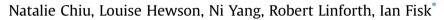
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Controlling salt and aroma perception through the inclusion of air fillers



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ABSTRACT

Global dietary sodium consumption significantly exceeds the WHO recommended intake levels, although strategies are available for sodium reduction, most are partial product-specific solutions. A wider range of approaches is urgently required to enable food manufacturers to reduce sodium within processed foods. In this study, the addition of air inclusions within hydrogels has been evaluated for its ability to enhance the delivery of sodium and perception of saltiness and was shown, on a volume basis, to achieve an 80% reduction in total sodium with no loss of saltiness perception; the addition of a congruent aroma volatile was shown to enhance overall flavour perception in foamed systems. Air inclusions were shown to increase both the delivery and perception of salt and aroma, in addition to increasing overall flavour perception. This work will be of interest to both academic researchers in this field and industrialists looking for new approaches to mitigate loss of taste quality with sodium reduction.

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1. Introduction

Sodium reduction remains as a primary strategy for the improvement of human health. National and international organisations encourage the general population to consume less salt and more importantly food manufacturers to lower sodium content in processed food products.

Although sodium is required for normal body functions, over consumption may lead to adverse health effects (He & MacGregor, 2007). Sodium chloride (salt) is the major source of sodium in human diets (He & MacGregor, 2010) and excessive consumption has been linked to the development of hypertension and adverse cardiovascular health (Brown, Tzoulaki, Candeias, & Elliott, 2009; Kempner, 1948; Page, Vandevert, Nader, Lubin, & Page, 1981; Ramsay et al., 1999). In most countries, the average salt intake is approximately 9–12 g/d (Brown et al., 2009), which far exceeds normal physiological needs and the current recommendations by the World Health Organisation of <5 g/d (WHO, 2007).

Processed and restaurant foods are estimated to account for up to 75% of sodium intake in developed countries, 10-12 % is naturally occurring in foods, whilst only 10-15 % is additionally added as salt during cooking or eating (Mattes & Donnelly, 1991). Thus,

participation by the food industry is vital in reducing sodium in the diet (He & MacGregor, 2003).

A range of different approaches have been used to reduce sodium levels, and in general multiple approaches are often the most successful when applied to food products. However, the developed approaches are often product specific and their application may not be applicable to all food products.

A frequent approach to salt reduction is the use of direct substitution with other ingredients to maintain sensory properties such as mineral salts (Desmond, 2006; Gou, Guerrero, Gelabert, & Arnau, 1996; Reddy & Marth, 1991). Although mineral salts are able to trigger a salty sensation, a common disadvantage of these substitutions is the presence of bitter off-tastes, which limits the level of substitution (Kilcast & den Ridder, 2007). The multifunctional nature and unique taste of sodium chloride has yet to be matched by any other chemical (Angus, 2007; Kilcast & den Ridder, 2007; McCaughey & Scott, 1998), adding to the complexity of sodium reduction.

Another approach is the use of congruent aroma volatiles, exploiting taste—aroma interactions (Batenburg & van der Velden, 2011; Bonorden, Giordano, & Lee, 2003; Cliff & Noble, 1990). This effect has been shown to be successful for both sweet (Hort & Hollowood, 2004) and salty tastes (Lawrence, Salles, Septier, Busch, & Thomas-Danguin, 2009). However, multiple factors can influence taste—aroma interactions, such as prior exposure and pleasantness (Djordjevic, Zatorre, & Jones-Gotman, 2004; Schifferstein & Verlegh, 1996).

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The influence of hydrocolloid thickeners on taste and aroma perception has received much attention. It has been reported that hydrocolloid thickened solutions above a specific viscosity (c*) supress both taste and aroma perception (Baines & Morris, 1987; Cook, Linforth, & Taylor, 2003).

