investigate whether lysine and calcium lactate could compensate the saltiness loss in a salt-reduced solution. A trained sensory panel rated solutions of 0.25% (w/v) NaCl, 1% (w/v) lysine and 0.75% (w/v) calcium lactate in single, binary, and ternary solutions, in comparison to 0.5 % (w/v) NaCl, for intensity of saltiness, bitterness and sourness. Results concluded that calcium lactate did not offer saltiness whereas lysine gave weak saltiness. When used with 0.25% (w/v) NaCl, lysine with/without calcium lactate had the same intensity of saltiness as control ($p > 0.05$), whereas the saltiness perceived from 0.25% NaCl with calcium lactate remained lower than control. This indicates that lysine can enhance saltiness whereas, within the levels tested, calcium lactate cannot. Moreover, whereas the bitterness of most tastants combinations were significantly higher than that of control, the bitterness of lysine with 0.25% (w/v) salt was lower than for lysine alone and not significantly different to the 0.5% (w/v) NaCl control. Additionally, saltiness increased with the increase in concentration of the composite solutions, while the perceived bitterness increased gradually at low and medium concentrations and reached a plateau at high concentration. In conclusion, 1% (w/v) lysine with/without 0.75% (w/v) calcium lactate could replace 50% salt in aqueous solution without compromising saltiness perception.

3.1 Introduction

Sodium chloride (NaCl) is frequently used in many foods as it provides a variety of functions. It is used to extend the shelf life of meat products as a preservative (Inguglia *et al.*, 2017), and has a beneficial effect on flavour, taste and texture of foods (De Marchi *et al.*, 2017). However, excess salt intake is associated with high blood pressure (He and MacGregor, 2010). According to a recommendation from the World Health Organization (WHO) in 2020, the average sodium consumption should be approximately 2 g sodium per day (equivalent to about 5 g salt per day) for adults to prevent chronic diseases, but current salt intake is much higher than the recommended standard by WHO for most populations. For example, in the UK the average sodium intake is estimated to be 9.2 g salt per day in men and 7.6 g salt per day in women (age 19-64 years) (Ashford, Jones and Collins, 2020). Therefore, it continues to be a rising demand for low sodium content foods.

It is widely accepted that dietary sodium reduction could be effectively achieved by reducing the sodium content of foods, rather than by merely giving dietary advice. However, complete salt replacement is almost unfeasible, even from the perspective of taste alone, due to the specificity of sodium in saltiness perception. The receptor mechanisms are hard to mimic by other molecules (Henney, Taylor and Boon, 2010). Although salty taste is elicited by many ionic species, it is sodium ions (Na^+) that are predominantly responsible for the salty taste of most foods (Lindemann, 1997). Saltiness is a distinctive sensory quality linked primarily to sodium or lithium containing compounds, while other cations like potassium and calcium can also exhibit

2036 salty taste, but it is not their dominant taste quality (Vanderklaauw and Smith, 1995).
2037 The epithelial sodium channel (ENaC) is considered as one of the most important
2038 receptors for saltiness perception. ENaC allows primarily sodium (and lithium) to move
2039 into the taste cell from outside the taste receptor cell, where it has been dissolved in
2040 saliva. In principle, Na^+ activates the ENaC to produce electrical pulses which are then
2041 transmitted via the sensory neurons to the brain to form salty taste (Yamamoto and
2042 Ishimaru, 2013).
2043 The popular strategy to reduce salt content by the food industry is to utilize salt
2044 substitutes, such as potassium chloride (KCl) (Tamm *et al.*, 2016). Although these
2045 compounds can contribute to saltiness perception, they often cause some unsatisfactory
2046 tastes, like bitterness, at high concentration (Sinopoli and Lawless, 2012). This is
2047 because these non-sodium cations can activate non-specific cation channels which are
2048 responsible of the off tastes (Liem, Miremadi and Keast, 2011). In addition, reducing
2049 NaCl levels below those typically used, without any other preservative measure, will
2050 reduce product shelf life. For example, Desmond (2006) reported that reducing the salt
2051 content of frankfurters by 40% (from 2.5 to 1.5 % w/v) without any salt substitutes
2052 caused the natural bacterial flora to grow more rapidly. Indeed, KCl has been proven to
2053 have the same antibacterial effect as NaCl against a wide range of pathogenic bacterial
2054 species, thus ensuring that the shelf life is not shortened in salt-reduced foods (Bidlas
2055 and Lambert, 2008). However, the substitution of salt with KCl in most foods must be
2056 limited to 30%, as higher levels can produce bitter and metallic tastes (Doyle and Glass,
2057 2010). Additionally, a high potassium load is associated with impairments in people

2058 with type 1 diabetes, renal disease and adrenal insufficiency (Khaw and Barrett-Connor,
2059 1984). Hence, these shortcomings have greatly limited the application of alternative
2060 metal salts in food manufacturing.
2061 Recently, lysine has been explored as a successful taste and flavour enhancer in meat
2062 products. Campagnol *et al.* (2011) indicated that 50% NaCl reduced fermented sausage
2063 (from 2.5% to 1.25% w/w NaCl), containing both KCl (1.25% w/w) and lysine (from
2064 0.313% w/w to 0.833% w/w) had a similar sensory aroma and taste to the control,
2065 whereas this was not achieved with KCl replacement alone. Dos Santos Alves *et al.*
2066 (2017) reported that 50% NaCl reduced low-fat Bologna-type sausage (from 2.5% to
2067 1.25% w/w NaCl) with KCl (1.25% w/w) and lysine (1% w/w) increased aroma, flavour
2068 and overall acceptability compared with KCl replacement alone. One of the most
2069 significant findings of lysine was that it could relieve the sensory defects caused by
2070 other salt substitutes, without introducing bitterness or sourness (Campagnol *et al.*,
2071 2011). However, some authors (Guo *et al.* (2020) and Vidal *et al.* (2020)) have found
2072 that lysine alone, at 3% w/w, was not able to compensate the saltiness lost in 50% salt-
2073 reduced ham or beef, although the physical-chemical characteristics were improved.
2074 There is limited literature on the use of calcium lactate as a salt replacer, however, it
2075 has three potential benefits. The calcium cation may confer some salty taste, although
2076 as noted above, this is not the primary taste, the Ca^{2+} salts were predominantly bitter
2077 (Vanderklaauw and Smith, 1995). Nevertheless, the most prominent advantage of
2078 calcium lactate is that the lactate ion can inhibit the growth of bacteria in meat products
2079 and provide anti-Listerial activity (Devlieghere *et al.*, 2009), which are not provided by

most other salt substitutes. Muchaamba *et al.* (2021) indicated that, in salami, a low salt (2.8% w/w NaCl) plus potassium lactate (1.6% w/w) combination had comparable anti-*Listeria monocytogene* activity to the high salt treatment (4% NaCl w/w). The third one is, the added benefit of calcium fortification. Irshad *et al.* (2016) reported that restructured buffalo meat loaves with 1.25% w/w calcium lactate could meet recommended dietary recommendations for calcium without affecting the textural and sensory properties of the product.

Previous studies have shown the individual benefits of either lysine or calcium lactate in salt-reduced foods, and their effects were tested in real food matrices without their modes of action proven in model systems. There is scarce information about how lysine and calcium lactate interact each other on salty taste perception in an aqueous solution. Therefore, the aim of this work was to investigate whether lysine and calcium lactate could compensate for the loss of salty taste in a reduced salt solution, without imparting off-tastes. Progressing the understanding from previous literature, it is hypothesised that lysine and/or calcium lactate could enhance the salty intensity in a salt-reduced aqueous solution.

3.2 Materials and Method

3.2.1 Panelists

A total of 12 sensory panelists participated in this study, all were screened and selected for their detection, discrimination and description ability, and had over 6 months sensory experience. There were 11 females and 1 male with age ranging from 35 to 65. All team members were healthy and had no defects or disorders in taste or olfaction.

All of them were trained and employed as sensory panelists and provided consent through their employment to taste foods and for their data to be used.

3.2.2 Stimulus

The taste stimuli used were aqueous solutions of sodium chloride (Co-op Food, Manchester, UK), L-lysine (Health Leads®, Llandysul, UK) and calcium lactate (Sigma-Aldrich, Gillingham, UK). Each tastant solution was prepared in mineral water (Harrogate Spa, UK) a day before the panel session and kept in the fridge (4 °C) overnight. All tastants solutions were taken out of the fridge prior to the test to equilibrate to ambient temperature, then 15 mL of the sample was poured into a 20 mL transparent polystyrene cups labeled with three-digit random codes and were served to the panel at room temperature (22 ± 2°C).

3.2.3 Training

Prior to the data collection, all panelists participated in a training in which they were trained on how to score the intensity of the taste on the general labelled magnitude scale (gLMS). The descriptor anchors on the gLMS logarithmic scale were “barely detectable” (0.14), “weak” (0.7), “moderate” (1.2), “strong” (1.5), “very strong” (1.7) and “strongest imaginable sensation of any sensation” (1.98) (exponentiated values 1.38, 5.01, 15.9, 31.6, 50.1 and 95, respectively) (Bartoshuk et al., 2004).

During the training session, the panellists were trained with NaCl (0.25% w/v), lysine (0.75% and 1.0% w/v) and calcium lactate (0.375% and 0.75% w/v) until they were familiar with the taste of each stimulus. These training samples were presented with blind code and in a random order, and panellists were asked to rate the salty taste

intensity of each stimulus respectively on gLMS. The higher level of each compound was selected according to the daily recommended intake level (about 0.8 g/day for lysine and 0.6 g/day for calcium lactate) (Tomé and Bos, 2007). Because red meat intake is recommended below 80 g/day (Islam et al., 2014; McAfee et al., 2010), the higher lysine and calcium lactate levels were selected (1.0% and 0.75% w/v respectively) for progression into the experiments as they approached "strong to very strong" on the gLMS scale when used in combination with 0.25% (w/v) NaCl.

3.2.4 Tastants preparation

3.2.4.1 Effect of lysine and calcium lactate on the perceived intensity of tastes

This first experiment contained a total of 8 treatments, including four single tastant solutions and four tastant mixtures (three binary, one ternary). The standard NaCl level was 0.5 % w/v, whereas lysine and calcium lactate were used at 1.0 and 0.75 % w/v respectively (levels are justified in section 3.2.4). The aim was to replace 50% NaCl using lysine and calcium lactate, which led to the 8 formulations detailed in table 3.1.

Table 3.1 Formulations used to evaluate the effects of calcium lactate and lysine on perceived taste intensity of aqueous solutions.

Treatment	Sodium chloride (% w/v)	Lysine (% w/v)	Calcium lactate (% w/v)
Control	0.5	-	-
H	0.25	-	-
L	-	1	-
CL	-	-	0.75
H+L	0.25	1	-
H+CL	0.25	-	0.75
L+CL	-	1	0.75
H+L+CL	0.25	1	0.75

H = half of control salt; L = lysine; CL = calcium lactate.

3.2.4.2 Relationship between concentration of composite solution and perceived taste intensity

3.2.4.2.1 Varying concentration of composite solution with a fixed ratio between components

The ratio between the three stimuli used in experiment 1 (Section 3.2.4.1) was maintained for the second experiment (0.25% NaCl: 1.0% Lysine: 0.75% Calcium Lactate), where the aim was to determine the psychophysical function between the concentration of this composite solution and perceived intensity of taste(s). Initially the concentration of composite was varied to identify, with the sensory panel, the approximate recognition threshold for salty taste and suprathreshold levels that led to "very strong" on the gLMS. An optimal dilution factor 1.7 was used to ensure that the perceived saltiness of the composite solution would cover the range of gLMS

descriptors, from barely detectable to very strong within six treatments. Table 3.2 shows the formulation of the six treatments.

Table 3.2 Formulation of treatments used to evaluate the relationship between concentration of composite solution with fixed ratio between components and the perceived intensity of tastes.

Treatment	Sodium chloride (% w/v)	Lysine (% w/v)	Calcium lactate (% w/v)
T1	0.05	0.21	0.15
T2	0.09	0.35	0.26
T3	0.15	0.59	0.44
T4	0.25	1.00	0.75
T5	0.43	1.70	1.28
T6	0.72	2.90	2.17

T4 was the standard solution which contained 0.25% w/v NaCl, 1% lysine w/v and 0.75% w/v calcium lactate. T1 = 1.7^{-3} x T4, T2 = 1.7^{-2} x T4, T3 = 1.7^{-1} x T4, T5 = 1.7 x T4, T6 = 1.7^{2} x T4.

3.2.4.2.2 Composite solution with varied lysine levels

The experiment contained 7 treatments. All composite solutions contained 0.25% w/v NaCl and 0.75% w/v calcium lactate, however the lysine level was either diluted or concentrated from the standard level of 1% w/v, using the dilution factor of 1.7. The purpose is to further investigate the psychophysical function between the perceived intensity of salty taste and the concentration of lysine. Table 3.3 shows the formulation of each treatment.

Table 3.3 Formulation of solutions used to evaluate the relationship between concentration of composite solution with varied lysine levels and perceived taste intensity.

Treatment	Sodium chloride (% w/v)	Lysine (% w/v)	Calcium lactate (% w/v)
L1	0.25	0.21	0.75
L2	0.25	0.35	0.75
L3	0.25	0.59	0.75
L4	0.25	1.00	0.75
L5	0.25	1.70	0.75
L6	0.25	2.90	0.75
L7	0.25	4.91	0.75

L4 is the standard solution containing 0.25% w/v NaCl, 1% w/v lysine and 0.75% w/v calcium lactate. The lysine level in L1 is 1.7^{-3} x L4, L2 is 1.7^{-2} x L4, L3 is 1.7^{-1} x L4, L5 is 1.7 x L4, L6 is 1.7^{2} x L4, L6 is 1.7^{3} x L4.

3.2.5 Sensory evaluation

The experiments were conducted within a standard sensory environment using individual sensory booths, artificial daylight and controlled room temperature (22 ± 2℃). All samples were blind-coded (3-digit random number codes) and presented monadically in a balanced order. During tasting sessions, panelists were instructed to sip and hold the stimulus in their mouths for five seconds before swallowing and then rate all attributes for each sample. In experiment 1 of saltiness perception of tastants, the attributes including overall taste intensity, saltiness, bitterness, sourness, and sweetness were rated. However, in experiments two and three, both investigating the psychophysical relationships between concentration of composite solutions and perceived taste intensity, the attributes were reduced to overall taste intensity, saltiness, and bitterness. Between samples, the panel was instructed to cleanse the palate with

plain crackers and water (filtered tap water at room temperature) to return the mouth back to a neutral state; an automatic reminder appeared during the countdown of ninety seconds between each stimulus after evaluating consecutive taste samples. The panel rated the samples in duplicate on separate days. Data were captured using the sensory software Compusense® (cloud version, Guelph, Ontario).

3.2.6 Data analysis

Log data from each panelist from the gLMS were anti-logged. Subsequently, two-way analysis of variance (ANOVA) was carried out using Senpaq (QI Statistics, Reading, UK) where panelists were treated as random effects and samples as fixed effects, the main effects were tested against the assessor by sample interaction. Multiple pairwise comparisons were carried out using Tukey's HSD at a significance level of 0.05. In order to evaluate the psychophysical relationship (experiment 2.4.2), the taste intensity (log data) was plotted against the concentration of the taste complex (log data) using Excel (Microsoft, version 16.68) and linear regression was applied. The concentration of the composite solution was presented with relative concentration to the standard (explained further in section 3.2.1) during plotting and regression.

3.3 Results and discussion

3.3.1 Saltiness perception of tastants

The mean log scores of perceived taste intensity for all single tastants and tastant mixtures are given in Table 3.4.

Table 3.4. Perceived taste intensity of sodium chloride, lysine and calcium lactate in single, binary and ternary solutions.

Perceived intensity (mean of gLMS intensity rating)

Treatment	Overall taste intensity	Saltiness	Bitterness	Sourness	Sweetness
Control	1.57^{b}	1.51^{a}	0.75^{c}	0.18^{d}	0.11^{c}
H	1.40^{c}	1.28^{cd}	0.97^{c}	0.36^{cd}	0.32^{bc}
L	1.55^{b}	1.05^{e}	1.40^{b}	0.70^{ab}	0.62^{a}
CL	1.47^{bc}	0.65^{f}	1.37^{b}	0.58^{bcd}	0.18^{c}
H+L	1.53^{b}	1.46^{ab}	1.13^{c}	0.61^{abc}	0.56^{ab}
H+CL	1.52^{bc}	1.37^{bc}	1.39^{b}	0.65^{abc}	0.30^{bc}
L+CL	1.71^{a}	1.13^{de}	1.63^{a}	0.79^{a}	0.46^{abc}
H+L+CL	1.70^{a}	1.45^{ab}	1.58^{a}	0.65^{abc}	0.41^{abc}

Means within a column which do not share a common superscript are significantly different in the perceived magnitude from Tukey's HSD test at the 95% confidence interval. C = NaCl at 0.5% w/v; H = NaCl at 0.25% w/v; L = lysine at 1.0% w/v; CL = calcium lactate at 0.75 % w/v.

Reducing the NaCl concentration by half (from 0.5 to 0.25 % w/v) significantly lowered saltiness intensity ($p < 0.05$), (reduced from "strong" to "moderate"). This confirms that, as expected, reduction of salt level in solution by 50% would lead to significant loss in saltiness perception. As shown in Table 4, the lysine (at 1 % w/v) did evoke a "weak" perception of saltiness (mean log value 1.05) which was significantly higher than that of calcium lactate (at 0.75 % w/v) at "barely detectable" (mean log value 0.65). Where lysine (1% w/v) was used with half NaCl (0.25% w/v), the resulting solution (H+L) was significantly saltier than the half salt (H) and the lysine alone (L) ($p < 0.05$), and importantly it has similar salty taste with the control salt solution ($p > 0.05$). However, where calcium lactate was used with half NaCl (0.25% w/v), the resulting solution (H+CL) was significantly saltier than calcium lactate alone (CL) ($p < 0.05$), but not significantly different to the half NaCl (H) ($p > 0.05$) and significantly less salty than

2221 the control salt solution ($p < 0.05$). The ternary solution (H+L+CL) was very similar to
2222 the binary solution of NaCl and lysine (H+L) ($p > 0.05$); it was significantly saltier than
2223 the half salt (H), the lysine alone (L), calcium lactate alone (CL) and their combination
2224 (L+CL) ($p < 0.05$), but not significantly different in salty taste than the control salt
2225 solution ($p > 0.05$). Therefore, in line with the study hypothesis, this indicates that 1%
2226 (w/v) lysine, with or without calcium lactate (H+L+CL or H+L), could make up the
2227 saltiness loss caused by 50% NaCl reduction. However, contrary to the study hypothesis,
2228 calcium lactate alone did not enhance any saltiness perception.
2229 Additional tastes were also perceived by the panel. Lysine (L) and calcium lactate (CL)
2230 solutions presented moderate bitterness, which was significantly higher than the control
2231 ($p < 0.05$). However, when lysine was used together with NaCl (H+L) the bitterness
2232 decreased (from “moderate” to “weak”) compared with the bitterness of lysine (L)
2233 alone ($p > 0.05$); resulting in a solution that was similar in both saltiness and bitterness
2234 intensity to the control NaCl ($p > 0.05$). Where calcium lactate was used with NaCl
2235 (H+CL) the bitterness was not significantly different from calcium lactate alone (CL)
2236 ($p > 0.05$) and it remained significantly higher in bitterness than the control ($p < 0.05$).
2237 Although sweetness and sourness also changed in different solutions, the effect could
2238 be ignored because the taste intensity were located between barely detectable to weak
2239 on the gLMS (1.38 to 5.01 antilog on gLMS). Although calcium lactate is weakly acidic,
2240 there are few free hydrogen ions in aqueous solution so that sour taste is difficult to
2241 perceive. Additionally, the overall taste intensity of 50% substitution of NaCl with
2242 lysine (H+L) or calcium lactate (H+CL) was similar to the control ($p > 0.05$), whereas

50% substitution of NaCl with both lysine and calcium lactate (H+L+CL) was significantly higher than the control ($p < 0.05$). In conclusion, the addition of 1% (w/v) lysine with or without 0.75% (w/v) calcium lactate into a 50% salt-reduced aqueous solution were optimal treatments for further investigation to establish the relationship between concentration and perceived intensity. Although the addition of calcium lactate increased bitterness it can provide additional benefits to shelf-life which was discussed earlier, therefore the treatment of 50% substitution of NaCl with 1% (w/v) lysine and 0.75% (w/v) calcium lactate was chosen for the subsequent experiments.

3.3.2 Relationship between concentration of composite solution and perceived taste intensity

3.3.2.1 Composite solution with fixed ratio of NaCl, lysine and calcium lactate

Since sweetness and sourness resulting from the tastants used were negligible (Table 4), only overall taste, salty and bitter were used to establish the psychophysical functions for this composite solution. The ratio of the tastants in the composite solutions was constant, with the standard levels used from the first experiment (0.25% NaCl: 1.0% lysine: 0.75% calcium lactate w/v). The series of composite solutions were developed by following a geometric progression of 1.7 in concentration. In order to illustrate the psychophysical relationship between the concentration in stimuli and the perceived intensity of taste, the concentration ratio relative to the standard (i.e., 0.21, 0.35, 0.59, 1.0, 1.7 and 2.89) was used to plot the curve. The resulting psychophysical relationship is shown in Figure 1.

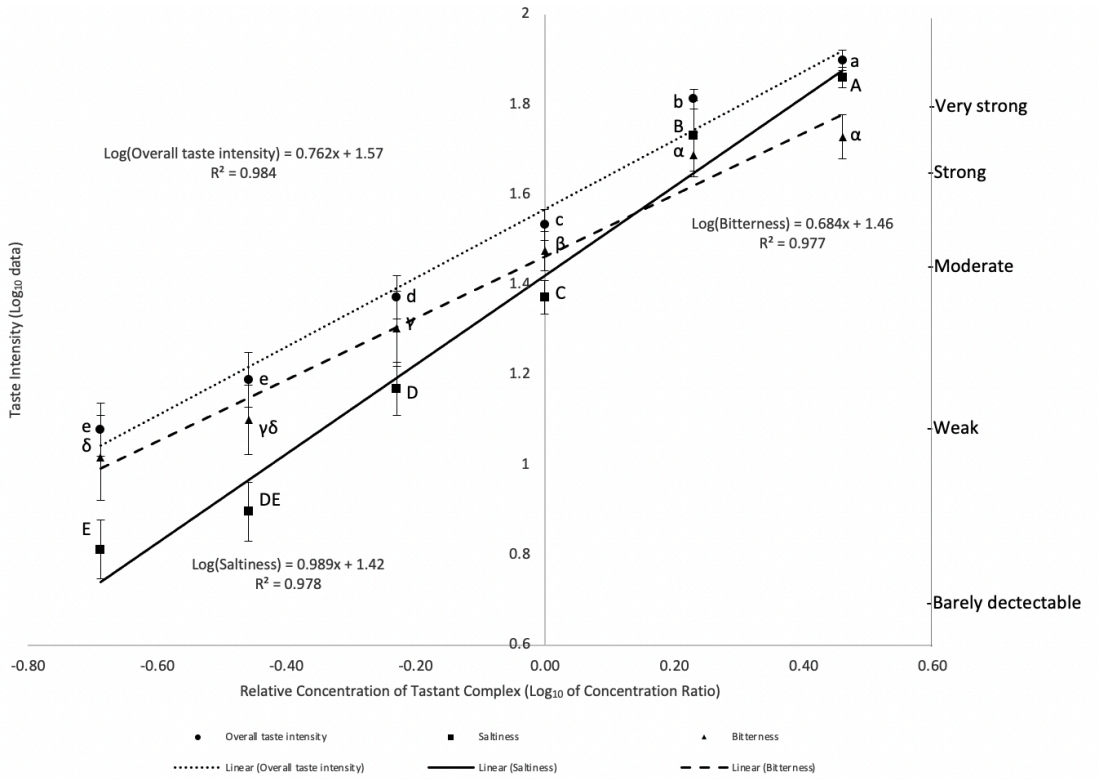

Figure 3.1 Logarithmic relationship between perceived intensity of overall taste, saltiness and bitterness, and the concentration of a composite tastant solution (fixed ratio of 0.25% NaCl: 1.0% lysine : 0.75% calcium lactate). The standard solution (0.25% NaCl, 1% lysine, 0.75% calcium lactate w/v) was denoted a concentration value of 1 (ie $\log_{10} = 0$). Within each intensity set, means that do not share a common letter denote samples are significantly different ($p < 0.05$). Lower case letters used for overall taste intensity, upper case letters use for saltiness, and Greek letters use for bitterness.

Steven's law describes the relationship between concentration and intensity as $I = kC^n$; where I is intensity, k is a constant, C is concentration and n is the exponent that describes the relationship between concentration and perceived intensity (Keast and Breslin, 2002). As shown in Figure 1, salty taste had a proportional relationship with the concentration of the mixture (n = 0.989). However, the overall taste intensity and bitter taste had slightly decelerating relationships with the concentration of the mixture, where the exponents were 0.762 and 0.684 respectively. The saltiness and bitterness of the standard solution (relative concentration 1.0, log (Concentration ratio) 0.0) were in

the "strong" region (Figure 3.1). This was in line with the first experiment (Table 4), where the H+L+CL sample had log values for salty and bitter of 1.45 and 1.58 respectively, both equivalent to "strong". However, the overall taste intensity was also in the "strong" region in this latter experiment, whereas it had been closer to "very strong" (log value 1.70) in the first experiment (Table 3.4). It could be beneficial that the concentration of tastant mixture had a proportionate relationship with perceived saltiness, whereas the perceived bitterness increased at a slower rate. As concentration increased, salty taste perception started to become stronger than the bitter taste perception (Figure 3.1). However, bitterness cannot be ignored as it was “strong” to “very strong” at the high concentrations of the tastant mixture.

3.3.2.2 Composite solution with fixed ratio of NaCl and calcium lactate but varied level of lysine

In this experiment the level of NaCl and calcium lactate were constant in each experiment, whereas the concentration of lysine was changed, in a geometric progression of 1.7. The psychophysical relationship between the concentration of lysine and the intensity of taste is presented in Figure 3.2.

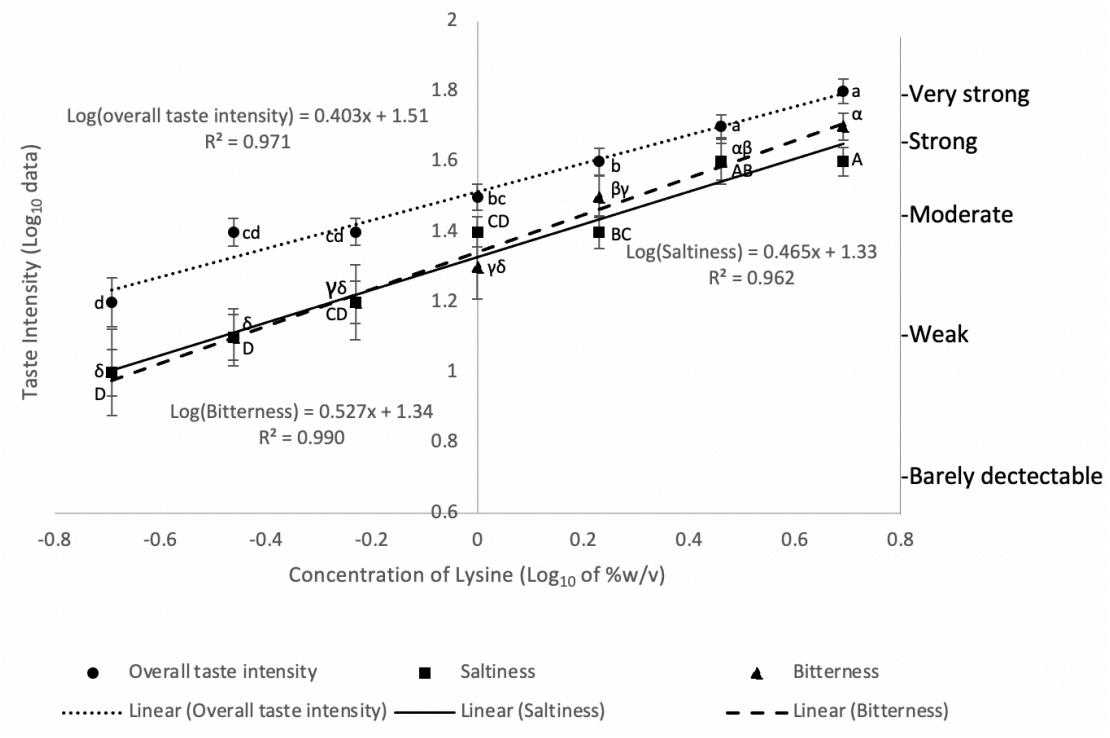

Figure 3.2 Logarithmic relationship between perceived intensity of overall taste, saltiness and bitterness, and concentration of lysine composite solution (each composite solution containing 0.25% NaCl and 0.75% calcium lactate w/v in addition to lysine). Within each intensity set means that do not share a common letter denote samples are significantly different ($p < 0.05$). Lower case letters used for overall taste intensity, upper case letters use for saltiness, and Greek letters use for bitterness.

According to the Steven's power law, the overall taste intensity, salty taste and bitter taste all had decelerating relationships with lysine concentration, where the exponents were 0.403, 0.465 and 0.527 respectively (Figure 3.2). Therefore, it was clear that lysine contributed similarly to both saltiness and bitterness, and the proportionate nature of the relationship between salty taste and concentration of the composite mixture seen in Figure 1 (sodium chloride with calcium lactate and lysine) must have been driven more by the sodium chloride than the lysine. However, this does not detract from the fact that lysine contributes to salty taste and the salty intensity evoked by lysine is dose-dependent (Figure 3.2).

3.4 Discussion

3.4.1 Salty taste of lysine solution

This study found 1% w/v lysine alone was perceived to have a weak saltiness intensity, however when used in combination with NaCl it could compensate for the saltiness loss in a 50% salt-reduced aqueous solution. Salty taste increased with the level of lysine, although the relationship was non-proportional. According to previous studies (Guo *et al.,* 2020, Vidal *et al.,* 2020) lysine was used as a salt substitute in 50% NaCl reduced meat products, yet the lysine could not compensate for the saltiness loss in salt-reduced ham or beef. One possible reason is that the concentration of lysine was too low. The highest concentration of lysine used by Guo *et al.* (2020) was 0.8% w/w, whereas in this experiment lysine at 1% w/v or more was used to have the ability to make up the salty taste loss caused by 50% salt reduction. Another reason may be the difference in food matrix. Previous studies have used solid food matrix, like meat, rather than pure aqueous systems to test the substitution effect of lysine. In fact, ingredients used in food products are often added at much higher concentrations than in the aqueous model systems to achieve the required taste intensity. The rheological properties of food matrices affect sensory perception, including taste; for example, tastants have greater mobility to reach taste receptors in liquids than that in solid foods (Liu *et al.*, 2017), as a result, the perceived taste intensity is much stronger than that in solid food. This could explain why in the experiment of Vidal *et al.* (2020) 3% w/w lysine addition in low-sodium salted beef was not detected with an increase in saltiness. Consequently, the perceived intensity of salt reduced system may vary greatly in different matrices.

Therefore, saltiness evaluation in aqueous solutions is only used for preliminary screening purposes (Kilcast and Den Ridder, 2007). In addition, individual recipes will require specific salt reduction strategies.

3.4.2 Bitter taste of calcium lactate and benefit/risk as salt substitute

The results found that calcium lactate did not offer saltiness in isolation, and it produced higher bitterness. Although 50% substitution of NaCl using combination of lysine and calcium lactate achieved similar intensity of saltiness in solution to the full NaCl control, bitterness resulting from this combination was increased. This is because the main taste characteristic of divalent cationic salts such as calcium and magnesium are bitterness, while other sensations are described as saltiness, metallic, astringent, sourness and sweetness, usually in decreasing order of intensity (Lawless *et al.*, 2003). However, Lawless *et al.* (2003) also found that compared to equimolar concentrations of calcium chloride, calcium lactate had a lower bitter response, even if the salty response was lower as well at the same time. Although calcium lactate brings some off-taste, it is still chosen as a salt substitute because it can be used to reinforce calcium content in food. Irshad *et al.* (2016) reported that restructured buffalo meat loaves with 1.25% w/w calcium lactate used as a calcium fortifier could meet recommended dietary allowance for calcium without affecting the textural and sensory properties. Another important reason is it can be used as a preservative, which may not be possessed by other salt substitutes. In meat products, lactic acid could pass across the cell membrane in their undissociated form and dissociate within the cell to acidify the cell interior.

Consequently, it could lower the water activity and inhibit the growth of bacteria in fresh and processed meat products to achieve longer shelf life (Shelef, 1994).

3.4.3 Psychophysical function between the lysine-calcium composite solutions and taste

In general, the perceived saltiness, bitterness and overall taste intensity increased with the concentration of tri-stimuli composite solution. For bitterness, although the sensation increased rapidly with the increase in concentration from weak to strong, the increase in bitterness was not proportional to concentration and could be considered to reach a plateau at a strong concentration range. This further confirms that bitterness has no effect on salty taste, but salty taste inhibits bitterness at any concentration intensity (Keast and Breslin, 2002). Due to the gradual increase of salty taste, the inhibition of bitterness became more obvious, so the relationship between bitterness and concentration was decelerating. In this experiment the relationship between salty taste and the concentration of composite solution was approximately proportional, and this is in line with the linear relationship between saltiness and NaCl reported by Moskowitz and Arabie (1970). They found that the saltiness increased linearly with the increase of NaCl from 0.05 mol/L to 1 mol/L. For an individual taste stimulus, as the physical concentration increases the perceived intensity elicited by that compound also increases, but at varying rates. For example, at very low concentrations of sapid compounds the taste intensity can grow in an exponential fashion. At medium concentration the perceived intensity can increase in linear fashion and at higher concentrations the perceived intensity may plateau (Keast and Breslin, 2002). In this experiment, the

relationship between bitterness and the composite solution seems to fit this pattern as the intensity of bitterness increased with increasing concentration up to 1.7 % w/v lysine (with 0.43% NaCl and 1.28 % calcium lactate; treatment T5 in Table 3.2; Supplementary table 8), while the bitterness did not increase beyond it. However, this is not supported by the experiment (Table 3.3) with fixed level of NaCl and calcium lactate but varied level of lysine where bitterness did significantly increase from 1.7 to 4.91 % lysine in Figure 2 (Supplementary table 10). It could be explained by the weak saltiness elicited by lysine compared to NaCl.

