



Strategies for sodium reduction in foods

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ABSTRACT

Urgent actions are needed to stall the sustained increase in the prevalence of hypertension, a major risk factor for cardiovascular diseases. Population based interventions to reduce sodium intake have proven to be highly cost-effective initiatives to accomplish this goal. In this line of action, endeavours have mainly focused on reducing the sodium content in processed food products, which are major contributors to dietary sodium. However, the implementation of salt (sodium chloride) reduction programmes wrestles with the negative impact that sizeable sodium reductions cause on consumer perception. In this context, the general aim of this thesis was to study different strategies for sodium reduction in foods from a sensory perspective. Two widely consumed food products that contribute a large proportion of dietary sodium in the Uruguayan population were considered as case study: white bread and white rice. The main focus was placed on product reformulation but the impact of communicating excessive sodium content on consumer perception was also addressed. Results added to the evidence that the salt content in food significantly affects consumer sensory and hedonic perception. The gradual salt reduction strategy proved promising for successfully reducing the sodium content in foods while maintaining consumers unaware of the change. Salt reductions close to 11% in rice and white bread could be regarded a safe criterion for removing salt without affecting consumer perception. While this approach has proved effective in reducing salt in food, its main disadvantage is the time needed to meet the target reduction level. Combining this strategy with partial replacement of 30% sodium chloride by potassium chloride could contribute to quicker reductions in sodium intake. The inclusion of front-of-pack sodium warnings nudged consumers towards food products with lower salt content and proved promising in shifting consumer hedonic perception. Results from the thesis are expected to provide guidance for the implementation of salt reduction strategies at the population level and contribute to improve public health.

RESUMEN

El sostenido aumento en la prevalencia de hipertensión, uno de los principales factores de riesgo para el desarrollo de enfermedades cardiovasculares, es motivo de creciente preocupación a nivel mundial. Para hacer frente a esta situación se ha exhortado a los países a tomar acciones urgentes orientadas a mejorar el estado de salud de la población. La reducción de la ingesta de sodio a nivel poblacional ha mostrado ser una iniciativa costo efectiva para lograr este objetivo, dado el estrecho vínculo entre el consumo de sodio y la presión arterial. En esta línea de acción, los esfuerzos se han focalizado principalmente en la reducción del contenido de sodio de productos procesados, que constituyen una fuente importante de sodio en la dieta. Sin embargo, la reducción del contenido de sal (cloruro de sodio) de los alimentos se enfrenta a una enorme dificultad: la preferencia del ser humano por el gusto salado y el impacto negativo de la reducción de sodio sobre la percepción del consumidor. En este contexto, el objetivo de la tesis fue estudiar diferentes estrategias de reducción de sodio en alimentos desde un enfoque sensorial. Para ello se consideraron dos productos extensamente consumidos por diversos segmentos de nuestra población y con gran aporte de sodio a la dieta: pan y arroz. La tesis se focalizó en la reformulación de alimentos hacia contenidos menores de sodio pero también se abordó el impacto de comunicar contenido excesivo de sodio sobre la percepción del consumidor. Los resultados reafirmaron que el contenido de sal de los alimentos afecta significativamente la percepción sensorial y hedónica del consumidor. La estrategia de reducción gradual mostró gran potencial para disminuir el contenido de sodio en alimentos sin que los consumidores perciban el cambio. Los resultados sugieren que pueden implementarse reducciones de sal cercanas al 11% en arroz y pan blanco sin afectar la percepción del consumidor. No obstante, la principal desventaja de la reducción gradual es el tiempo requerido para disminuir la ingesta diaria de sodio a los niveles establecidos por las metas regionales y globales. En este sentido, la combinación de esta estrategia con la sustitución parcial de 30% de cloruro de sodio por cloruro de potasio mostró ser una alternativa prometedora para lograr reducciones más rápidas. La inclusión de advertencias nutricionales en el frente del paquete favoreció la elección de productos con bajo contenido de sodio y mostró enorme potencial para modificar la percepción hedónica del consumidor. Se espera que los resultados de esta tesis proporcionen insumos valiosos para el diseño e implementación de programas de reducción de sodio a nivel poblacional.