Studies have explored oil-in-water emulsions, using oil droplets as fillers thereby replacing the water phase within the system (Bayarri, Smith, Hollowood, & Hort, 2007; Drewnowski & Schwartz, 1990; Yamamoto & Nakabayashi, 1999). The addition of oil replaces the water, causing an increase in taste perception as the concentration of tastant effectively increases in the aqueous phase. However, the additional oil can lead to mouth coating (Yamamoto & Nakabayashi, 1999) and additional oil in products is undesirable for health, therefore limiting its application.

Control over the temporal delivery of tastants from the bolus to saliva has been previously shown (Rama et al., 2013; Tian & Fisk, 2012) to control taste perception and could act as one approach to enhance sodium perception. It is well known that increasing the concentration of a tastant within a liquid phase will increases perceived taste intensity, therefore techniques where tastant concentrations are effectively increased through substitution of the water phase is an appealing way to reduce sodium in the diet. A previous study reported that overall taste perception was dependent on the tastant concentration in the aqueous phase and was irrespective of the volume fraction of the air inclusions (Goh, Leroux, Groeneschild, & Busch, 2010).

The objective of this study is to evaluate the impact of air as a filler particle within hydrogels and its impact on the temporal delivery of salt and congruent aroma delivery in-nose, and its resulting impact on saltiness and overall flavour perception.

2. Material and methods

2.1. Sample preparation

An aqueous solution of containing 2 g/100 mL type B bovine gelatine (Sigma-Aldrich Ltd., Dorset, UK), 5 g/100 mL whey protein isolate (Davisco, Minnesota, USA) and between 0.6 g/ 100 mL and 3.3 g/100 mL sodium chloride (Sigma-Aldrich Ltd., Dorset, UK) was stirred and heated to 60 °C. 1-octen-3-ol, a mushroom aroma volatile (Log P 2.73), was then added to samples S6, S7 and S8 as indicated in Table 1 producing a concentration of 0.8 μ L/L. The solution was immediately placed into an ice bath and sheared at 3000 rpm using a high shear overhead mixer (L5M, emulsor screen, Silverson, Chesham, UK). Different durations were used to achieve the required volume. Air inclusion fraction was calculated by subtracting the original volume of the solution before shearing from the volume of the solution after shearing. The foams were stored at 4 °C and were stable for up to 24 h, all samples were prepared and analysed within 14 h Table 1 displays the formulations of the gel samples that were used for sensory testing.

2.2. Sodium concentration

Flame photometry (Sherwood Scientific Ltd., Model 410, Cambridge, UK) was used to evaluate sodium concentration, sodium standards ($0-1 \mu L/L$) were prepared for calibration. The calibration curve demonstrated repeatability ($R^2 > 0.99$), and linearity up to 1 $\mu L/L$, wavelength 589 nm. The sodium data was collated in triplicate and converted to sodium chloride concentration (×2.5).

2.3. Sensory evaluation

96 untrained panellists (aged 19–61; 55 females and 41 males) were recruited to conduct a series of paired comparison (PC) tests (BS EN ISO 5495:2007). The same volume of sample (6 mL) was prepared individually on plastic spoons and left to equilibrate at room temperature (21 °C). For each sample, panellists were required to place the sample in their mouth for 10 s, the tongue was moved up and down three times before swallowing. The samples were presented in pairs on plastic spoons each labelled with a random three-digit code and panellists were asked to select the sample they perceived as saltiest overall, each sample was evaluated against every other sample in a balanced design resulting in 3 PC tests. The first set of samples, S1, S2 and S3, contained equal concentrations (6.6 g salt/L) of salt in the aqueous phase and 0%, 40% and 80% air inclusions respectively, (S1, S2 and S3 contained total sodium contents of 198 mg, 118.8 mg and 39.6 mg respectively). The following series of samples, S1, S4 and S5, contained increasing air inclusions from 0 % to 40 % and 80% respectively whilst overall weight amount of salt was kept constant (198 mg). Again each sample was evaluated against every other resulting in a further 3 PCs. Finally, panellists were asked to perform PCs selecting the sample that was perceived to be most intense in overall flavour, where a mushroom aroma volatile was applied. Samples S6, S7 and S8 were used in these tests and contained the same concentration of 1-octen-3-ol aroma volatile (0.8 μ L/L) and the same overall salt concentration (6.6 g/L) in all samples (samples varied in air inclusion, 0%, 40% and 80% respectively).