3.5 Conclusion

The results indicated that 1% w/v lysine produced a weak saltiness, and 0.75 % w/v calcium lactate did not offer saltiness alone. However, 0.75% w/v calcium lactate with 1% w/v lysine was successful in replacing 50% of salt in solution whilst maintaining saltiness of a control full salt sample, although additional bitterness was introduced. Furthermore, saltiness increased proportionally with the increase in concentration of the composite mixture (lysine, calcium lactate and NaCl), while the bitterness increase was less than proportionate. This suggests that at high concentration the saltiness increased to a greater extent than the bitter taste. In terms of application in real food matrix, lysine alone may face the issue of shortened shelf life caused by salt reduction, although the saltiness loss could be compensated. Therefore, the antibacterial effect of calcium lactate could be utilized to combine with lysine to offer practical application for food industry, i.e.to ensure both saltiness and shelf life of the food products could be maintained/enhanced in a salt reduced scenario. What is more, ingredients used in

food products are often added at much higher concentrations than in aqueous model systems to achieve the desired taste intensity. Therefore, applying lysine and calcium lactate to food matrices should be further investigated on verify their effects. Overall, the findings of this study fill a gap in the literature regarding the role of lysine as a salt substitute in terms of saltiness perception, providing new ideas for salt reduction in subsequent food products development through using lysine and calcium lactate blends. In addition, this study has used lysine with calcium lactate as a proposed mixture to replace salt in various food matrices, with the main roles of the two constituents being salty taste and antimicrobial activity respectively.

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Reference

Ashford, R., Jones, K. and Collins, D., 2020. National Diet and Nutrition Survey: Assessment of salt intake from urinary sodium in adults (aged 19 to 64 years) in England, 2018 to 2019. GOV. UK, pp.133-155.

Bartoshuk, L.M., Duffy, V.B., Green, B.G., Hoffman, H.J., Ko, C.W., Lucchina, L.A., Marks, L.E., Snyder, D.J. and Weiffenbach, J.M. (2004). Valid across-group comparisons with labeled scales: the gLMS versus magnitude matching. *Physiology & behavior*, 82(1), 109-114.

Bidlas, E. and Lambert, R.J., 2008. Comparing the antimicrobial effectiveness of NaCl and KCl with a view to salt/sodium replacement. *International Journal of Food Microbiology*, 124(1), pp.98-102.

Campagnol, P.C.B., dos Santos, B.A., Morgano, M.A., Terra, N.N. and Pollonio, M.A.R., 2011. Application of lysine, taurine, disodium inosinate and disodium guanylate in fermented cooked sausages with 50% replacement of NaCl by KCl. *Meat science*, 87(3), pp.239-243.

Desmond, E., 2006. Reducing salt: A challenge for the meat industry. *Meat science,* 74(1), pp.188-196.

De Marchi, M., Manuelian, C., Ton, S., Manfrin, D., Meneghesso, M., Cassandro, M. and Penasa, M., 2017. Prediction of sodium content in commercial processed meat products using near infrared spectroscopy. *Meat Science*, 125, pp.61-65.

Devlieghere, F., Vermeiren, L., Bontenbal, E., Lamers, P.P. and Debevere, J., 2009. Reducing salt intake from meat products by combined use of lactate and diacetate salts without affecting microbial stability. *International journal of food science & technology,* 44(2), pp.337-341.

Dos Santos Alves, L., Lorenzo, J., Gonçalves, C., dos Santos, B., Heck, R., Cichoski, A. and Campagnol, P., 2017. Impact of lysine and liquid smoke as flavor enhancers on the quality of low-fat Bologna-type sausages with 50% replacement of NaCl by KCl. *Meat Science*, 123, pp.50-56.

2438 Doyle, M.E. and Glass, K.A., 2010. Sodium reduction and its effect on food safety,
2439 food quality, and human health. *Comprehensive reviews in food science and food safety,*
2440 9(1), pp.44-56.
2441 Guo, X., Tao, S., Pan, J., Lin, X., Ji, C., Liang, H., Dong, X. and Li, S., 2020. Effects
2442 of L-Lysine on the physiochemical properties and sensory characteristics of salt-
2443 reduced reconstructed ham. *Meat science*, 166, p.108133.
2444 He, F. and MacGregor, G., 2010. Reducing Population Salt Intake Worldwide: From
2445 Evidence to Implementation. *Progress in Cardiovascular Diseases*, 52(5), pp.363-382.
2446 Henney, J.E., Taylor, C.L. and Boon, C.S., 2010. Taste and flavor roles of sodium in
2447 foods: A unique challenge to reducing sodium intake. *Strategies to Reduce Sodium*
2448 *Intake in The United States; National Academies Press: Washington, DC, USA.*
2449 Inguglia, E., Zhang, Z., Tiwari, B., Kerry, J. and Burgess, C., 2017. Salt reduction
2450 strategies in processed meat products – A review. *Trends in Food Science &*
2451 *Technology*, 59, pp.70-78.
2452 Irshad, A., Sharma, B., Ahmed, S., Talukder, S., Malav, O. and Kumar, A., 2016. Effect
2453 of incorporation of calcium lactate on physico-chemical, textural, and sensory
2454 properties of restructured buffalo meat loaves. *Veterinary World*, 9(2), pp.151-159.
2455 Islam, M. Z., Shamim, A. A., Ahmed, A., Akhtaruzzaman, M., Kärkkäinen, M., and
2456 Lamberg-Allardt, C., 2014. Effect of vitamin D, calcium and multiple micronutrients
2457 supplementation on lipid profile in pre-menopausal Bangladeshi garment factory
2458 workers with hypovitaminosis D. *Journal of health, population, and nutrition,* 32(4),
2459 687.

Keast, R. S. J., Breslin, P. A. S., 2002. An overview of binary taste-taste interactions. *Food Quality and Preference.*,14:111-24.

Khaw, K.T. and Barrett-Connor, E., 1984. Dietary potassium and blood pressure in a population. *The American journal of clinical nutrition,* 39(6), pp.963-968.

Kilcast, D. and Den Ridder, C., 2007. Sensory issues in reducing salt in food products. *In Reducing salt in foods* (pp. 201-220). Woodhead publishing.

Lawless, H., Rapacki, F., Horne, J. and Hayes, A., 2003. The taste of calcium and magnesium salts and anionic modifications. *Food Quality and Preference*, 14(4), pp.319-325.

Liem, D., Miremadi, F. and Keast, R., 2011. Reducing Sodium in Foods: The Effect on Flavor. *Nutrients*, 3(12), pp.694-711.

Lindemann, B., 1997. Sodium taste. *Curr. Opin. Nephrol.* Hypertension 6, 425–429.

Liu, D., Deng, Y., Sha, L., Abul Hashem, M. and Gai, S., 2017. Impact of oral processing on texture attributes and taste perception. J*ournal of food science and technology,* 54(8), pp.2585-2593.

McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W., Wallace, J. M., Bonham, M. P., and Fearon, A. M., 2010. Red meat consumption: An overview of the risks and benefits. *Meat science,* 84(1), 1-13.

Moskowitz, H. and Arabie, P., 1970. Taste intensity as a function of stimulus concentration and solvent viscosity. *Journal of Texture Studies*, 1(4), pp.502-510.

2480 Muchaamba, F., Stoffers, H., Blase, R., Ah, U. V., and Tasara, T., 2021. Potassium
2481 lactate as a strategy for sodium content reduction without compromising salt-associated
2482 antimicrobial activity in salami. Foods, 10(1), 114.
2483 Pangborn, R. and Pecore, S., 1982. Taste perception of sodium chloride in relation to
2484 dietary intake of salt. *The American Journal of Clinical Nutrition*, 35(3), pp.510-520.
2485 Shelef, L.A., 1994. Antimicrobial effects of lactates: a review. *Journal of Food*
2486 *Protection*, 57(5), pp.445-450.
2487 Sinopoli, D. A., and Lawless, H. T., 2012. Taste properties of potassium chloride alone
2488 and in mixtures with sodium chloride using a check-all-that-apply method. Journal of
2489 food science, 77(9), S319-S322.
2490 Tamm, A., Bolumar, T., Bajovic, B. and Toepfl, S., 2016. Salt (NaCl) reduction in
2491 cooked ham by a combined approach of high pressure treatment and the salt replacer
2492 KCl. *Innovative Food Science & Emerging Technologies*, 36, pp.294-302.
2493 Tomé, D. and Bos, D., 2007. Lysine Requirement through the Human Life Cycle, *The*
2494 *Journal of Nutrition,* 137(6), 1642S–1645S,
2495 World Health Organisation, 2020. *Salt Reduction, Fact Sheets*. Retrieved 3 November
2496 2020 from https://www.who.int/news-room/fact-sheets/detail/salt-reduction
2497 Vanderklaauw, N. J., and Smith, D. V., 1995. Taste quality profiles for 15 organic and
2498 inorganic salts. *Physiology & Behavio*r, 58(2), 295-306.
2499 Vidal, V.A., Santana, J.B., Paglarini, C.S., da Silva, M.A., Freitas, M.Q., Esmerino,
2500 E.A., Cruz, A.G. and Pollonio, M.A., 2020. Adding lysine and yeast extract improves
2501 sensory properties of low sodium salted meat. *Meat Science*, 159, p.107911.

2502 Yamamoto, K. and Ishimaru, Y., 2013. Oral and extra-oral taste perception. *Seminars*

2503 *in Cell & Developmental Biology,* 24(3), pp.240-246.

Chapter 4. Effect of lysine and calcium lactate in physicochemical characteristics, sensory properties and shelf-life in salt-reduced pork patty

Abstract

The aim of this study was to evaluate the effects of calcium lactate and lysine on the physicochemical characteristics and sensory properties of pork patties that had 50% of salt (sodium chloride) replaced. The use of 0, 1.5%, 3% (w/w) calcium lactate and 0%, 3%, 6% (w/w) lysine as salt substitutes were added into the pork patties and compared to the full salt (2% w/w) control patty. The results showed that both calcium lactate and lysine increased texture attributes, decreased water holding capacity and water activity of a salt-reduced pork patty ($p < 0.05$). Additionally, lysine increased the yield, and calcium lactate improved shelf-life ($p < 0.05$). The combination of calcium lactate (3% w/w) and lysine (3% w/w, 6% w/w) or 1.5% w/w calcium lactate with 3% w/w lysine could compensate the loss in saltiness caused by 50% salt reduction in pork patty. Considering the effects of lysine and calcium lactate on physical-chemical characteristic, shelf-life and sensory traits, it was recommended that the addition of 3% w/w lysine and 1.5% w/w calcium lactate could be used to develop pork patty with 50% NaCl reduction with comparable eating quality.

4.1 Introduction

Sodium chloride (NaCl), known as salt, has been used as an ingredient or food preservative for thousands of years. It plays a beneficial role on flavour, taste, and texture (Rios-Mera et al., 2021; De Marchi et al., 2017; Inguglia et al., 2017). In Europe, around 70% of salt consumption comes from processed foods, among which 20% is

derived from meat products (Ruusunen and Puolanne, 2005). For example, fresh pork typically contains only 0.18 g salt/100 g, but bacon contains about 3.2 g salt/100 g (Inguglia et al., 2017). According to the recommendation of World Health Organization (WHO) in 2020, the average sodium consumption should be approximately 2 g sodium per day (equivalent to about 5 g salt per day) for adults to prevent chronic diseases. However, current salt intake is much higher than the standard recommended by WHO in most counties. In the UK, the dietary intake for salt reached 8.4 g per day (equivalent to about 3.4 g sodium per day) in 2018/2019 (Ashford, Jones and Collins, 2020). Numerous literatures have reported that the consumption of sodium in excess is directly related to the increase of blood pressure, which is a risk factor for cardiovascular diseases including heart diseases and stroke (Rybicka et al., 2022; Rucker, Rudemiller and Crowley, 2018; He and MacGregor, 2010). Moreover, it can also lead to calcium losses and impairment of skeletal mass (Tiyasatkulkovit et al., 2021). Therefore, an increasing number of countries have implemented various initiatives to reduce the use of sodium salt in the food industry in the last decade.

Current approaches to reduce the sodium level in processed foods and meat products have consisted of the following strategies: complete or partial replacement of NaCl; replacement with a low-sodium mixture; use of flavour enhancers such as monosodium glutamate or yeast extract; changes in the physical form of salt; improvement of salt diffusion via high pressure treatment or ultrasound technology (Fellendorf, O'Sullivan and Kerry, 2016; Ojha et al., 2016; Dos Santos et al., 2014; Emorine et al., 2014; Paulsen et al., 2014). Among them, utilization of salt substitutes such as potassium

chloride (KCl) has been considered as the most popular and effective method to reduce sodium level in food products (Tamm et al., 2016). Although such compounds make a contribution to saltiness perception, they may also cause some unsatisfactory taste like bitterness at high concentration or shorten the shelf life of products (Inguglia et al., 2017; Van Der Klaauw and Smith, 1995), which limits their application in food manufacturing. It should be noted that ideal salt substitutes should replace the role of salts in meat products without compromising the eating quality of meat products.

Recently, lysine has been added into meat products to improve its eating quality. Lysine is one of the nine essential amino acids in the human body that cannot be produced by the body and therefore must come from food (Blemings and Benevenga, 2007). It has been used as flavour enhancer in low-sodium sausage (Dos Santos Alves et al., 2014; Campagnol et al., 2012). Both Guo et al. (2020) and Vidal et al. (2020) also reported that lysine could improve the physical-chemical characteristics in salt-reduced ham or beef. However, the saltiness loss caused by salt reduction could not be compensated even at 3% w/w lysine. Calcium lactate could be another effective salt substitute, although few studies have tested this. Calcium lactate is associated with saltiness because Ca2+ the divalent metal cations are mainly perceived with saltiness and bitterness, but calcium lactate also has a considerable sour component (Lawless et al., 2003; Kilcast and Den Ridder, 2007). In addition, it could also be used in salt reduced formulations as a preservative because it can inhibit the growth of bacteria (Irshad et al., 2016; Shelef and Potluri, 1995), a property not delivered by some other salt substitutes. Weaver and Shelef (1993) found that 2% w/w calcium lactate could inhibit

the growth of Listeria monocytogenes (L. monocytogenes) which is relatively common in the meat products. Calcium lactate would also provide a function of calcium fortification to improve the nutrition value of meat products because the calcium content in the meat is relatively poor at about 10 mg/100 g whereas adults require a daily intake of calcium of l000 mg/day (Okuskhanova et al., 2016).

Our previous research in aqueous solutions (chapter 3) found that 1% w/v lysine had a very weak salty taste, however when used together with sodium chloride it could enhance salty taste to enable a 50% salt reduction, with or without calcium lactate. This research aimed to test whether the salt taste enhancement tested in aqueous solution is still effective in a real food matrix and further to evaluate their effects on physicochemical characteristics, sensory properties and microbial load of food product. Progressing understanding from previous literature and our previous research, this study specifically hypothesized that combination of lysine and calcium lactate could achieve a 50% salt-reduced pork patty without reducing salty taste and shelf-life. If salt substitution using lysine and calcium lactate is successful in meat products, this could offer health benefits to consumer through decreasing dietary sodium intake and increasing calcium intake from processed meat products.

4.2 Materials and Methods

4.2.1 Pork raw meat

All the ground lean pork leg and pork back fat was purchased from a local supplier (Solent Butchers & Co. Limited, UK) on three occasions to provide material for three replicates (section 2.2). All the meat was vacuum packaged (A300/52, Multivac

Gastrovac, Germany) and stored at -18 °C in a freezer until further use. The samples were thawed at 4 °C in a refrigerator for 24 h before use.

4.2.2 Experiment design

The salt content of meat products is usually around 1.5% - 2.5% (Guo *et al.*, 2020), hence, for the control sample a salt concentration of 2% w/w sodium chloride was used. In addition, Public Health England (2020) has set 2024 ideal salt content for pork sausages as 1.08 g salt per 100g, so a 50% salt reduction was chosen in order to target 1% w/w sodium chloride contained. To develop sodium reduced pork patties calcium lactate (Merck, USA) and lysine (Health Leads, UK), were combined with each at three levels. Because ingredients used in food products are often added at much higher concentrations than in aqueous model systems to achieve the desired taste intensity, a higher concentration of lysine and calcium lactate were used in the preliminary trials. According to the results of these preliminary trials, for calcium lactate, levels at 0%, 1.5% and 3% (w/w) were used, and lysine was added at 0%, 3% and 6% (w/w). According to the factorial design for two factors and three levels, 9 treatments plus one control sample were prepared as detailed in Table 4.1. Each treatment was prepared in triplicate, each using a different batch of pork.

Table 4.1. Formulation of pork patties used to investigate the effects of calcium lactate and lysine.

Treatment*	Lean pork leg (% w/w)	Pork back fat (% w/w)	Distilled water (% w/w)	Sodium Chloride (% w/w)	Lysine (%w/w)	Calcium lactate (% w/w)
Control	70	10	18	2	-	-
C0L0	70	10	18	1	-	-
C0L3	70	10	18	1	3	-
C0L6	70	10	18	1	6	-
C1.5L0	70	10	18	1	-	1.5
C1.5L3	70	10	18	1	3	1.5
C1.5L6	70	10	18	1	6	1.5
C3L0	70	10	18	1	-	3
C3L6	70	10	18	1	3	3
C3L6	70	10	18	1	6	3

*Control = 2% w/w NaCl; C0L0 = 1% w/w NaCl; C0L3 = 1% w/w NaCl + 3% w/w lysine; C0L6 = 1% w/w NaCl + 6% w/w lysine; C1.5L0 = 1% w/w NaCl + 1.5% w/w calcium lactate; C1.5L3 = 1% w/w NaCl + 1.5% w/w calcium lactate + 3% w/w lysine; C1.5L6 = 1% w/w NaCl + 1.5% w/w calcium lactate + 6% w/w lysine; C3L0 = 1% w/w NaCl + 3% w/w calcium lactate; C3L3 = 1% w/w NaCl + 3% w/w calcium lactate + 3% w/w lysine; C3L6 = 1% w/w NaCl + 3% w/w calcium lactate + 6% w/w lysine.

4.2.3 Preparation of pork patties

The formulation of pork patties was adapted from the work of Lu, Kuhnle and Cheng (2017) with slight modification to include lean pork leg (700 g/kg), pork back fat (100 g/kg), and distilled water (180 g/kg). For each formulation (Table 1), the ground meat and all ingredients (distilled water, salt, calcium lactate and lysine) were homogenized at 5000 rpm for 5min until uniformity was reached using a food processor (Titanium Major KMM020, Kenwood Limited, UK). Each pork patty was formed with 100 g

mixture in a foil cup (8 cm diameter). In order to assess the impact of calcium lactate and lysine on the quality of raw patties over shelf life, the samples were packed in resealable dual-track food freezer bags (Co-op, UK) and stored at 4 ℃ for 1, 3, 5 and 7 days. Samples were cooked at 200°C in an oven (B1542, Naff, Germany) until the centre temperature reached 75°C. After cooking, samples were covered with foil and chilled at 4°C in a refrigerator for 24 h before physical analysis (i.e., yield, colour and texture). A portion of the chilled cooked samples were ground to a powder using a blender (AT640, Kenwood Limited, UK), then vacuum packed and stored at -18°C in a freezer, for further chemical analysis (pH after cooking, water holding capacity and moisture content).

4.2.4 Microbial analysis

4.2.4.1 Water activity

Water activity measurements were carried out on the raw samples at 1, 3, 5 and 7 day of storage and using a water activity meter (HYGROLAB C1, Rotronic, USA) at room temperature (20°C). The raw ground pork patties were added to sample container without exceeding half height of the container. The analysis was performed in triplicate.

4.2.4.2 pH

pH was measured on both raw (1, 3, 5 and 7 day of storage) and cooked ground pork patties. 10 g patty sample was added into 100 ml distilled water and mixed using a magnetic stirrer (SS3H STIRRER-HOTPLATE, hemLab, Netherlands) for 90 s at a medium speed. pH was measured using a pH meter (Orion star A111, Thermo scientific, USA). Analysis was performed in triplicate.

4.2.4.3 Total viable count (TVC)

TVC was carried out at 1, 3, 5 and 7 days of storage. 10 g of raw ground pork patty were aseptically weighted and mixed with 90 ml of buffered peptone water. After 2 min mixing in a stomacher blender (Stomacher 400 circulator, Seward, UK), appropriate decimal dilutions were plated in duplicate on Plate Count Agar (PCA) (Oxiod Ltd, UK) for TVC. Plates were incubated at 37°C (constant temperature room) for 48 h. All microbial counts were converted to logarithms of colony-forming units per gram (log cfu/g).

4.2.5 Physical-chemical characteristics of pork patties

4.2.5.1 Moisture content

According to AOAC method, 3 g ground sample was put into the aluminium moisture dish, then dried in an oven (GALLENKAMP, UK) at 100 ° C for 24 h. Samples were cooled in a desiccator at least 30 min and reweighed to calculate the weight difference. The moisture content was calculated by the weight difference divided by the starting weight of sample before drying and expressed as %. The analysis was performed in triplicate.

4.2.5.2 Yield

The cooking loss was calculated using the formula as follows:

Yield (%) = 1 - $(W_b - W_a)/W_b$ x 100

W_b means weight of pork patty before cooking, and W_a means weight of pork patty after chilling.

4.2.5.3 Water holding capacity

This method was based up that of Zhou, Li and Tan (2014). Ground sample (5g) was wrapped with filter paper and put into a centrifuge tube. The tube was centrifuged at 3800 g for 10 min (Sorvall X Pro/ST plus series, Thermo Scientific, USA) at room temperature (20°C). The water holding capacity was determined as follows: Water holding capacity (%) = (1 - (W_a – W_b)/5) x 100%, where W_a was filter paper weight after centrifuge, and W_b was the filter paper weight before centrifuge. The analysis was performed in triplicate.

4.2.5.4 Texture profile analysis

The texture profile analysis was measured by the Texture Analyser (TA-XT2, Stable Micro Systems, USA). Cooked pork patties were equilibrated for 30 min at room temperature (20 °C) before sampled using a cork borer. Each sample was 1.8 cm height and 2.2 cm diameter. A 30 kg load cell was used, and test speed was 2 mm/s with the strain at 30%. The samples were compressed twice, and the interval time between each compression was 5 s, for texture profile analysis to calculate the hardness, springiness, cohesiveness, and chewiness. Hardness (N) was defined as the peak force that occurs during the first compression; springiness was expressed as a ratio or percentage of a product's original height; cohesiveness was the area of work during the second compression divided by the area of work during the first compression; chewiness (N cm) was calculated as the product of hardness x cohesiveness x springiness (Del Pulgar, Gázquez and Ruiz-Carrascal, 2012.). At least 5 patties per sample were used to measure the texture attributes of each sample, and the average was recorded as the value of the sample.

4.2.5.5 Colour

A chroma meter (CR-400, Konica minolta, Japan) with 8mm diameter measuring aperture, illuminant D65, 2° standard observer was used to determine the colour of cooked pork patty. The instrument was calibrated using white calibration plate (CR-A43, Y = 93.5, x = 0.3140, y = 3318) and CIELAB colour space was selected. Colour characteristics, including L* (lightness), a* (redness) and b* (yellowness), were measured at three surface locations, and the average was recorded as the value of the sample.

4.2.6 Sensory evaluation

Sensory profiling is a method that is used to determine a food product's specific sensory profile, and such profiling relies on the panelist's ability to evaluate the specific attributes of the product by describing and quantitative rating them, followed by statistical analysis (Fauza *et al.*, 2021). An employed trained sensory profiling panel were used for the sensory evaluation. There were 11 females and 1 male with age ranging from 35 to 65 years. They are all screened for sensory acuity, as well as descriptive and discrimination ability, and each has a minimum of 6 months' experience. The consent to taste foods as part of the employment contract as sensory panellists. The panel developed a consensus vocabulary to describe the attributes of the pork patties. Where possible reference standards were used to ensure panellists were in agreement over the attribute descriptions, where an appropriate reference standard could not be found then the panel agreed on a descriptor for the attribute (see final attribute list in the results section, Table 4). Five samples were selected for sensory evaluation by

principal component analysis (PCA) based upon the physio-chemical and microbiological analysis results (see detail explanation in the section 4.3.3). The scoring of samples was carried out in a quiet, air-conditioned room (21°C) under artificial daylight lighting, with panelists sitting in separate booths. To ensure that each meat sample was served to the panel within 1 h of cooking at same temperature (50°C), a bain-marie was used to serve food. Each sample with gold curst (approximately 5 g) was coded with three-digit random number and presented to the panellists sequentially in a balanced order. The panellists were asked to use warm water to clean the palate between samples, and the time delay between samples (post after-effects scoring) was 30 s. Samples were assessed using unstructured line scales and panellists rated attribute based on their perception with 'not' for '0' and 'very' for '100'. Different anchors were used for following attributes: overall intensity of colour used '0' for 'pale' and '100' for 'intense'; golden crust used '0' for 'none' and '100' for 'lots'; dense used '0' for 'open structure ' and '100' for 'dense structure'; moist used '0' for 'dry ' and '100' for 'moist '; smooth used '0' for ' rough' and '100' for 'smooth '. All samples were scored in duplicate on separate days.

4.2.7 Statistical analysis

For all analysis other than the sensory evaluation, two factors (lysine and calcium lactate), each at three levels (0%, 3%, 6% w/w for lysine and 0%, 1.5%, 3% w/w for calcium lactate) were used to evaluate the impact of lysine and calcium lactate on the quality of salt reduced pork patties. This resulted in 9 treatments plus one control treatment, and each treatment had 3 replicates. SPSS Statistics 27 (IBM, USA) was

used to carry out the statistical analysis. One-way analysis of variance (ANOVA) was used to evaluate the significant difference between 10 treatments in physical-chemical and microbial analysis at the significant level 0.05, while two-way ANOVA was used to examine the effect of factors (lysine, calcium lactate) and the interaction between factors at significant level 0.05. Duncan test was selected for multiple comparisons if equal variances were assumed, otherwise, Tamhane's T2 test was used. PCA was carried out by XLSTAT Version 2022.4.1 (Addinsoft, Paris, France) on the correlation matrix from the physicochemical and microbiological results to visualise the main differences the different formulations.

For the sensory profiling a partial design was used where 5 treatments were selected from the physical-chemical analysis alongside control treatment, all samples assessed in two replicates. Two-way analysis of variance (ANOVA) was carried out using Senpaq (QI Statistis, Reading, UK) where panelists were treated as random effects and samples as fixed effects, main effects were tested against the assessor by sample interaction. Multiple pairwise comparisons were carried out using Tukey's HSD at a significance level of 0.05.

4.3 Results and discussion

4.3.1 Shelf Life

The effect of calcium lactate and lysine on factors influencing the shelf life of pork patties are shown in Table 4.2.

2748 Table 4.2. Analysis related to the shelf life of salt-reduced pork patties formulated with calcium lactate and lysine.

2749 Table 4.2a. The significant difference for each treatment on shelf life of salt-reduced pork patties.

Treatment	Day 1			Day 3			Day 5			Day 7		
	WA	pH	TVC (Log cfu g^{-1})	WA	pH	TVC (Log cfu g^{-1})	WA	pH	TVC (Log cfu g^{-1})	WA	pH	TVC (Log cfu g^{-1})
Control	0.980±0.005^{cde}	5.57±0.07^{a}	5.56±0.28^{ab}	0.980±0.005^{bc}	5.55±0.10^{ab}	6.18±0.07^{ab}	0.978±0.004^{cd}	5.58±0.05^{ab}	6.81±0.37^{a}	0.979±0.004^{cd}	5.64±0.06^{ab}	8.56±0.28^{a}
C0L0	0.986±0.003^{a}	5.57±0.12^{a}	5.60±0.24^{ab}	0.985±0.004^{a}	5.52±0.09^{abc}	6.38±0.11^{a}	0.983±0.003^{a}	5.48±0.34^{cd}	6.82±0.27^{a}	0.987±0.005^{a}	5.55±0.14^{bc}	8.93±0.46^{a}
C0L3	0.981±0.005^{bcd}	5.56±0.06^{a}	5.59±0.23^{ab}	0.980±0.005^{bc}	5.53±0.09^{abc}	6.44±0.35^{a}	0.979±0.002^{bcd}	5.58±0.08^{ab}	7.08±0.26^{a}	0.979±0.004^{cd}	5.75±0.21^{a}	8.63±0.77^{a}
C0L6	0.977±0.003^{de}	5.59±0.05^{a}	5.57±0.31^{ab}	0.973±0.006^{d}	5.53±0.11^{abc}	6.50±0.44^{a}	0.975±0.003^{e}	5.56±0.08^{abc}	6.97±0.31^{a}	0.978±0.005^{de}	5.73±0.13^{a}	7.83±0.83^{bc}
C1.5L0	0.985±0.003^{ab}	5.57±0.07^{a}	5.43±0.31^{ab}	0.982±0.006^{ab}	5.41±0.18^{c}	6.38±0.14^{a}	0.982±0.004^{ab}	5.52±0.10^{abcd}	6.83±0.14^{a}	0.985±0.005^{ab}	5.53±0.17^{bc}	7.87±0.63^{b}
C1.5L3	0.979±0.004^{cde}	5.59±0.05^{a}	5.48±0.52^{ab}	0.974±0.006^{d}	5.55±0.15^{ab}	5.89±0.44^{b}	0.978±0.004^{cd}	5.50±0.12^{bcd}	7.06±0.20^{a}	0.978±0.004^{de}	5.51±0.15^{bc}	7.46±0.33^{bcd}
C1.5L6	0.976±0.004^{e}	5.58±0.09^{a}	5.70±0.37^{a}	0.973±0.004^{d}	5.58±0.11^{a}	5.95±0.56^{b}	0.974±0.002^{e}	5.54±0.04^{abc}	6.80±0.28^{a}	0.973±0.006^{e}	5.49±0.09^{c}	7.31±0.36^{cd}
C3L0	0.982±0.004^{bc}	5.58±0.04^{a}	5.30±0.14^{b}	0.981±0.005^{abc}	5.44±0.09^{bc}	5.48±0.17^{c}	0.981±0.002^{abc}	5.44±0.15^{d}	6.03±0.46^{b}	0.984±0.005^{abc}	5.44±0.06^{c}	7.06±0.45^{d}
C3L3	0.977±0.003^{de}	5.59±0.05^{a}	5.44±0.18^{ab}	0.976±0.005^{cd}	5.47±0.15^{abc}	5.36±0.24^{c}	0.976±0.003^{de}	5.54±0.12^{abc}	5.85±0.52^{bc}	0.980±0.005^{bcd}	5.52±0.02^{bc}	7.21±0.38^{d}
C3L6	0.967±0.005^{f}	5.59±0.07^{a}	5.28±0.16^{b}	0.965±0.004^{e}	5.48±0.15^{abc}	5.30±0.33^{c}	0.970±0.003^{f}	5.60±0.06^{a}	5.64±0.48^{c}	0.973±0.006^{e}	5.52±0.07^{bc}	7.01±0.16^{d}

2750 Table 4.2b. Effect of calcium lactate and lysine on shelf life of salt-reduced pork patties.

Substitutes	Dosage	Day 1			Day 3			Day 5			Day 7		
		WA	pH	TVC ((Log cfu g^{-1})	WA	pH	TVC ((Log cfu g^{-1})	WA	pH	TVC ((Log cfu g^{-1})	WA	pH	TVC ((Log cfu g^{-1})
Calcium lactate	0	0.982±0.005^{a}	5.57±0.08^{a}	5.59±0.26^{a}	0.979±0.007^{a}	5.53±0.09^{a}	6.44±0.32^{a}	0.979±0.005^{a}	5.54±0.08^{a}	6.96±0.29^{a}	0.981±0.006^{a}	5.68±0.18^{a}	8.46±0.86^{a}
	1.5	0.980±0.005^{a}	5.58±0.07^{a}	5.54±0.41^{a}	0.976±0.006^{b}	5.52±0.16^{a}	6.07±0.46^{b}	0.978±0.005^{a}	5.52±0.09^{a}	6.90±0.24^{b}	0.979±0.007^{a}	5.51±0.14^{b}	7.55±0.51^{b}
	3	0.975±0.008^{b}	5.58±0.05^{a}	5.35±0.17^{b}	0.974±0.008^{b}	5.47±0.13^{a}	5.38±0.26^{c}	0.975±0.005^{b}	5.52±0.13^{a}	5.83±0.50^{b}	0.979±0.007^{a}	5.49±0.07^{b}	7.11±0.35^{c}

P (C)		<0.001	0.879	0.014	0.002	0.199	<0.001	<0.001	0.648	<0.001	0.133	<0.001	<0.001
Lysine	0	0.985±0.004^{a}	5.57±0.08^{a}	5.46±0.26^{a}	0.983±0.005^{a}	5.46±0.13^{a}	6.11±0.44^{a}	0.982±0.003^{a}	5.47±0.10^{a}	6.60±0.48^{a}	0.985±0.005^{a}	5.50±0.14^{a}	8.00±0.91^{a}
	3	0.979±0.004^{b}	5.58±0.05^{a}	5.51±0.34^{a}	0.977±0.005^{b}	5.52±0.14^{a}	5.90±0.57^{b}	0.978±0.003^{b}	5.54±0.10^{b}	6.67±0.68^{a}	0.979±0.004^{b}	5.60±0.18^{b}	7.77±0.81^{a}
	6	0.973±0.006^{c}	5.59±0.07^{a}	5.52±0.33^{a}	0.970±0.006^{c}	5.53±0.13^{a}	5.91±0.66^{b}	0.973±0.004^{c}	5.57±0.06^{b}	6.47±0.70^{a}	0.975±0.006^{c}	5.58±0.15^{b}	7.38±0.66^{b}
P (L)		<0.001	0.849	0.687	<0.001	0.112	0.103	<0.001	<0.001	0.124	<0.001	0.023	<0.001
P (I)		0.058	0.938	0.323	0.063	0.307	0.097	0.619	0.062	0.187	0.416	0.095	0.072

*Control = 2% w/w NaCl; C0L0 = 1% w/w NaCl; C0L3 = 1% w/w NaCl + 3% w/w lysine; C0L6 = 1% w/w NaCl + 6% w/w lysine; C1.5L0 = 1% w/w NaCl + 1.5% w/w calcium lactate; C1.5L3 = 1% w/w NaCl + 1.5% w/w calcium lactate + 3% w/w lysine; C1.5L6 = 1% w/w NaCl + 1.5% w/w calcium lactate + 6% w/w lysine; C3L0 = 1% w/w NaCl + 3% w/w calcium lactate; C3L3 = 1% w/w NaCl + 3% w/w calcium lactate + 3% w/w lysine; C3L6 = 1% w/w NaCl + 3% w/w calcium lactate + 6% w/w lysine. P(D) = significance level of days; P(C) = significance level for calcium lactate; P(L) = significance level for lysine; P(I) = significance of any interaction between lysine and calcium lactate; WA = water activity; TVC = total viable count. Averages within the same column followed by the same letter in Table 2a for each salt substitute are not significantly different ($P > 0.05$); Within each sample set statistically significant differences between samples for the primary taste quality are indicated by different letters above the bar ($p < 0.05$). Values represented as the Mean ± standard deviation (SD), n = 3.