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LIST OF PUBLICATIONS

The following articles have been published in refereed international journals during the development of this thesis:

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The following abstracts were presented in scientific congress during the development of this thesis:

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- Antúñez, L., Giménez, A., Alcaire, F., Vidal, L., Ares, G. Sustitución parcial de sodio por cloruro de potasio como estrategia de reducción de sodio en pan. Poster presented at the II Congreso de la Asociación Española de Profesionales del Análisis Sensorial, Velence, Spain, 18-20 October, 2017.
- Antúñez, L., Giménez, A., Alcaire, F., Vidal, L., Ares, G. Do single and multiple bite evaluations provide the same information? A case study with salt reduced bread samples. Poster presented at the 12th Pangborn Sensory Science Symposium, Providence, RI, United States, 20-24 August, 2017.
- Antúñez, L., Giménez, A., Alcaire, F., Vidal, L., Ares, G. Exploring consumers' reaction toward salt reduction in white rice: contributions from consumer segmentation (2018). Poster presented at Eighth European Conference on Sensory and Consumer Research, Verona, Italy, 2-5 September, 2018.
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- Rojas-Rivas, E., Cuffia, F., Antúñez, L., Otterbring, T., Aschemann-Witzel, J., Giménez, A., Ares, G. Time orientation and risk perception modulate the influence of nutritional warnings on food choice: Case study with bread packages. Poster presented at ISBNPA Annual Meeting, Prague, Czech Republic, 4-7 June, 2019.
- Antúñez, L., Alcaire, F., Giménez, A., Ares, G. Can sodium warnings modify preferences? A case study with bread. Accepted as a Poster Presentation for the ISBNPA 2020 Annual Meeting in Auckland, New Zealand. The meeting has been cancelled due to COVID-19 but all accepted abstracts will still be published in the ISBNPA 2020 Abstract Book.

TABLE OF CONTENTS

ABSTRACT	I
RESUMEN	III
ACKNOWLEDGEMENTS	V
LIST OF PUBLICATIONS	VII
TABLE OF CONTENTS	IX
INTRODUCTION	XI
LITERATURE REVIEW	1
OBJECTIVES	27
STRUCTURE OF THE THESIS	29
CHAPTER 1	31
CHAPTER 2	53
CHAPTER 3	65
CHAPTER 4	83
CHAPTER 5	111
CHAPTER 6	137
CHAPTER 7	149
CONCLUSIONS	175
REFERENCES	179

INTRODUCTION

Sodium is an essential nutrient for the normal physiological function of mammalian (Albraham et al., 1975; Elliot & Brown, 2007; McCaughey, 2007; World Health Organization [WHO], 2012). While humans evolved on low sodium diets (de Wardener & MacGregor 2002; He & MacGregor, 2007), the availability of this mineral in the food environment has markedly increased throughout history, mainly associated to the use of added salt (sodium chloride) in food manufacturing. This has triggered a deep change in sodium consumption patterns, leading to a daily intake well above nutritional requirements with adverse effects on health, particularly high blood pressure and cardiovascular diseases (He & MacGregor, 2007; WHO, 2012). In light of the current worldwide health situation, urgent action has been deemed necessary to stall the sustained increase in the prevalence of hypertension, a major risk factor for cardiovascular diseases. Population-based interventions to reduce sodium intake have proven to be highly cost-effective and feasible initiatives to accomplish this goal. In this context, endeavors have mainly focused on reducing sodium content in processed food products, that are major contributors to dietary sodium intake among most populations around the world (Campbell, Johnson, & Campbell, 2012; He & MacGregor, 2009; Stuckler, McKee, Ebrahim, & Basu, 2012; Trieu et al., 2015).

However, product reformulation towards lower salt contents faces the challenge of removing sizeable amount of salt while maintaining its essential functions in food products. Practical difficulties in finding salt substitutes that deliver the same functions have added further complexity to the topic (McCaughey, 2007). While salt has multiple functionalities as food ingredient, the present thesis mainly focuses on the sensory aspects of salt reduction. The microbiological stability and safety as well as other technological challenges arising from sodium reduction are beyond the scope of this work.

From a sensory perspective, the hedonic appeal of salty taste is likely the major barrier to sodium reduction (Bobowski, 2015). This gains more complexity in the current food environment, where individuals are repeatedly exposed to high salt processed food products which further contributes to develop preferences for high salt contents (Bobowski, 2015; Bobowski, Rendahl & Vickers, 2015b; Zandstra, Lion, & Newson, 2015). In this sense, a plethora of evidence have shown that salt reduction has a negative impact on overall flavour, affecting consumers acceptability (Beauchamp, Bertino, & Moran, 1982; Phelps et al., 2006; Rødbotten et al., 2015).

Different salt reduction strategies have been developed in attempts to overcome the main difficulties associated to sodium reduction and achieve sizeable salt reductions without negatively affecting consumer sensory and hedonic perception (Kilcast & den Ridder, 2007). There is now broad agreement that preferences for the salt content in food are malleable through experience, and that consumers will adapt, eventually, to lower salt contents (Ventura & Worobey, 2013). This lays the groundwork for the gradual salt reduction approach, which basically consists on the implementation of unnoticeable cumulative changes to modify the composition of food products without affecting consumer perception (Bobowski, Rendahl, & Vickers, 2015a). The gradual nature of this approach provides a means to cope with the negative effect of sodium removal on consumer perception. However, recommendations for the implementation of gradual sodium reduction strategies are still lacking.