The test was used in forced-choice mode, so panellists were required to give an answer even if the perceived difference was negligible. Plain crackers (99% Fat Free, Rakusen's, Leeds, UK) and mineral water (Evian, France) was supplied for panellists to palate cleanse between samples, rest breaks were given between every 3 PC. All tests were carried out within individual sensory booths under northern hemisphere lighting and controlled temperature and humidity. Consensual answers were compared to data tables to determine significance (BS EN ISO 5495:2007), $\alpha = 0.05$ for difference testing, $\alpha = 0.2$, $\beta = 0.05$ and $p_D = 30\%$ for similarity testing.

2.4. Sodium ion release

The rate of dissolution of sodium from a 6 mL sample to a beaker of deionised water (200 mL) was evaluated every 10 s over 150 s,

Table 1	l
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Formulation of samples used for sensory testing

Sample code	Air inclusion (%v/v)	Solution (mL)	Salt (mg)	Air inclusion (mL)	Total volume (mL)	Salt concentration in aqueous phase (g/L)	1-octan-3-ol (µL/L)
S1	0	30	198	0	30	6.6	0
S2	40	18	118.8	12	30	6.6	0
S3	80	6	39.6	24	30	6.6	0
S4	40	18	198	12	30	11	0
S5	80	6	198	24	30	33	0
S6	0	30	198	0	30	6.6	8.0
S7	40	18	118.8	12	30	6.6	8.0
S8	80	6	39.6	24	30	6.6	8.0

conductivity (Hanna Instruments, Michigan, USA) was used to determine the concentration of ions released over time in triplicate. The temperature was maintained at 37 $^{\circ}$ C using a water bath and the solution was stirred at 500 rpm.

2.5. In-vivo aroma release

Gas chromatography – mass spectrometry (GC–MS) analysis was used to quantify 1-octen-3-ol. The concentration of 1-octen-3-ol in all samples was compared to ensure volatile concentration was maintained at similar levels during preparation, thereby removing concentration differences of aroma volatiles as a variable of perceivable difference. An equal concentration of 0.8 μ L/L 3-heptanone (Sigma–Aldrich, Dorset, UK) was added in dichloromethane to the sample and left on a roller mixer to equilibrate for 3 h.

2 mL of the solvent was pipetted into a 2 mL GC vials (SLS Ltd, Nottingham, UK) and 1 μ L of the sample was injected into the injector port of a Trace 2000 Series GC (Thermo Scientific, Massachusetts, USA) using an AS 3000 autosampler (Thermo Scientific, Massachusetts, USA). The column was ZB WAX, 30 m \times 0.25 mm i.d. x 1 μ m film thickness (Phenomenex, Macclesfield, UK). The temperature programme for the oven was maintained at 50 °C for 1 min after injection and then ramped at 10 °C/min to 250 °C over 3 min. Analytes were detected in triplicate using a DSQ II mass spectrometer (Thermo Scientific, Massachusetts, USA) operating in full scan mode from 50 to 250 *m*/*z* at 1.6 scans/s. 1-octen-3-ol peak area was compared to that of the internal standard, 3-heptanone, to calculate the concentration of 1-octen-3-ol, and a two-tailed t-test showed no significant differences between the samples.

Real-time gas phase release of 1-octen-3-ol was measured using the atmospheric pressure chemical ionisation mass spectrometry (APCI-MS-NOSE) (Fernández-Vázquez et al., 2013). Seven panellists were recruited from the University of Nottingham (aged 19–30; 4 females and 3 males) and asked to follow a fixed predefined oral processing protocol, panellists consumed two different samples (S6, and S8) with equal concentration of 1-octen-3-ol. Panellists were asked to place their nose on a sampling tube, breath in, consume the whole sample, close their mouths, breath out and in at a specific time, swallow the sample and continue breathing out and in as indicated. Data was collected in triplicate. Exhaled aroma was monitored at m/z 129 and the relative abundances of 1-octen-3-ol within the gas phase were calibrated against known standards prior to and at the end of each analysis run.