4.3.1.1 Water activity

Water activity plays an important role in meat preservation, as it is negatively correlated with the growth and metabolic activity of microorganisms. Its measurement has been a valuable tool for predicting the microbial stability (and safety) of meat and meat products (Fernández-Salguero *et al.*, 1993). The water activity of all pork patties except C3L6 was unchanged over the 7 days storage (Figure 4.1, $p > 0.05$). Significant increase in water activity was observed when the salt content was reduced by 50% at all storage days (Table 2, $p < 0.05$) because the water binding ability was decreased due to the reduction of salt (Albarracín *et al.*, 2011). It further confirmed that 50% salt reduction would reduce the suppression of bacterial growth and deteriorate the shelf life. Irshad *et al.* (2016) found that water activity of fortified restructured buffalo meat loaves with 1% calcium lactate was significantly lower than their control product. Similar results were also achieved in this work. Lysine was also found with the ability to reduce the water activity because of its polarity. Campagnol *et al*. (2011) reported that an increasing concentration of lysine had no effect on the water activity of 50% salt - reduced fermented cooked sausage. This contradicts the current study where lysine was found to significantly decrease water activity. While the difference could be explained by the concentration difference in lysine. In this work, the higher concentration of lysine (3% w/w) was used compared to 0.139 - 0.833% in their work. Although the addition of calcium lactate and lysine decreased water activity of patties (Table 2, $p < 0.05$), the water activities in all samples were still above 0.96, which is much higher than the maximum water activity of 0.85 recommended to inhibit growth of microorganisms in

food products (Houtsma *et al.,* 1993). The there was no significance of any interaction between lysine and calcium lactate observed as shown in Table 2 ($p > 0.05$).

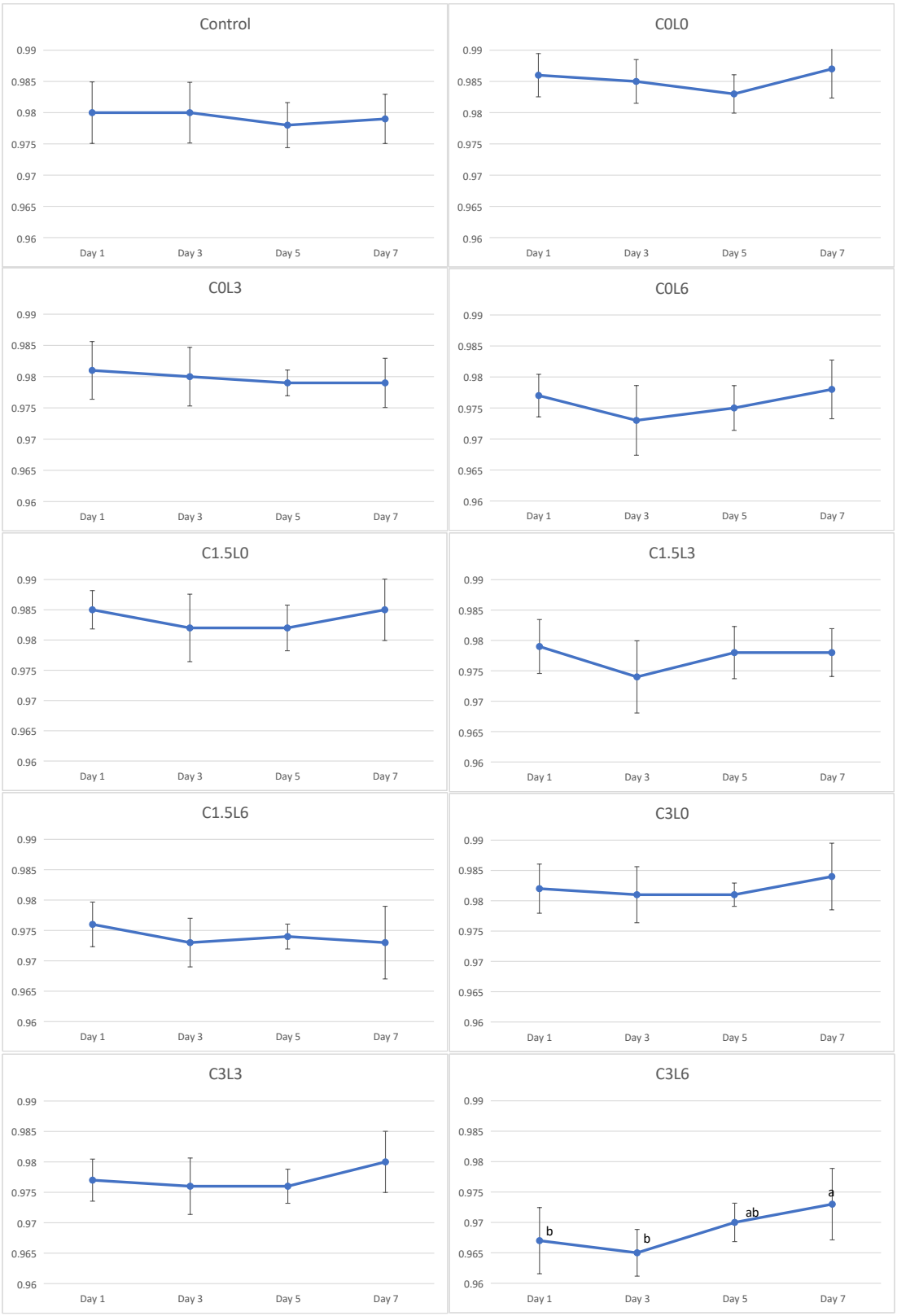

Figure 4.1 The changing of water activity within a week for different treatments. Error bars representing the standard error indicate the variability of the sample mean or estimate. Different letters mean significantly different ($p < 0.05$).

4.3.1.2 pH before cooking

A reduction in pH generally improves food safety or shelf life of meat products as it reduces or inhibits microbial growth associated with food deterioration or pathogenicity. The pH range of fresh meat is around 5.5-6.0 (Calkins and Hodgen, 2007). pH in the raw control pork patty and 50% salt reduction patty (C0L0) remained stable during 7 days of storage (Figure 4.2, $p > 0.05$). However, when lysine only was added to salt-reduced pork patty (C0L3, C0L6), the pH increased significantly on day 7 (Figure 4.2, $p < 0.05$), and the pH increased with the increasing concentration of lysine from day 5 (Table 2, $p < 0.05$). This result is consistent with Vidal *et al.* (2020) experimental results, where they found that adding lysine to low sodium salted meat significantly increased pH. This may be because the amino acid side chain of lysine is basic (Watanabe, Kadowaki, and Fujimura, 2005). In contrast, when 3% w/w calcium lactate was added to salt-reduced pork patty (C3L0), there was a significant drop in pH on the third day compared to the first day (Figure 4.2, $p < 0.05$). In addition, the addition of calcium lactate did not have an impact on pH of the raw salt-reduced pork patties over the first 5 days of storage ($p > 0.05$), but it significantly reduced the pH value on day 7 (Table 2, $p < 0.05$). Lawrence *et al.* (2004) reported that the addition of 2.4% calcium lactate to beef muscle led to a significant decrease in pH on day 7 of storage because the calcium lactate had thoroughly dispersed through the meat over the seven days. Table 2 also shown that there was no interaction between lysine and calcium lactate in terms of pH ($p > 0.05$).

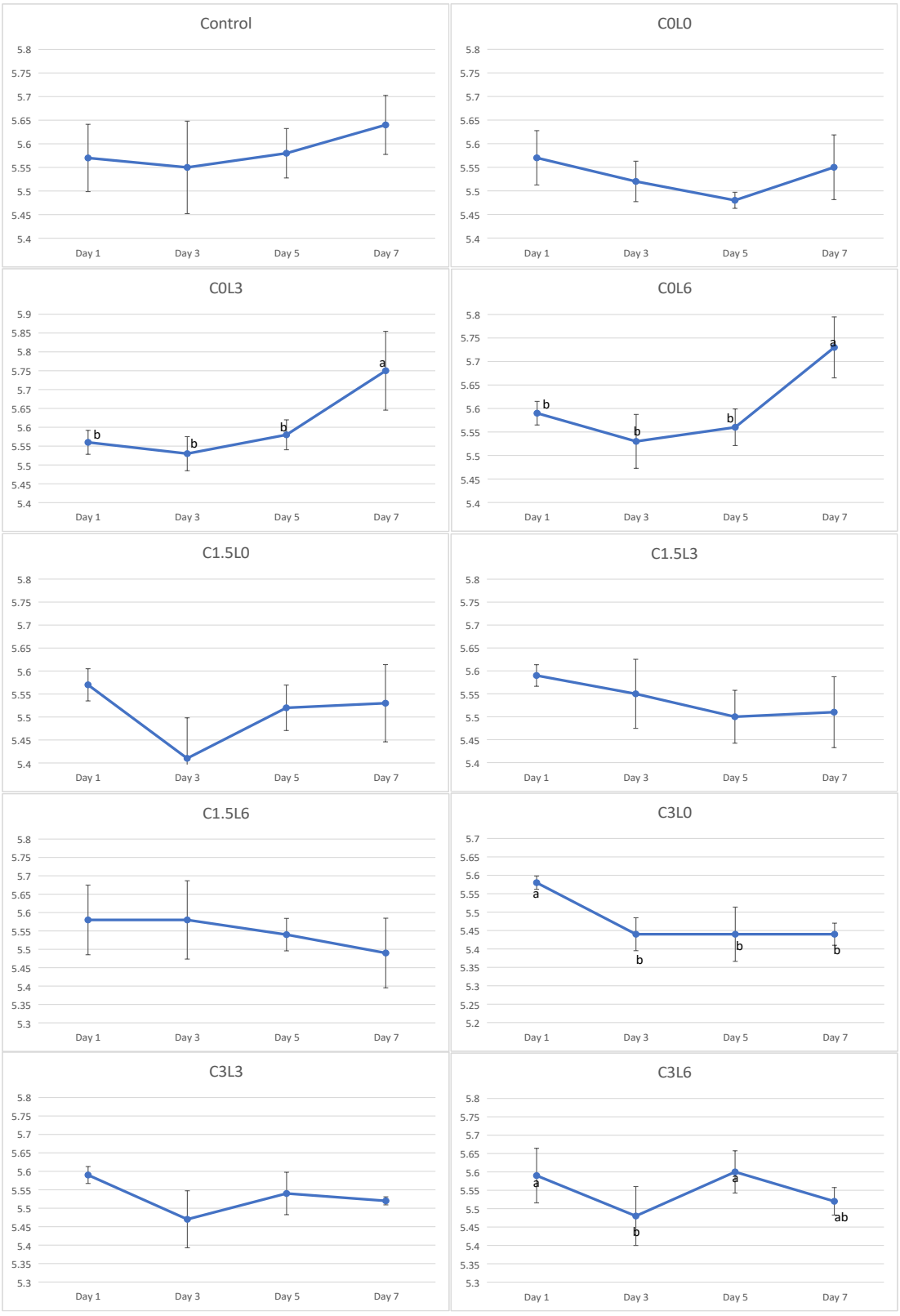

Figure 4.2 The changing of pH before cooking within a week for different treatments. Error bars representing the standard error indicate the variability of the sample mean or estimate. Different letters mean significantly different ($p < 0.05$).

4.3.1.3 Total viable count

The total viable count was increased from 5.28 to 8.93 log cfu g^{-1} during seven days storage (Figure 4.3). No treatment presented a higher TVC than the control patty. At day 3, 5, 7, the addition of 3% w/w calcium lactate significantly reduced the TVC compared to both 2% salt control and the 50% salt reduced patty only (C0L0) ($p < 0.05$); 1.5% w/w calcium lactate treatments reduced TVC but only at day 7 of storage compared with 2% salt control (Table 2a, $p<0.05$). Such a reduction in TVC can be explained through the decrease in pH and water activity caused by calcium lactate. As shown in Table 2a, at day 7 the highest concentration of lysine (6% w/w) did inhibit the growth of bacteria compared to 2% salt control ($p < 0.05$). The finding that lysine had a smaller inhibiting effect on microbiological growth than calcium lactate can be expected because lysine had less of an effect on water activity and did not reduce pH. However, Vidal *et al.* (2020) found 3% lysine added into low sodium salted meat significantly reduced water activity which did result in low total counts was observed for their treatments. However, the water activity of their low sodium salted meat was much lower at 0.753, while the water activity in this study was more than 0.97. In addition, it should be noted that although lysine alone can guarantee the same shelf life, the addition of calcium lactate can significantly increase the shelf life of salt-reduced meat products. This is a distinctive advantage for developing reduced-salt meat products.

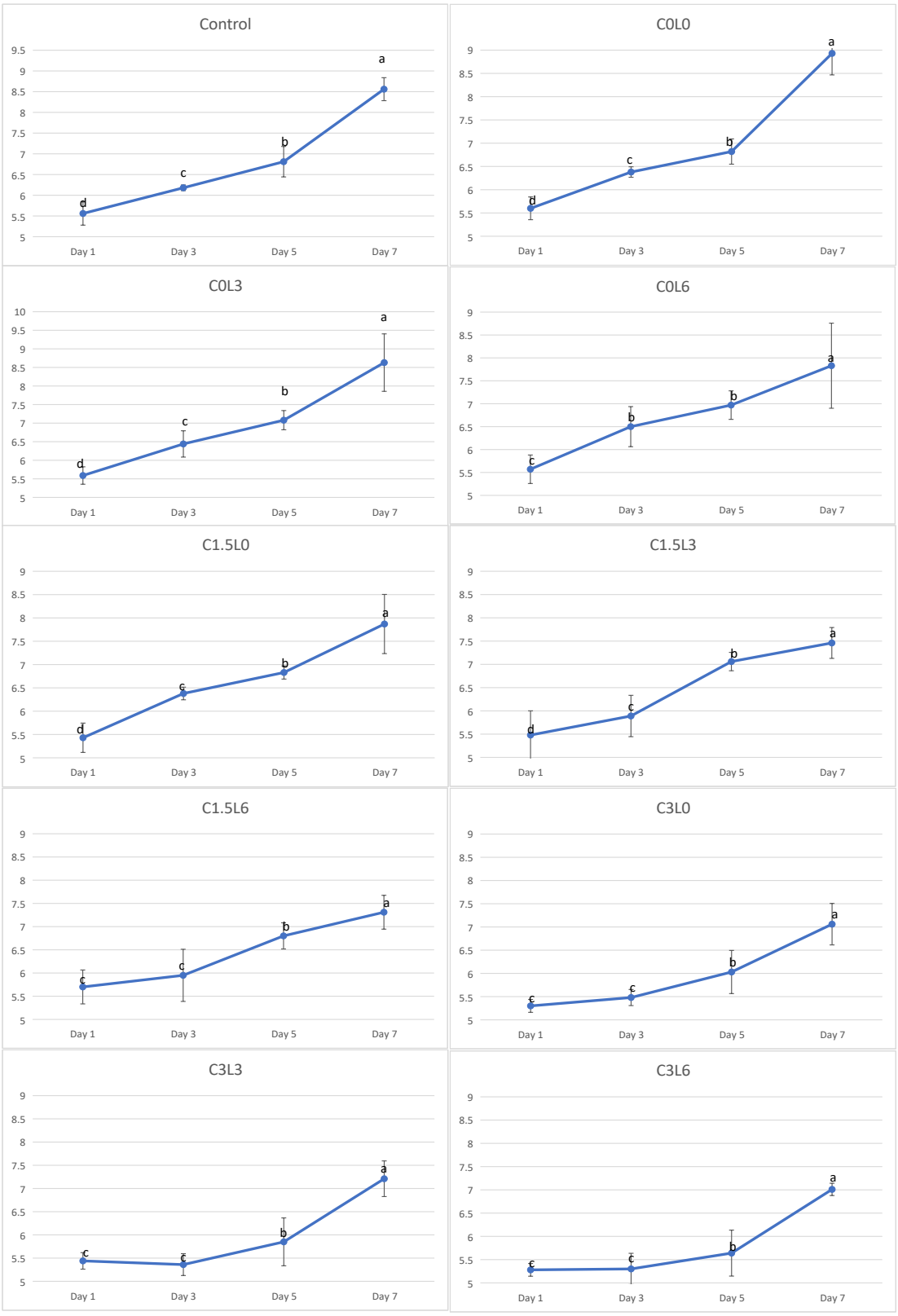

Figure 4.3 The changing of total viable count within a week for different treatments. Error bars representing the standard error indicate the variability of the sample mean or estimate. Different letters mean significantly different ($p < 0.05$).

4.3.2 Physical-chemical analysis

The effect of calcium lactate and lysine on physical-chemical properties of pork patties are shown in Table 4.3.

2837 Table 4.3. Effect of calcium lactate and lysine on physical-chemical characteristics in a salt-reduced pork patty.

2838 Table 4.3a. The significant difference for each treatment on physical-chemical characteristics of salt-reduced pork patties.

Treatment	pH after cooking	Moisture	Yield	WHC	Hardness	Chewiness	Springiness	Cohesiveness	L*	a*	b*
Control	5.99±0.08^{a}	59.50±7.84^{a}	74.89±5.75^{a}	92.70±1.55^{abc}	20.60±2.34^{c}	9.35±1.21^{cde}	0.80±0.03^{ab}	0.52±0.04^{b}	56.49±4.50^{a}	4.74±0.74^{bcde}	17.56±0.91^{a}
C0L0	6.00±0.07^{a}	59.69±3.14^{a}	64.74±6.44^{c}	93.67±0.67^{a}	16.13±1.03^{d}	5.39±0.29^{f}	0.73±0.02^{d}	0.44±0.03^{c}	59.05±3.77^{a}	4.15±1.16^{de}	16.56±1.25^{ab}
C0L3	5.98±0.04^{a}	61.46±3.17^{a}	76.24±4.31^{a}	92.37±1.11^{abcd}	21.49±2.72^{c}	9.00±1.75^{de}	0.81±0.02^{a}	0.54±0.03^{b}	51.50±3.31^{b}	5.75±1.20^{ab}	17.40±1.28^{a}
C0L6	5.88±0.11^{a}	61.00±3.64^{a}	77.55±5.52^{a}	92.21±1.73^{abcd}	21.44±3.13^{c}	8.36±2.08^{e}	0.80±0.03^{ab}	0.51±0.07^{b}	48.08±4.06^{b}	6.66±0.77^{a}	16.62±0.80^{ab}
C1.5L0	5.70±0.19^{b}	60.09±3.69^{a}	69.61±2.25^{b}	93.33±1.48^{ab}	20.56±2.23^{c}	7.95±1.47^{e}	0.76±0.03^{c}	0.52±0.04^{b}	60.36±6.96^{a}	3.52±1.53^{e}	15.59±0.90^{bc}
C1.5L3	5.62±0.14^{bc}	60.48±2.34^{a}	74.54±6.91^{ab}	91.49±0.83^{cde}	24.45±2.79^{ab}	10.17±2.28^{bcd}	0.79±0.02^{ab}	0.52±0.09^{b}	56.58±3.54^{a}	4.78±1.11^{bcd}	16.51±1.38^{ab}
C1.5L6	5.58±0.16^{bc}	59.68±3.47^{a}	77.33±4.58^{a}	91.11±1.72^{de}	25.04±2.58^{a}	10.76±0.90^{abc}	0.78±0.04^{bc}	0.56±0.06^{ab}	56.36±3.76^{a}	3.65±0.51^{de}	13.96±1.09^{d}
C3L0	5.65±0.18^{b}	58.96±4.21^{a}	72.71±5.01^{ab}	91.95±1.09^{bcd}	22.61±1.96^{bc}	9.10±1.67^{de}	0.78±0.02^{bc}	0.52±0.08^{b}	60.55±5.60^{a}	3.97±1.03^{de}	15.43±1.19^{bc}
C3L3	5.60±0.12^{bc}	60.38±2.77^{a}	75.13±4.26^{a}	91.37±1.95^{cde}	26.24±1.87^{a}	12.03±2.06^{a}	0.80±0.02^{ab}	0.61±0.04^{a}	55.98±4.57^{a}	5.36±1.27^{bc}	16.21±1.53^{ab}
C3L6	5.50±0.17^{c}	59.14±2.20^{a}	76.58±4.44^{a}	90.44±1.52^{e}	26.06±0.78^{a}	11.61±1.43^{ab}	0.79±0.02^{ab}	0.55±0.06^{b}	56.81±3.41^{a}	4.31±1.70^{cde}	14.38±2.82^{cd}

2839 Table 4.3b. Effect of calcium lactate and lysine on shelf life of salt-reduced pork patties.

Substitutes	Dosage	Yield	Moisture	WHC	Hardness	Chewiness	Springiness	Cohesiveness	L	a	b	pH after
Calcium lactate	0	72.84±7.89^{a}	60.72±3.28^{a}	92.75±1.37^{a}	19.68±3.49^{a}	7.58±2.21^{a}	0.780±0.044^{a}	0.50±0.06^{a}	52.88±5.89^{a}	5.52±1.47^{a}	16.86±1.15^{a}	5.95±0.09^{a}
	1.5	73.83±5.77^{a}	60.08±3.11^{a}	91.97±1.66^{b}	23.35±3.17^{b}	9.63±2.01^{b}	0.778±0.031^{a}	0.53±0.07^{b}	57.77±5.16^{b}	3.98±1.23^{b}	15.35±1.54^{b}	5.64±0.17^{b}
	3	74.90±4.76^{a}	59.34±3.07^{a}	91.22±1.65^{b}	25.18±2.09^{c}	11.08±1.99^{c}	0.795±0.019^{b}	0.57±0.07^{c}	58.19±4.49^{b}	4.62±1.42^{b}	15.39±2.07^{b}	5.57±0.16^{b}
P (C)		0.37	0.284	0.001	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001
Lysine	0	68.89±5.83^{a}	59.43±3.57^{a}	93.02±1.34^{a}	19.77±3.33^{a}	7.51±2.04^{a}	0.757±0.032^{a}	0.50±0.06^{a}	60.48±4.89^{a}	3.92±1.24^{a}	15.94±1.15^{a}	5.78±0.22^{a}

	3	75.30±5.15[b]	60.94±2.72[a]	91.74±1.40[b]	24.06±3.12[b]	10.40±2.34[b]	0.803±0.019[b]	0.56±0.07[b]	54.69±4.36[b]	5.30±1.22[b]	16.71±1.44[a]	5.73±0.21[a]
	6	77.15±4.70[b]	59.94±3.15[a]	91.26±1.76[b]	24.18±3.06[b]	10.24±2.05[b]	0.791±0.030[b]	0.54±0.06[b]	53.75±5.45[b]	4.87±1.70[b]	14.98±2.10[b]	5.65±0.22[b]
P (L)		<0.001	0.293	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.008
P (I)		0.055	0.915	0.698	<0.001	0.649	<0.001	<0.001	<0.001	<0.001	0.355	0.946

*Control = 2% w/w NaCl; C0L0 = 1% w/w NaCl; C0L3 = 1% w/w NaCl + 3% w/w lysine; C0L6 = 1% w/w NaCl + 6% w/w lysine; C1.5L0 = 1% w/w NaCl + 1.5% w/w calcium lactate; C1.5L3 = 1% w/w NaCl + 1.5% w/w calcium lactate + 3% w/w lysine; C1.5L6 = 1% w/w NaCl + 1.5% w/w calcium lactate + 6% w/w lysine; C3L0 = 1% w/w NaCl + 3% w/w calcium lactate; C3L3 = 1% w/w NaCl + 3% w/w calcium lactate + 3% w/w lysine; C3L6 = 1% w/w NaCl + 3% w/w calcium lactate + 6% w/w lysine. P(C) = significance level for calcium lactate; P(L) = significance level for lysine; P(I) = significance of any interaction between lysine and calcium lactate; WHC = water holding capacity. Averages within the same column followed by the same letters for each salt substitute did not show any significant difference (P > 0.05). Values represented as the Mean ± standard deviation (SD), n = 3.

4.3.2.1 pH after cooking

The pH of the patties increased by approximately 0.4 unit after cooking (from 5.56 to 5.60up to 5.88 to 6.00), except samples with calcium lactate addition. Fletcher, Qiao and Smith (2000) also found the similar tendency in chicken breast meat, and they reported pH of cooked chicken breast had about 0.3 unit of pH increase compared with raw meat. The pH increase could be attributed to the bond breaking of imidazole, sulfhydryl and hydroxyl groups during cooking (Oz and Celik, 2015). The addition of lysine showed a tendency to lower pH, however, this was to a lesser effect than calcium lactate. The significant difference was only observed when lysine was combined with 3% w/w calcium lactate, where 6% w/w lysine resulted in a drop of 0.15 unit of pH than 0% lysine addition. In this experiment, the L-lysine used is in the form of Lysine HCl (Hydrochloride). During the cooking of the sample, the degree of ionization of hydrochloric acid increases due to the increased temperature, released more hydrogen ions (H+), leaded to a pH dropping. Adding calcium lactate without lysine reduced approximately 0.3 unit of the final pH value of cooked pork patties. This was unsurprising as calcium lactate is acidic and is used as a pH regulator in the food industry. The results of this study were consistent with the experimental results of Irshad *et al.* (2016), where the final pH of cooked restructured buffalo meat loaves with 1.5% added calcium lactate was dropped by 0.32 comparing to control. Calcium lactate did not affect the pH of the raw material (up to day 5 shown on Table 4.2), because it has a weak dissolving capacity and can dissolve in cold water at very slow speed (Chen and Shelef, 1992). Therefore, the hydrogen ion may not have been fully released into

the raw meat. However, the subsequent cooking process led to release of hydrogen ions into the meat matrix, thereby lowering the pH. Table 4.3 also showed that there was no significance of any interaction between lysine and calcium lactate ($p > 0.05$).

4.3.2.2 Moisture content

The moisture content of salt-reduced pork patties ranged from 59.1% to 61.5%. Neither calcium lactate nor lysine ($p > 0.05$) had an impact on moisture content of a pork patty ($p > 0.05$). Zhang *et al.* (2018) reported that 0.6% lysine added as a salt substitute with KCl and histidine to dry-cured loin did not impact final moisture content. Similarly, Seyfert *et al.* (2007) reported a similar finding that beef patties treated with high concentrations of calcium lactate (2.6%, 4.4%) did not change the final moisture content. Table 3 also showed that there was no significance of any interaction between lysine and calcium lactate ($p > 0.05$).

4.3.2.3 Yield

The yield of the control pork patty was 74.89%, however, this reduced substantially to 64.74% in the 50% NaCl reduced pork patty when no substitutes were added ($p < 0.05$). Ideally, salt reduction leads to lower water content so that the yield was decreased (Desmond and Vasilopoulos, 2019). However, the moisture content of control and 50% NaCl reduced pork patty was similar ($P > 0.05$). This may be due to cooking losses in addition to moisture loss, other substrates from the meat may also be lost in large quantities which cause a reduction of yield. Table 4.3 shown that the addition of lysine substantially increased cooking yield of the salt-reduced pork patties ($p < 0.05$) and could completely compensate the cooking loss caused by salt reduction ($p > 0.05$). This

is in agreement with Guo *et al.* (2020), yield of low-salt ham was increased with the level of lysine addition from 0.2% to 0.8%. The reason for this phenomenon is that lysine is a positively charged and polar amino acid which can bind with anions to form hydrogen bonds, that then retain water within the structure (Betts and Russell, 2003). Addition of calcium lactate did not affect the yield ($p > 0.05$). This disagreed with Irshad *et al.* (2016) who found that addition of 1.5% calcium lactate eventually resulted in a huge loss (12.53%) of yield in fortified restructured buffalo meat loaves, whereas 1.5% w/w calcium lactate only reduced 5.38%. The difference in results may be because phosphate was also used in Irshad's experiments. Calcium competes with phosphate for protein binding sites resulting in more water loss from the product (Lawrence *et al.*, 2004). Table 4.3 also shown that there was no significance of any interaction between lysine and calcium lactate ($p > 0.05$).

4.3.2.4 Water holding capacity

Water holding capacity is one of the most important quality attributes of meat products, as it influences both cooking yield and juiciness. Table 4.3 indicated that both lysine and calcium lactate had decrease effect in WHC ($p < 0.05$). Swift and Berman (1959) found that an increased cation concentration lowers water-binding ability. But Zhou, Li and Tan (2014) reported a negative relationship between lysine level and water holding capacity of pork sausage, i.e., a lower level of lysine addition would result in a higher level of WHC. The L-lysine (0.4% - 0.8%) used in Zhou, Li and Tan's experiments significantly increased the pH of the pork sausage, whereas the lysine hydrochloride used in this experiment did not increase the pH, but rather tended to decrease it.

Especially when used in combination with calcium lactate, the pH decreased significantly. Because lower pH leads to higher protein-protein interactions, reducing the space within and between myofilaments, resulting in a reduced immobilization of water (Honikel, 2004), so the WHC was decreased.

4.3.2.5 Texture

50% NaCl reduction without any substitutes substantially reduced all textural properties (hardness, chewiness springiness and cohesiveness) compared to the full salt control ($p < 0.05$). Both calcium lactate and lysine significantly increased the values of texture attributes of salt-reduced pork patty (Table 4.3, $p < 0.05$). However, the addition of lysine (at either 3 or 6% w/w) or calcium lactate (at 1.5% or 3% w/w), was able to achieve comparable results with 2% control samples in all textural attributes ($p > 0.05$). Overall, there was no interaction between calcium lactate and lysine on both hardness and chewiness ($p > 0.05$); but interaction between them was found in springiness and cohesiveness ($p < 0.05$). Guo *et al*. (2020) showed a similar result using L-lysine (0.2% - 0.8%), where they were able to maintain hardness, chewiness and springiness in a 50% salt-reduced reconstructed ham. What is more, lysine caused an increase in cohesiveness between lysine added samples and the control with an increased substitution ratio. It was proposed that lysine could increase the solubility of porcine myosin even at the low ionic strength solution (Guo *et al.*, 2015). During ham production, myosin protein extractability can be further enhanced by tumbling to ensure better textural properties (Maddock, 2014). As a result, better cohesion would be expected in lysine added pork patties because it is the main binder in muscles. In

agreement with results, Irshad *et al.* (2016) found that hardness was increased with increase in calcium lactate levels (1% - 1.5%) in restructured buffalo meat loaves. The presence of calcium promotes the mutual bonding between myosin to form a stronger network, which results in an increase of hardness (Jimenez *et al.*, 2012). However, they also indicated that chewiness, springiness and cohesiveness were not affected by different level of calcium lactate. But Mehta *et al.* (2015) found similar results with this work that the texture values of low-fat and low-salt chicken meat patties fortified with calcium lactate (1.5% - 2%) were marginally higher compared to that of the control, because calcium salts provided an increased gelling effect. This may imply that chewiness, springiness and cohesiveness would only be affected at higher levels of calcium lactate (above 1.5%). Hence, significant increase in texture attributes would be expected when (1.5%, 3% w/w) of calcium lactate was added at 1.5% and 3% in this work.

4.3.2.6 Colour

Table 4.3 found that lysine did decrease lightness and yellowness in a salt-reduced pork patty, but the redness was increased ($p < 0.05$; Supplementary table 12). Campagnol *et al*. (2011) discovered that using a low concentration of lysine (< 1.25%) as a salt substitute with 50% replacement of salt by KCl in fermented cooked sausage had no significant difference in colour compared with the control group. But the results showed that lysine had an impact on colour at higher concentration is higher (3%, 6%) ($p < 0.05$). The main reason for such colour difference is likely to be that as one kinds of amino acid, lysine can promote the generation of colour through the Maillard Reaction

(Martins, Jongen and Van Boekel, 2000). As for calcium lactate, it was shown that calcium lactate did decrease redness and yellowness in a salt-reduced pork patty, but increased lightness ($p < 0.05$). According to the experiments of Kim *et al.* (2006), lactate dehydrogenase (LDH) in the meat can convert exogenous lactic acid into pyruvate and NADH (nicotinamide adenine dinucleotide), and then NADH can effectively promote the reduction of metmyoglobin to myoglobin (oxy- or deoxy-), thereby improving the stability of flesh color. Yang *et al.* (2021) reported that the L* values gradually increased in the cooked sausage with calcium lactate addition at 0.2%, 0.4% and 0.7%, and b* values gradually declined ($p < 0.05$) which disagreed with the findings in this work. As mentioned above, exogenous lactic acid needs to react with a series of substances inside the meat, so as to achieve the purpose of improving the stability of meat colour. However, the quality of meat products in the experiment is not constant. The activity of substances was not clear, which may be the reason why lactic acid did not maintain or improve the stability of meat colour in this test. In addition, the different concentration of calcium lactate was used, the level used in this work was 1.5% and 3%, vs 0.2-0.7% in their work. That may imply that the colour changed by calcium lactate will be dependent on the concentration. Significant interaction was found between lysine and calcium lactate on the redness of pork patties ($p = 0.013$). As lysine and calcium lactate had opposite effects on the redness, this may mean that their combination will tend to leave the redness unchanged. This is probably because the addition of calcium lactate lowers the pH, which further inhibited the Maillard reaction during cooking (Ames, 1998).

4.4.4 Sensory evaluation

In order to improve sensory analysis for better focus, all treatments need to be screened. A PCA based upon the physio-chemical and microbiological analysis result was done.

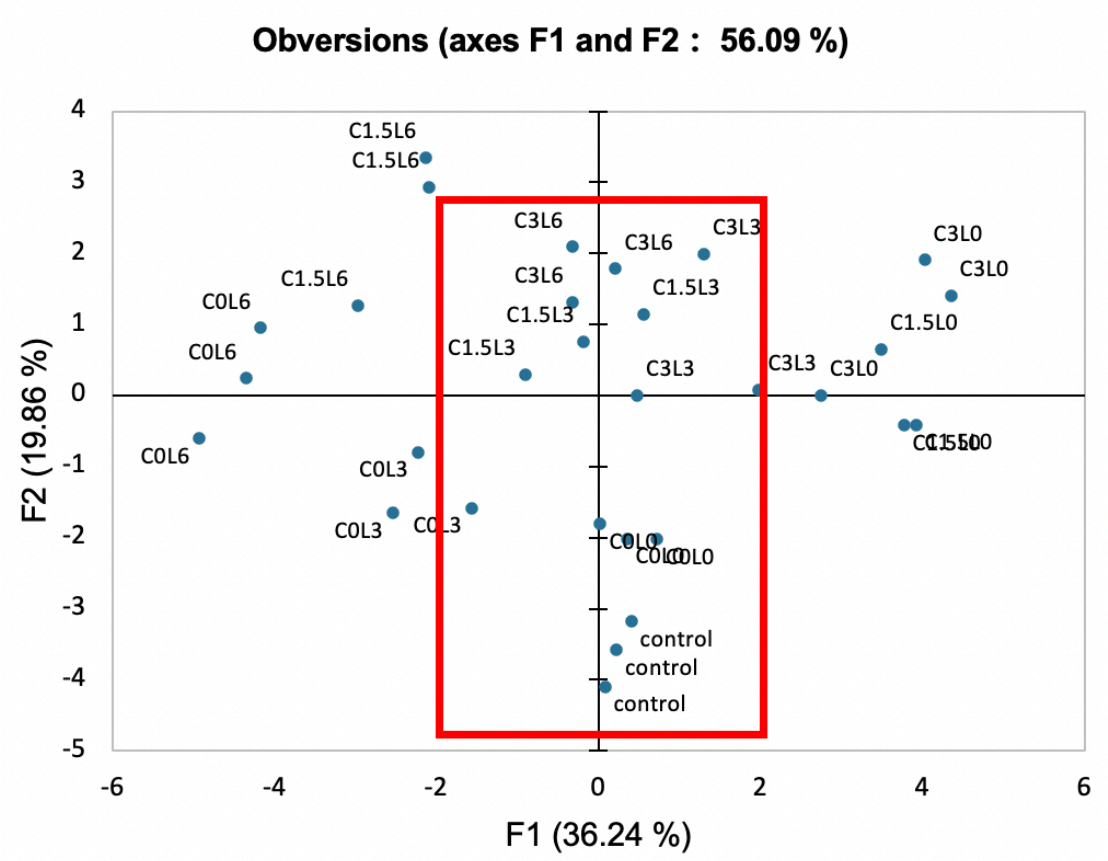

Figure 4.4. PCA plot for physical-chemical and microbiological results of cooked salt-reduced pork patty. Control = 2% w/w NaCl; C0L0 = 1% w/w NaCl; C0L3 = 1% w/w NaCl + 3% w/w lysine; C0L6 = 1% w/w NaCl + 6% w/w lysine; C1.5L0 = 1% w/w NaCl + 1.5% w/w calcium lactate; C1.5L3 = 1% w/w NaCl + 1.5% w/w calcium lactate + 3% w/w lysine; C1.5L6 = 1% w/w NaCl + 1.5% w/w calcium lactate + 6% w/w lysine; C3L0 = 1% w/w NaCl + 3% w/w calcium lactate; C3L3 = 1% w/w NaCl + 3% w/w calcium lactate + 3% w/w lysine; C3L6 = 1% w/w NaCl + 3% w/w calcium lactate + 6% w/w lysine.