While the gradual approach has proven effective for reducing salt content in foods, its main limitation is the time needed to meet the sodium target. The combination of this strategy with other product reformulation approaches provides an opportunity to circumvent the apparent shortcomings of gradual reduction (Busch, Yong, & Goh, 2013). Different salt reduction strategies have been proposed to fulfil this goal, including the replacement of sodium chloride with other salts, such as potassium chloride (Liem, Miremedi, & Keast, 2011; Phelps et al., 2006) and the non-homogeneous spatial distribution of salt in food products (Noort, Bult, & Stieger, 2012; Noort, Bult, Stieger, & Hamer, 2010; Mosca, Bult, & Stieger, 2013).

In addition, the inclusion of front-of-pack (FOP) nutritional labelling to make information more accessible has been regarded a key pillar for empowering consumers to make informed food choices and nudge them towards lower sodium intakes (Campbell et al., 2012; Downs et al., 2015; Regan, et al., 2017; Webster, 2015). In particular, nutritional warnings have emerged as a promising alternative to discourage unhealthy food choices (Khandpur, Swinburn, & Monteiro, 2018). Yet, further research is needed to shed light on how sodium warnings impacts on the consumer perception.

In this context, this thesis aimed to explore different strategies for salt reduction in foods, striving to offer a comprehensive discussion on their potentialities and drawbacks. Results from the thesis are expected to provide guidance for the design of salt reduction strategies and contribute to improve public health.

LITERATURE REVIEW

LITERATURE REVIEW

Sodium: An essential nutrient

Sodium is the most prevalent cation in the extracellular fluid in the body (Albraham et al., 1975). It is an essential nutrient for the normal physiological function of mammalian, being involved in vital processes, such as the generation of the membrane potential of cells, normal cell function, maintenance of plasma volume and water balance in the body (Albraham et al., 1975; Elliot & Brown, 2007; McCaughey, 2007; WHO, 2012). Although the minimum sodium intake necessary for proper bodily function has not yet been defined, it is estimated to be between 200 and 500 mg/day (WHO, 2012).

To date, the reasons why humans desire and consume sodium in the absence of biological need remains unknown (Schulkin, 1991). Humans have evolved on low sodium diets, being exposed only to sodium occurring naturally in foods (plants and animals) and water, which typically account for less than 200-250 mg/day (Denton, 1982; Elliot & Brown, 2007; Campbell et al., 2012). The characteristics of the food environment determined humans to be genetically programmed to low sodium intakes and to develop efficient mechanisms to safeguard the organism against sodium deficiency (de Wardener and MacGregor, 2002; He & MacGregor, 2007). Two body functions play a key role in fulfilling this goal: (i) physiological retention and conservation of the limited sodium naturally present in food within the body, and (ii) identification of sodium sources in the environment (McCaughey, 2007). Physiological retention is accomplished by sodium reabsorption in the kidney, and other organs, reducing to almost zero sodium excretion in urine and sweat. The identification of sodium sources is achieved through salty taste, a specialized mechanism for detecting sodium chloride (NaCl) and salts of a few other cations (Lewandowski, Sukumaram, Margolskee, & Bachmanov, 2016).

Salty taste perception

Taste is a sensory modality that involves the oral perception of food-derived molecules which stimulate receptors located in the oral cavity and pharynx (Breslin, 2013). In order to understand the mechanisms that may be involved in salty taste transduction, a basic understanding of the oral anatomy and physiology is necessary. Taste buds are the functional units of taste transduction and constitutes the first step of gustatory signal processing. These structures are located in three different types of gustatory papillae (circumvallate, foliate and fungiform), which are distributed in different parts of the tongue and also reside in the flat epithelium of the soft palate and pharynx (Breslin, 2013; Lawless & Heymann, 2010). Taste buds consist of groups of 30-50 taste receptor cells (TRCs) that are clustered in a layered ball. TRCs are not independent receptors within the taste bud, rather they communicate with each other for common

LITERATURE REVIEW

signalling functions (Breslin, 2013). There are three morphological types of taste receptor cells: Type 1 TRCs; Type 2 TRCs (express T1R or T2R receptors for detection of sweet, bitter, and umami substances) and Type 3 TRCs (involved in sour taste transduction) (Menon & Chen, 2019). These cells transmit the taste signal to higher processing centres of the brain through different peripheral nerves that innervate the oral cavity: the chorda tympani branches of the facial nerves (cranial nerve VII), the glossopharyngeal nerves (cranial nerve IX) and the vagus nerve (cranial X) (Breslin, 2013; Lawless & Heymann, 2010).