3. Results and discussion

3.1. Sensory evaluation

Samples with up to 80% reduction in total sodium content were directly compared, the results of the paired comparison tests for overall saltiness perception with equal concentrations of salt in the aqueous phase (Fig. 1) indicated no significant difference in salt perception (P > 0.05). Indeed, evaluation of results provided sufficient evidence to conclude similarity between the samples despite the highly significant reduction in total sodium (198 mg reduced to 39.6 mg). It was therefore shown that less salt per product volume was required to maintain overall perceived saltiness after the inclusion of air as a filler particle. This suggests that salt perception is not driven by the amount of salt in the total volume but rather the concentration of salt in the continuous phase and that inclusion of air did not obstruct taste perception of the samples. The results contrast the findings from previous studies where oil and solid fillers were applied as a mouth coating effect and solid content obstructs the diffusion of tastants for perception, respectively

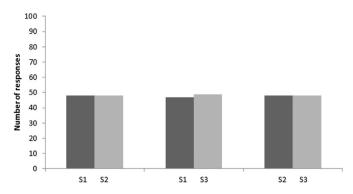


Fig. 1. Two-sided paired comparison tests for saltiness where samples contained equal salt concentrations in the aqueous phase (6.6 g/L) and varying total salt amounts per sample. S1 contained 198 mg salt, S2 had a 40% sodium reduction (118.8 mg) and S3 had an 80% sodium reduction (39.6 mg).

(Kokini, Bistany, Poole, & Stier, 1982; Yamamoto & Nakabayashi, 1999). It is suggested that the air bubbles rupture during consumption, removing any physical barrier, and thus allowing tastants to successfully reach the taste buds and be perceived (Goh et al., 2010). Panellists were asked to follow a specific protocol, including specific tongue movement, however if actions including free movement and chewing was permitted, the disruption of the structure and consequently perception is presumed to increase.

The overall saltiness perception of samples with equal weight of salt and different air inclusions is shown in Fig. 2. The results indicate that samples containing air inclusions were perceived to be significantly saltier compared to the sample containing no air (P < 0.05). Despite having the same overall weight of salt, the saltiness increased significantly with increasing inclusions of air.

When comparing a non-aerated and aerated system of equal volumes with the same amount of salt on a volume basis, the latter system uses air to replace the aqueous phase, making the salt concentration in the aqueous phase more concentrated in comparison. Thus samples with more air inclusions were perceived as overall more salty which supports similar results previously reported by Goh et al., (2010).

Panellists were asked to rate the overall flavour intensity of the samples containing 1-octen-3-ol, 1-octen-3-ol was selected as it is commonly encountered in many salty food products and can be considered congruent with saltiness (Cook et al., 2003). Samples were comparable to those presented in Fig. 1 but contained 1-octen-3-ol at 0.8 μ L/L. Whilst it was previously shown that there was no difference in saltiness across the three samples, the

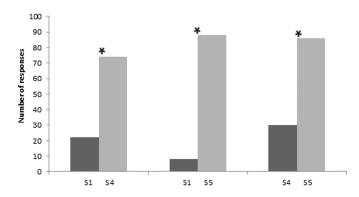


Fig. 2. Two-sided paired comparison tests for saltiness where samples contained equal weights of salt (198 mg) and varying total sodium concentrations in the aqueous phase. S1, S4 and S5 contained 6.6 g/L, 11 g/L and 33 g/L continuous phase respectively. *P < 0.05.

aeration (40% and 80%) of samples containing 1-octen-3-ol resulted in a significant (P < 0.05) enhancement in flavour intensity (Fig. 3). The concentration of salt in the continuous phase was constant across all samples presented in Fig. 3, therefore the difference in flavour intensity is proposed to be due to the enhanced delivery of 1-octen-3-ol.