It clearly presented from the score plot (Figure 4.4) that C0L0, C0L3, C1.5L3, C3L3 and C3L6 were the samples with overall similarity for all the variables compared to control. Therefore, these treatments were selected for following sensory evaluation.

The Effects of salt reduction, calcium lactate and lysine on the sensory profile of salt-reduced pork patties are shown in Table 4.4.

Table 4.4. Sensory profile of pork patties varying in levels of salt, calcium lactate and lysine.

Treatment	Control	C0L0	C0L3	C1.5L3	C3L3	C3L6	p
Appearance							
Overall intensity of colour	37.9^{a}	28.9^{b}	33.7^{ab}	27.3^{b}	30.8^{ab}	32.6^{ab}	0.006
Golden crust	38.5^{ab}	24.2^{c}	39.5^{ab}	30.9^{bc}	42^{ab}	48.9^{a}	<0.001
Rubbery	41.6^{a}	19.3^{b}	42.1^{a}	43.3^{a}	48.4^{a}	49.3^{a}	<0.001
Dense	54.7^{b}	28.7^{c}	61.9^{ab}	62.3^{ab}	64.6^{ab}	67.6^{a}	<0.001
Moist	45.7^{a}	19.8^{b}	50.6^{a}	44.0^{a}	42.8^{a}	48.1^{a}	<0.001
Smooth	51.2^{b}	22.1^{c}	53.8^{b}	55.6^{ab}	66.3^{a}	65.3^{a}	<0.001
Aroma							
Boiled meat/pork	37.5^{a}	37.5^{a}	38.7^{a}	39.1^{a}	38.8^{a}	36.4^{a}	0.949
Roasted meat/pork	23.8^{a}	15.3^{a}	23.3^{a}	19.3^{a}	19.8^{a}	24.9^{a}	0.065
Blood	15.2^{a}	20.5^{a}	16.3^{a}	17.7^{a}	17.7^{a}	15.4^{a}	0.408
Rancid/stale	4.0^{a}	4.6^{a}	7.5^{a}	4.5^{a}	5.2^{a}	4.0^{a}	0.644
Taste and flavour							
Salty	54.9^{ab}	35.1^{c}	44.7^{bc}	50.0^{ab}	49.0^{ab}	56.7^{a}	<0.001
Umami	34.2^{a}	22.1^{b}	27.4^{ab}	30.6^{ab}	29.0^{ab}	29.0^{ab}	0.107
Sour	5.5^{bc}	2.9^{c}	6.3^{bc}	13.1^{ab}	20.3^{a}	20.0^{a}	<0.001
Sweet	12.3^{a}	13.3^{a}	17.7^{a}	14.4^{a}	10.2^{a}	11.1^{a}	0.083
Bitter	7.1^{b}	4.3^{b}	6.1^{b}	12.4^{b}	24.3^{a}	27.4^{a}	<0.001
Metallic	15.6^{a}	17.2^{a}	14.7^{a}	17.0^{a}	20.6^{a}	21.8^{a}	0.205
Boiled meat/pork	33.4^{ab}	32.7^{ab}	33.3^{ab}	37.0^{a}	28.5^{ab}	24.8^{b}	0.016
Roasted meat/pork	22.5^{a}	17.2^{a}	18.1^{a}	16.1^{a}	19.5^{a}	19.0^{a}	0.657
Fatty	14.6^{a}	11.0^{a}	14.8^{a}	14.5^{a}	13.2^{a}	11.9^{a}	0.772
Mouthfeel							

Soft	46.5[a]	48.7[a]	54.6[a]	51.5[a]	54.1[a]	45.6[a]	0.078
Chewy	51.4[a]	53.2[a]	41.3[a]	50.8[a]	43.5[a]	47.4[a]	0.094
Moist	45.9[a]	22.0[b]	50.05[a]	44.6[a]	41.4[a]	41.0[a]	<0.001
Rubbery	35.2[abc]	22.5[c]	32.0[bc]	38.7[ab]	44.3[ab]	46.6[a]	<0.001
Dense	54.8[a]	31.2[b]	52.6[a]	53.9[a]	57.8[a]	60.6[a]	<0.001
Greasy	24.4[a]	13.3[b]	27.0[a]	18.7[ab]	18.6[ab]	20.8[ab]	0.003
Sticky	6.0[b]	17.1[a]	6.0[b]	9.8[ab]	6.0[b]	9.1[ab]	0.002
Bitty	37.9[b]	57.0[a]	28.4[b]	37.5[b]	30.1[b]	29.9[b]	<0.001
After taste							
Salty	42.7[ab]	27.0[d]	35.1[c]	36.3[bc]	39.3[bc]	47.4[a]	<0.001
Metallic	14.8[a]	16.4[a]	16.1[a]	14.4[a]	18.0[a]	16.7[a]	0.841
Meaty	27.5[a]	26.8[a]	25.2[a]	25.3[a]	19.8[a]	18.9[a]	0.074
Residue	19.7[b]	31.1[a]	16.4[b]	22.5[b]	16.3[b]	17.7[b]	<0.001
Salivating	29.2[ab]	20.5[b]	27.3[ab]	23.4[ab]	29.1[ab]	32.4[a]	0.004
Drying	23.6[a]	26.9[a]	25.8[a]	26.1[a]	29.3[a]	28.5[a]	0.366

*Control = 2% NaCl; C0L0 = 1% NaCl; C0L3 = 1% NaCl + 3% lysine; C1.5L3 = 1% NaCl + 1.5% calcium lactate + 3% lysine; C3L3 = 1% NaCl + 3% calcium lactate + 3% lysine; C3L6 + 3% calcium lactate + 6% lysine. References: boiled meat/pork was boiled pork belly; roasted meat/pork was roasted pork belly; blood/metallic was iron sulfate; rancid/stale was butyric acid; salty was sodium chloride solution. Averages within the same row followed by the same letters for each salt substitute are not significantly different (p> 0.05). Values represented as the Mean ± standard deviation (SD), n = 3.

The colour of fresh red meat is crucial in meat marketing as it is the first quality attribute perceived by the consumer and is considered as an indicator of freshness, shelf life and eating quality. In terms of appearance, the full salt control had the highest overall intensity of surface colour; while 3% lysine alone (C0L3), or with 3% lysine combined with 3% or 6% (C3L3, C3L6) achieved similar colour intensity with the control (p > 0.05). This was consistent with colour results (a* value) measured by instruments

(Table 4.3). Considering the golden crust, all of the salt reduced patties containing lysine or calcium lactate were able to maintain the same golden crust as the control ($p > 0.05$), whereas the salt reduced patty without any salt substitutes had a significantly less golden crust ($p < 0.05$). This may be related to the Maillard reaction. This experiment found that lysine and calcium lactate lowered the water activity of salt-reduced pork patty, and that reduction from high water activity resulted in increased reaction rates (Van Boekel, 2001).

Perception of texture was assessed through both visual appearance (including rubbery, dense, moist and smooth) and mouthfeel. The salt reduced pork patty without substitutions was significantly less smooth in appearance ($p < 0.05$); less rubbery, dense and moist than 2% salt control both in visually and in the mouthfeel ($p < 0.05$); as well as less greasy, stickier and bittier in mouthfeel ($p < 0.5$). The substituted formulations had comparable values with 2% control samples in any of the four visual texture attributes ($p > 0.05$), although the high calcium lactate formulations had significantly higher value in dense appearance (C3L6) and smooth (C3L3 and C3L6) than the full salt control ($p<0.05$). Similarly, none of the substituted formulations were significantly lower than the control in mouthfeel texture ($p < 0.05$). Although the instrument analysis in colour and texture showed difference in specific texture attributes, the mouthfeel texture changes were not reflected in the sensory analysis.

There were no differences between any treatments in the aroma of the patties ($p > 0.05$), inferring that salt reduction did not affect aroma. The most noteworthy sensory result, saltiness, is in line with previous findings with aqueous solutions (Chapter 3). The

saltiness of 50% salt-reduced pork patty was significantly reduced compared to the full salt control ($p < 0.05$), however lysine effectively mitigated the loss of saltiness ($p > 0.05$). The salty taste of salt reduced patties substituted with lysine were same with that of the full salt control, although patties with high levels of lysine and calcium lactate (C3L6) was reported significantly saltier than the ones with lower level of lysine without calcium lactate (C0L3) ($p < 0.05$). The source of the salty taste is because calcium lactate is associated with saltiness. Ca^{2+} the divalent metal cations are mainly perceived with saltiness and bitterness, but calcium lactate also has a considerable sour component (Lawless et al., 2003). However, the mechanism for lysine eliciting saltiness is unknown, and follow-up experiments are needed to explore, for example, whether the salty taste signal is also generated through ENaC. The umami taste of 50% salt-reduced pork patty was significantly reduced compared to the full salt control ($p < 0.05$), and again all samples with the substituted formulations were not significantly different than the full salt control in umami taste. This may be because that umami is a less recognized taste in Western countries and consumers may confuse it with the perceived saltiness (Cecchini *et al.*, 2019). None of samples differed in sweetness ($p > 0.05$). One obvious disadvantage, however, is a significantly higher bitter and sour taste observed in samples with calcium lactate at the higher concentration of 3% w/w ($p < 0.05$). The chloride ions existing in the patty's matrix could explain the high bitterness in the sample, as the binding of calcium ion and Cl^- could generate stronger bitterness sensation compared to calcium lactate (Lawless *et al*., 2003). High level (3%) of calcium lactate addition in the meat would create more opportunity for calcium ions to

bind chloride ions. As a result, a higher bitterness would be expected in samples with 3% calcium lactate addition. The increased sourness was expected due to the increased H+ in the matrix (a decrease in pH) due to calcium lactate addition, while similar results were reported by Lawrence *et al.* (2004), and Devatkal and Mendiratta (2001). None of the products differed significantly from the control in metallic taste, boiled or roasted meat flavour and fatty flavour.

For after effect, almost all treatments showed similar results to the full salt control ($p > 0.05$), except that the salt-reduced pork patty with no substitutions or only lysine was significantly lower in salty aftertaste ($p < 0,05$). The salt-reduced pork patty with no substitutions also led to a significantly higher residue in the mouth than the control and all other treatments. This is probably due to its lower off-taste (sour and bitter) and smoother mouthfeel (open structure). In general, all treatments were in line with the requirements of the full salt control patty and did not substantially change the original sensory properties of the pork patty, except that the highest concentration of calcium lactate brought tastes normally perceived as unpleasant (bitter and sour).

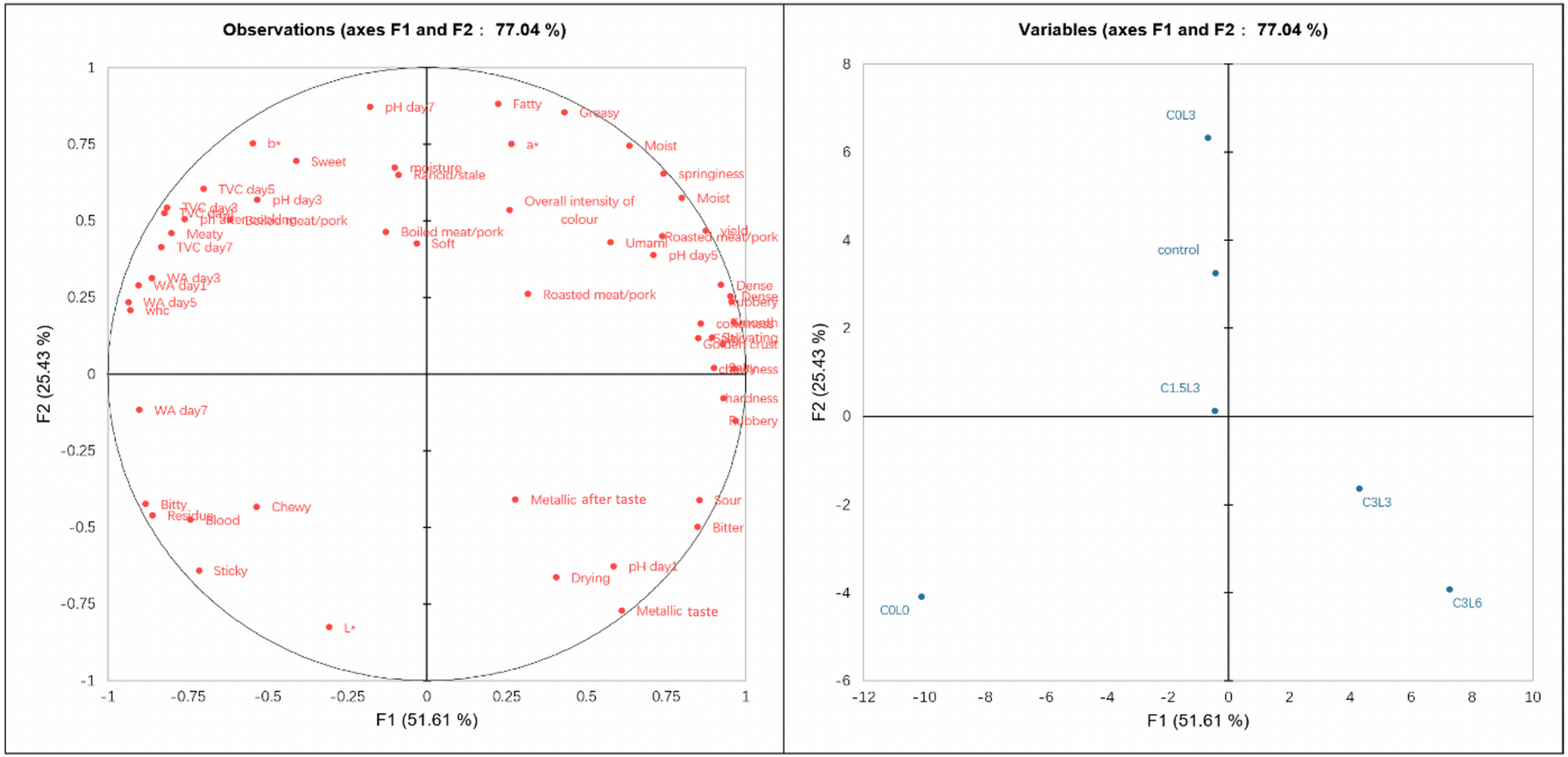

Figure 4.5. Principal component analysis of pork patties varying in physical-chemical characteristics, shelf-life and sensory evaluation. Control = 2% w/w NaCl; C0L0 = 1% w/w NaCl; C0L3 = 1% w/w NaCl + 3% w/w lysine; C0L6 = 1% w/w NaCl + 6% w/w lysine; C1.5L0 = 1% w/w NaCl + 1.5% w/w calcium lactate; C1.5L3 = 1% w/w NaCl + 1.5% w/w calcium lactate + 3% w/w lysine; C1.5L6 = 1% w/w NaCl + 1.5% w/w calcium lactate + 6% w/w lysine; C3L0 = 1% w/w NaCl + 3% w/w calcium lactate; C3L3 = 1% w/w NaCl + 3% w/w calcium lactate + 3% w/w lysine; C3L6 = 1% w/w NaCl + 3% w/w calcium lactate + 6% w/w lysine. WA = water activity; TVC = total viable count.

PCA was performed to offer visual compare the physical-chemical characteristics, shelf-life and sensory quality for the 10 samples (Figure 4.5), and to observe the correlations between lysine, calcium lactate and physiochemical data, sensory data. The PCA results clearly showed that the salt-reduced pork with 3% lysine with/without 1.5% calcium lactate had similar food quality with control. Salt-reduced pork patty containing high concentration of calcium lactate (3% w/w) was furthest away from control. They had higher sourness and bitterness, and it was negatively correlated with most of the attributes including meaty flavour, WHC, etc. In contrast, reduced-salt pork patty containing low concentration of lysine (3% w/w) had similar food quality to the control. They had higher moisture, meaty flavour, yield, etc., and it was positively correlated with most of the attributes including redness, softeness, etc. It was worth noting that the higher the concentration of lysine combined with calcium lactate in the salt-reduced pork patty, the worse the food quality compared to the control. In addition, PCA also clearly reflected the correlation between physical-chemical properties and sensory indicators. For example, salt-reduced pork patties with high moisture content were positively associated with juicy and negatively associated with drying and bitty. This means that the salt-reduced pork patty needs to have an increased moisture content in order o be perceived as juicier. PCA also reflected a negative correlation between metallic taste and TVC, which means that the salt-reduced pork patty with higher shelf-life had more metallic taste, due to the addition of high concentration of calcium lactate.

4.4 Conclusion

In this study, utilization of calcium lactate and lysine influenced colour, texture and water activity of pork patty with 50% salt reduction. Although lysine increased the pH value of the raw salt-reduced pork patty, the elevating effect could be cancelled out by addition of calcium lactate. Hence comparable yield could be achieved for patties with 50% salt reduction by combining lysine and calcium lactate and 2% full salt control. The addition of calcium lactate decreased water activity of the salt-reduced pork patty, which inhibited the growth of bacteria. According to the sensory result, lysine and calcium lactate could effectively compensate the saltiness loss in a salt-reduced pork patty. Therefore, it is recommended that a 50% salt reduced pork patty can be successfully processed with 3% lysine and 1.5% calcium lactate, although costs need to be considered. This combination is the optimal choice for the meat industry based on physical-chemical characteristics, shelf-life and sensory profile. In addition, it should be noted that although lysine alone can guarantee the same shelf life, the addition of calcium lactate can significantly increase the shelf life of salt-reduced meat products. This is a substantial advantage for reduced-salt meat products. However, high level of calcium lactate addition significantly increased the bitterness, and balancing the shelf life and bitter taste should be carefully considered.

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References

Aaron, K. and Sanders, P. (2013). Role of Dietary Salt and Potassium Intake in Cardiovascular Health and Disease: A Review of the Evidence. Mayo Clinic Proceedings, 88(9), pp.987-995.

Aaslyng, M., Bejerholm, C., Ertbjerg, P., Bertram, H. and Andersen, H., 2003. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food Quality and Preference, 14(4), pp.277-288.

Albarracín, W., Sánchez, I.C., Grau, R. and Barat, J.M., 2011. Salt in food processing; usage and reduction: a review. International Journal of Food Science & Technology, 46(7), pp.1329-1336.

Ames, J.M., 1998. Applications of the Maillard reaction in the food industry. Food Chemistry, 62(4), pp.431-439.

Ashford, R., Jones, K. and Collins, D., 2020. National Diet and Nutrition Survey: Assessment of salt intake from urinary sodium in adults (aged 19 to 64 years) in England, 2018 to 2019. GOV. UK, pp.133-155.

Betts, M.J. and Russell, R.B., 2003. Amino acid properties and consequences of substitutions. Bioinformatics for geneticists, 317, p.289.

Blemings, K. and Norlin J. Benevenga, N., 2007. Unique Aspects of Lysine Nutrition and Metabolism. American Society for Nutrition, 137(6), pp.1610S-1615S.

Campagnol, P.C.B., dos Santos, B.A., Morgano, M.A., Terra, N.N. and Pollonio, M.A.R., 2011. Application of lysine, taurine, disodium inosinate and disodium

guanylate in fermented cooked sausages with 50% replacement of NaCl by KCl. Meat science, 87(3), pp.239-243.

Campagnol, P., Dos Santos, B., Terra, N. and Pollonio, M., 2012. Lysine, disodium guanylate and disodium inosinate as flavor enhancers in low-sodium fermented sausages. Meat Science, 91(3), pp.334-338.

Calkins, C.R. and Hodgen, J.M., 2007. A fresh look at meat flavor. Meat science, 77(1), pp.63-80.

Cecchini, M. P., Knaapila, A., Hoffmann, E., Boschi, F., Hummel, T., and Iannilli, E., 2019. A cross-cultural survey of umami familiarity in European countries. Food Quality and Preference, 74, 172-178.

Chen, N. and Shelef, L., 1992. Relationship Between Water Activity, Salts of Lactic Acid, and Growth of Listeria monocytogenes in a Meat Model System. Journal of Food Protection, 55(8), pp.574-578.

De Marchi, M., Manuelian, C., Ton, S., Manfrin, D., Meneghesso, M., Cassandro, M. and Penasa, M., 2017. Prediction of sodium content in commercial processed meat products using near infrared spectroscopy. Meat Science, 125, pp.61-65.

Desmond, E. and Vasilopoulos, C., 2019. Reducing salt in meat and poultry products. In Reducing salt in foods (pp. 159-183). Woodhead Publishing.

Devatkal, S. and Mendiratta, S., 2001. Use of calcium lactate with salt-phosphate and alginate-calcium gels in restructured pork rolls. Meat Science, 58(4), pp.371-379.

Del Pulgar, J. S., Gázquez, A., and Ruiz-Carrascal, J., 2012. Physico-chemical, textural and structural characteristics of sous-vide cooked pork cheeks as affected by vacuum, cooking temperature, and cooking time. Meat science, 90(3), 828-835.

Dos Santos, B., Campagnol, P., Morgano, M. and Pollonio, M., 2014. Monosodium glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl with KCl. Meat Science, 96(1), pp.509-513.

Dos Santos Alves, L., Lorenzo, J., Gonçalves, C., Dos Santos, B., Heck, R., Cichoski, A. and Campagnol, P., 2017. Impact of lysine and liquid smoke as flavor enhancers on the quality of low-fat Bologna-type sausages with 50% replacement of NaCl by KCl. Meat Science, 123, pp.50-56.

Doyle, M. and Glass, K., 2010. Sodium Reduction and Its Effect on Food Safety, Food Quality, and Human Health. Comprehensive Reviews in Food Science and Food Safety, 9(1), pp.44-56.

Emorine, M., Septier, C., Thomas-Danguin, T. and Salles, C., 2014. Ham particle size influences saltiness perception in flans. Journal of food science, 79(4), pp.S693-S696.

Fauza, G., Muhammad, D.R.A., Affandi, D.R. and Ariviani, S., 2021, July. Sensory profile analysis of steamed brownies using Quantitative Descriptive Analysis (QDA). In *IOP Conference Series: Earth and Environmental Science* (Vol. 828, No. 1, p. 012058). IOP Publishing.

3174 Fellendorf, S., O'Sullivan, M.G. and Kerry, J.P., 2016. Impact of ingredient replacers
3175 on the physicochemical properties and sensory quality of reduced salt and fat black
3176 puddings. Meat Science, 113, pp.17-25.
3177 Fernández-Salguero J, Gómez R, Carmona MA. Water activity in selected high-
3178 moisture foods. J Food Compost Anal. 1993; 6:364–369.
3179 Fletcher, D.L., Qiao, M. and Smith, D.P., 2000. The relationship of raw broiler breast
3180 meat color and pH to cooked meat color and pH. Poultry science, 79(5), pp.784-788.
3181 Guo, X., Tao, S., Pan, J., Lin, X., Ji, C., Liang, H., Dong, X. and Li, S., 2020. Effects
3182 of L-Lysine on the physiochemical properties and sensory characteristics of salt-
3183 reduced reconstructed ham. Meat science, 166, p.108133.
3184 He, F. and MacGregor, G., 2010. Reducing Population Salt Intake Worldwide: From
3185 Evidence to Implementation. Progress in Cardiovascular Diseases, 52(5), pp.363-382.
3186 Honikel, K.O., 2004. Water-holding capacity of meat. Muscle development of livestock
3187 animals: Physiology, genetics and meat quality, pp.389-400.
3188 Houtsma, P. C., De Wit, J. C., and Rombouts, F. M., 1993. Minimum inhibitory
3189 concentration (MIC) of sodium lactate for pathogens and spoilage organisms occurring
3190 in meat products. International Journal of Food Microbiology, 20(4), 247-257.
3191 Inguglia, E., Zhang, Z., Tiwari, B., Kerry, J. and Burgess, C., 2017. Salt reduction
3192 strategies in processed meat products – A review. Trends in Food Science &
3193 Technology, 59, pp.70-78.

3194 Irshad, A., Sharma, B., Ahmed, S., Talukder, S., Malav, O. and Kumar, A., 2016. Effect
3195 of incorporation of calcium lactate on physico-chemical, textural, and sensory
3196 properties of restructured buffalo meat loaves. Veterinary World, 9(2), pp.151-159.
3197 Jimenez-Colmenero F, Herrero A, Cofrades S, Capillas C.R. Meat and functional foods.
3198 Handbook of Meat and Meat Processing. 2nd ed. Boca Raton, London, New York: CRC
3199 Press, Taylor &Francis Group; 2012. pp. 225–242.
3200 Kilcast, D. and Den Ridder, C., 2007. Sensory issues in reducing salt in food products.
3201 In Reducing salt in foods (pp. 201-220). Woodhead publishing.
3202 Kim, Y. H., Hunt, M. C., Mancini, R. A., Seyfert, M., Loughin, T. M., Kropf, D. H.,
3203 and Smith, J. S., 2006. Mechanism for lactate-color stabilization in injection-enhanced
3204 beef. Journal of agricultural and food chemistry, 54(20), 7856-7862.
3205 Kountouras, J., Zavos, C. and Chatzopoulos, D. (2004). Salt intake and Helicobacter
3206 pylori infection. Journal of Hypertension, 22(12), p.2397.
3207 Lawrence, T.E., Dikeman, M.E., Hunt, M.C., Kastner, C.L. and Johnson, D.E., 2003.
3208 Staged injection marination with calcium lactate, phosphate and salt may improve beef
3209 water-binding ability and palatability traits. Meat Science, 65(3), pp.967-972.
3210 Lawrence, T.E., Dikeman, M.E., Hunt, M.C., Kastner, C.L. and Johnson, D.E., 2004.
3211 Effects of enhancing beef longissimus with phosphate plus salt, or calcium lactate plus
3212 non-phosphate water binders plus rosemary extract. Meat Science, 67(1), pp.129-137.
3213 Lu, F., Kuhnle, G.K. and Cheng, Q., 2017. Vegetable oil as fat replacer inhibits
3214 formation of heterocyclic amines and polycyclic aromatic hydrocarbons in reduced fat
3215 pork patties. Food Control, 81, pp.113-125.

3216 Maddock, R., 2014. Meats–Beef and pork based. Food processing: Principles and
3217 applications, 535-548.
3218 Martins, S.I., Jongen, W.M. and Van Boekel, M.A., 2000. A review of Maillard reaction
3219 in food and implications to kinetic modelling. Trends in food science & technology,
3220 11(9-10), pp.364-373.
3221 Mehta, N., Sharma, B.D., Kumar, R.R., Kumar, P., Malav, O.P. and Verma, A.K., 2015.
3222 Fortification of low-fat chicken meat patties with calcium, vitamin E and vitamin C.
3223 Nutrition & Food Science.
3224 Ojha, K.S., Keenan, D.F., Bright, A., Kerry, J.P. and Tiwari, B.K., 2016. Ultrasound
3225 assisted diffusion of sodium salt replacer and effect on physicochemical properties of
3226 pork meat. International journal of food science & technology, 51(1), pp.37-45.
3227 Okuskhanova, E.; Assenova, B.; Rebezov, M.; Yessimbekov, Z.; Kulushtayeva, B.;
3228 Zinina, O.; Stuart, M., 2016. Mineral composition of deer meat pate. Pak. J. Nutr., 15,
3229 217–222
3230 Oz, F. and Celik, T., 2015. Proximate composition, color and nutritional profile of raw
3231 and cooked goose meat with different methods. Journal of food processing and
3232 preservation, 39(6), pp.2442-2454.
3233 Paulsen, M.T., Nys, A., Kvarberg, R. and Hersleth, M., 2014. Effects of NaCl
3234 substitution on the sensory properties of sausages: Temporal aspects. Meat Science,
3235 98(2), pp.164-170.
3236 Powles, J., Fahimi, S., Micha, R., Khatibzadeh, S., Shi, P., Ezzati, M., Engell, R., Lim,
3237 S., Danaei, G. and Mozaffarian, D. (2013). Global, regional and national sodium intakes

in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. BMJ Open, 3(12), p.e003733.

Public Health England. Salt Reduction Targets for 2024; Public Health England: London, UK, 2020

Rios-Mera, J.D., Selani, M.M., Patinho, I., Saldaña, E. and Contreras-Castillo, C.J., 2021. Modification of NaCl structure as a sodium reduction strategy in meat products: An overview. Meat Science, 174, p.108417.

Rucker, A.J., Rudemiller, N.P. and Crowley, S.D., 2018. Salt, hypertension, and immunity. Annual review of physiology, 80, p.283.

Ruusunen, M. and Puolanne, E., 2005. Reducing sodium intake from meat products. Meat Science, 70(3), pp.531-541.

Rybicka, I., Gonçalves, A., Oliveira, H., Marques, A. and Nunes, M.L., 2022. Salt reduction in seafood–A review. Food Control, p.108809.

Seyfert, M., Hunt, M., Lundesjö Ahnström, M. and Johnson, D., 2007. Efficacy of lactic acid salts and sodium acetate on ground beef colour stability and metmyoglobin-reducing activity. Meat Science, 75(1), pp.134-142.

Shelef, L.A. and Potluri, V., 1995. Behaviour of foodborne pathogens in cooked liver sausage containing lactates. Food Microbiology, 12, pp.221-227.

Swift C E, Berman M D. Factors affecting the water retention of beef. 1. Variations in composition and properties among 8 muscles[J]. Food Technology, 1959, 13(7): 365-370.

3259 Tamm, A., Bolumar, T., Bajovic, B. and Toepfl, S. (2016). Salt (NaCl) reduction in
3260 cooked ham by a combined approach of high pressure treatment and the salt replacer
3261 KCl. Innovative Food Science & Emerging Technologies, 36, pp.294-302.
3262 Tiyasatkulkovit, W., Aksornthong, S., Adulyaritthikul, P., Upanan, P., Wongdee, K.,
3263 Aeimlapa, R., Teerapornpuntakit, J., Rojviriya, C., Panupinthu, N. and
3264 Charoenphandhu, N., 2021. Excessive salt consumption causes systemic calcium
3265 mishandling and worsens microarchitecture and strength of long bones in rats.
3266 Scientific reports, 11(1), pp.1-15.
3267 Van Boekel, M.A.J.S., 2001. Kinetic aspects of the Maillard reaction: a critical review.
3268 Food/Nahrung, 45(3), pp.150-159.
3269 Van Der Klaauw, N. and Smith, D., 1995. Taste quality profiles for fifteen organic and
3270 inorganic salts. Physiology & Behavior, 58(2), pp.295-306.
3271 Vidal, V.A., Santana, J.B., Paglarini, C.S., da Silva, M.A., Freitas, M.Q., Esmerino,
3272 E.A., Cruz, A.G. and Pollonio, M.A., 2020. Adding lysine and yeast extract improves
3273 sensory properties of low sodium salted meat. Meat Science, 159, p.107911.
3274 Watanabe, Y., Kadowaki, M. and Fujimura, S., 2005. Regulation of Taste-active
3275 Components of Meat by Dietary Lysine Levels. In 51th International Congress of Meat
3276 Science and Technology (pp. 444-447).
3277 Weaver, R.A. and Shelef, L.A., 1993. Antilisterial activity of sodium, potassium or
3278 calcium lactate in pork liver sausage. Journal of Food Safety, 13(2), pp.133-146.
3279 World Health Organisation, 2020. Salt Reduction, Fact Sheets. Retrieved 3 November
3280 2020 from https://www.who.int/news-room/fact-sheets/detail/salt-reduction

Yang, X., Sebranek, J.G., Luo, X., Zhang, W., Zhang, M., Xu, B., Zhang, Y. and Liang, R., 2021. Effects of calcium salts on the physicochemical quality of cured beef sausages during manufacturing and storage: A potential calcium application for sausages with alginate casings. Foods, 10(11), p.2783

Zhang, Y., Guo, X., Liu, T. and Peng, Z., 2018. Effects of substitution of NaCl with KCl, L-histidine, and L-lysine on instrumental quality attributes of cured and cooked pork loin. CyTA-Journal of Food, 16(1), pp.877-883.

Zhou, C., Li, J., and Tan, S., 2014. Effect of L-lysine on the physicochemical properties of pork sausage. Food Science and Biotechnology, 23(3), 775-780.

Chapter 5. Effect of pH on physio-chemical characteristics and volatile flavour compounds in a salt-reduced pork patty with lysine and calcium lactate

Abstract

The Maillard reaction is an important route to many of the aroma volatiles found in cooked meat. Previous work has identified that lysine and calcium lactate can be used together to partially replace sodium chloride in pork patties. Since lysine is highly reactive substrate for the Maillard reaction during heating processes, so may therefore contribute to flavour generation which could further impact perception of salty taste. However, the Maillard reaction is very pH dependent. Therefore, this study was designed to test the effects of lysine (3%), calcium lactate (1.5%) and pH (5.5, 6.0, and 6.5, controlled through addition of dipotassium phosphate) on physio-chemical characteristics and volatile compounds of salt-reduced pork patties, while 2% NaCl and 1% pork patty were used as conventional control and 50% salt reduction control, respectively. Cooking loss, colour, moisture content and pH were measured as physio-chemical characteristics; GC-MS was used to analysis the volatile compounds. Increasing pH significantly decreased cooking loss and resulted in a high moisture product. Redness and yellowness increased with increasing pH, whereas lightness decreased. Almost all volatile compounds came from lipid degradation, whereas very few Maillard reaction-derived volatile flavour compounds were detected after heating, and these were only in relatively small amounts with increased pH. Therefore, where lysine is added as a partial salt replacer in meat patties, this can be carried out without concern that it will substantially change the flavour profile of the product. In conclusion,

lysine and calcium lactate could be used as salt substitute to develop salt reduced meat products without substantial change of their flavour profile.