The current body of evidence suggests that different salt (NaCl) concentrations elicit two distinct behavioural responses: appetitive low concentration (<100 mM) and aversive high concentration (>300 mM) responses (Menon & Chen, 2019). At least two distinct salt transduction mechanisms are thought to be involved in salty taste perception in humans. These mechanisms have been classified into two categories according to their sensitivity to amiloride, a diuretic drug that blocks the epithelial sodium channel (ENaC): amiloride-sensitive (AS) and amiloride-insensitive (AI) (McGregor, 2007; Menon & Chen, 2019; Roper, 2015). While important contributions have been done in elucidating the molecular machinery underlying salt transduction mechanisms, the full picture remains unknown and it continues to be an active area of research.

The amiloride-sensitive (AS) mechanism was elucidated the first. This pathway mediates low salt detection (Menon & Chen, 2019) and selectively responds to sodium (and lithium) salts. It has been theorized that this mechanism is mediated by the interaction of sodium ions with a membrane channel, identified as the epithelial sodium channel (ENaC) (Roper, 2015). Several studies in mice have shown that amiloride hydrochloride causes a downshift in the chorda tympani nerve responses when NaCl solutions are applied on the tongue, giving further support that ENaC is involved in sodium transduction (McGregor, 2007).

On the other hand, the underlying transduction mechanism for the amiloride-insensitive (AI) pathway has remained elusive, as are the identity of the taste cell types involved in this pathway (Lewandowski, et al., 2016, Roper, 2015). What is well-established is that this mechanism is related to high-salt responses and is cation non-selective (Lu, Breza, & Contreras, 2016).

Lyall et al. (2004) have suggested that the mechanism underlying amiloride-insensitive salty taste transduction may be associated to the TRPV1 receptor variants. However, later research provided evidence that high-salt responses persisted in mice lacking these channels, creating uncertainty on the role this receptor plays in the AI salty

taste transduction (Ruiz, Gutknecht, Delay, & Kinnamon, 2006; Treesukosol, Lyall, Heck, DeSimone, & Spector, 2007). Other studies have postulated that Type 3 TRCs of the circumvallate papillae were involved in this mechanism (Ninomiya, 1998; Lewandowski et al., 2016). In line with this, Oka, Butnaru, von Buchholtz, Ryba and Zuker (2013) found that blocking the synaptic transmission of Type 3 TCRs in mice caused a depletion in amiloride-insensitive high salt responses in the chorda tympani nerve, suggesting these receptors not only play a key role in sour transduction but also may be involved in the AI salty taste mechanism. More recently, Roebber, Roper, and Chaudhari (2019) suggested that Type 3 TCRs may modulate high salt responses through interaction with other types of TRCs but they are not directly involved as salt receptors (Roper & Chaudhari, 2017). In addition, these authors provided evidence that Type 2 TRCs in fungiform papillae may mediate the AI high salt transduction mechanism and that this mechanism is markedly dependent on the presence of the Cl⁻ anion. This result is consistent with previous studies reporting a modulating effect of the anion size on the nerve response for the AI pathway (Ye, Heck, & DeSimone, 1991). While the actual contribution of the cation to this pathway remains to be elucidated, evidence has shown that substituting KCl for NaCl reduced high salt responses by 50% (Roebber et al., 2019). Based on the latest evidence, it has been theorized that the mechanism underlying salty taste coding varies across different types of TRCs in anatomically and functionally different gustatory papillae.

Beyond uncertainties regarding the AI salty taste transduction mechanisms, it is well-recognized that sodium ions cause the depolarization of TRCs, lead to neurotransmitter release and the gustatory signal is sent to the brain via branches of the facial, glossopharyngeal, and vagus nerves (McCaughey, 2007). Studies in rats revealed that despite all these nerves respond to the NaCl stimuli, they are not equally important for saltiness perception (McCaughey, 2007). Further research on the mechanism behind sodium perception may provide valuable insights for the development of sodium reduction strategies.

Added salt consumption throughout history

Although the human organism has successfully developed highly efficient protection mechanisms from sodium deficiency, the availability of sodium in the food environment markedly increased throughout the history. The development of agriculture and animal husbandry, about 6000-8000 years ago, brought the need to have food reserves (Stamler, 1993). In this context, salt (NaCl) became one of the most traded commodities in the world and make major contributions to the development of settled communities, due to its preservation properties. It was called '*natron*' (divine salt) by

LITERATURE REVIEW

Egyptians, while in ancient Rome the Latin term '*salarium*' meant the amount of salt a worker or Roman legionary received as payment for his job (Albarracín, Sánchez, Grau, & Barat, 2011). Initially, salt production was expensive and, therefore, it represented a luxury. Then, with the advent of salt mining it became a widely used commodity. This set a turning point in sodium intake at the population level. Salt was not only used for preservation purposes, but it also started to be added to fresh food, which began to taste bland relative to highly salted preserved foods (Stamler, 1993). Moreover, salt addition to food going putrid was found to mask bitterness, which further contributed to increase sodium intake (He & MacGregor, 2007).