The addition of air altered the appearance and texture of the samples. Visual differences were observed between the samples, as samples with 0% air inclusion were noted to be clear compared to samples with air inclusions which were opaque foams. Texture differences were also found in a previous study although minimal influence on taste perception was reported (Goh et al., 2010). In the current study, panellists were instructed to focus only on either overall saltiness or total flavour intensity, although multimodal interactions involving appearance and textural variation cannot be ruled out. It is important to consider the type of product this method of salt reduction could be utilised for, such that appearance and texture differences due to air inclusions do not impact on the consumer acceptance of the product whilst allowing the enhanced mixing offered by the bubble collapse to maximise sodium perception.

3.2. Sodium ion release

Conductivity measures the concentration of dissolved sodium, which has been ionised in a solution (Ge, Hardacre, Nancarrow, & Rooney, 2007). Fig. 4 shows the conductivity readings at 37 °C of the samples as they dissolve over time. Samples increase in conductivity over time indicating that the sodium and chloride ions are moving from the sample into the continuous phase. As air inclusion increases, the rate of dissolution increases and samples containing air, regardless of volume inclusion, reached peak conductivity prior to the sample with no air included. As air inclusion increases, the surface area within each sample increases proportionately. Assuming that the samples perform similarly inmouth, this would increase the amount of sample that is in contact with the saliva. This allows more sodium ions to reach the taste receptor cells and subsequently be perceived at an increased rate, resulting in the samples with more air included being perceived as overall more salty, within the timeframe of oral processing. It is also important to consider differences in-mouth, such as the surface roughness of the tongue and palate and level of saliva in-mouth which can contribute to the extent of structure disruption and perception.

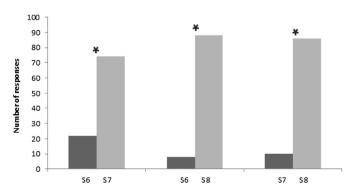


Fig. 3. Two-sided paired comparison tests for overall flavour perception where samples contained 1-octen-3-ol at 8 μ L/L and equal salt concentrations in the aqueous phase (6.6 g/L) and varying total salt amounts per sample. S6 contained 198 mg salt, S7had a 40% sodium reduction (118.8 mg) and S8 had an 80% sodium reduction (39.6 mg). *P < 0.05.

3.3. In-vivo aroma release

Prior to measuring *in-vivo* release of the aroma compounds, the concentration of aroma volatile in samples consisting 0% and 80% air inclusion must have a similar aroma concentration present for comparison. Sheering of the solution to incorporate air had no significant effect on the relative abundance of 1-octen-3-ol (P > 0.05) and the aroma concentration within the two systems was comparable.

MS-Nose was used to measure the concentration of 1-octen-3-ol in exhaled breath during consumption. The maximum in nose concentration occurred after swallowing samples containing 0% and 80% air inclusion (Fig. 5a). Although individual variations were detected, similar trends were observed for all panellists where the maximum in nose concentration was greater for 80% air inclusion. The maximum in nose concentration is significantly higher in the sample with 80% air inclusion compared to 0% air inclusion (P < 0.05). A possible explanation for the maximum in nose concentration being greater in the aerated sample could be due a faster liberation of aroma volatiles as the sample is less dense resulting in a quicker breakdown of the matrix (Mestres, Moran, Jordan, & Buettner, 2005; Wilson & Brown, 1997). This subsequently leads to a greater flux of aroma compounds being delivered retronasally to the oronasal cavity (Buettner, Beer, Hannig, & Settles, 2001).