5.1 Introduction

Sodium chloride (NaCl) is an important ingredient in meat products, such as enhance product texture and ensure shelf-life (Desmond and Vasilopoulos, 2019; Inguglia *et al.*, 2017). However, high intake of salt increases the risk of hypertension and cardiovascular disease (Petit *et al.,* 2019; Rucker, Rudemiller and Crowley, 2018). Due to the health concern, salt reduction has attracted lots of attention from both industry and academia. One of the most common strategies to reduce salt content in meat products is to use salt substitutes (Inguglia *et al.*, 2017). In addition to the most commonly used metal salts (e.g., potassium chloride), many alternatives have been explored. Lysine had been successfully used to enhance the aroma, flavour and suppress off-flavour of meat products (Guo *et al.*, 2020; Dos Santos Alves *et al.*, 2017; Campagnol *et al.*, 2011). Calcium lactate has been added to meat products for calcium fortification and as a preservative (Irshad *et al.*, 2016; Lawrence *et al.*, 2003). In previous work (Chapter 4), 3% w/w lysine and 1.5% w/w calcium lactate were proven to be effective in retaining salty taste, physicochemical properties and shelf life of a 50% salt reduced pork patty. However, as a reactive amino acid, lysine can be involved in Maillard reaction during heating processes, which may generate volatile compounds and subsequently affect salty taste (Martins, Jongen and Van Boekel, 2000).

Flavour is one of the most important factors influencing consumer buying behaviour and preference on meat products (Robbins *et al.*, 2003). Generally speaking, raw meat

has little aroma and only a bloody flavour (Jayasena *et al.*, 2013). However, due to the complex interaction of precursors from the lean and fatty components of the meat, it can develop a series of volatile flavour compounds during cooking (Van Ba, Amna and Hwang, 2013). Typically, the volatile flavour compounds produced during cooking are mainly due to the Maillard reaction, thermal degradation of lipids and Maillard-lipid interactions (Sun *et al.*, 2022). Maillard reaction, also known as non-enzymatic browning, is a reaction between carbonyl compounds (reducing sugars) and amino compounds (amino acids and proteins) (Ames, 1992). The Maillard derived flavour compounds include many sulphur-containing compounds which are important for the flavour of meat (Van Boekel, 2006). In addition, thermal degradation of thiamin produces a few sulfur compounds, such as thiols, sulphides and disulphide compounds which contribute to the meaty flavour (Grosch, 2001). Cysteine is one of the most important sulphur-containing amino acids contributing to meaty flavour through Maillard reactions (Aaslyng and Meinert, 2017). Several compounds produced by lipid oxidation contribute to the overall flavor of cooked meat, especially typical fatty fried notes (Parker, 2013). Although the flavour detection threshold of the meaty-flavored compounds produced by lipid oxidation are much higher than that of the sulfur- and nitrogen-containing heterocyclic compounds formed by the Maillard reaction of water-soluble precursors, however, some aldehydes which produced by lipid oxidation, including 6 – 10 saturated and unsaturated aldehydes of 10 carbon atoms, are the main volatile constituents of all cooked meats (Mottram, 1998). In addition, amino acids can undergo the Strecker degradation process in Maillard reaction, and then generate some

reactive radicals, such as ammonia, hydrosulfide, and these free radicals can further react with the secondary oxidation products of lipids to generate volatile flavour compounds such as thiols and thiophenes, thiazoles (Van Ba *et al.*, 2012).

The formation of Maillard derived flavour compounds is dependent on the type of sugars and amino acids involved, as well as temperature, time, pH and water content (Van Boekel, 2006). As pH increases, colour and polymeric compounds increase and nitrogen-containing compounds like pyrazines are favoured (Calkins and Hodgen, 2007). At low pH (for example pH < 5), flavour is readily generated by Strecker degradation of amino acids. From non-sulfur amino acids this can lead to compounds such as methylbutanals (malty aromas), whereas from the sulfur amino acids this leads to highly reactive intermediates (including hydrogen sulfide and methanethiol), which interact to form a many odourless compounds; At high pH (for example pH > 7) more nitrogen-containing volatiles are formed, particularly the pyrazines as well as more brown pigment (melanoidin) (Parker, 2013). The pH value of muscle is now recognized as an important factor affecting the rate and extent of lipid oxidation in meat (Tichivangana, and Morrissey, 1985). The oxidative stability is more stable at a neutral or acidic pH (pH = 4, 7), but the rate of lipid degradation can be increased at an alkaline condition (pH = 10) (Kim *et al.*, 2016). In addition, thiamin is considered as a source of meat flavour generated on heating, and it is affected by temperature and pH (Madruga, 1997). 2-methyl-3-furanthiol and bis (2-methyl-3-furyl) disulfide (meaty aromas) and thiophene are the main aroma volatile compounds at pH 5 and 7; however,

when the pH is increased to 9, the levels of these meaty flavour compounds decrease (Van Ba, Amna and Hwang, 2013).

Although previous literature has confirmed the role of pH in the formation of flavour through the Maillard reaction in model systems, less research has investigated the effect of pH on Maillard products within meat where the pH is buffered and relatively low (pH 5.5-6.5) (Calkins and Hodgen, 2007). Therefore, the aim of this study was to investigate whether relatively small changes in pH, at below pH 7, would affect the physicochemical quality and volatile flavour compounds of pork patties varying in salt (sodium chloride), lysine and calcium lactate. Based on the understanding of previous literature, this study specifically hypothesised that addition of lysine and calcium lactate would modify the flavour profile of salt reduced meat products due to involvement of Maillard reaction at different pH values.

5.2 Method & materials

5.2.1 Raw pork meat

All the lean pork leg and pork back fat was purchased from a local supplier (Solent Butchers & Co. Limited, UK) on three occasions in considering the batch effect. All the meat were vacuum packaged (A300/52, Multivac Gastrovac, Germany) and stored at -18 °C in a freezer until further use. The sample was thawed at 4 °C in a refrigerator for 24 h before use.

5.2.2 Experiment design

For the control sample, a salt (sodium chloride, NaCl) concentration at 2% (w/w) was used, while 1% NaCl was used to target 50% sodium reduction for the sodium reduced

meat samples. The sodium reduced pork patties, contained 3% lysine (Health Leads, UK) and 1.5% calcium lactate (Merck, USA) based on previous work (Chapter 4). Dipotassium phosphate (Merck, USA) was used to adjust meat pH to 5.5, 6 and 6.5 respectively. Overall, 12 treatments plus one control sample were prepared as detailed in Table 1. Each treatment preparation was repeated three times.

5.2.3 Preparation of pork patties

The formulation of pork patties was adapted from the previous work (Chapter 4). All the ground meat and ingredients (distilled water, salt, calcium lactate, lysine and dipotassium phosphate) were homogenized at 5000 rpm for 5 min until uniformity was reached using a food processor (Titanium Major KMM020, Kenwood Limited, UK), according to the formulation described in Table 5.1. Each pork patty was formed with 100 g batter in a foil cup (8 cm diameter, 3 cm thickness). Samples were cooked at 200°C in an oven (B1542, Naff, Germany) until the centre temperature reached 75°C. After cooking, samples were covered up by foil and chilled at 4 °C in a refrigerator for 24 h before physical analysis (cooking loss and colour). Some of the chilled samples were ground by a blender (AT640, Kenwood Limited, UK), then vacuum packed and stored at -18 °C in a freezer for further chemical analysis (pH after cooking, moisture content). At each sampling point samples were withdrawn in triplicate for subsequent analyses.

Table 5.1. Formulation of pork patties varying in salt, lysine, calcium lactate and pH.

Treatment Code	Lean pork leg (%)	Pork back fat (%)	Distilled water (%)	Sodium Chloride (%)	Lysine (%)	Calcium lactate (%)	Dipotassium phosphate (%)
Control	70	10	18	2	-	-	-
S5.5	70	10	18	1	-	-	-
S6	70	10	18	1	-	-	0.4
S6.5	70	10	18	1	-	-	0.8
SL5.5	70	10	18	1	3	-	-
SL6	70	10	18	1	3	-	0.4
SL6.5	70	10	18	1	3	-	0.8
SC5.5	70	10	18	1	-	1.5	-
SC6	70	10	18	1	-	1.5	0.9
SC6.5	70	10	18	1	-	1.5	1.9
SLC5.5	70	10	18	1	3	1.5	-
SLC6	70	10	18	1	3	1.5	0.9
SLC6.5	70	10	18	1	3	1.5	1.9

*Control = 2% NaCl, pH = 5.5; S5.5 = 1% NaCl, pH = 5.5; S6 = 1% NaCl, pH 6; S6.5 = 1% NaCl, pH = 6.5; SL5.5 = 1% NaCl + 3% lysine, pH = 5.5; SL6 = 1% NaCl + 3% lysine, pH = 6; SL6.5 = 1% NaCl + 3% lysine, pH = 6.5; SC5.5 = 1% NaCl + 1.5% calcium lactate, pH = 5.5; SC6 = 1% NaCl + 1.5% calcium lactate, pH = 6; SC6.5 = 1% NaCl + 1.5% calcium lactate, pH = 6.5; SLC5.5 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 5.5; SLC6 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 6; SLC6.5 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 6.5.

5.2.4 Physical-chemical characteristics of pork patties

5.2.4.1 pH

The pH was measured on raw and cooked ground pork patties. The patty sample (10g) was added to 100 ml distilled water and mixed using a magnetic stirrer (SS3H stirrer hot plate, hemLab, Netherlands) for 90 s at a medium speed. The pH was measured using an electrode meter (Orion star A111, Thermo scientific, USA).

5.2.4.2 Moisture content

According to AOAC method, 3 g ground sample was put into the aluminium moisture dish, then dried in an oven (Gallenkamp, UK) at 100 ° C for 24 h. Samples were cooled in a desiccator at least 30 min and reweighed to calculate the weight difference. The moisture content was calculated by the weight difference (before and after drying) divided by the starting weight of sample before drying and expressed as % (w/w).

5.2.4.3 Cooking loss

The cooking loss was calculated using the formula as follows: cooking loss (%) = (W_b – W_a)/W_b x 100%. W_b means weight of pork patty before cooking, and W_a means weight of pork patty after chilling.

5.2.4.4 Colour

A chroma meter (CR-400, Konica minolta, Japan) with 8mm diameter measuring aperture, illuminant D65, 2° standard observer was used to determine the colour of cooked pork patty. The instrument was calibrated using white calibration plate (CR-A43, Y = 93.5, x = 0.3140, y = 3318) and CIELAB color space was selected to describe the colour feature of pork patties. Colour characteristics including L* (lightness), a* (redness) and b* (yellowness) were measured at three surface and internal locations and the average was calculated to present the colour characteristics of the pork patty.

5.2.5 Analysis of volatile compounds

The pork patties were immediately ground after cooking, and ground meat (2 g) was transferred into 20 mL headspace sample vials which were rapidly fitted with a screw cap. Analyses were conducted by automated headspace SPME using an Agilent 110 PAL injection system and a 7890A gas chromatography system with 5975C mass spectrometer (Agilent, Santa Clara, CA, USA). An SPME fiber coated with polydimethylsilocane/divinylbenzene/carboxen (PDMS/DVB/CAR) was used for extraction (Supelco, Bellefonte, PA). The samples were equilibrated by constant agitation at 500 rpm for 10 mins at 50°C, and then extracted at the same temperature for 30 mins. After extraction, the SPME device was inserted into the injection port (260 °C) of the GC instrument and immediately desorbed for 20 mins. An Agilent capillary column DB-5 (30 m × 0.32 mm × 0.25 μm thickness) (Agilent, Santa Clara, CA, USA) was used for chromatographic separation. The initial oven temperature was held at 40°C for 5 minutes, and subsequently increased to 260°C at 4°C/min before holding isothermal for 5 minutes. The inlet was a splitless injection with a helium carrier gas introduced at a constant flow rate of 0.9 mL/min (pressure pulse of 6.2035 psi). Mass spectra were measured in electron ionization mode with ion source temperatures at 230 °C and scanned from m/z 20 to m/z 350. Volatile compounds were identified by comparing each mass spectrum with the NIST mass spectral database (NIST/EPA/NIH Mass Spectral database, 2011). The retention times of the homologous series of C6-C25 n-alkanes were used to calculate a linear retention index (LRI) for each volatile compound to confirm the identification. Measurement of the GC peak area for each compound was used to provide semi-quantitative relative values in order to compare the volatile profile of different samples.

5.2.6 Statistical analysis

The data of physical-chemical characteristics of pork patties and quantitative data for each compound identified in the SPME GC-MS analysis were analysed by both one-way and two-way analysis of variance (ANOVA) using SPSS Statistics 27 (IBM, USA). One-way analysis of variance (ANOVA) was used to evaluate the significant difference between treatments at the significant level 0.05, while two-way ANOVA was used to examine the effect of factors (ingredients, pH) at significant level 0.05. Duncan test was selected for multiple comparisons if equal variances were assumed, otherwise, Tamhane's T2 test was used. Principal component analysis (PCA) was carried out by XLSTAT Version 2022.4.1 (Addinsoft, Paris, France) on the correlation matrix from the volatile data to visualise the main differences in volatile profile between the different formulations.

5.3 Results and discussion

5.3.1 Physical-chemical characteristics

The effect of pH on physical-chemical characteristics of the pork patties are shown in Table 5.2. It demonstrates that both the variation in ingredients and the initial pH had significant effects on the pH after cooking, cooking loss, moisture and colour ($p < 0.05$).

Table 5.2. Physical-chemical characteristics of pork patties varying in salt, lysine, calcium lactate and pH

Treatment	pH before cooking	pH after cooking	Moisture	Cooking loss	L* surface	a* surface	b* surface	L* internal	a* internal	b* internal
Control	5.57±0.10^{e}	6.11±0.04^{c}	65.13±3.37^{bcde}	25.05±1.60^{c}	56.49±4.50^{bcd}	4.74±0.74^{bcd}	17.56±0.91^{a}	67.72±2.43^{bcd}	4.98±1.00^{bcd}	8.96±0.17^{fg}
S5.5	5.56±0.07^{e}	6.11±0.06^{c}	60.31±1.12^{f}	34.37±1.73^{a}	59.05±3.77^{b}	4.08±0.27^{def}	14.47±1.01^{ef}	69.53±2.89^{ab}	3.61±1.14^{f}	10.36±0.21^{b}
S6	6.00±0.09^{cd}	6.17±0.04^{c}	66.18±4.92^{abcd}	29.45±3.53^{b}	56.06±4.35^{bcd}	4.48±0.86^{cde}	15.22±0.78^{de}	67.90±0.58^{bcd}	5.03±0.25^{abc}	10.50±0.14^{b}
S6.5	6.55±0.04^{a}	6.57±0.11^{a}	68.69±3.20^{a}	21.42±2.59^{de}	54.60±1.26^{cde}	4.95±0.72^{bc}	16.56±1.25^{abc}	66.15±0.59^{def}	5.71±0.39^{a}	10.88±0.83^{a}
SL5.5	5.56±0.06^{e}	6.11±0.11^{c}	63.59±0.88^{de}	24.04±2.45^{cd}	54.63±1.15^{cde}	5.10±0.35^{bc}	13.92±0.58^{f}	66.61±2.79^{cde}	4.89±0.82^{bcd}	8.85±0.12^{g}
SL6	5.97±0.07^{d}	6.19±0.07^{c}	66.64±4.04^{abcd}	20.45±7.40^{ef}	51.50±3.31^{ef}	5.43±0.35^{ab}	14.84±1.40^{ef}	64.57±0.38^{efg}	5.37±1.14^{ab}	9.37±0.13^{de}
SL6.5	6.47±0.05^{b}	6.54±0.08^{ab}	68.43±5.64^{ab}	17.64±4.93^{f}	49.96±3.62^{f}	5.91±0.64^{a}	17.40±1.28^{ab}	63.37±0.41^{g}	5.70±0.33^{a}	9.60±0.31^{d}
SC5.5	5.55±0.07^{e}	5.58±0.08^{e}	60.23±2.54^{f}	29.55±1.28^{b}	62.58±3.48^{a}	2.28±0.51^{g}	14.93±1.19^{ef}	70.50±4.56^{a}	4.28±0.43^{def}	8.85±0.14^{g}
SC6	6.05±0.08^{c}	6.00±0.10^{d}	63.85±4.50^{cde}	25.41±2.81^{c}	58.16±5.39^{bc}	2.74±0.16^{g}	15.59±0.90^{cde}	68.70±1.09^{abc}	4.42±0.33^{cde}	9.65±0.30^{d}
SC6.5	6.51±0.12^{ab}	6.49±0.07^{b}	65.88±1.76^{abcde}	20.08±3.39^{ef}	55.87±4.32^{bcd}	3.52±1.52^{f}	16.59±1.24^{abc}	67.23±0.75^{bcd}	5.34±0.98^{ab}	9.99±0.16^{c}
SLC5.5	5.57±0.04^{e}	5.58±0.05^{e}	62.65±1.99^{ef}	24.50±2.10^{cd}	59.07±0.77^{b}	3.85±0.42^{ef}	16.18±1.14^{bcd}	67.42±1.47^{bcd}	3.71±0.18^{f}	8.03±0.18^{h}
SLC6	6.01±0.04^{c}	5.99±0.10^{d}	67.17±1.63^{abc}	20.11±2.56^{ef}	56.58±3.54^{bcd}	4.10±1.24^{def}	16.55±1.99^{abc}	66.40±0.83^{cde}	4.04±0.30^{ef}	9.18±0.40^{ef}
SLC6.5	6.50±0.07^{ab}	6.48±0.07^{b}	69.07±1.36^{a}	18.51±1.75^{ef}	53.84±3.05^{de}	4.78±1.11^{bcd}	16.91±1.57^{ab}	64.02±4.37^{fg}	4.45±0.91^{cde}	9.33±0.31^{de}
P (ingredient)	0.143	<0.001	0.003	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001
P (pH)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P (interaction)	0.187	<0.001	0.751	0.042	0.951	0.986	0.055	0.992	0.016	<0.001

*Control = 2% NaCl, pH = 5.5; S5.5 = 1% NaCl, pH = 5.5; S6 = 1% NaCl, pH 6; S6.5 = 1% NaCl, pH = 6.5; SL5.5 = 1% NaCl + 3% lysine, pH = 5.5; SL6 = 1% NaCl + 3% lysine, pH = 6; SL6.5 = 1% NaCl + 3% lysine, pH = 6.5; SC5.5 = 1% NaCl + 1.5% calcium lactate, pH = 5.5; SC6 = 1% NaCl + 1.5% calcium lactate, pH = 6; SC6.5 = 1% NaCl + 1.5% calcium lactate, pH = 6.5; SLC5.5 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 5.5; SLC6 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 6; SLC6.5 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 6.5. Averages within the same column followed by the same letters are not significantly different (P > 0.05). Values represented as the Mean ± standard deviation (SD), n = 3

5.3.1.1 pH

As shown in Table 5.2, the pH of the cooked patties was significantly affected by the ingredients when the pH of the raw patties was same ($p < 0.05$; Supplementary table 12). Pork patties without addition of calcium lactate or dipotassium phosphate (control, S5.5 and SL5.5) increased in pH during cooking from 5.5 to 6.11. This may be due to thermally induced dynamic changes in the acidic and basic groups in the denatured protein (Yang *et al.*, 2021). However, where calcium lactate was added, the pH seemingly did not increase with cooking. Although calcium lactate (1.5% w/w) is acidic it did not lower the pH of the uncooked patties (SC5.5, SC6, SC6.5, SLC5, SLC6, SLC6.5), and yet their pH did not increase over cooking unlike for the other patties; therefore, it is likely that the water solubility of calcium lactate was improved by the increasing temperature during cooking (Kubantseva and Hartel, 2002).

5.3.1.2 Moisture content

The moisture content of the control (2% salt) cooked patty was 65.1% (w.v), whereas the moisture content of the salt-reduced pork patty (1%) without any salt substitutes (S5.5) was 4.82% (w/v) lower ($p < 0.05$; Supplementary table 12). This is in agreement with Tobin *et al.* (2013) where the higher salt samples were correlated with lower moisture content in pork breakfast sausages. This is because salt reduction leads to lower solubilisation of functional myofibrillar protein in meat (actin and myosin), which reduces protein hydration and water holding capacity, resulting in a lower water content (Desmond and Vasilopoulos, 2019). However, where the pH was raised 6 or above this additional moisture loss was avoided (S6, S6.5). This is supported by an earlier study of Guerrero, Gou and Arnau (1999) where cooked ham at pH 6.2 had a higher water content than that at pH 5.8, because high pH far away from isoelectric

point of muscle protein would create more space between thin filament and thick filament to allow more water retained in the muscle structure (Honikel, 2004).

The lowest moisture content was measured in pork patty with calcium lactate at pH 5.5(SC5.5), and the highest moisture content was measured in pork patty with lysine and calcium lactate at pH 6.5 (SLC6.5). Lysine may have reduced the water loss due to its positively charged polar amino acid which can bind with anions to form hydrogen bonds in order to retain water within the structure (Betts and Russell, 2003). This result is consistent with the work of Vidal et al. (2020) where a 50% salt-reduced meat with 3% lysine had similar moisture content compared to the non-salt reduced meat.

According to Table 2, SC5.5 and SLC5.5 were significantly lower in moisture than that of the control ($p < 0.05$), whereas SC6, SC6.6, SLC6 and SLC 6.5 had similar moisture content with control ($p > 0.05$). It indicates that, to avoid excess moisture loss where calcium lactate was used in the salt-reduced formulation, the pH needed to be adjusted 6 or above. Irshad *et al.* (2016) found that calcium lactate (1% - 1.5%) reduced moisture in a restructured buffalo meat loaf and they proposed that an increase in tightly bound multivalent cations could result in a lower water binding ability (Yang *et al.*, 2004). Consequently, low moisture content would be expected in calcium lactate added samples. While high pH (6 or above) would create more charged anions within the muscle structure due to far away from the isoelectrical point of muscle protein (pH5.2), which could cancel out the effect of cations effect of calcium to achieve similar moisture level of control meat patties. Overall, the water holding capacity of raw meat increased with the increasing pH. Lower pH leads to higher protein-protein interactions, reducing the space within and between myofilaments, resulting in a lower level of immobilization of water (Honikel, 2004).

5.3.1.3 Cooking loss

The cooking loss of the standard salt control patty was 25.1%, whilst the highest loss (34.4%) was in the salt-reduced pork patty without any salt substitutes (S5.5) which is 9.32% higher than that of control. The cooking loss is in-line with the moisture loss, indicating the moisture loss formed main part of the cooking loss. The cooking loss was reduced significantly when the pH was raised to 6.0 and 6.5. The lowest cooking loss was achieved by the pork patty with lysine at pH 6.5 (SL6.5) where the yield was 7.5% higher than the control (ie cooking loss 17.6% compared to 25.1%). This was consistent with moisture content results that lysine reduced the cooking loss of the salt-reduced pork patties. For calcium lactate addition, the cooking loss decreased with the increase of pH ($p < 0.05$; Supplementary table 12), sample with pH 6 (SC6) achieved similar cooking loss with control sample ($p > 0.05$). The result is in line with the moisture content result whereas the salt-reduced pork patties with calcium lactate needed to be adjusted to a pH above 6 in order to avoid excess moisture loss and hence cooking loss. These results are in agreement with Tobin *et al.* (2013), Guo *et al.* (2020) and Irshad *et al.* (2016), where they indicated respectively that increasing either the concentration of salt (0.8% - 2.4%) or lysine (0.2% - 0.8%) decreased cooking loss in a pork breakfast sausage or salt-reduced restructure ham, while calcium lactate (1% - 1.5%) increased cooking loss in a restructured buffalo meat loaf. Aaslyng *et al.* (2003) reported that pork steak at high pH (pH > 5.8) had a lower cooking loss, whereas the cooking loss was higher at low pH (pH < 5.4). The conclusion of the current study (see Table 2) is that cooking loss is affected by the interaction of ingredients and pH, meaning that when salt substitutes are used such as calcium lactate, the pH of the meat may need to be raised to ensure a constant yield.

5.3.1.4 Colour

The lightness (L*) of the standard salt patty (control) was 56.5 on the surface and 67.7 inside. The measurement data also shown that red colour (a*) was consistent between the surface and inside, whereas the yellow colour (b*) was almost halved compared to the surface. When the salt content was reduced by 50% (S5.5), the lightness and surface redness were not influenced, but the internal redness and surface yellowness were significantly decreased ($p < 0.05$; Supplementary table 12), whereas internal yellowness increased ($p < 0.05$; Supplementary table 12). These differences were mostly consistent across the salt reduced patties at higher pH (S6, S6.5). Tobin *et al.* (2013) previously found a 50% salt-reduced pork breakfast sausage to have a paler colour than the higher salt control and concluded this was because salt has the ability to reduce the oxygen solubility in food matrix and then alleviate the oxidation of myoglobin. The reduction of salt would promote myoglobin to be oxidized into metmyoglobin and colour would shift from purple colour to brown colour which resulted in a reduction of redness (Petit *et al.,* 2019). The addition of lysine (SL5.5) resulted in a similar colour with the 2% control ($p > 0.05$), except for the surface yellowness. But the addition of calcium lactate (SC5.5) only ensured that the internal redness and internal yellowness were similar with the control ($p > 0.05$), while all other colour measurements were significantly different. The effects of lysine and calcium lactate on colour were in agreement with the findings reported by Zhou and Tan (2014) and Yang *et al.* (2021) in sausages. The variation in L* is related to water content of pork products that higher water content leads to a lower L* in colour (Hong *et al.*, 2016). It is because that high moisture content indicates swelling of muscle fiber, and a bigger space within the myofiber lattice. while the increased myofiber lattice and space would reduce the light scattering as a result a low L* would be expected (Ruedt, Gibis, and Weiss, 2022). The redness (a*) depends on the amount of deoxymyoglobin and/or oxymyoglobin and oxidation of myoglobin to

metmyoglobin, while lysine was reported promoting their oxidation (Zhou and Tan, 2014). With the addition of lysine and calcium lactate, the lightness was increased from 8 to 18 units, and the yellowness reduced around 6 to 8 units from surface to inside which were in the same trend with the control sample (L* increased around 12 units and b* dropped around 7 units). Differences in measured values for interior and surface may be due to a small surface area to volume ratio, meaning that very little of the patty would have reached temperature of over 100 °C at low water activity that is required for greater Maillard reaction (Van Ba, Amna and Hwang, 2013).

5.3.2 Volatile composition

In total, 29 compounds were identified in the headspace by GC-MS of the different pH pork patties varying in salt, lysine, calcium lactate and pH, as listed in Table 5.3. These included 2 acids, 1 alkane, 6 alcohols, 12 aldehydes, 1 furan, 5 ketones, 1 phenol and 1 pyrazine. The formation of these volatile compounds is mainly associated with the degradation of lipids and, to a lesser extent, the Maillard reaction. Volatile compounds originating from lipid degradation usually have low thresholds and play a major role in flavour development (Wen *et al.*, 2019). The aldehydes contributed almost average of 89.1% of the flavour composition and clearly dominate. Similar results were reported by Xie *et al.* (2008), who indicated that the major volatile compounds in roasted pork was the aldehyde group, accounting for 52.6% of the total flavour profile. In the current study, hexanal, which is considered to be the most abundant lipid oxidation product in meat, was found in the largest quantities within the aldehyde group (typically accounting for 88.6% of aldehydes). Other straight chain aldehydes such as pentanal, heptanal and nonanal, which were present at relatively high quantities, are also derived from the oxidation of unsaturated fatty acids and are known to contribute to the characteristic fatty aroma of meat (Wen *et al.*, 2019). Ketones and alcohols were also

abundant lipid derived volatiles in the pork patties. Volatile alcohols can be derived from lipid oxidation or Maillard reaction in meat products and can provide a wide variety of aromatic compounds by reacting with themselves or other compounds. Ketones are often regarded as secondary products formed during lipid oxidation, alkane degradation and dehydrogenation of secondary alcohols (Deng *et al.*, 2021). Relative quantitative differences were observed between the different pH levels (5.5, 6, 6.5) and ingredients (50% salt, lysine, calcium lactate) used in this study. Changes to the ingredients (salt, lysine and calcium lactate) had significant effects ($p < 0.05$; Supplementary table 13) on the relative amounts of most aldehydes, alcohols, ketones, hexanoic acid and phenols. Likewise, the adjustment of pH also significantly affected ($p < 0.05$; Supplementary table 13) the relative amounts of most aldehydes, alkanes and ketones in addition to acids, 1-heptanol 1-octen-3-ol, 1-octanol, phenols and pyrazines. There was a significant interaction of pH and ingredients on a limited number of volatiles: hexanoic acid, 1-pentanol, 2-methylbutanal, 3-methylbutanal, pentanal, hexanal and 2-phenoxyethanol ($p < 0.05$; Supplementary table 13). Kim *et al.* (2016) reported that the protonation state of the lipid molecule can influence the stability of the molecule and the ease with which it undergoes chemical reactions. Consequently, a low pH (acidic conditions) can promote lipid oxidation by creating a more favourable environment for oxidation reactions to occur. Conversely, a high pH (basic or alkaline conditions) can inhibit lipid oxidation by reducing the rate of oxidation reactions.

Table 5.3. Volatile flavour compounds in the headspace above pork patties (by SPME GC–MS), relative amounts are mean peak areas (/1000). Patties varied in salt, lysine, calcium lactate and pH.

Compound	Code	LRI	Control	s5.5	s6	s6.5	sl5.5	sl6	sl6.5	sc5.5	sc6	sc6.5	slc5.5	slc6	slc6.5	p(ingredient)	p(pH)	p(interaction)
Acids (2)																		
Butanoic acid	AC1	780^{b}	2,211^{ab}	4,231^{ab}	3,633^{ab}	2,507^{ab}	1,550^{b}	3,061^{ab}	1,753^{b}	5,295^{a}	2,335^{ab}	1,294^{b}	4,420^{ab}	4,494^{ab}	2,525^{ab}	0.208	0.041	0.354
Hexanoic acid	AC2	974^{b}	3,615^{bc}	5,424^{a}	1,968^{cdef}	1,766^{def}	3,251^{bcd}	2,920^{bcde}	1,983^{cdef}	4,478^{ab}	675^{f}	353^{f}	4,177^{ab}	1,291^{ef}	416^{f}	0.027	<0.001	0.037
Total			5,825	9,655	5,602	4,274	4,801	5,981	3,736	9,773	3,010	1,647	8,597	5,785	2,941			
Alkanes (1)																		
2-Pentyloxirane	ALK1	917^{b}	1,703^{ab}	943^{d}	1,338^{abcd}	1,047^{bcd}	1,639^{abcd}	1,737^{a}	784^{d}	ND	ND	ND	1,002^{cd}	ND	ND	0.147	0.016	0.063
Alcohols (6)																		
1-Penten-3-ol	ALC1	678^{a}	2,370^{ab}	1,430^{b}	1,611^{ab}	2,797^{ab}	2,173^{ab}	3,575^{a}	1,902^{ab}	1,520^{b}	ND	ND	2,077^{ab}	ND	ND	0.676	0.438	0.205
1-Pentanol	ALC2	765^{a}	29,707^{ab}	22,440^{b}	35,969^{ab}	47,985^{a}	32,191^{ab}	29,725^{ab}	25,597^{b}	16,456^{bc}	ND	ND	21,954^{b}	512	ND	0.013	0.411	0.024
1-Hexanol	ALC3	878^{a}	6,430^{a}	5,723^{ab}	4,590^{abc}	3,466^{abc}	4,920^{ab}	4,830^{ab}	3,208^{bc}	1,716^{c}	ND	ND	3,072^{bc}	ND	ND	0.044	0.094	0.85
1-Heptanol	ALC4	973^{a}	2,589^{ab}	2,858^{a}	2,576^{ab}	1,437^{bc}	2,250^{abc}	2,711^{ab}	1,261^{c}	ND	ND	ND	1,563^{abc}	ND	ND	0.165	0.013	0.628
1-Octen-3-ol	ALC5	982^{a}	40,424^{a}	33,550^{ab}	27,551^{abc}	24,109^{bc}	38,337^{ab}	23,550^{bc}	14,047^{cd}	4,399^{d}	1,310^{d}	ND	25,046^{bc}	915^{d}	386^{d}	<0.001	<0.001	0.132
1-Octanol	ALC6	1070^{a}	3,467^{a}	2,851^{ab}	2,689^{abc}	2,535^{abc}	1,665^{cd}	2,540^{abc}	1,015^{d}	ND	ND	ND	1,823^{bcd}	ND	ND	0.011	0.06	0.123
Total			84,987	68,851	74,986	82,330	81,536	66,931	47,029	24,091	1,310	0	55,535	1,427	386			
Aldehydes (12)																		
Butanal	ALD1	580^{a}	2,251^{ab}	ND	ND	ND	3,097^{a}	2,399^{ab}	1,300^{b}	ND	ND	ND	1,428^{b}	ND	ND	0.024	0.054	/
2-Methylbutanal	ALD2	656^{a}	ND	ND	ND	ND	ND	ND	1,114^{b}	ND	690^{b}	3,320^{a}	ND	1,075^{b}	1,428^{b}	0.016	0.002	0.008
3-Methylbutanal	ALD3	644^{a}	ND	ND	ND	ND	ND	ND	209^{b}	ND	618^{b}	2,405^{a}	ND	810^{b}	938^{b}	0.002	0.005	0.011
Pentanal	ALD4	697^{a}	136,736^{a}	83,322^{a}	108,737^{a}	88,174^{a}	130,695^{a}	109,443^{a}	106,912^{a}	74,278^{a}	1,223^{b}	835^{b}	97,924^{a}	2,526^{b}	ND	<0.001	0.007	0.04
Hexanal	ALD5	800^{a}	1,670,382^{a}	1,433,613^{ab}	1,349,386^{ab}	1,329,246^{ab}	1,471,910^{ab}	1,279,524^{ab}	1,160,749^{ab}	1,077,237^{b}	52,146^{c}	11,308^{c}	1,208,683^{ab}	28,670^{c}	7,059^{c}	<0.001	<0.001	0.007
2-Hexenal, (E)-	ALD6	862^{a}	1,671^{a}	1,502^{a}	1,618^{a}	ND	1,483^{a}	1,761^{a}	ND	1,175^{a}	ND	ND	1,677^{a}	ND	ND	0.328	0.293	0.654

Heptanal	ALD7	913[a]	32,777[ab]	41,189[a]	30,746[abc]	20,111[bc]	24,101[bc]	18,686[bc]	15,645[c]	23,055[bc]	ND	ND	23,055[bc]	ND	ND	0.033	0.022	0.403
2-Heptenal, (E)-	ALD8	963[a]	6,843[a]	5,247[abc]	3,345[cd]	2,413[d]	6,019[ab]	3,142[cd]	1,635[d]	2,821[cd]	ND	ND	3,975[bcd]	ND	ND	0.087	0.001	0.609
Benzaldehyde	ALD9	970[a]	3,191[a]	3,243[a]	2,097[bc]	1,209[cd]	2,711[ab]	1,442[cd]	1,169[cd]	807[d]	ND	ND	1,501[cd]	573[d]	ND	<0.001	<0.001	0.703
Octanal	ALD10	1004[a]	16,602[ab]	23,081[a]	15,383[abc]	10,432[bcd]	11,006[bcd]	17,482[ab]	7,857[cd]	5,515[d]	ND	ND	7,801[cd]	ND	ND	0.006	0.012	0.055
2-Octenal, (E)-	ALD11	1061[a]	4,708[b]	6,891[a]	2,140[c]	1,496[c]	4,605[b]	2,061[c]	1,303[c]	1,495[c]	ND	ND	2,130[c]	ND	ND	<0.001	<0.001	0.258
Nonanal	ALD12	1105[a]	23,797[b]	33,213[a]	15,183[c]	13,512[c]	14,725[c]	14,776[c]	10,347[cde]	7,410[cde]	2,571[de]	2,084[de]	10,886[c]	3,037[de]	1,839[de]	<0.001	<0.001	0.058
Total			1,898,958	1,631,302	1,528,634	1,466,594	1,670,353	1,450,716	1,308,240	1,193,793	57,248	19,952	1,359,061	36,692	11,264			
Furans (1)																		
2-Pentylfuran	F1	994[a]	8,434[a]	7,737[a]	7,606[a]	6,502[a]	9,331[a]	6,932[a]	6,668[a]	6,943[a]	ND	ND	7,648[a]	ND	ND	0.709	0.278	0.635
Ketones (5)																		
Acetol	K1	670[b]	643[d]	ND	1,063[cd]	1,395[bcd]	523[d]	2,669[b]	4,546[a]	ND	311[d]	2,061[bc]	227[d]	698[d]	2,042[bc]	<0.001	<0.001	0.06
2,3-Pentanedione	K2	694[a]	4,341[a]	4,019[a]	3,857[a]	ND	5,126[a]	4,033[a]	5,175[a]	4,995[a]	ND	ND	5,033[a]	ND	ND	0.747	0.52	0.51
Acetoin	K3	714[a]	1,743[def]	1,231[f]	1,435[f]	2,840[cde]	719[f]	1,856[def]	2,973[cd]	788[f]	3,789[bc]	4,700[ab]	1,501[ef]	3,738[bc]	5,420[a]	<0.001	<0.001	0.057
2-Heptanone	K4	903[a]	3,533[ab]	3,123[ab]	2,105[abc]	1,985[bc]	3,695[a]	2,939[ab]	1,880[bc]	983[c]	ND	ND	2,267[abc]	ND	ND	0.011	0.024	0.523
2,3-Octanedione	K5	985[b]	94,291[ab]	100,517[ab]	95,944[ab]	91,352[ab]	121,744[a]	82,482[ab]	62,129[b]	66,295[b]	638[c]	456[c]	77,943[ab]	719[c]	ND	<0.001	<0.001	0.176
Total			104,551	108,889	104,406	97,571	131,808	93,979	76,703	73,061	4,738	7,216	86,970	5,154	7,463			
Phenols (1)																		
2-Phenoxyethanol	PH1	1227[b]	1,742[cd]	2,120[bc]	2,775[ab]	423[f]	990[def]	1,595[cd]	1,324[cdef]	3,042[a]	1,118[def]	1,077[def]	1,379[cde]	1,470[cd]	514[ef]	0.021	<0.001	<0.001
Pyrazines (1)																		
2-methylpyrazine	PY1	830[a]	ND	ND	ND	ND	ND	349[b]	1,457[a]	ND	ND	ND	ND	ND	ND	/	0.044	/

*Control = 2% NaCl, pH = 5.5; S5.5 = 1% NaCl, pH = 5.5; S6 = 1% NaCl, pH 6; S6.5 = 1% NaCl, pH = 6.5; SL5.5 = 1% NaCl + 3% lysine, pH = 5.5; SL6 =1% NaCl + 3% lysine, pH = 6; SL6.5 = 1% NaCl + 3% lysine, pH = 6.5; SC5.5 = 1% NaCl + 1.5% calcium lactate, pH = 5.5; SC6 = 1% NaCl + 1.5% calcium lactate, pH = 6; SC6.5 = 1% NaCl + 1.5% calcium lactate, pH = 6.5; SLC5.5 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 5.5; SLC6 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 6; SLC6.5 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 6.5. ND means not detected. The letters in LRI column presented the reliability of identification, a means identification by mass spectrum and by coincidence with the LRI on a DB-5 column of an authentic standard; b means tentatively identification by mass spectrum. Averages within the same crow followed by the same letters are not significantly different (P > 0.05).