The invention of refrigeration systems in the late nineteenth century marked a shift in food preservation methods and led to a decrease in sodium consumption. However, some years later, the development of the food industry gave back to salt an important role in food manufacturing, resulting in a gradual increase in sodium consumption at the population level (He & MacGregor, 2007). Salt has been linked to the evolution of food processing due to its multiple functionalities. The most widespread use of salt is to impart saltiness, to enhance flavour and suppress bitterness (Kilcast & den Ridder, 2007; Man, 2007; McGregor, 2007). However, salt also has other technological properties. It reduces water activity, contributes to antioxidant or prooxidant effects, improves water-holding capacity and preserves food (Breslin & Beauchamp, 1995; Gillette, 1985; Man, 2007). Besides, salt is involved in both the promotion and inhibition of enzymatic reactions, mainly related to the development of texture, colour, taste and aroma that are characteristic of a wide variety of food products (Albarracín et al., 2011). In addition, apart from NaCl, other sodium salts are added to food products for specific technological reasons. That is the case of the use of sodium bicarbonate as a leavening agent or sodium benzoate as a preservative (Busch et al., 2013).

Effect of excessive sodium consumption on health

The changes in sodium consumption patterns that have occurred late in human evolution represent a major challenge for the human physiological system. Not enough time has elapsed to adapt the organism to the excretion of large quantities of sodium through the kidneys into the urine. For this reason, humans continue to retain most of the sodium they consume (Denton, 1982; Stamler, 1993; He & MacGregor, 2007). The increase in sodium consumption has been linked to negative health consequences, particularly high blood pressure and cardiovascular diseases.

LITERATURE REVIEW

Scientific evidence for a link between salt intake and blood pressure was reported for the first time in the early 1900s (He & MacGregor, 2007). Based on data from five different population groups, Dahl (1960) established a positive linear relationship between the prevalence of hypertension and average salt intake. Moreover, a strong correlation between mortality from strokes and salt consumption was reported: death rates from strokes were markedly higher in the northern region compared to the southern region of Japan (27 vs. 14 g salt/day) (Elliot & Brown, 2007). Since then, a plethora of evidence supporting a causal relationship between salt intake and blood pressure (BP) has emerged from different lines of research, including epidemiology (Elliott et al. 1996), migration (Poulter et al. 1990), population-based intervention (Forte, Miguel, Miguel, De Padua, & Rose, 1989), clinical trials (He & MacGregor, 2002), animal studies (Denton et al., 1995) and genetic studies (Lifton, 1996). In particular, a stream of research in experimental animals has shown a dose-response curve between salt (sodium) intake and blood pressure: the higher the sodium intake the higher the blood pressure (Dahl, Knudsen, Heine, & Leitl, 1968). A study with chimpanzees, in which the usual salt intake (0.5 g/day) was altered towards higher salt levels (5, 10 and 15 g/day) provided further evidence on the dose-response relationship between sodium intake and blood pressure (Denton et al. 1995). A meta-analysis of randomized trials revealed that moderate reductions in dietary salt intake during a one-month period or longer led to a significant fall in blood pressure in both, normotensives and hypertensives individuals (He & MacGregor, 2002). Moreover, a consistent dose-response curve between salt intake and blood pressure was found for salt concentrations ranging from 3 g salt/day to 12 g salt/day (i.e. the lower the salt intake, the lower the blood pressure) (He & MacGregor, 2003). The reduction in systolic and diastolic blood pressure was independent of baseline sodium intake (WHO, 2012). In line with this, a systematic review and meta-analyses on children data provided evidence that reducing daily sodium intake results in a decrease in systolic and diastolic blood pressure in this population. Similar results have been found in two previous systematic reviews of the literature in children (WHO, 2012).

Hypertension, defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, has been widely recognized as a major risk factor for both stroke and coronary heart disease (CHD) (WHO, 2010). There is conclusive scientific evidence showing a positive, strong and linear relationship between blood pressure and risk of vascular mortality for systolic blood pressures equal to or higher than 115 mmHg. So far, there is no evidence on this trend to occur below this threshold (WHO, 2012).

The impact of sodium consumption on health is not limited to blood pressure. Other independent but additive harmful effects have been reported. High sodium intake

LITERATURE REVIEW

directly affects the cardiovascular system: a close association with stroke and left ventricular hypertrophy have been established (He & MacGregor, 2007). Thus, the effect of sodium reduction on cardiovascular disease may be larger than expected if only the effect on blood pressure was taken into account. Other health problems associated with high sodium consumption are stomach cancer, bone demineralization as well as the progression of renal disease and albuminuria (He & MacGregor, 2007). Excessive sodium intake represents a challenge for excretion by the kidneys and cause a higher urinary protein excretion which may in turn increase the rate of deterioration of renal function (WHO, 2012).