In Fig. 5b the total release (total area under the curve) of 1octen-3-ol was recorded and similarly to the maximum in nose concentration the overall release of 1-octen-3-ol was significantly greater for the sample containing 80% air inclusion (P < 0.05). The increase in aroma release could be due to an increased surface area between the sample and oral cavity where air is introduced to the sample creating air pockets, which increases volatile release (Van Ruth et al., 1994; Yu et al., 2012). The action of movement of the tongue followed in the protocol will further increase the exposed surface area enhancing aroma volatile release. The microstructure of the foam also allows aroma volatiles to be entrapped within the air pockets and the volatile therefore is rapidly released during mastication (Zúñiga & Aguilera, 2008), thus accelerating aroma release from the matrix prior to swallowing. Diffusion rates in air are much faster than those shown in solid/semi-solid gels, this could additionally contribute to an increased rate of release of 1octen-3-ol from the aerated samples.

The work presented demonstrates enhancement of both salt and flavour perception solely by increasing the proportion of air inclusions, this is consistent with previous reports by Goh et al. (2010). Similar results have also been shown in other studies by increasing the oil phase volume in oil-in-water emulsions (Bayarri et al., 2007; Drewnowski & Schwartz, 1990; Malone, Appelqvist, & Norton, 2003; Yamamoto & Nakabayashi, 1999); the limitation of the mouth coating effects shown previously to suppress saltiness are not observed using air inclusions. This study demonstrates the rate of diffusion of tastant increases with increasing air inclusion, contributing to greater salt being made available at the receptor and hence an increased perception of saltiness. The presentation of a complimentary aroma was able to enhance total flavour perception in samples with increasing air inclusions. The rupture of the air bubbles during oral processing is believed to increase delivery of aroma and tastants and consequentially saltiness and flavour perception. Minor, Vingerhoeds, Zoet, De Wijk, and Van Aken (2009) and Weel et al. (2002) demonstrated that texture-aroma interactions could altered perceived flavour intensity in gels of different hardness (harder gels were perceived as less intense in flavour perception). Aerated food gels have been shown within this work to have the potential to control tastant and aroma delivery and are directly applicable to food applications (Zúñiga & Aguilera, 2008), this would be especially applicable in multiphase foods

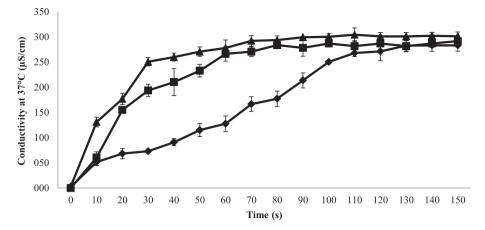


Fig. 4. Conductivity over time of samples with 0% (\diamond), 40% (\blacksquare) and 80% (\blacktriangle) air inclusion and equal weights of salt, referring to samples S1, S2 and S3, respectively. Data is expressed means of three replicates \pm standard deviation.

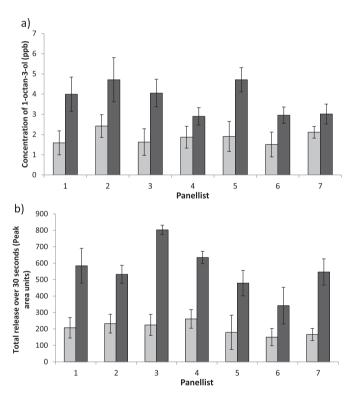


Fig. 5. A maximum aroma release (a) and total aroma release (b) from nose of 1-octen-3-ol during consumption of samples consisting 0% (**II**) and 80% (**II**) air inclusion. Data is expressed as means of three replicates \pm standard deviation.

containing hydrogels however careful consideration of the nature of the volatile, tastant and matrix is important for future research or industrialisation of the results.

4. Conclusions

In this study, the impact of salt reduction (80% salt reduction, volume basis) on loss of saltiness perception has been effectively mitigated through the use of air inclusions (40% and 80%); air inclusions were also shown to enhance sodium perception for equal sodium contents which was explained by the enhanced sodium delivery rates, this was further demonstrated *in vitro*. When applying an aroma to taste, air inclusions were able to enhance

flavour perception and the delivery of aroma was shown to be more efficient with air inclusions. In conclusion, the use of air inclusions has been shown to increase both the delivery and perception of salt and aroma, leading to increasing overall flavour perception.

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