A total of 26 volatile compounds were detected in the control; notable the Maillard derived 2-methylbutanal, 3-methylbutanal, and 2-methylpyrazine were not observed in the control. 2-Methylpyrazine from the Maillard reaction was only released in the salt-reduced pork patties with added lysine, and the amount increased with increasing pH ($p < 0.05$; Supplementary table 13). This is not surprising since lysine as an amino acid is an efficient reactant for the Maillard reaction, and the higher the pH, the more reactive the protonated amino groups are with sugars, resulting in increasing the products of the Maillard reaction (Martins, Jongen and Van Boekel, 2000). The Strecker aldehydes 2-methylbutanal and 3-methylbutanal, also from the Maillard reaction, were only released in the salt-reduced pork patties containing lysine and calcium lactate, and their levels also increased with raising pH ($p < 0.05$; Supplementary table 13). The salt-reduced pork patties with lysine alone only contained these Strecker aldehydes at the highest pH (SL6.5), whilst the salt-reduced pork patties with calcium lactate began to show 2-methylbutanal and 3-methylbutanal from pH 6, and the amount was higher than that from the lysine only patty ($p < 0.05$; Supplementary table 13). Table 4.3 also shows that when lysine and calcium lactate were added together to salt-reduced pork patties, the amount of 2-methylbutanal and 3-methylbutanal were lower than when only calcium lactate was added ($p < 0.05$). According to Jane's (2013) work, it could be explained that Strecker aldehydes are produced more at lower pH and pyrazines more at higher pH, and the calcium lactate kept the pH lower during cooking (and there may well still be sufficient of the amino acids that lead to 3 and 2 methyl butanal in the meat itself).

The reduction in salt alone (without salt replacers) had no effect on the relative amounts of alcohols, furans, hydrocarbons, phenols, and most of aldehydes and ketones; however, only hexanoic acid, 2-pentyloxirane, butanal, nonanal, 2-octenal and acetol were significantly decreased compared with the control ($p > 0.05$; Supplementary table

9). Hu *et al.* (2020) report a similar result that different NaCl levels almost not vary volatile flavour compounds derived from lipid oxidation in treatments. When lysine was added to the salt-reduced pork patty alone as a salt substitute, there was little change in the amount of lipid-derived flavour compounds compared to the control ($p > 0.05$), except for a significant reduction in the amount of 1-octanol and nonanal ($p < 0.05$; Supplementary table 13). However, the addition of calcium lactate alone had a substantial and significant impact in reducing the majority of lipid-derived volatile flavour compounds compare to control ($p < 0.05$; Supplementary table 13), the only compound not affected by calcium lactate were acids, 1-penten-3-ol, 1-pentanol, pentanal, heptanal, 2-hexenal, furans, and some ketones ($p > 0.05$; Supplementary table 13). When these two salt substitutes were added together to the salt-reduced pork patty, the amounts of phenols, ketones, furans, benzenes and acids were not significantly different compared to the control ($p > 0.05$), half of the alcohols and aldehydes were significantly reduced ($p < 0.05$). It may be explained by the pH and moisture content. According to Kim's work (2016), an acidic environment can slow down lipid oxidation by limiting the formation of free radicals and decreasing the solubility of oxygen. In addition, In the presence of water, lipid oxidation reactions can occur faster, as water can participate in the reactions and enhance the formation of peroxides (Shahidi and Zhong, 2010). Therefore, the addition of calcium lactate resulted in a low water content and pH after cooking, thereby reducing the rate of lipid oxidation, so lipid-derived flavor compounds were less relative to other treatments. In addition, it is worth noting that table 3 clearly shows that there are interactions between the type of salt substitute and pH, affecting the level of hexanoic acid, 1-pentanol, 2-methylbutanal, 3-methylbutanal, pentanal, hexanal and 2-phenoxyethanol. Therefore, the effect of salt substitutes on these chemical compounds are dependent on the pH. Apart from 2-

methylbutanal and 3-methylbutanal which increased with the level of salt substitutes at high pH only, the levels of the other flavor compounds decreased significantly with level of salt substitutes at all pHs. So far the mechanism of action remains unclear, and further experiments are needed to elucidate potential mechanisms.

PCA was performed to visually compare the volatile profile from the 13 treatments (Figure 5.1) and to observe the correlations between ingredients, pH and volatile compounds. The PCA results (Figure 1a) clearly showed that the salt-reduced pork with different ingredients and pH were well differentiated. In total, principal components one (F1) and two (F2) explained 79.61% of the variation present in the data, F1 explained 68.05% of the variance and 11.56% for F2. The first component (F1) separated samples predominantly on the different ingredients (50% salt, lysine, calcium lactate), while the second component (F2) separated samples predominantly by pH (5.5, 6, 6.5). Salt-reduced pork containing calcium lactate was positioned on the left and furthest away from the standard salt control. These sample were characterised by containing fewer volatile compounds overall, but by being higher in the Strecker aldehydes (2- and 3-methylbutanal) and acetoin. Salt-reduced pork treated with lower pH (5.5, 6) with calcium lactate or no added salt substitutes were inversely associated with F2. These sample mainly produced acids, phenols and lots of lipid-derived aldehydes like heptanal and nonanal. In contrast, any reduced-salt pork containing lysine alone and at the highest pH (6.5) were positively associated with F2, and these sample mainly presented alkanes, alcohols, pyrazines and pentanal, hexanal, octanal. The volatile compounds in salt-reduced pork containing lysine only or without any substitutes at lower pH (5.5, 6) were similar with control, especial only contain lysine at pH 5.5 (SL5.5). It could be that the volatile flavour compounds of pork were like alcohols, ketones, aldehydes when heated. A strong significant relationship between

compound groups were also found, such as alcohols and alkanes showed a strong positive correlation, while most of the aldehydes and ketones showed a strong negative correlation. Whereas the addition of lysine led to the formation and release of the one pyrazine identified, and only in the higher pH samples (pH 6 and 6.5), which fits with the expect. ntified, and only in the higher pH samples (pH 6 and 6.5), which fits with the expect. It is worth noting that most of the low pH samples had more volatile compounds. This is because low pH accelerates lipid oxidation and releases more flavor compounds (Parker, 2013). Acidic conditions can promote chemical reactions to generate more volatile compounds. For example, under acidic conditions esterification and hydrolysis reactions were accelerated, leading to the formation or breakdown of volatile esters or other volatile compounds (Khan *et al.*, 2021). In addition, functional groups on organic compounds can become protonated in low pH conditions. Protonation can alter the polarity and reactivity of molecules, making them more volatile (Petukh, Stefl and Alexov, 2013). This is particularly relevant for compounds containing amine groups, which can be protonated to form ammonium ions that are more volatile (Zhu, Riskowski and Torremorell, 1999).

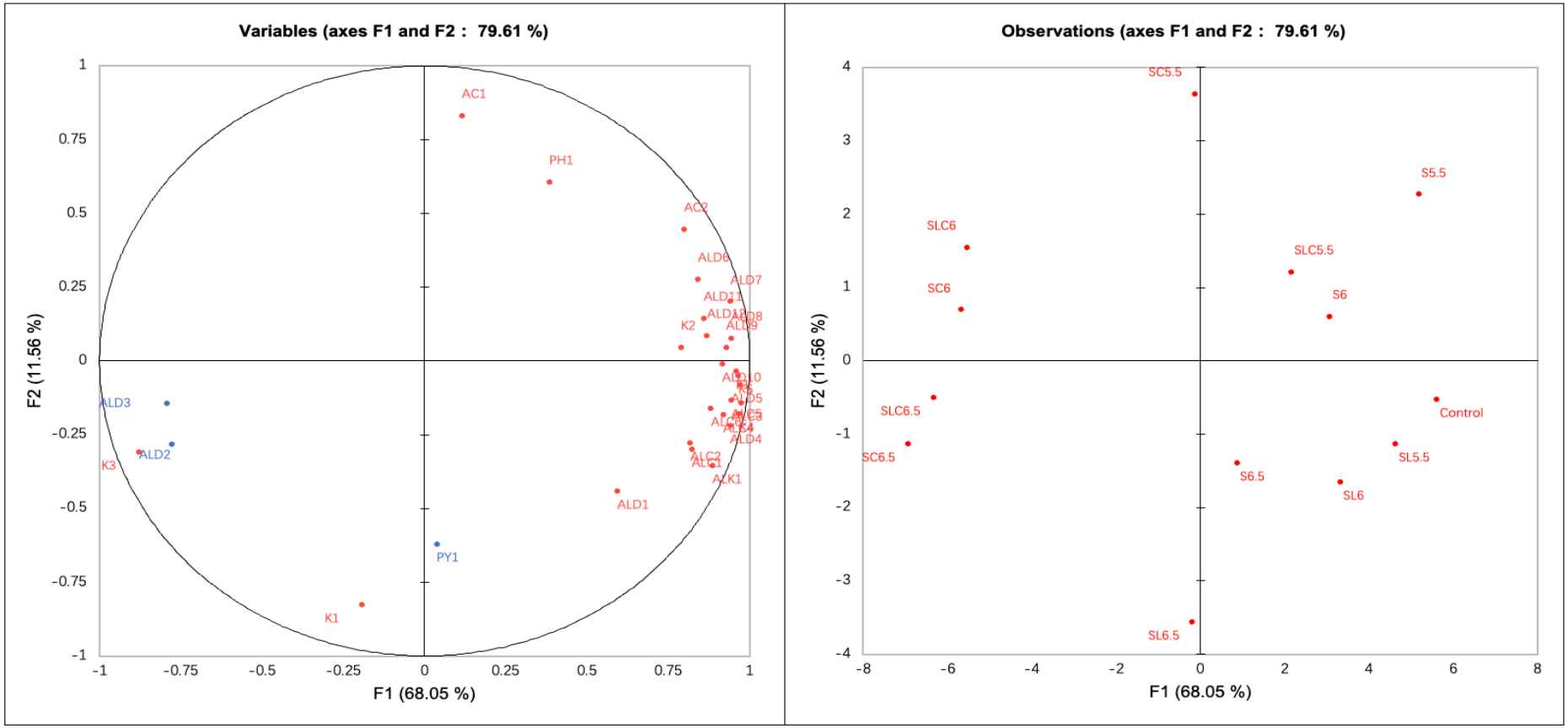

Figure 5.1. Principal component analysis of pork patties varying in salt, lysine, calcium lactate and pH. Control = 2% NaCl, pH = 5.5; S5.5 = 1% NaCl, pH = 5.5; S6 = 1% NaCl, pH 6; S6.5 = 1% NaCl, pH = 6.5; SL5.5 = 1% NaCl + 3% lysine, pH = 5.5; SL 6 1% NaCl + 3% lysine, pH = 6; SL6.5 = 1% NaCl + 3% lysine, pH = 6.5; SC5.5 = 1% NaCl + 1.5% calcium lactate, pH = 5.5; SC6 = 1% NaCl + 1.5% calcium lactate, pH = 6; SC6.5 = 1% NaCl + 1.5% calcium lactate, pH = 6.5; SLC5.5 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 5.5; SLC6 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 6; SLC6.5 = 1% NaCl + 3% lysine + 1.5% calcium lactate, pH = 6.5. AC1 = butanoic acid; AC2 = hexanoic acid; ALK1 = 2-pentyloxirane; ALC1 = 1-penten-3-ol; ALC2 = 1-pentanol; ALC3 = 1-hexanol; ALC4 = 1-heptanol; ALC5 = 1-octen-3-ol; ALC6 = 1-octanol; ALD1 = butanal; ALD2 = 2-methylbutanal; ALD3 = 3-methylbutanal; ALD4 = pentanal; ALD5 = hexanal; ALD6= 2-hexenal,(E)-; ALD7 = heptanal; ALD8 = 2-heptenal, (E)-; ALD9 = benzaldehyde; ALD10 = octanal; ALD11 = 2-octenal, (E)-; ALD12 = nonanal; F1 = 2-pentylfuran; K1 = acetol; K2 = 2,3-pentanedione; K3 = acetoin; K4 = 2-heptanone; K5 = 2,3-octanedione; PH1= 2-phemoxyethanol; PY1 = 2-methylpyrazine. Compounds in red were produced by lipid degradation, compounds in blue were produced by Maillard reaction.

5.4 Conclusion

This work analysed the changes in the physicochemical properties and volatile flavour compounds of pork patty at different levels of pH and using different salt substitutes. The results showed that increasing the pH significantly increased the moisture content post processing, thus reducing cooking loss. According to the analysis of GC-MS, only a small amount of volatile flavour compounds associated with the Maillard reaction were produced in pork patties at increased pH, with almost all other volatile compounds coming from lipid degradation. Therefore, this means that lysine is not heavily involved in the Maillard reaction in an acidic environment (5.5 – 6.5) when added to pork patty as a salt substitute without additional adjustment of pH. This provides an idea of the content of lysine to be added to different type of meat products, while subsequent experiments can further analyse the flavour compounds corresponding to the salty taste produced by lysine. There are also some limitations in this experiment, which need to be improved in future experiment. Since the experimental sample (salt-reduced pork patty) was not extracted, and minced meat was directly used for the analysis of flavour compounds, some interfering compounds existing in the product may interfere result and reduce the accuracy of measurement. Hence extraction methodd other than SPME should be explored. In addition, the flavour compounds generated due to addition of lysine and calcium lactate at pH 5.5 - 6.5 may be odour-active compounds that may be present at much lower level, that may affect the consumers' eating experience, hence further sensory tests should be conducted to verify the result.

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References

3768 Ames, J.M., 1992. The Maillard reaction. *In Biochemistry of food proteins* (pp. 99-153).
3769 Springer, Boston, MA.
3770 Aaslyng, M.D., Bejerholm, C., Ertbjerg, P., Bertram, H.C. and Andersen, H.J., 2003.
3771 Cooking loss and juiciness of pork in relation to raw meat quality and cooking
3772 procedure. *Food quality and preference*, 14(4), pp.277-288.
3773 Aaslyng, M.D. and Meinert, L., 2017. Meat flavour in pork and beef–From animal to
3774 meal. *Meat science*, 132, pp.112-117.
3775 Betts, M.J. and Russell, R.B., 2003. Amino acid properties and consequences of
3776 substitutions. *Bioinformatics for geneticists*, 317, p.289.
3777 Calkins, C.R. and Hodgen, J.M., 2007. A fresh look at meat flavor. *Meat science*, 77(1),
3778 pp.63-80.
3779 Campagnol, P.C.B., dos Santos, B.A., Morgano, M.A., Terra, N.N. and Pollonio,
3780 M.A.R., 2011. Application of lysine, taurine, disodium inosinate and disodium
3781 guanylate in fermented cooked sausages with 50% replacement of NaCl by KCl. *Meat*
3782 *science*, 87(3), pp.239-243.
3783 Deng, S., Liu, Y., Huang, F., Liu, J., Han, D., Zhang, C. and Blecker, C., 2021.
3784 Evaluation of volatile flavor compounds in bacon made by different pig breeds during
3785 storage time. *Food Chemistry*, 357, p.129765.
3786 Desmond, E. and Vasilopoulos, C., 2019. Reducing salt in meat and poultry products.
3787 In *Reducing salt in foods* (pp. 159-183). Woodhead Publishing.
3788 Dos Santos Alves, L., Lorenzo, J., Gonçalves, C., dos Santos, B., Heck, R., Cichoski,
3789 A. and Campagnol, P., 2017. Impact of lysine and liquid smoke as flavor enhancers on
3790 the quality of low-fat Bologna-type sausages with 50% replacement of NaCl by KCl.
3791 *Meat Science*, 123, pp.50-56.

3792 Grosch, W., 2001. Evaluation of the key odorants of foods by dilution experiments,
3793 aroma models and omission. *Chemical senses*, 26(5), 533-545.
3794 Guerrero, L., Gou, P. and Arnau, J., 1999. The influence of meat pH on mechanical and
3795 sensory textural properties of dry-cured ham. *Meat science*, 52(3), pp.267-273.
3796 Guo, X., Tao, S., Pan, J., Lin, X., Ji, C., Liang, H., Dong, X. and Li, S., 2020. Effects
3797 of L-Lysine on the physiochemical properties and sensory characteristics of salt-
3798 reduced reconstructed ham. *Meat science*, 166, p.108133.
3799 Hong, G. P., Park, S. H., Kim, J. Y., and Min, S. G., 2006. The effects of high pressure
3800 and various binders on the physico-chemical properties of restructured pork meat.
3801 *Asian-australasian journal of animal sciences,* 19(10), 1484-1489.
3802 Honikel, K.O., 2004. Water-holding capacity of meat. *Muscle development of livestock*
3803 *animals: Physiology, genetics and meat quality*, pp.389-400.
3804 Hu, Y., Zhang, L., Zhang, H., Wang, Y., Chen, Q. and Kong, B., 2020. Physicochemical
3805 properties and flavour profile of fermented dry sausages with a reduction of sodium
3806 chloride. *Lwt*, 124, p.109061.
3807 Inguglia, E., Zhang, Z., Tiwari, B., Kerry, J. and Burgess, C., 2017. Salt reduction
3808 strategies in processed meat products – A review. *Trends in Food Science &*
3809 *Technology*, 59, pp.70-78.
3810 Irshad, A., Sharma, B., Ahmed, S., Talukder, S., Malav, O. and Kumar, A., 2016. Effect
3811 of incorporation of calcium lactate on physico-chemical, textural, and sensory
3812 properties of restructured buffalo meat loaves. *Veterinary World*, 9(2), pp.151-159.
3813 Jayasena, D.D., Ahn, D.U., Nam, K.C. and Jo, C., 2013. Factors affecting cooked
3814 chicken meat flavour: A review. *World's Poultry Science Journal,* 69(3), pp.515-526.
3815 Khan, Z., Javed, F., Shamair, Z., Hafeez, A., Fazal, T., Aslam, A., Zimmerman, W.B.
3816 and Rehman, F., 2021. Current developments in esterification reaction: A review on

process and parameters. *Journal of Industrial and Engineering Chemistry*, *103*, pp.80-101.

Kim, J.Y., Yi, B., Lee, C., Gim, S.Y., Kim, M.J. and Lee, J., 2016. Effects of pH on the rates of lipid oxidation in oil–water system. *Applied Biological Chemistry*, *59*(2), pp.157-161.

Kubantseva, N. and Hartel, R.W., 2002. Solubility of calcium lactate in aqueous solution. *Food Reviews International,* 18(2-3), pp.135-149.

Lawrence, T., Dikeman, M., Hunt, M., Kastner, C. and Johnson, D., 2003. Effects of calcium salts on beef longissimus quality. *Meat Science*, 64(3), pp.299-308.

Lawrence, T.E., Dikeman, M.E., Hunt, M.C., Kastner, C.L. and Johnson, D.E., 2004. Effects of enhancing beef longissimus with phosphate plus salt, or calcium lactate plus non-phosphate water binders plus rosemary extract. *Meat Science*, 67(1), pp.129-137.

Madruga, M.S., 1997. Studies on some precursors involved in meat flavour formation. *Food Science and Technology*, *17*, pp.148-153.

Martins, S.I., Jongen, W.M. and Van Boekel, M.A., 2000. A review of Maillard reaction in food and implications to kinetic modelling. *Trends in food science & technology*, *11*(9-10), pp.364-373.

Mottram, D.S., 1998. Flavour formation in meat and meat products: a review. *Food chemistry*, *62*(4), pp.415-424.

Parker, J.K., 2013. The kinetics of thermal generation of flavour. *Journal of the Science of Food and Agriculture*, *93*(2), pp.197-208.

Petit, G., Jury, V., de Lamballerie, M., Duranton, F., Pottier, L. and Martin, J.L., 2019. Salt intake from processed meat products: Benefits, risks and evolving practices. *Comprehensive Reviews in Food Science and Food Safety*, 18(5), pp.1453-1473.

Petukh, M., Stefl, S. and Alexov, E., 2013. The role of protonation states in ligand-receptor recognition and binding. *Current pharmaceutical design*, *19*(23), pp.4182-4190.

Richards, M.P., 2013. Redox reactions of myoglobin. *Antioxidants & redox signaling,* 18(17), pp.2342-2351.

Robbins, K., Jensen, J., Ryan, K. J., Homco-Ryan, C., McKeith, F. K., & Brewer, M. S., 2003. Consumer attitudes towards beef and acceptability of enhanced beef. Meat Science, 65(2), 721-729.

Ruedt, C., Gibis, M. and Weiss, J., 2022. Effect of varying salt concentration on iridescence in precooked pork meat. *European Food Research and Technology*, pp.1-12.

Rucker, A.J., Rudemiller, N.P. and Crowley, S.D., 2018. Salt, hypertension, and immunity. *Annual review of physiology*, *80*, p.283.

Shahidi, F. and Zhong, Y., 2010. Lipid oxidation and improving the oxidative stability. *Chemical society reviews*, *39*(11), pp.4067-4079.

Sun, A., Wu, W., Soladoye, O.P., Aluko, R.E., Bak, K.H., Fu, Y. and Zhang, Y., 2022. Maillard reaction of food-derived peptides as a potential route to generate meat flavor compounds: A review. *Food Research International*, 151, p.110823.

Tichivangana, J.Z. and Morrissey, P.A., 1985. The influence of pH on lipid oxidation in cooked meats from several species. *Irish Journal of Food Science and Technology,* pp.99-106.

Tobin, B.D., O'Sullivan, M.G., Hamill, R.M. and Kerry, J.P., 2013. The impact of salt and fat level variation on the physiochemical properties and sensory quality of pork breakfast sausages. *Meat Science*, 93(2), pp.145-152.

Van Ba, H., Hwang, I., Jeong, D. and Touseef, A., 2012. Principle of meat aroma flavors and future prospect. *Latest research into quality control*, *2*, pp.145-176.

Van Ba, H., Amna, T. and Hwang, I., 2013. Significant influence of particular unsaturated fatty acids and pH on the volatile compounds in meat-like model systems. Meat Science, 94(4), pp.480-488.

Van Boekel, M. A. J. S., 2006. Formation of flavour compounds in the Maillard reaction. *Biotechnology advances,* 24(2), 230-233.

Vidal, V.A., Santana, J.B., Paglarini, C.S., da Silva, M.A., Freitas, M.Q., Esmerino, E.A., Cruz, A.G. and Pollonio, M.A., 2020. Adding lysine and yeast extract improves sensory properties of low sodium salted meat. *Meat Science*, 159, p.107911.

Wen, R., Hu, Y., Zhang, L., Wang, Y., Chen, Q. and Kong, B., 2019. Effect of NaCl substitutes on lipid and protein oxidation and flavor development of Harbin dry sausage. *Meat Science*, 156, pp.33-43

World Health Organisation, 2020. Salt Reduction, Fact Sheets. Retrieved 3 November 2020 from https://www.who.int/news-room/fact-sheets/detail/salt-reduction

Xie, J., Sun, B., Zheng, F. and Wang, S., 2008. Volatile flavor constituents in roasted pork of Mini-pig. *Food Chemistry,* 109(3), pp.506-514.

Yang, X., Sebranek, J.G., Luo, X., Zhang, W., Zhang, M., Xu, B., Zhang, Y. and Liang, R., 2021. Effects of calcium salts on the physicochemical quality of cured beef sausages during manufacturing and storage: A potential calcium application for sausages with alginate casings. *Foods*, 10(11), p.2783.

Zhou, C., Li, J., and Tan, S., 2014. Effect of L-lysine on the physicochemical properties of pork sausage. Food Science and Biotechnology, 23(3), 775-780.

Zhu, J., Riskowski, G.L. and Torremorell, M., 1999. Volatile fatty acids as odor indicators in swine manure—acritical review. *Transactions of the ASAE*, *42*(1), pp.175-182.

Chapter 6 General discussion and conclusion

As explained in the previous chapter (Chapter 1), salt has important roles in meat products, such as improving texture, extending shelf life and contributing to salty taste (Liem Miremadi and Keast, 2011; Hutton, 2002). However, excessive salt intake will increase the risk of high blood pressure and cardiovascular disease (Aaron and Sanders, 2013; He and MacGregor, 2010). Therefore, there are some widely used salt reduction strategies, including changing the physical form of salt, using flavor enhancers and replacing sodium chloride with potassium chloride (Campagnol, Dos Santos and Rodriguez-Pollonio, 2017; Moncada *et al.*, 2015; Doyle and Glass, 2010). However, each of these strategies has its limitations. Salt reduction by changing form only and can changes salt taste intensity over time in solid food (Kilcast and Den Ridder, 2007); KCl leads to salty taste, but also bring off- taste like bitterness (Wu *et al.*, 2014); use of flavour enhancers is usually achieved through ingredients high in umami taste (e.g. soy sauce) (Maluly *et al.*, 2017), which questions whether salty taste is really enhanced or whether it is the taste quality that has changed. With this in mind, in order to better select a more suitable new salt substitute, one of the first aspects of this thesis was to address the role of umami, and later an amino acid (lysine) in salt-taste interactions.

In Chapter 2, five aqueous solutions presenting the 5 basic tastes at equi-intense levels, were used to evaluate the relationship between umami and other tastes, by scoring their specific and overall taste intensity using the general labeled magnitude scale. The results concluded that the addition of umami taste did not enhance or suppress any other taste; but the addition of sweet, salty, sour and bitter did significantly suppress umami

taste. Although this experiment filled the gap in the literature concerning the relationship between umami and other taste sensations, the experimental results rejected the hypothesis that umami could be used as a salt substitute. Although there are many studies claiming that umami taste can increase salty taste in food (Maluly *et al.*, 2017; Dos Santos Alves *et al.*, 2014; Yamaguchi & Takahashi, 1984), the results could be conflicted due to the difference in methodology, tastant concentration or sensory group. Trained sensory panelists, such as the assessors in this study, "dissect" a product into its component attributes for rating, whereas consumers "synthesise" the information from the foods they are tasting (Ares and Varela, 2017). So, where a trained panel might be better at discriminating between salty and umami taste (and therefore not conclude that umami enhances salty taste), consumers may be more inclined to notice the overall increase in salty or savoury taste where umami and salty are used together.

Since it was confirmed in Chapter 2 that umami could not increase saltiness, new salt substitutes were further explored. Lysine and calcium lactate were considered as viable options. Previous studies have used lysine as a flavour enhancer, and it could effectively improve the physical-chemical properties of meat products like high yield and cover the off-taste by KCl (Guo *et al.*, 2020; Dos Santos Alves *et al.*, 2014; Campagnol *et al.*, 2012); whereas calcium ions are perceived with a weak salty taste, and lactic acid can inhibit the growth of bacteria (Kilcast and Den Ridder, 2007; Shelef and Potluri, 1995). Therefore, the combination of lysine and calcium lactate could offer great potential to replace salt in terms of salty taste and ionic function, and at the same time it may even

effectively prevent the reduction in shelf life usually caused by salt reduction. In order to find the taste of the potential salt replacers, a simple aqueous solutions system was used. Chapter 3 used a trained sensory panel with same method as Chapter 2 to assess the replacers in an aqueous system. The result indicated that 1% w/v lysine produced a very weak saltiness, and 0.75 % w/v calcium lactate alone did not offer saltiness, while the combination of 0.75% w/v calcium lactate and 1% w/v lysine or 1% lysine alone could replace 50% of salt in solution as they offered comparable saltiness with the control full salt sample (0.5%), although bitterness was perceived by the sensory panel. Therefore, lysine can be considered as an effective salt substitute.

Although Calcium lactate did not confer any salty taste, it can offer the benefit of antimicrobial function to address key issue of shelf life for salt reduced food products along with function of calcium fortification. Hence, the combination of lysine and calcium lactate were considered as great potential for developing salt reduction strategy for food production, hence their effects were further validated in a real food matrix. In chapter 4, varied levels of lysine (3% and 6% w/w) and calcium lactate (1.5% and 3% w/w) were added into a 50% salt-reduced pork patty, and physical-chemical properties, sensory and microbiological tests were carried out to determine whether they can be effectively used in meat products. The results showed that both calcium lactate and lysine improved texture and colour but decreased water holding capacity of a salt-reduced pork patty. Additionally, lysine increased the yield, and calcium lactate improved shelf-life. Most importantly, the combination of 1.5% w/w calcium lactate and 3% w/w lysine could compensate the loss in saltiness caused by 50% salt reduction

in pork patty. This provides a good strategy for the meat processing industry to reduce salt content while maintaining the quality of the final product. However, the cost needs to be considered, as lysine and calcium lactate are more expensive than salt. Research reported that consumers were willing to pay extra for the health benefit of salt reduced products, hence it would be worthwhile to perform market research to confirm this in the future.

The Maillard reaction is one of the most important routes forming aroma volatiles in cooked meat (Van Boekel, 2006). As one of the basic active amino acids, lysine could participate in the Maillard reaction during the heating process, resulting in a decrease in lysine content and affecting its function of compensating salty taste in salt reduced products. In addition, the Maillard reaction is very pH dependent (Calkins and Hodgen, 2007). Therefore, Chapter 5 explored the effects of normal meat pH levels (5.5, 6, 6.5) and substrates (lysine and calcium lactate) on the physico-chemical properties of salt-reduced pork patty, especially the volatile flavor compounds. The results showed that increasing the pH significantly decreased cooking loss, thus increasing the moisture content. Most volatile compounds within the patties were attributed to lipid degradation, whereas there were very few Maillard reaction-derived volatile flavour compounds detected after heating, and they were only in relatively small amounts within the observed meat pH range. Therefore, this means that lysine was not heavily involved in the Maillard reaction in the meat products which typically have a weak acidic environment (5.5 - 6.5). In addition, the patties had a small surface area to volume ratio, meaning that very little of the patty would have reached temperature of over 100 °C at

low water activity that is required for greater Maillard reaction. Combined with the sensory results from the previous chapters (Chapter 4), thus, it is feasible to use lysine as a salt substitute in meat products without substantially altering the flavor profile of the food.