Downshifting sodium intake at the population level has not been reported to cause any adverse effect on health. In particular, reducing sodium has been shown not to significantly affect blood lipids, catecholamine levels or renal function (WHO, 2012). Thus, there is scientific community that reducing sodium intake is likely to be beneficial for most individuals, regardless of their current sodium consumption level (WHO, 2012).

Current sodium intake: A serious threat for public health worldwide

Globally, non-communicable diseases (NCDs) are responsible for about 60% of all death and 43% of disease burden (WHO, 2010). In particular, cardiovascular disease (CVD) is responsible for 48% of all deaths caused by NCDs and accounts for as much deaths as infectious disease, nutritional deficiency, maternal and perinatal conditions taken all together (WHO, 2005). About 55% of annual deaths from cardiovascular disease have been related to raised blood pressure (Lim et al., 2012). High blood pressure has been identified to be responsible for 62% of strokes and 49% of coronary heart disease (WHO, 2012). According to the latest report from the World Health Organization, hypertension affects nearly one out of three adults (WHO, 2014). In Uruguay, the prevalence of high blood pressure in adults aged between 25 and 64 have shifted from 30.4% in 2006 to 38.7% in 2013 (Ministerio de Salud Pública, 2015). Moreover, there is a global trend towards risen blood pressure in children (Estragó et al., 2018; He & MacGregor, 2007; WHO, 2012).

In light of the current global health situation, actions to tackle the sustained increase in the prevalence of hypertension are paramount (He & MacGregor, 2007; WHO, 2012). Population-based interventions aimed at reducing sodium intake have proven to be highly cost-effective and affordable initiatives to fulfil this goal. In this line of action, initiatives towards sodium reduction in food products have been recognized as one of the most feasible approaches to substantially improve population health in short time frames (Campbell et al., 2012; He & MacGregor, 2009; Trieu et al., 2015; Stuckler,

et al., 2012). Even small population-wide dietary salt reductions may be highly beneficial to public health, translating into major cost-savings for individuals, their families and the health service (He, Brown, & Tan, 2019)

The current WHO recommendation for adults is to reduce sodium intake to < 2 g (< 5 g salt) per day (WHO, 2012). When referring to children, this value should be adjusted taking into consideration their energy requirements relative to those of adults (WHO, 2012). Albeit the current recommendation is expected to cause a major benefit to public health, the current body of evidence suggests that a larger effect would be obtained if sodium intake were reduced to 3 g salt/day (WHO, 2012). In this sense, the current recommendation is more likely a compromise between the ideal sodium intake and feasibility, but there is still scope to further adjust this target (He & MacGregor, 2007; WHO, 2012).

Although dietary sodium intake may vary across populations, today, nearly all populations around the world consume sodium far in excess of nutritional requirements (Campbell et al., 2012). In Uruguay, daily sodium intake is estimated to be 3.7 g/day, about twice the WHO recommendation for this nutrient (Bove & Cerruti, 2008; Taroco, 2011).

World Health Organization (WHO, 2013) has made a strong recommendation to take immediate actions towards sodium intake reduction and encouraged all countries around the world to set it as a health priority. The member states of the WHO committed to a 30% reduction in average salt (sodium) intake at the population level by 2025 (Trieu et al., 2015; WHO, 2013). Besides, the WHO's 13th General Program of Work has given added momentum to the worldwide efforts towards salt reduction actions. Salt reduction efforts are aligned with the Sustainable Development Goal 3, which aims at reducing by one-third premature mortality from NCDs by 2030 (He et al., 2019).

Individual countries and regions have also set their own targets for downshifting sodium intake at the population level (He et al., 2019; SACN 2003). In particular, all MERCOSUR members have firmly committed to take actions towards reducing the sodium content in processed food products to achieve a mean population sodium intake below 2000 mg/day by 2020 (MERCOSUR, 2015).

LITERATURE REVIEW

Sodium sources

Efforts geared towards reducing sodium intake at the population level should adopt a comprehensive and intersectoral approach that is culturally relevant and contemplates the entire population (Regan et al., 2017). In this sense, getting insights into which are the main contributors to sodium intake is imperative and constitutes a first step for the design and implementation of public programmes to fulfil this goal.

Dietary sodium mainly derives from salt (sodium chloride) added to food, either discretionary salt (i.e. salt added during cooking or at the table) or salt in prepared or processed food products (Purdy & Armstrong, 2007; Zandstra et al., 2015). In addition, sodium salts other than sodium chloride are in some cases added to food products for specific technological purposes (e.g. food additives) (Bussell & Hunt, 2007). Another source is sodium occurring naturally in foods such as milk, meat and shellfish. However, this source of sodium contributes to a lesser extent to daily sodium intake (He & MacGregor, 2007).

Customary sodium intake is strongly influenced by the cultural context and the population's eating habits, which are driven by socioeconomic status and sociodemographic characteristics (Brown, Tzoulaki, Candeias, & Elliott, 2009; Purdy & Armstrong, 2007; Quader et al., 2016). While sodium sources in diet are thought to be region and country-specific (Anderson et al., 2010; Elliot & Brown, 2007), large international studies providing objective, detailed and complete data on dietary sodium sources remain scarce (Zandstra et al., 2015).