In conclusion, lysine and calcium lactate could effectively compensate the saltiness loss in 50% salt reduction pork patty as salt substitutes. Additionally, it also provides important directions for future research. Of course, this study also has many limitations. Although the experiment in Chapter 2 showed that umami had no effect on other tastes, the results are only limited to a specific concentration range and trained panelists. Hence, future research should further explore the relationship between umami and saltiness, and more complex food models should also be used, such as real food systems, different concentrations, etc. Chapter 3 found that 1% w/v lysine had weak salty taste, but the mechanism of lysine eliciting saltiness is not clear, this deserves a more in-depth study. Therefore, the future work needs to understand the mode of action of lysine in terms of salty taste. For example, lysine could produce saltiness through ENaC, or there may be another specific channel or multi-pathways involved for lysine to stimulate the brain to release salty signals. In Chapter 4, the combination of 1.5% w/w calcium lactate and 3% w/w lysine could compensate the loss in saltiness caused by 50% salt reduction in pork patty and achieve comparable or better shelf life. However, this combination is verified in pork patties, further validation in other food matrices, such as bread, etc. should be conducted before application. In addition, the potentially positive effect os calcium fortification using calcium lactate needs further analysis. Overall, the

combination of lysine and calcium lactate offers a viable option for meat industry to develop salt reduced meat products, while validation of the salt substitution effect should be conducted before applying to other foods. At the same time, the content of lysine and calcium should also be optimised to confirm if they can provide health benefits to consumers. Although the experiment in Chapter 5 confirmed that lysine hardly participates the Maillard reaction when heated in a typical meat product environment (pH = 5.5 ~ 6.5), the surface area and thickness of salt-reduced pork patties may also affect the extent of Maillard reaction due to high temperature/low moisture condition (the optimum reaction environment). So, future experiments can further refine the experiments by considering the product dimension in this aspect. For example, the flavor compounds of different parts (surface, centre) can also be analyzed separately considering the difference in the degree of Maillard reaction. Since Maillard reaction only happens on the surface of the meat products, it would be useful to investigate the effect of ratio of surface area to mass on the flavour formation, because meat products differ in size and shape. The involvement in Maillard reaction could directly affect the efficiency of lysine imparting its saltiness. In addition, it is useful to measure the content of lysine in pork patty before and after heating in order to further confirm the extent of lysine participating the Maillard reaction. In the follow-up experiments, the flavor compounds corresponding to the salty taste produced by lysine can be further studied and analyzed.

Reference

4022 Aaron, K. and Sanders, P., 2013. Role of Dietary Salt and Potassium Intake in
4023 Cardiovascular Health and Disease: A Review of the Evidence. *Mayo Clinic*
4024 *Proceedings*, 88(9), pp.987-995.
4025 Ares, G. and Varela, P., 2017. Trained vs. consumer panels for analytical testing:
4026 Fueling a long lasting debate in the field. *Food Quality and Preference*, 61, 79-86.
4027 Calkins, C.R. and Hodgen, J.M., 2007. A fresh look at meat flavor. *Meat science*, 77(1),
4028 pp.63-80.
4029 Campagnol, P., Dos Santos, B., Terra, N. and Pollonio, M., 2012. Lysine, disodium
4030 guanylate and disodium inosinate as flavor enhancers in low-sodium fermented
4031 sausages. *Meat Science*, 91(3), pp.334-338.
4032 Campagnol, P.C.B., dos Santos, B.A. and Rodriguez-Pollonio, M.A., 2017. Strategies
4033 to reduce the salt content in fermented meat products. *Strategies for Obtaining*
4034 *Healthier Foods*, p.291.
4035 Dos Santos, B., Campagnol, P., Morgano, M. and Pollonio, M., 2014. Monosodium
4036 glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the
4037 sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl
4038 with KCl. *Meat Science,* 96(1), 509-513.
4039 Doyle, M.E. and Glass, K.A., 2010. Sodium reduction and its effect on food safety,
4040 food quality, and human health. *Comprehensive reviews in food science and food safety,*
4041 9(1), pp.44-56.

4042 Guo, X., Tao, S., Pan, J., Lin, X., Ji, C., Liang, H., Dong, X. and Li, S., 2020. Effects
4043 of L-Lysine on the physiochemical properties and sensory characteristics of salt-
4044 reduced reconstructed ham. *Meat science*, 166, p.108133.
4045 He, F.J. and MacGregor, G.A., 2010. Reducing population salt intake worldwide: from
4046 evidence to implementation. *Progress in cardiovascular diseases*, 52(5), pp.363-382.
4047 Hutton, T. 2002. Sodium: Technological functions of salt in the manufacturing of food
4048 and drink products. *British Food Journal*, 104(2):126-152.
4049 Kilcast, D. and Den Ridder, C., 2007. Sensory issues in reducing salt in food products.
4050 In *Reducing salt in foods* (pp. 201-220). Woodhead publishing.
4051 Liem, D., Miremadi, F. and Keast, R., 2011. Reducing Sodium in Foods: The Effect on
4052 Flavor. *Nutrients*, 3(6), pp.694-711.
4053 Maluly, H.D., Arisseto-Bragotto, A.P. and Reyes, F.G., 2017. Monosodium glutamate
4054 as a tool to reduce sodium in foodstuffs: Technological and safety aspects. *Food science*
4055 *& nutrition*, 5(6), 1039-1048.
4056 Moncada, M., Astete, C., Sabliov, C., Olson, D., Boeneke, C., and Aryana, K. J., 2015.
4057 Nano spray-dried sodium chloride and its effects on the microbiological and sensory
4058 characteristics of surface-salted cheese crackers. *Journal of dairy science*, 98(9), 5946-
4059 5954.
4060 Shelef, L.A. and Potluri, V., 1995. Behaviour of foodborne pathogens in cooked liver
4061 sausage containing lactates. *Food Microbiology*, 12, pp.221-227.
4062 Van Boekel, M. A. J. S., 2006. Formation of flavour compounds in the Maillard reaction.
4063 *Biotechnology advances,* 24(2), 230-233.

Wu, H., Zhang, Y., Long, M., Tang, J., Yu, X., Wang, J., and Zhang, J., 2014. Proteolysis and sensory properties of dry-cured bacon as affected by the partial substitution of sodium chloride with potassium chloride. Meat science, 96(3), 1325-1331.

Yamaguchi, S., and Takahashi, C., 1984. Interactions of monosodium glutamate and sodium chloride on saltiness and palatability of clear soup. *Journal of Food Science,* 49(1), 82– 85.

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Statement

The COVID-19 pandemic has had a profound impact on various aspects of academic research, including the pursuit of a PhD. As a PhD student, my own research and progress have been significantly affected by the pandemic. The pandemic has caused widespread disruptions in research activities. Laboratory access, fieldwork, and data collection have been severely limited or halted altogether due to lockdowns, travel restrictions, and physical distancing measures. This has led to delays in conducting experiments, gathering essential data, and executing planned research methodologies. What is more, libraries, archives, and research facilities have been closed or limited in their operations, making it challenging to access critical resources and references necessary for comprehensive literature reviews and data analysis. This limited access to resources has hampered the depth and breadth of research that could be conducted during this time. I was in China during the lockdown, and the restrictions were more sever then in the UK.

4104 **Appendix**

4105 Supplementary table 1. Assessors performance of perceived intensity (antilogged
4106 values) of overall taste, sweet, salty, sour, bitter and umami where MSG was used as
4107 the umami tastant without sodium balance.

4108 Table 1a. Assessor mean scores with significance of assessor differences for each
4109 attribute (showing different use of scale).

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	43.2	14	8.3	6.7	18.2	11.1
Assessor 2	42.6	12.7	11.5	11.8	13.3	11
Assessor 3	42.7	12.5	13	1	9.7	6.7
Assessor 4	47.4	14.3	17.1	7	17.7	13
Assessor 5	37.7	11.2	6.8	10.9	7	15.1
Assessor 6	36.3	9.1	7.7	7.3	13.2	14.9
Assessor 7	45.5	14.4	11.4	14.9	12.3	13
Assessor 8	41.5	12.6	8.6	7	12.3	14.1
Assessor 9	49.2	12.9	19.7	8.5	16.1	5.2
HSD	13.8	8.4	11	9.5	14.5	10.8
p - value	0.0758	0.592	0.0029	0.002	0.2579	0.0388

4110 Table 1b. F values for Assessor Discrimination

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	19.9	2.5	177.4	20.6	96.4	10.5
Assessor 2	10.9	332.2	3.7	598.4	22.6	4.1
Assessor 3	1.7	14.1	4.8	NA	8.4	4.6
Assessor 4	2	27.2	11.1	8.3	7.6	4.8
Assessor 5	0.6	24.9	26.5	46.4	308.1	9.1
Assessor 6	9	25.1	35.4	34.9	53.1	29.4
Assessor 7	3.8	14.8	3.4	6.8	2.5	9.1
Assessor 8	5.8	1109.8	4.2	295.2	37.7	377.8
Assessor 9	5	6.6	31.3	12.3	6.4	13.6

4111 Table 1c. p-values for Assessor Discrimination

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	<.0001	0.0837	<.0001	<.0001	<.0001	0.0005
Assessor 2	0.0004	<.0001	0.0271	<.0001	<.0001	0.0197
Assessor 3	0.1989	0.0001	0.0114	NA	0.0013	0.0126
Assessor 4	0.1543	<.0001	0.0004	0.0013	0.0019	0.011
Assessor 5	0.7906	<.0001	<.0001	<.0001	<.0001	0.0009
Assessor 6	0.001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 7	0.0252	0.0001	0.0346	0.0031	0.0815	0.0009

Assessor 8	0.0056	<.0001	0.0171	<.0001	<.0001	<.0001
Assessor 9	0.0099	0.0033	<.0001	0.0003	0.0038	0.0002

Table 1d. Correlations of each assessor's mean scores with panel average

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.72	0.84	0.87	0.99	0.95	0.97
Assessor 2	0.62	0.98	0.95	0.99	0.96	0.87
Assessor 3	0.78	0.94	0.72	0	0.92	0.78
Assessor 4	0.87	0.99	0.96	0.9	0.85	0.93
Assessor 5	0.01	0.99	0.87	0.98	0.97	0.87
Assessor 6	0.77	0.95	0.95	0.99	0.98	0.92
Assessor 7	-0.1	0.99	0.83	0.91	0.37	0.79
Assessor 8	0.82	0.99	0.97	0.97	0.91	0.87
Assessor 9	0.84	0.97	0.99	0.96	0.99	0.7

Table 1e. Assessor's repeatability standard deviation

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	4.75	13.33	1.39	3.41	4.08	4.17
Assessor 2	4.01	1.47	8.7	1.07	6.36	7.73
Assessor 3	9.87	7.5	9.82	NA	6.68	7.03
Assessor 4	9	5.43	7.66	6.19	9.98	8.34
Assessor 5	7.83	4.63	3.11	3.46	1.01	7.62
Assessor 6	6.34	3.98	2.69	2.53	4.55	3.9
Assessor 7	10.39	7.95	7.99	9.36	8.76	7.38
Assessor 8	4.97	0.77	7.7	1.02	4.24	1.15
Assessor 9	8.27	10.83	6.05	6.02	11.85	3.12

Table 1f. Test of each assessor's repeatability (replicate variability) against the Panel average repeatability (F value)

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.4	3.3	0	0.5	0.3	0.5
Assessor 2	0.3	0	1.7	0.1	0.8	1.6
Assessor 3	1.7	1	2.1	NA	0.9	1.3
Assessor 4	1.4	0.5	1.3	1.8	2	1.9
Assessor 5	1.1	0.4	0.2	0.6	0	1.6
Assessor 6	0.7	0.3	0.2	0.3	0.4	0.4
Assessor 7	1.9	1.2	1.4	4.1	1.5	1.5
Assessor 8	0.4	0	1.3	0	0.4	0
Assessor 9	1.2	2.2	0.8	1.7	2.8	0.3

Table 1g. Test of each assessor's repeatability (replicate variability) against the Panel average repeatability (p - value)

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.9475	0.0011	1	0.8589	0.972	0.9074
Assessor 2	0.9844	1	0.1014	1	0.6339	0.1173

Assessor 3	0.0963	0.4126	0.0305	NA	0.5584	0.2277
Assessor 4	0.1918	0.8511	0.2476	0.0765	0.0476	0.0599
Assessor 5	0.3994	0.9436	0.9947	0.8465	1	0.1315
Assessor 6	0.7249	0.9808	0.9984	0.9801	0.9398	0.9397
Assessor 7	0.0598	0.3179	0.1921	0.0001	0.1495	0.1662
Assessor 8	0.9293	1	0.241	1	0.9628	1
Assessor 9	0.3118	0.0259	0.6251	0.0986	0.0052	0.9878

4118 Table 1h. F-values for Assessor contribution to the interaction

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	5	3.7	2.1	0.5	7.7	0.5
Assessor 2	2.1	0.8	0.7	4.7	2.3	2
Assessor 3	1.3	2	5.6	15.4	2.1	3.1
Assessor 4	0.8	0.6	2.3	3.2	4.8	1.5
Assessor 5	2.3	0.2	2	2.8	1.4	4.5
Assessor 6	3.5	1.5	0.9	0.6	2.4	2.4
Assessor 7	10.3	1.2	2.5	6.6	11.6	5.7
Assessor 8	0.9	0.4	0.5	1	2.7	4
Assessor 9	2.7	1.1	6.6	2.2	1.2	3.7
Interaction F	3.2	1.3	2.6	4.1	4	3.1

4119 Table 1i. p-values for Assessor contribution to the interaction

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	<.0001	0.0009	0.0403	0.8309	<.0001	0.8603
Assessor 2	0.0441	0.6218	0.6571	0.0001	0.0295	0.0588
Assessor 3	0.2456	0.0496	<.0001	<.0001	0.0469	0.0035
Assessor 4	0.5884	0.8056	0.0275	0.003	0.0001	0.167
Assessor 5	0.0266	0.9869	0.0562	0.0081	0.1941	0.0001
Assessor 6	0.0016	0.1762	0.5513	0.7636	0.02	0.0204
Assessor 7	<.0001	0.3159	0.015	<.0001	<.0001	<.0001
Assessor 8	0.5062	0.9384	0.8675	0.4363	0.0117	0.0004
Assessor 9	0.0096	0.3628	<.0001	0.0372	0.3121	0.0008
Interaction p-value	<.0001	0.1404	<.0001	<.0001	<.0001	<.0001

4120 *NA means not applicable.

Supplementary Table 2. Assessor performance of perceived intensity (antilogged values) of overall taste, sweet, salty, sour, bitter and umami where MSG was used as the umami tastant with sodium balance.

Table 2a. Assessor mean scores with significance of assessor differences for each attribute (showing different use of scale).

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	43.3	10.7	7.4	7.1	18.5	9.4
Assessor 2	46.2	14.2	7.1	9.9	4.1	23.5
Assessor 3	49.1	14.8	22.3	12.6	16.2	15.4
Assessor 4	46.1	10.4	17	1.9	8.6	6.1
Assessor 5	43.9	15.5	15.6	6.9	11.6	14.3
Assessor 6	54.7	14.7	11.1	7.2	9.6	14.4
Assessor 7	25.9	9.1	6	7	5.7	9.3
Assessor 8	34.7	9.8	8	6.8	12.9	10.8
Assessor 9	52.3	16.8	17.7	16.7	10.8	15.2
Assessor 10	41.1	14.3	11	7.2	11.2	10.5
Assessor 11	53.6	15	19	11.1	18.2	6.6
HSD	15.1	8.7	11.9	10.9	15.4	11.5
p - value	<.0001	0.0246	<.0001	0.003	0.0251	0.0001

Table 2b. F values for Assessor Discrimination

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	3.4	40.2	28.8	4	18.1	52.6
Assessor 2	4.6	15	2.2	11.2	1.7	3.2
Assessor 3	2.4	25.2	11.8	27.3	6.2	5.7
Assessor 4	1	16.9	10.6	17.5	5.3	3.1
Assessor 5	3.6	36.4	2.1	22.1	5.4	3.9
Assessor 6	NA	NA	NA	NA	NA	NA
Assessor 7	10.3	33.1	9.1	6.4	15.4	2
Assessor 8	6.4	40.8	12	2.3	29.8	11.7
Assessor 9	2.4	9.7	10.4	6.8	4.2	3.4
Assessor 10	7.4	39.4	6	196.7	33.8	4.3
Assessor 11	5.3	19.4	56.3	23.6	69	1

Table 2c. p-values for Assessor Discrimination

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.0346	<.0001	<.0001	0.02	<.0001	<.0001
Assessor 2	0.0124	0.0001	0.1176	0.0004	0.2158	0.0409
Assessor 3	0.0911	<.0001	0.0003	<.0001	0.0043	0.0059
Assessor 4	0.505	0.0001	0.0005	0.0001	0.0078	0.0472
Assessor 5	0.0282	<.0001	0.1311	<.0001	0.0071	0.0219

Assessor 6	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 7	0.0005	<.0001	0.0009	0.0038	0.0001	0.1505
Assessor 8	0.0038	<.0001	0.0003	0.1019	<.0001	0.0003
Assessor 9	0.09	0.0007	0.0005	0.0031	0.0182	0.0337
Assessor 10	0.0022	<.0001	0.0048	<.0001	<.0001	0.0158
Assessor 11	0.0076	<.0001	<.0001	<.0001	<.0001	0.4941

Table 2d. Correlations of each assessor's mean scores with panel average

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.53	0.97	0.92	0.97	0.98	0.88
Assessor 2	0.53	0.97	0.99	0.77	0.67	0.93
Assessor 3	0.54	0.99	0.55	0.93	0.88	0.71
Assessor 4	0.74	0.94	0.97	0.96	0.99	0.85
Assessor 5	0.75	1	0.91	0.96	0.97	0.97
Assessor 6	0.83	0.92	0.74	0.88	0.77	0.72
Assessor 7	0.67	1	0.8	1	0.94	0.83
Assessor 8	0.14	0.98	0.94	0.87	0.99	0.93
Assessor 9	0.78	0.98	0.94	0.95	0.31	0.89
Assessor 10	0.95	0.99	0.94	0.96	0.95	0.89
Assessor 11	0.6	0.99	0.93	0.97	0.97	0.73

Table 2e. Assessor's repeatability standard deviation

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	10.04	3.23	3.02	8.74	9.07	2.27
Assessor 2	11.04	7.45	7.79	6.45	4.23	14.38
Assessor 3	7.81	6.31	6.42	5.49	12.24	9.32
Assessor 4	8.06	5.61	6.94	0.58	7.3	7.01
Assessor 5	6.45	4.8	11.48	3.46	7.39	9.75
Assessor 6	NA	NA	NA	NA	NA	NA
Assessor 7	4.5	3.25	4.09	5.7	2.95	7.83
Assessor 8	6.07	3.14	4.22	7.31	5.21	4.8
Assessor 9	12.21	10.83	6.31	8.71	5.91	10.2
Assessor 10	5.09	4.14	6.42	1.21	4.01	8
Assessor 11	7.78	7.49	4.25	5.66	4.3	11.09

Table 2f. Test of each assessor's repeatability (replicate variability) against the Panel average repeatability (F value)

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	1.5	0.3	0.2	2.1	1.8	0.1
Assessor 2	1.8	1.5	1.4	1.2	0.4	2.5
Assessor 3	0.9	1.1	1	0.8	3.2	1.1
Assessor 4	1	0.8	1.1	0	1.2	0.6
Assessor 5	0.6	0.6	3.1	0.3	1.2	1.2
Assessor 6	NA	NA	NA	NA	NA	NA

Assessor 7	0.3	0.3	0.4	0.9	0.2	0.7
Assessor 8	0.5	0.3	0.4	1.5	0.6	0.3
Assessor 9	2.2	3.2	0.9	2.1	0.8	1.3
Assessor 10	0.4	0.5	1	0	0.3	0.8
Assessor 11	0.9	1.5	0.4	0.9	0.4	1.5

4132 Table 2g. Test of each assessor's repeatability (replicate variability) against the Panel
4133 average repeatability (p-vaule)

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.1597	0.9842	0.9945	0.0272	0.075	1
Assessor 2	0.0728	0.1524	0.1784	0.3205	0.9503	0.0093
Assessor 3	0.5421	0.3908	0.4734	0.5842	0.0012	0.399
Assessor 4	0.4915	0.5848	0.3442	1	0.3328	0.8097
Assessor 5	0.803	0.793	0.0018	0.9693	0.3144	0.3261
Assessor 6	NA	NA	NA	NA	NA	NA
Assessor 7	0.9804	0.9834	0.9469	0.5239	0.9968	0.6774
Assessor 8	0.8581	0.9872	0.9341	0.1489	0.8226	0.984
Assessor 9	0.0247	0.0015	0.5029	0.0286	0.6719	0.2567
Assessor 10	0.9529	0.9107	0.4738	1	0.9654	0.6461
Assessor 11	0.5485	0.1461	0.9313	0.5366	0.9439	0.1495

4134 Table 2h. F-values for Assessor contribution to the interaction

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	4	2	1.2	0.5	9.2	0.8
Assessor 2	6.7	1.7	0.7	5.9	6.6	2.3
Assessor 3	1.9	1.2	9.2	6.7	6.1	3.3
Assessor 4	0.7	2.3	1.6	6.2	0.3	0.9
Assessor 5	1.1	0.4	1.3	0.7	0.7	0.5
Assessor 6	3.6	2.7	3	1	2.2	2.5
Assessor 7	1.9	1.7	2.5	0.2	2.3	1
Assessor 8	4.8	1.6	0.8	2.3	2	0.5
Assessor 9	2.6	2.7	1.5	2.2	9.3	1.1
Assessor 10	0.4	0.5	0.8	0.7	1.4	0.7
Assessor 11	3.3	2	8.2	4.5	6.8	1.4
Interaction F	2.8	1.7	2.8	2.8	4.3	1.4

4135 Table 2i. p-values for Assessor contribution to the interaction

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.0003	0.0545	0.3329	0.8271	<.0001	0.6098
Assessor 2	<.0001	0.1161	0.7043	<.0001	<.0001	0.0253
Assessor 3	0.0641	0.3242	<.0001	<.0001	<.0001	0.0018
Assessor 4	0.6544	0.0231	0.1354	<.0001	0.9568	0.5509
Assessor 5	0.3855	0.9201	0.243	0.6554	0.7324	0.872
Assessor 6	0.0009	0.0098	0.0039	0.4662	0.033	0.0161

Assessor 7	0.0739	0.118	0.0172	0.9918	0.0244	0.4755
Assessor 8	0.0001	0.1327	0.6369	0.0255	0.0496	0.8465
Assessor 9	0.0108	0.0102	0.168	0.0307	<.0001	0.3996
Assessor 10	0.9045	0.8372	0.6388	0.7155	0.1955	0.6528
Assessor 11	0.002	0.0546	<.0001	0.0001	<.0001	0.1915
Interaction p-value	<.0001	0.0051	<.0001	<.0001	<.0001	0.0663

*NA means not applicable.

Supplementary Table 3. Assessor performance of perceived intensity (antilogged values) of overall taste, sweet, salty, sour, bitter and umami where MPG was used as the umami tastant.

Table 3a. Assessor mean scores with significance of assessor differences for each attribute (showing different use of scale).

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	29.8	7.5	5.2	6.6	11.3	13.4
Assessor 2	39.4	13	12.7	7.4	7.2	16.1
Assessor 3	43.7	9.3	9.5	14.1	14	9.3
Assessor 4	31.6	7.5	8	5.5	8.4	4.4
Assessor 5	31.4	8.5	8.4	7.5	10.8	10.4
Assessor 6	26.6	8.7	8.8	5.9	8.2	7.1
Assessor 7	18.9	4.4	4.5	6.7	6.5	7.1
Assessor 8	29	7.6	6.5	5.2	9	12.2
Assessor 9	30.6	7.4	8.4	11.7	4.7	10.7
Assessor 10	31.6	9.2	6.5	7.1	8.8	14
Assessor 11	50.2	7.5	15.2	15.1	20.3	10.4
Assessor 12	37	12.3	9.7	8.1	7.6	12.4
HSD	14.8	8.2	8.7	9.8	12.2	10.8
p - value	<.0001	0.1336	0.0201	0.0058	0.0366	0.0157

Table 3b. F values for Assessor Discrimination

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	2	49	15.7	21.6	16.2	3.9
Assessor 2	NA	NA	NA	NA	NA	NA
Assessor 3	4.2	10.8	6.2	141.9	5.6	107.6
Assessor 4	0.5	9.4	8.4	14.9	6.7	10.3
Assessor 5	16.2	21.9	10.7	31.1	23.3	8.3
Assessor 6	0.3	8.3	14.6	7.6	0.8	2.3
Assessor 7	NA	NA	NA	NA	NA	NA
Assessor 8	17.5	65.2	11.1	51.7	49.3	12.4
Assessor 9	NA	NA	NA	NA	NA	NA
Assessor 10	14.4	46	458.6	35.6	74.2	18.8
Assessor 11	NA	NA	NA	NA	NA	NA
Assessor 12	3.4	185.1	4.2	2.2	1.8	4.2

Table 3c. p-values for Assessor Discrimination

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.1455	<.0001	0.0001	<.0001	0.0001	0.0217
Assessor 2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 3	0.0178	0.0004	0.0043	<.0001	0.0063	<.0001

Assessor 4	0.8595	0.0008	0.0013	0.0001	0.0032	0.0006
Assessor 5	0.0001	<.0001	0.0005	<.0001	<.0001	0.0014
Assessor 6	0.9382	0.0014	0.0001	0.002	0.6009	0.1055
Assessor 7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 8	0.0001	<.0001	0.0004	<.0001	<.0001	0.0003
Assessor 9	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 10	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 11	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 12	0.0352	<.0001	0.0176	0.1197	0.189	0.0176

4144 Table 3d. Correlations of each assessor's mean scores with panel average

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.71	0.98	0.85	0.95	0.97	0.95
Assessor 2	0.37	0.92	0.88	0.56	0.95	0.79
Assessor 3	0.46	0.94	0.96	0.99	0.88	0.92
Assessor 4	0.68	0.97	0.98	0.94	0.95	0.65
Assessor 5	0.88	0.96	0.95	0.99	0.89	0.83
Assessor 6	0.18	0.94	0.9	0.89	0.64	0.88
Assessor 7	-0.04	0.88	0.8	0.92	0.98	0.86
Assessor 8	0.53	0.91	0.89	0.94	0.99	0.94
Assessor 9	0.22	0.93	0.78	0.94	0.12	0.48
Assessor 10	0.93	0.98	0.99	1	0.92	0.85
Assessor 11	0.84	0.9	0.85	0.96	0.83	0.83
Assessor 12	0.18	0.99	0.93	0.98	0.91	0.94

4145 Table 3e. Assessor's repeatability standard deviation

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	7.94	2.23	2.49	3.02	5.65	7.87
Assessor 2	NA	NA	NA	NA	NA	NA
Assessor 3	9.36	6.54	6.08	2.63	12.22	1.21
Assessor 4	12.27	4.84	5.67	3.5	7.01	4.03
Assessor 5	4.11	3.66	4.26	2.74	4.05	4.53
Assessor 6	14.98	6.06	3.24	2.73	8.34	5.55
Assessor 7	NA	NA	NA	NA	NA	NA
Assessor 8	3.88	1.86	3.67	1.75	3.19	5.14
Assessor 9	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
Assessor 10	3.55	2.74	0.58	2.38	2.08	4.31
Assessor 11	NA	NA	NA	NA	NA	NA
Assessor 12	7.4	1.87	7.3	10.76	6.58	7.69

4146 Table 3f. Test of each assessor's repeatability (replicate variability) against the Panel
4147 average repeatability (F value)

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.8	0.3	0.3	0.4	0.7	2.1

Assessor 2	NA	NA	NA	NA	NA	NA
Assessor 3	1.1	2.5	1.7	0.3	3.2	0
Assessor 4	1.9	1.4	1.5	0.6	1	0.6
Assessor 5	0.2	0.8	0.8	0.4	0.4	0.7
Assessor 6	2.9	2.2	0.5	0.4	1.5	1
Assessor 7	NA	NA	NA	NA	NA	NA
Assessor 8	0.2	0.2	0.6	0.1	0.2	0.9
Assessor 9	NA	NA	NA	NA	NA	NA
Assessor 10	0.2	0.4	0	0.3	0.1	0.6
Assessor 11	NA	NA	NA	NA	NA	NA
Assessor 12	0.7	0.2	2.5	5.5	0.9	2

Table 3g. Test of each assessor's repeatability (replicate variability) against the Panel average repeatability (p-vaule)

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.6223	0.9808	0.9815	0.9247	0.7372	0.0331
Assessor 2	NA	NA	NA	NA	NA	NA
Assessor 3	0.3561	0.0108	0.0883	0.9709	0.0017	1
Assessor 4	0.0533	0.2047	0.153	0.8232	0.411	0.8468
Assessor 5	0.9942	0.6386	0.585	0.9611	0.9637	0.7221
Assessor 6	0.0041	0.0285	0.8907	0.9622	0.1596	0.4109
Assessor 7	NA	NA	NA	NA	NA	NA
Assessor 8	0.9964	0.9954	0.7841	0.9989	0.994	0.5392
Assessor 9	NA	NA	NA	NA	NA	NA
Assessor 10	0.9983	0.9218	1	0.9861	0.9999	0.7815
Assessor 11	NA	NA	NA	NA	NA	NA
Assessor 12	0.721	0.9953	0.0118	<.0001	0.5135	0.0429

Table 3h. F-values for Assessor contribution to the interaction

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.9	0.8	2.6	1.1	1.5	1.1
Assessor 2	2.8	6	8.2	5.3	0.4	7.1
Assessor 3	4.1	4	1.1	13.7	6.4	1
Assessor 4	0.6	1.3	1	1.4	0.7	4.4
Assessor 5	1.5	1.7	0.9	0.2	1.9	2.1
Assessor 6	1.6	2.4	1.8	4.6	3.8	1.8
Assessor 7	1.2	3.7	1.7	0.9	0.1	0.9
Assessor 8	2.7	3.1	1.9	1.7	1.1	2
Assessor 9	2.1	1.2	2.3	1.5	3.1	3.6
Assessor 10	0.7	1	0.3	0.1	1.1	3.9
Assessor 11	1.4	2	4.9	5.3	3.3	3.1
Assessor 12	2.9	4.7	1.5	0.6	1.9	1.1
Interaction F	1.9	2.7	2.3	3	2.1	2.7

Table 3i. p-values for Assessor contribution to the interaction

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.5384	0.6474	0.0137	0.3412	0.1727	0.4
Assessor 2	0.0076	<.0001	<.0001	<.0001	0.8998	<.0001
Assessor 3	0.0004	0.0004	0.4052	<.0001	<.0001	0.4532
Assessor 4	0.7931	0.2571	0.4448	0.1911	0.6869	0.0002
Assessor 5	0.1544	0.1213	0.5146	0.9933	0.0751	0.0438
Assessor 6	0.15	0.0199	0.0933	0.0001	0.0006	0.0927
Assessor 7	0.2956	0.0009	0.1029	0.4842	0.9971	0.5395
Assessor 8	0.0113	0.0035	0.0746	0.1129	0.3884	0.0531
Assessor 9	0.0388	0.3014	0.0281	0.1789	0.0041	0.0011
Assessor 10	0.71	0.4334	0.9738	0.9995	0.3505	0.0006
Assessor 11	0.2187	0.0553	<.0001	<.0001	0.0022	0.0037
Assessor 12	0.0067	0.0001	0.1695	0.7482	0.069	0.3437
Interaction p-value	0.002	<.0001	0.0001	<.0001	0.0003	<.0001

*NA means not applicable.

4153 Supplementary table 4. Ratings and significance testing (ANOVA) results of perceived
4154 intensity (antilogged values) of overall taste, sweet, salty, sour, bitter and umami where
4155 MSG was used as the umami tastant without sodium balance.

	Perceived intensity (mean of antilogged gLMS intensity ratings)					
Sample	Total intensity	Sweet	Salty	Sour	Bitter	Umami
S	36.2^{cd}	34.7^{a}	2.5^{c}	2.2^{c}	1.9^{c}	1.2^{d}
S+U	45.1^{abc}	39.9^{a}	6.3^{c}	1.6^{c}	1.9^{c}	13.5^{c}
N	37.9^{cd}	1.1^{b}	31.4^{ab}	1.1^{c}	4.1^{c}	2.8^{d}
N+U	44.6^{abc}	4.5^{b}	32.8^{a}	1.3^{c}	2.5^{c}	23.5^{b}
C	38.7^{cd}	1.4^{b}	3.6^{c}	31.4^{a}	9.3^{c}	1.0^{d}
C+U	41.3^{bcd}	2.2^{b}	5.0^{c}	29.8^{a}	8.3^{c}	18.5^{bc}
Q	49.6^{ab}	1.0^{b}	1.1^{c}	1.9^{c}	45.6^{a}	1.0^{d}
Q+U	49.2^{ab}	1.1^{b}	2.7^{c}	1.4^{c}	43.6^{a}	16.6^{bc}
U	33.4^{d}	1.4^{b}	5.6^{c}	1.1^{c}	1.5^{c}	32.2^{a}
S+N+C+Q+U	53.2^{a}	39.1^{a}	24.7^{b}	11.7^{b}	14.2^{b}	5.3^{d}
df of Sample	9	9	9	9	9	9
df of Interaction	72	72	72	72	72	72
F-value of Sample Effect	4.08	80.81	24.8	29.93	25.45	19.22
Sample significance (p)	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

4156 ^{abcde} Values within a column which do not share a common superscript are significantly different in means ratings
4157 of the perceived magnitude from Tukey's HSD test at the 95% confidence interval. S = sucrose; N = sodium chloride;
4158 C = citric acid; Q = quinine hemisulfate salt monohydrate; U = monosodium glutamate (MSG). *df* = degrees of
4159 freedom of interaction, noting that the main effect of sample (F-value of sample) was determined by dividing the
4160 variance of sample by the variance of the interaction (MSsample/MSinteraction) hence both the *df* of sample and
4161 interaction are given.

Supplementary Table 5. Ratings and significance testing (ANOVA) results of perceived intensity (antilogged values) of overall taste, sweet, salty, sour, bitter and umami where MSG was used as the umami tastant with sodium balance.

	Perceived intensity (mean of antilogged gLMS intensity ratings)					
Sample	Total intensity	Sweet	Salty	Sour	Bitter	Umami
S	43.5^{bcd}	41.9^{a}	4.9^{c}	1.2^{c}	1.1^{c}	1.0^{e}
S+U	49.9^{ab}	44.4^{a}	5.3^{c}	2.2^{c}	1.6^{c}	14.4^{c}
N	41.0^{cde}	2.1^{c}	35.4^{a}	2.8^{c}	3.0^{c}	6.2^{e}
N+U	47.5^{bc}	2.4^{c}	30.7^{a}	2.8^{c}	3.0^{c}	22.4^{b}
C	37.9^{de}	2.0^{c}	5.0^{c}	31.2^{a}	6.0^{c}	1.3^{e}
C+U	42.8^{bcde}	1.7^{c}	7.4^{c}	29.1^{a}	6.7^{c}	13.3^{cd}
Q	34.6^{e}	1.4^{c}	5.2^{c}	2.5^{c}	33.0^{a}	1.5^{e}
Q+U	50.4^{ab}	1.4^{c}	8.3^{c}	1.5^{c}	37.7^{a}	23.3^{b}
U	36.2^{de}	1.9^{c}	8.1^{c}	2.8^{c}	1.5^{c}	31.2^{a}
S+N+C+Q+U	57.5^{a}	32.3^{b}	20.1^{b}	10.5^{b}	23.1^{b}	7.4^{de}
df of Sample	9	9	9	9	9	9
df of Interaction	90	90	90	90	90	90
F-value of Sample Effect	2.64	113.66	23.5	28.39	21.03	21.16
Sample significance (p)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{abcde} Values within a column which do not share a common superscript are significantly different in means ratings of the perceived magnitude from Tukey's HSD test at the 95% confidence interval. S = sucrose; N = sodium chloride; C = citric acid; Q = quinine hemisulfate salt monohydrate; U = monosodium glutamate (MSG). *df* = degrees of freedom of interaction, noting that the main effect of sample (F-value of sample) was determined by dividing the variance of sample by the variance of the interaction (MSsample/MSinteraction) hence both the *df* of sample and interaction are given.