Western consumer culture, characterized by its modern lifestyle patterns, has triggered a high demand for processed and ready-to-eat foods (Purdy & Armstrong, 2007). In many high-income countries, manufactured foods and foods eaten away from home account for 75% of dietary salt intake (Campbell et al., 2012; Dötsch et al., 2009; Downs et al., 2015; He, Campbell, & MacGregor, 2012). Still, the major contributions to dietary sodium intake vary greatly among countries (He et al., 2012). The amount of salt added during food processing is largely outside of the direct control of individuals and is often referred to as 'hidden salt', hinting that consumers are often not aware of how much salt they are consuming when eating these products (Campbell et al., 2012). In food environments characterized by a large variety and availability of processed food products, it is difficult for consumers to estimate their sodium intake but, easy to consume excessive amount of salt (SACN, 2003). A completely different scenario occurs when the focus is put on some Asian countries. In China and Japan, sodium added while cooking and from sauces, including soy sauce, contributes to the largest proportion of

sodium intake (Elliot & Brown, 2007). In these countries, public health efforts towards sodium intake reduction at the population level should be mainly approached through education and behaviour change interventions (Campbell et al., 2012).

In low and middle-income countries populations, discretionary salt has historically been the main contributor to sodium intake. In particular, Uruguayan data from 2005-2006 has shown that discretionary salt is the largest contributor to dietary sodium among households in the lowest income quintile, contributing 57.4% of the total dietary sodium intake. In households in the highest income quintile, discretionary salt contributes a smaller, but still significant, proportion of dietary sodium (38.8%). This could be ascribed to a higher consumption of processed foods as the income per capita increases (Bove & Cerruti, 2008). In recent years, there has been a massive influx of processed foods into developing countries, which has been associated to the global shift in sodium sources towards processed food products (WHO, 2014; Pan American Health Organization [PAHO], 2015; Stuckler, 2012). In this sense, the ubiquity of high salt products in the current food supply has been identified as one of the major barriers for consumer to maintain a low-sodium-diet (Campbell et al., 2012). In particular, given the growth consumption of ultra-processed products in Uruguay (PAHO, 2015), it can be hypothesized that nowadays processed products account for the largest proportion of sodium intake at the population level.

Global actions towards sodium intake reduction at the population level

A total of 75 countries (including territories and areas) from all over the world have already implemented national salt reduction strategies and nine countries are currently undergoing the planning stage. This reflects a considerable increase in the number of countries undergoing salt reduction interventions compared to 2010 (Trieu et al., 2015). Yet, actual progress of individual countries has been slow, especially in light of the current sodium intake, far in excess nutritional recommendations (He et al., 2019).

Several countries worldwide have taken multifaceted actions towards sodium reduction. Efforts have mainly focused on reducing the sodium content of products available in the food environment, as well as raising consumer awareness about the sodium content of processed foods seeking to promote the selection of healthier food products (Campbell et al., 2012; Downs et al., 2015; Regan, et al., 2017; Webster, 2015; WHO, 2007). The most widely used strategies are: product reformulation, setting sodium targets for foods products, consumer education programmes, the inclusion of front-of-pack (FOP) nutritional labelling on food packages, imposing taxes on high-salt foods, as well as interventions in public institutions (Trieu et al., 2015). Globally, consumer

LITERATURE REVIEW

education, focused on improving people's understanding of the linkage between salt and health, has been the most widely used strategy all over the world, followed by product reformulation and the provision of FOP nutritional labelling on food packages. In Latin America, product reformulation has been the most widely used strategy to approach sodium reduction (Trieu et al., 2015).

Most sodium reduction initiatives to date have been voluntary (Webster, Dunford, Hawkes, & Neal, 2011) although mandatory interventions have been also implemented. In Europe, Bulgaria, Belgium, Hungary, The Netherlands and Portugal have established mandatory salt standards for a reduced number of widely consumed food products. Seeking to maximize the effect of reducing salt content on downshifting sodium intake at the population level, the priority has been put into products with the largest market share (European Commission, 2010). Africa has also taken a mandatory approach by setting salt standards for a larger number of products (Downs et al., 2015; Webster, Trieu, Dunford, & Hawkes, 2014). In Latin America, many countries have established salt reduction targets for key product categories, setting deadlines and periodic compliance reviews to ensure that targets were met. While Brazil and México adopted voluntary approaches, Argentina has opted for a mandatory approach (Trieu et al., 2015) and has set salt standards for a long list of processed products and food in restaurants (Downs et al., 2015; Webster et al., 2014). Paraguay, Chile and Ecuador have combined both mandatory and voluntary approaches (Campbell et al., 2012). Uruguay, in turn, is in an early stage of the implementation of interventions geared towards reducing sodium intake at the population level. Through a voluntary programme, named "Less Salt, More Health" ("Menos Sal, Más Salud"), bakeries are being encouraged to implement gradual salt reductions in bread. So far, bread is the only food product for which sodium reduction targets have been proposed in the country.