Supplementary Table 6. Ratings and significance testing (ANOVA) results of perceived intensity (antilogged values) of overall taste, sweet, salty, sour, bitter and umami where MPG was used as the umami tastant.

	Perceived intensity (mean of antilogged gLMS intensity ratings)					
Sample	Total intensity	Sweet	Salty	Sour	Bitter	Umami
S	29.2^{cd}	28.6^{a}	2.2^{c}	1.2^{c}	1.3^{c}	1.1^{d}
S+U	35.3^{abc}	28.1^{a}	4.2^{c}	2^{c}	3.4^{a}	14.5^{b}
N	23.5^{d}	1^{c}	22.5^{a}	1.6^{c}	3.3^{c}	2.4^{cd}
N+U	34.2^{bc}	1.3^{c}	25.2^{a}	1.6^{c}	2^{a}	18.9^{b}
C	29.6^{cd}	1.4^{c}	1.5^{c}	26.3^{a}	5.8^{bc}	1.1^{d}
C+U	36^{abc}	1.3^{c}	3.7^{c}	28.8^{a}	6.1^{bc}	15.7^{b}
Q	32.8^{bc}	1.1^{c}	1.5^{c}	1.2^{c}	29.7^{a}	1.4^{d}
Q+U	38.5^{ab}	1.1^{c}	2.7^{c}	3.4^{c}	32.4^{a}	15.8^{b}
U	29^{cd}	1.3^{c}	3.1^{c}	1.3^{c}	3.8^{bc}	27.2^{a}
S+N+C+Q+U	42.2^{a}	21.6^{b}	16.7^{b}	13.2^{b}	9.7^{b}	7.2^{c}
df of Sample	9	9	9	9	9	9
df of Interaction	99	99	99	99	99	99
F-value of Sample Effect	3.98	65.36	34.69	37.19	26.64	21.49
Sample significance (p)	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^{abcde} Values within a column which do not share a common superscript are significantly different in means ratings of the perceived magnitude from Tukey's HSD test at the 95% confidence interval. S = sucrose; N = sodium chloride; C = citric acid; Q = quinine hemisulfate salt monohydrate; U = potassium L-glutamate monohydrate (MPG). *df* = degrees of freedom of interaction, noting that the main effect of sample (F-value of sample) was determined by dividing the variance of sample by the variance of the interaction (MSsample/MSinteraction) hence both the *df* of sample and interaction are given.

Supplementary table 7. Assessor performance of perceived taste intensity of sodium chloride, lysine and calcium lactate in single, binary and ternary solutions.

Table 7a. Assessor mean scores with significance of assessor differences for each attribute (showing different use of scale).

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	37.1	1.3	26	4.7	22.2	37.1
Assessor 2	42.4	3	23.4	1.2	32.8	42.4
Assessor 3	49	6.8	19.6	6	38.1	49
Assessor 4	37.4	1	20.4	2.2	17.6	37.4
Assessor 5	36.7	4.1	21.3	2.5	25.6	36.7
Assessor 6	47.4	2.2	10.3	6.5	39.2	47.4
Assessor 7	24.4	1.9	12.5	4.7	12.6	24.4
Assessor 8	27.8	1	17.2	3.1	16.7	27.8
Assessor 9	41.1	3.7	25.4	9.4	17.7	41.1
Assessor 10	12.6	1.3	9.6	1.6	1.5	12.6
Assessor 11	39.5	1.1	26.8	1.5	19.4	39.5
Assessor 12	44.7	1.4	24	1.6	30.2	44.7
HSD	10.7	2.1	8.1	3	11.1	10.7
p - value	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001

Table 7b. F values for Assessor Discrimination

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	2	1.4	2.9	0.5	8.9	2
Assessor 2	NA	NA	NA	NA	NA	NA
Assessor 3	6.6	0.7	5.4	0.5	15.3	6.6
Assessor 4	2.2	4.6	0.6	0.9	1.7	2.2
Assessor 5	3	2.5	8.1	1.2	1.8	3
Assessor 6	NA	NA	NA	NA	NA	NA
Assessor 7	0.7	3.6	1.4	1.6	7.2	0.7
Assessor 8	3.8	1	4.4	1.6	6.8	3.8
Assessor 9	5.8	0.6	13.9	0.7	5.9	5.8
Assessor 10	NA	NA	NA	NA	NA	NA
Assessor 11	NA	NA	NA	NA	NA	NA
Assessor 12	12.1	2.4	17.2	1	40.7	12.1

Table 7c. p-values for Assessor Discrimination

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.1771	0.3272	0.0793	0.8308	0.0031	0.1771
Assessor 2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 3	0.0082	0.6493	0.0154	0.837	0.0005	0.0082
Assessor 4	0.1457	0.0241	0.777	0.5488	0.2343	0.1457

Assessor 5	0.0754	0.1114	0.0043	0.4191	0.2229	0.0754
Assessor 6	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 7	0.6545	0.0479	0.3128	0.2731	0.0062	0.6545
Assessor 8	0.0408	0.4934	0.0269	0.2507	0.0076	0.0408
Assessor 9	0.012	0.7161	0.0007	0.6809	0.0115	0.012
Assessor 10	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 11	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Assessor 12	0.0011	0.1255	0.0003	0.4868	<.0001	0.0011

4186 Table 7d. Correlations of each assessor's mean scores with panel average

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.71	0.62	0.91	0.56	0.9	0.71
Assessor 2	0.68	0.76	0.81	0.04	0.84	0.68
Assessor 3	0.4	0.82	0.94	0.83	0.73	0.4
Assessor 4	0.74	0.67	0.53	0.6	0.9	0.74
Assessor 5	0.77	0.52	0.84	0.12	0.87	0.77
Assessor 6	0.73	-0.3	0.85	-0.24	0.87	0.73
Assessor 7	0.81	0.58	0.87	0.5	0.93	0.81
Assessor 8	0.97	-0.2	0.94	0.69	0.95	0.97
Assessor 9	0.72	0.74	0.74	0.8	0.59	0.72
Assessor 10	0.72	0.49	0.95	0.4	0.01	0.72
Assessor 11	0.73	0.06	0.86	0.3	0.68	0.73
Assessor 12	0.57	0.81	0.82	0.31	0.89	0.57

4187 Table 7e. Assessor's repeatability standard deviation

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	9.9	0.63	10.19	6.2	7.5	9.9
Assessor 2	NA	NA	NA	NA	NA	NA
Assessor 3	8.85	8.07	5.54	8.81	6.37	8.85
Assessor 4	10.56	0.07	20.59	3.76	14.24	10.56
Assessor 5	10.7	2.34	6.09	2.3	15.29	10.7
Assessor 6	NA	NA	NA	NA	NA	NA
Assessor 7	14.01	1.46	10.71	4.68	6.01	14.01
Assessor 8	10.71	0.05	7.54	3.13	9.08	10.71
Assessor 9	7.6	4.19	5.63	8.74	6.04	7.6
Assessor 10	NA	NA	NA	NA	NA	NA
Assessor 11	NA	NA	NA	NA	NA	NA
Assessor 12	4.81	0.5	5.43	0.95	4.81	4.81

4188 Table 7f. Test of each assessor's repeatability (replicate variability) against the Panel
4189 average repeatability (F value)

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	1	0	1	1.3	0.6	1
Assessor 2	NA	NA	NA	NA	NA	NA

Assessor 3	0.8	5.7	0.3	2.5	0.5	0.8
Assessor 4	1.1	0	4.1	0.5	2.3	1.1
Assessor 5	1.2	0.5	0.4	0.2	2.6	1.2
Assessor 6	NA	NA	NA	NA	NA	NA
Assessor 7	2	0.2	1.1	0.7	0.4	2
Assessor 8	1.2	0	0.5	0.3	0.9	1.2
Assessor 9	0.6	1.5	0.3	2.5	0.4	0.6
Assessor 10	NA	NA	NA	NA	NA	NA
Assessor 11	NA	NA	NA	NA	NA	NA
Assessor 12	0.2	0	0.3	0	0.3	0.2

4190 Table 7g. Test of each assessor's repeatability (replicate variability) against the Panel
4191 average repeatability (p-vaule)

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.4541	1	0.4429	0.2809	0.7474	0.4541
Assessor 2	NA	NA	NA	NA	NA	NA
Assessor 3	0.6135	<.0001	0.9647	0.0181	0.8817	0.6135
Assessor 4	0.3603	1	0.0005	0.8775	0.0325	0.3603
Assessor 5	0.3409	0.8644	0.9385	0.9937	0.0149	0.3409
Assessor 6	NA	NA	NA	NA	NA	NA
Assessor 7	0.0638	0.9918	0.3703	0.677	0.913	0.0638
Assessor 8	0.34	1	0.8149	0.9557	0.5002	0.34
Assessor 9	0.7895	0.1601	0.9612	0.0198	0.9108	0.7895
Assessor 10	NA	NA	NA	NA	NA	NA
Assessor 11	NA	NA	NA	NA	NA	NA
Assessor 12	0.9832	1	0.9685	1	0.9763	0.9832

4192 Table 7h. F-values for Assessor contribution to the interaction

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	1.2	0.1	0.6	0.4	1.2	1.2
Assessor 2	2.1	0.5	1.6	0.1	1.3	2.1
Assessor 3	4.9	3.3	0.2	0.7	3.5	4.9
Assessor 4	1.2	0.2	2.1	0.3	0.9	1.2
Assessor 5	1.5	1	0.9	0.3	1.2	1.5
Assessor 6	2.8	0.6	0.3	1	5.9	2.8
Assessor 7	0.7	0.5	0.5	0.9	0.6	0.7
Assessor 8	0.9	0.2	0.3	0.3	0.9	0.9
Assessor 9	1.8	0.6	2.1	1.2	2.9	1.8
Assessor 10	0.4	0.1	0.2	0.1	2.1	0.4
Assessor 11	1.3	0.1	0.6	0.1	1.5	1.3
Assessor 12	2.2	0.1	2	0.1	3.4	2.2
Interaction F	1.7	0.6	1	0.5	2.1	1.7

4193 Table 7i. p-values for Assessor contribution to the interaction

	Total intensity	Sweet	Salty	Sour	Bitter	Umami
Assessor 1	0.332	0.9952	0.7715	0.8538	0.3327	0.332
Assessor 2	0.0628	0.8401	0.1488	0.998	0.2625	0.0628
Assessor 3	0.0003	0.0063	0.9672	0.6641	0.0036	0.0003
Assessor 4	0.2953	0.9877	0.0599	0.9455	0.5376	0.2953
Assessor 5	0.1761	0.4436	0.5	0.9251	0.2991	0.1761
Assessor 6	0.0157	0.72	0.9474	0.4636	<.0001	0.0157
Assessor 7	0.6987	0.8251	0.82	0.4818	0.748	0.6987
Assessor 8	0.5201	0.9814	0.9248	0.936	0.5003	0.5201
Assessor 9	0.1147	0.7438	0.0598	0.3364	0.0137	0.1147
Assessor 10	0.8602	0.9989	0.9762	0.999	0.0638	0.8602
Assessor 11	0.2903	0.9974	0.749	0.9989	0.1908	0.2903
Assessor 12	0.0516	0.9986	0.0759	0.9913	0.005	0.0516
Interaction p-value	0.0115	0.9849	0.5773	0.9994	0.0012	0.0115

*NA means not applicable.

Supplementary Table 8. Ratings of perceived intensity of overall taste, salty, bitter and the concentration of a composite tastant solution (fixed ratio of 0.25% NaCl : 1.0% lysine : 0.75% calcium lactate).

	Perceived intensity (mean of gLMS intensity ratings)		
Concentration ratio	Total intensity	Saltiness	Bitterness
0.21	1.08^{e}	0.81^{e}	1.02^{d}
0.35	1.19^{e}	0.90^{de}	1.10^{cd}
0.59	1.37^{d}	1.17^{d}	1.30^{c}
1	1.54^{c}	1.37^{c}	1.48^{b}
1.7	1.81^{b}	1.73^{b}	1.69^{a}
2.89	1.90^{a}	1.86^{a}	1.73^{a}
df of sample	9	9	9
df of interaction	45	45	45
F-value of sample effect	141.48	94.81	45.17
Sample significance (p)	<0.001	<0.001	<0.001

Means within a column which do not share a common superscript are significantly different in the perceived magnitude from Tukey's HSD test at the 95% confidence interval. 1 means the fixed ratio of 0.25% NaCl : 1.0% lysine : 0.75% calcium lactate. Other means the composite tastant solution (1) was concentrated or diluted by 1.7, and the concentration of NaCl, lysine and calcium lactate were concentrated or diluted at the same time. *df* = degrees of freedom of interaction, noting that the main effect of sample (F-value of sample) was determined by dividing the variance of sample by the variance of the interaction (MSsample/MSinteraction) hence both the *df* of sample and interaction are given.

Supplementary Table 9. Assessor performance of perceived intensity of overall taste, salty, bitter and the concentration of a composite tastant solution (fixed ratio of 0.25% NaCl : 1.0% lysine : 0.75% calcium lactate).

Table 9a. Assessor mean scores with significance of assessor differences for each attribute (showing different use of scale).

	Total intensity	Salty	Bitter	Sour
Assessor 1	32.2	29.5	24.4	4.9
Assessor 2	54.6	30.3	54.7	1.1
Assessor 3	48.1	38.4	42.5	3.9
Assessor 4	39	36.2	30.5	2.1
Assessor 5	19.9	21	14.1	7
Assessor 6	34.3	30.9	25.5	1
Assessor 7	36.4	15.7	27.2	6.5
Assessor 8	35.1	25.8	12.3	1.2
Assessor 9	36.7	33.4	19.3	2.4
Assessor 10	43.5	36	27.5	1.2
HSD	9.1	10.9	10.7	3.1
p - value	<.0001	0.0005	<.0001	0.0001

Table 9b. F values for Assessor Discrimination

	Total intensity	Salty	Bitter	Sour
Assessor 1	170.1	134.8	7.7	0.8
Assessor 2	13	15.4	11.5	0.4
Assessor 3	27.8	17.6	6.4	2
Assessor 4	93	94.9	13.8	0.9
Assessor 5	21.8	20.2	3.4	1.5
Assessor 6	17.1	14.8	3.6	NA
Assessor 7	12.9	19.7	4.2	0.7
Assessor 8	NA	NA	NA	NA
Assessor 9	116.2	103.4	20.6	0.6
Assessor 10	36.2	33.5	4.5	1.8

Table 9c. p-values for Assessor Discrimination

	Total intensity	Salty	Bitter	Sour
Assessor 1	<.0001	<.0001	0.0018	0.6
Assessor 2	0.0002	0.0001	0.0003	0.823
Assessor 3	<.0001	<.0001	0.0041	0.1463
Assessor 4	<.0001	<.0001	0.0001	0.5397
Assessor 5	<.0001	<.0001	0.0391	0.2498
Assessor 6	<.0001	0.0001	0.0321	NA
Assessor 7	0.0002	<.0001	0.0201	0.6265

Assessor 8	<.0001	<.0001	<.0001	<.0001
Assessor 9	<.0001	<.0001	0.001	0.7349
Assessor 10	<.0001	<.0001	0.0158	0.1815

4212 Table 9d. Correlations of each assessor's mean scores with panel average

	Total intensity	Salty	Bitter	Sour
Assessor 1	0.99	1	1	0.53
Assessor 2	0.98	0.99	0.98	0.06
Assessor 3	1	0.99	0.98	0.71
Assessor 4	0.99	1	0.96	0.83
Assessor 5	0.99	0.99	0.95	0.74
Assessor 6	0.98	0.97	0.96	0
Assessor 7	0.99	0.83	0.98	0.71
Assessor 8	0.99	0.89	0.88	0.39
Assessor 9	1	0.99	0.98	0.89
Assessor 10	0.98	1	0.69	-0.93

4213 Table 9e. Assessor's repeatability standard deviation

	Total intensity	Salty	Bitter	Sour
Assessor 1	4.1	4.83	12.94	7.16
Assessor 2	12.1	11.79	11.96	0.13
Assessor 3	9.5	13.39	14.16	4.09
Assessor 4	6.04	6.05	13.47	3.22
Assessor 5	6.84	8.03	8.08	4.18
Assessor 6	8.18	9.45	15.97	NA
Assessor 7	12.81	8.26	16.17	11.55
Assessor 8	NA	NA	NA	NA
Assessor 9	4.81	4.57	6.82	1.85
Assessor 10	9.16	9.99	13.38	0.12

4214 Table 9f. Test of each assessor's repeatability (replicate variability) against the Panel
4215 average repeatability (F value)

	Total intensity	Salty	Bitter	Sour
Assessor 1	0.2	0.3	1	1.9
Assessor 2	1.9	1.7	0.8	0
Assessor 3	1.2	2.1	1.2	0.6
Assessor 4	0.5	0.4	1	0.4
Assessor 5	0.6	0.8	0.4	0.6
Assessor 6	0.9	1.1	1.5	NA
Assessor 7	2.1	0.8	1.5	4.9
Assessor 8	NA	NA	NA	NA
Assessor 9	0.3	0.3	0.3	0.1
Assessor 10	1.1	1.2	1	0

Table 9g. Test of each assessor's repeatability (replicate variability) against the Panel average repeatability (p-value)

	Total intensity	Salty	Bitter	Sour
Assessor 1	0.9974	0.9914	0.4876	0.0443
Assessor 2	0.046	0.0864	0.6263	1
Assessor 3	0.3243	0.02	0.3265	0.8244
Assessor 4	0.9289	0.9442	0.4147	0.9673
Assessor 5	0.8385	0.678	0.9694	0.8005
Assessor 6	0.5923	0.3946	0.1482	NA
Assessor 7	0.0229	0.6332	0.1345	<.0001
Assessor 8	NA	NA	NA	NA
Assessor 9	0.9371	0.9582	0.9506	0.9928
Assessor 10	0.3886	0.2981	0.4273	1

Table 9h. F-values for Assessor contribution to the interaction

	Total intensity	Salty	Bitter	Sour
Assessor 1	1.2	1.3	0.2	1.2
Assessor 2	1.4	0.8	0.7	0.2
Assessor 3	0.3	1.7	0.3	0.8
Assessor 4	2	2	2.9	0.1
Assessor 5	3.9	2	2.2	0.6
Assessor 6	3.7	3.1	0.5	0.2
Assessor 7	0.6	9.1	0.3	2.8
Assessor 8	1.1	2.1	0.5	0.1
Assessor 9	2.5	1.4	0.4	0
Assessor 10	2.1	1.7	3.7	0.3
Interaction F	1.9	2.5	1.2	0.6

Table 9i. p-values for Assessor contribution to the interaction

	Total intensity	Salty	Bitter	Sour
Assessor 1	0.3147	0.2602	0.9626	0.33
Assessor 2	0.2375	0.5632	0.5752	0.9338
Assessor 3	0.8904	0.144	0.8701	0.536
Assessor 4	0.0925	0.0898	0.0208	0.9851
Assessor 5	0.0042	0.0852	0.0688	0.6968
Assessor 6	0.0056	0.0154	0.7659	0.9335
Assessor 7	0.6855	<.0001	0.9088	0.0268
Assessor 8	0.3691	0.0842	0.7743	0.9941
Assessor 9	0.0404	0.225	0.8589	0.9985
Assessor 10	0.0849	0.1572	0.0054	0.9157
Interaction p-value	0.0049	0.0001	0.26	0.9596

*NA means not applicable.

Supplementary Table 10. Ratings of perceived intensity of overall taste, salty, bitter and concentration of lysine composite solution (each composite solution containing 0.25% NaCl and 0.75% calcium lactate w/v in addition to lysine).

	Perceived intensity (mean of gLMS intensity ratings)		
Concentration ratio	Total intensity	Saltiness	Bitterness
0.21	1.33^{d}	1.13^{d}	1.21^{d}
0.35	1.40^{cd}	1.17^{d}	1.26^{d}
0.59	1.43^{cd}	1.26^{cd}	1.36^{cd}
1	1.56^{bc}	1.42^{cd}	1.44^{cd}
1.7	1.63^{b}	1.47^{bc}	1.54^{bc}
2.89	1.75^{a}	1.60^{ab}	1.66^{ab}
4.91	1.82^{a}	1.67^{a}	1.75^{a}
df of sample	10	10	10
df of interaction	60	60	60
F-value of sample effect	24.85	18.09	18.55
Sample significance (p)	<0.001	<0.001	<0.001

Means within a column which do not share a common superscript are significantly different in the perceived magnitude from Tukey's HSD test at the 95% confidence interval. 1 means the fixed ratio of 0.25% NaCl : 1.0% lysine : 0.75% calcium lactate. Other means the composite tastant solution (1) was concentrated or diluted by 1.7, but only the concentration of lysine was concentrated or dilute, the concentration of NaCl and calcium lactate did not change. *df* = degrees of freedom of interaction, noting that the main effect of sample (F-value of sample) was determined by dividing the variance of sample by the variance of the interaction (MSsample/MSinteraction) hence both the *df* of sample and interaction are given.

4231 Supplementary Table 11. Assessor performance of perceived intensity of overall taste,
4232 salty, bitter and concentration of lysine composite solution (each composite solution
4233 containing 0.25% NaCl and 0.75% calcium lactate w/v in addition to lysine).
4234 Table 11a. Assessor mean scores with significance of assessor differences for each
4235 attribute (showing different use of scale).

	Total intensity	Salty	Bitter
Assessor 1	1.5	1.4	1.5
Assessor 2	1.6	1.4	1.5
Assessor 3	1.5	1.3	1.4
Assessor 4	1.5	1.4	1.4
Assessor 5	1.7	1.3	1.6
Assessor 6	1.3	1.2	0.6
Assessor 7	1.3	1.2	1.4
Assessor 8	1.5	1.4	1.4
Assessor 9	1.6	1.3	1.5
Assessor 10	1.6	1.5	1.1
Assessor 11	1.7	1.1	1.6
HSD	0.1	0.2	0.2
p - value	<.0001	0.0022	<.0001

4236 Table 11b. F values for Assessor Discrimination

	Total intensity	Salty	Bitter
Assessor 1	15.7	2.9	8
Assessor 2	6.5	4.5	4.5
Assessor 3	7.5	3.7	3.1
Assessor 4	10.3	14.1	3.7
Assessor 5	2.6	27.4	1.9
Assessor 6	3.6	6.5	10.5
Assessor 7	NA	NA	NA
Assessor 8	10.4	3.7	18.7
Assessor 9	NA	NA	NA
Assessor 10	3.2	1.5	1.8
Assessor 11	15.5	5	9.5

4237 Table 11c. p-values for Assessor Discrimination

	Total intensity	Salty	Bitter
Assessor 1	0.001	0.0979	0.0074
Assessor 2	0.0131	0.0348	0.0344
Assessor 3	0.0088	0.0539	0.083
Assessor 4	0.0035	0.0014	0.0562
Assessor 5	0.1186	0.0002	0.2063

Assessor 6	0.0582	0.0133	0.0033
Assessor 7	<.0001	<.0001	<.0001
Assessor 8	0.0034	0.0543	0.0006
Assessor 9	<.0001	<.0001	<.0001
Assessor 10	0.0759	0.2962	0.2283
Assessor 11	0.001	0.0264	0.0045

4238 Table 11d. Correlations of each assessor's mean scores with panel average

	Total intensity	Salty	Bitter
Assessor 1	0.8	0.92	0.75
Assessor 2	0.9	0.71	0.97
Assessor 3	0.97	0.84	0.95
Assessor 4	0.97	0.9	0.98
Assessor 5	0.82	0.83	0.74
Assessor 6	0.85	0.91	0.92
Assessor 7	0.77	0.69	0.75
Assessor 8	0.98	0.87	0.88
Assessor 9	0.74	0.74	0.74
Assessor 10	0.78	0.68	0.88
Assessor 11	0.94	0.86	0.86

4239 Table 11e. Assessor's repeatability standard deviation

	Total intensity	Salty	Bitter
Assessor 1	0.09	0.24	0.15
Assessor 2	0.11	0.11	0.13
Assessor 3	0.16	0.22	0.33
Assessor 4	0.11	0.1	0.21
Assessor 5	0.09	0.11	0.12
Assessor 6	0.18	0.17	0.21
Assessor 7	NA	NA	NA
Assessor 8	0.12	0.13	0.17
Assessor 9	NA	NA	NA
Assessor 10	0.19	0.38	0.4
Assessor 11	0.05	0.16	0.06

4240 Table 11f. Test of each assessor's repeatability (replicate variability) against the Panel
4241 average repeatability (F value)

	Total intensity	Salty	Bitter
Assessor 1	0.5	1.4	0.4
Assessor 2	0.7	0.3	0.3
Assessor 3	1.5	1.3	2.2
Assessor 4	0.8	0.2	0.9
Assessor 5	0.4	0.3	0.3
Assessor 6	2	0.7	0.9

Assessor 7	NA	NA	NA
Assessor 8	0.8	0.4	0.6
Assessor 9	NA	NA	NA
Assessor 10	2.1	3.6	3.3
Assessor 11	0.2	0.7	0.1

Table 9g. Test of each assessor's repeatability (replicate variability) against the Panel average repeatability (p-value)

	Total intensity	Salty	Bitter
Assessor 1	0.8099	0.2109	0.8731
Assessor 2	0.6653	0.9401	0.9407
Assessor 3	0.1807	0.2774	0.0459
Assessor 4	0.5977	0.9729	0.5388
Assessor 5	0.8727	0.9555	0.9607
Assessor 6	0.0725	0.6387	0.5351
Assessor 7	NA	NA	NA
Assessor 8	0.5957	0.8874	0.7421
Assessor 9	NA	NA	NA
Assessor 10	0.0574	0.0024	0.0045
Assessor 11	0.9913	0.6997	0.9989

Table 11h. F-values for Assessor contribution to the interaction

	Total intensity	Salty	Bitter
Assessor 1	3.3	0.8	1.8
Assessor 2	1	1.4	0.4
Assessor 3	1.9	1.6	1.4
Assessor 4	0.9	0.7	0.1
Assessor 5	2.4	3.3	1.8
Assessor 6	2.1	1.1	2.8
Assessor 7	4.3	1.2	0.8
Assessor 8	0.8	0.7	4.6
Assessor 9	1.3	1	0.8
Assessor 10	2.9	3.3	1.7
Assessor 11	0.9	1	1.2
Interaction F	2	1.5	1.6

Table 11i. p-values for Assessor contribution to the interaction

	Total intensity	Salty	Bitter
Assessor 1	0.0091	0.5921	0.1268
Assessor 2	0.4253	0.2184	0.8579
Assessor 3	0.1035	0.1702	0.2246
Assessor 4	0.4661	0.6139	0.9898
Assessor 5	0.0452	0.0089	0.1252
Assessor 6	0.0669	0.3947	0.022

Assessor 7	0.0016	0.3245	0.5451
Assessor 8	0.5754	0.6293	0.0009
Assessor 9	0.2771	0.4456	0.5979
Assessor 10	0.018	0.0091	0.1481
Assessor 11	0.5006	0.4478	0.3127
Interaction p-value	0.0042	0.0727	0.0379

*NA means not applicable.

Supplementary Table 12. Physical-chemical characteristics of pork patties varying in salt, lysine, calcium lactate and pH.

Factor	pH before cooking	pH after cooking	Cooking loss	Moisture	L surface	a* surface	b* surface	L internal	a* internal	b* internal
Salt	6.035±0.09[a]	6.284±0.12[a]	28.412±6.03[a]	65.0574.88[a]	56.572±3.77[b]	4.506±0.73[c]	15.416±1.33[b]	67.859±2.19[a]	4.783±1.12[b]	10.58±0.53[a]
CL	6.034±0.09[a]	6.021±0.08[b]	25.012±4.69[b]	63.317±3.85[b]	58.87±5.14[a]	2.847±1.03[b]	15.704±1.28[b]	68.809±2.97[a]	4.678±0.78[b]	9.495±0.53[b]
LY	5.999±0.08[a]	6.28±0.06[a]	20.712±5.77[c]	66.218±4.38[a]	52.029±3.42[c]	5.481±0.56[a]	15.38±1.86[b]	64.849±2.09[b]	5.32±0.74[a]	9.274±0.38[c]
LY+CL	6.042±0.08[a]	6.017±0.08[b]	21.04±3.59[c]	66.296±3.18[a]	56.496±3.41[b]	4.24±1.03[c]	16.548±1.57[a]	65.949±2.98[b]	4.063±0.62[c]	8.849±0.67[d]
p(ingredients)	0.143	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
pH 5.5	5.559±0.06[c]	5.847±0.08[c]	28.114±4.65[a]	61.693±2.24[c]	58.834±3.83[a]	3.827±1.09[c]	14.876±1.28[c]	68.515±3.39[a]	4.121±0.79[c]	9.021±0.87[c]
pH 6	6.017±0.08[b]	6.085±0.12[b]	23.855±5.94[b]	65.958±4.02[b]	55.575±4.76[b]	4.188±1.22[b]	15.55±1.45[b]	66.892±1.76[b]	4.712±0.80[b]	9.677±0.57[b]
pH 6.5	6.506±0.08[a]	6.519±0.09[a]	19.413±3.55[c]	68.015±3.51[a]	53.567±3.84[c]	4.791±1.15[a]	16.866±1.33[a]	65.193±2.67[c]	5.299±0.86[a]	9.95±0.75[a]
p(pH)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
p(interaction)	0.187	<0.001	0.042	0.751	0.951	0.986	0.055	0.992	0.016	<0.001

CL = calcium lactate; LY = lysine. Averages within the same column followed by the same letters are not significantly different (P > 0.05). Values represented as the Mean ± standard deviation (SD), n = 3.

Supplementary Table 13. Volatile flavour compounds in the headspace above pork patties (by SPME GC–MS), relative amounts are mean peak areas (/1000). Patties varied in salt, lysine, calcium lactate and pH.

Compound	Salt	LY	CL	LY+CL	p(ingredients)	pH 5.5	pH 6	pH 6.5	p(pH)	p(interaction)
Acids (2)										
Butanoic acid	3,457[a]	2,121[a]	2,975[a]	3,813	0.208	3,874[a]	3,381[a]	2,020[b]	0.041	0.354
Hexanoic acid	3,053[a]	2,718[ab]	1,835[b]	1,961[b]	0.027	4,338[a]	1,713[b]	1,130[b]	<0.001	0.037
Alkanes (1)										
2-Pentyloxirane	1,109[a]	1,387[a]	ND	1,002[a]	0.147	1,195[ab]	1,538[a]	916[b]	0.016	0.063
Alcohols (6)										
1-Penten-3-ol	1,952[a]	2,279[a]	1,798[a]	2,077[a]	0.676	1,822[a]	2,187[a]	2,235[a]	0.438	0.205
1-Pentanol	35,465[c]	29,171[b]	16,456[ab]	11,233[a]	0.013	23,260[a]	22,069[a]	36,791[a]	0.411	0.024
1-Hexanol	4,593[a]	4,319[a]	1,716[b]	3,072[ab]	0.044	3,858[a]	4,710[a]	3,337[a]	0.094	0.85
1-Heptanol	2,290[a]	2,074[a]	ND	1,563[a]	0.165	2,227[a]	2,643[a]	1,349[c]	0.013	0.628
1-Octen-3-ol	28,403[a]	25,311[a]	2,854[b]	8,782[b]	<0.001	25,333[a]	13,332[b]	12,847[b]	<0.001	0.132
1-Octanol	2,692[a]	1,740[b]	ND	1,823[b]	0.011	2,113[a]	2,615[a]	1,775[a]	0.06	0.123
Aldehydes (12)										
Butanal	ND	2,265[a]	ND	1,428[b]	0.024	2,263[a]	1,910[a]	1,300[a]	0.054	/
2-Methylbutanal	ND	1,114[b]	2,005[a]	1,251[ab]	0.016	ND	883[b]	1,954[a]	0.002	0.008
3-Methylbutanal	ND	209[b]	1,512[a]	874[a]	0.002	ND	714[b]	1,184[a]	0.005	0.011
Pentanal	93,411[a]	115,684[a]	25,445[b]	50,225[b]	<0.001	96,555[a]	55,482[b]	65,307[b]	0.007	0.04
Hexanal	1,370,748[a]	1,304,061[a]	380,231[b]	414,804[b]	<0.001	1,297,861[a]	677,431[b]	627,091[b]	<0.001	0.007
2-Hexenal, (E)-	1,560[a]	1,622[a]	1,175[a]	1,677[a]	0.328	1,459[a]	1,690[a]	ND	0.293	0.654
Heptanal	30,682[a]	19,477[b]	23,055[ab]	23,055[ab]	0.033	27,850[a]	24,716[ab]	17,878[b]	0.022	0.403
2-Heptenal, (E)-	3,668[a]	3,599[a]	2,821[a]	3,975[a]	0.087	4,516[a]	3,243[ab]	2,024[b]	0.001	0.609

Benzaldehyde	2,183[a]	1,774[ab]	807[c]	1,037[bc]	<0.001	2,065[a]	1,371[b]	1,189[b]	<0.001	0.703
Octanal	16,299[a]	12,115[ab]	5,515[b]	7,801[b]	0.006	11,851[b]	16,433[a]	9,145[b]	0.012	0.055
2-Octenal, (E)-	3,509[a]	2,657[a]	1,494[b]	2,130[b]	<0.001	3,780[a]	2,101[b]	1,400[b]	<0.001	0.258
Nonanal	20,636[a]	13,283[b]	4,021[c]	5,254[c]	<0.001	16,559[a]	8,891[b]	6,945[b]	<0.001	0.058
Furans (1)										
2-Pentylfuran	7,282[a]	7,643[a]	6,943[a]	7,648[a]	0.709	7,915[a]	7,269[a]	6,585[a]	0.278	0.635
Ketones (5)										
Acetol	1,229[b]	2,579[a]	1,186[b]	989[b]	<0.001	375[c]	1,185[b]	2,511[a]	<0.001	0.06
2,3-Pentanedione	3,938[a]	4,778[a]	4,995[a]	5,033[a]	0.747	4,793[a]	3,945[a]	5,175[a]	0.52	0.51
Acetoin	1,835[b]	1,849[b]	3,092[a]	3,553[a]	<0.001	1,060[c]	2,705[b]	3,983[a]	<0.001	0.057
2-Heptanone	2,404[a]	2,838[a]	983[b]	2,267[a]	0.011	2,517[a]	2,522[a]	1,933[b]	0.024	0.523
2,3-Octanedione	95,938[a]	88,785[a]	22,463[b]	38,498[b]	<0.001	91,208[a]	44,946[b]	51,312[b]	<0.001	0.176
Phenols (1)										
2-Phenoxyethanol	1,773[a]	1,303[b]	1,746[a]	1,121[b]	0.021	1,882[a]	1,740[a]	834[c]	<0.001	<0.001
Pyrazines (1)										
2-methylpyrazine	ND	903[a]	ND	ND	/	ND	349[b]	1,459[a]	/	/

4254 CL = calcium lactate; LY = lysine. ND means not detected. Averages within the same crow followed by the same letters are not significantly different (P > 0.05).