Many countries worldwide have selected bread as a target product category for sodium reduction, embarking on either voluntary or mandatory programmes to reformulate this product towards lower salt contents (He et al., 2019; La Croix et al., 2014; Pflaum, Konitzer, Hofmann, & Koehler, 2013; Rødbotten, et al., 2015; Spina et al., 2015). Two characteristics make bread a particularly appealing target for sodium reduction: its widespread consumption across all population income levels and the fact that is a leading source of dietary sodium in many places around the world (He et al., 2019; Trieu et al., 2015). In Uruguay, bread has been identified as one of the largest contributors to dietary sodium (Bove & Cerruti, 2008), accounting for 19-23% of average daily sodium intake (Taroco, 2011). Apart from bread, other food products, such as

bakery products, processed meats, dairy products, sauces and convenience meals, have been targeted for sodium reduction (Trieu et al., 2015)

Main challenges associated with sodium reduction in processed food products

Product reformulation towards lower salt contents has been widely promoted as a best-buy strategy for reducing sodium intake at the population level. However, this process wrestles with a major challenge: human preference for salty taste (Mennella, 2014). Preference is a key driver of food choices in both children and adults (Ventura & Worobey, 2013). Consumers are usually not willing to giving up their hedonic experience with products in return for potential health benefits (Civille & Oftedal, 2012; Tuorila & Cardello, 2002; Verbeke, 2006). In this sense, the hedonic appeal of salty taste is likely the biggest barrier towards sodium reduction (Bobowski, 2015).

In addition, other sensory challenges also arise from salt removal in food. For instance, salt reduction may enhance bitterness intensity as a result of the loss of bitterness inhibition by salt. In addition, the use of salt replacers to compensate for salt reduction may also contribute to enhance bitterness, as most of these ingredients elicit bitterness. Bitterness has been regarded as an undesirable attribute in a wide range of product categories and is generally associated with negative hedonic reactions (Kilcast, & den Ridder, 2007). Besides, several studies have provided evidence that salt reduction has a negative impact on overall flavour across a wide range of product categories, affecting consumers acceptability (Beauchamp et al., 1982; Kilcast, & den Ridder, 2007; Phelps et al., 2006; Rodbotten et al., 2015). While great advances have been made in overcoming other technological difficulties associated to sodium reduction, the effect of any sizeable salt reduction on flavour remains a major challenge for the food industry (Kilcast & den Ridder, 2007). Along with safety, ensuring that salt reduction does not negatively affect consumers perception and acceptance of the product is paramount in a marketplace characterized by food variety (Kilcast & den Ridder, 2007)

Evolving in low sodium environments, humans have developed innate preference for salty taste as a means to ensure survival (salty taste signal the presence of sodium, an essential mineral). At early ages, preference is mainly driven by unlearned components, but, as we grow up, experiential learning plays a major role in shaping food preferences, even to the point of reversing innate responses. Food preferences are developed throughout the life course in response to a complex interplay of multiple social and environmental factors (Ventura & Worobey, 2013). Dietary patterns are considered key determinants of food preferences in adulthood, being the most preferred level of salt highly dependent on dietary sodium intake (Bobowski, 2015).

LITERATURE REVIEW

Nowadays, individuals are repeatedly exposed to high salt foods due to the wide availability of processed products with high salt content. In the current food environment, individuals may develop preferences for high salt products through associative learning (Bobowski, 2015; Bobowski et al., 2015b; Zandstra et al., 2015). However, the malleability of food preferences through experiential learning provides an opportunity to move towards healthier eating patterns (Ventura & Worobey, 2013). A large body of evidence suggests that consumer preference for the sodium content in food could be modified through exposure to either low (Bertino, Beauchamp, & Engelman, 1982; Blais et al., 1986; Elmer, 1988) or high (Bertino, Beauchamp, & Engelman, 1986) sodium diets. Bertino, Beauchamp and Engelman (1982) explored the effect of a low sodium diet on consumer liking for salt, considering saline solutions and two food products (soup and crackers) at varying salt concentration as case study. The authors divided consumers into two groups: a control group (unrestricted sodium intake) and a consumer group exposed to a self-maintained low-sodium diet for a 5-month period. Exposure to a low salt diet heightened consumers perception of saltiness intensity in crackers and caused a downshift in the salt level considered to be optimum in soup and crackers after a two-month period. However, no significant changes were observed for the control group, which led to the conclusion that habituation to lower salt contents occurred as a consequence of the overall dietary context (Bertino et al., 1982). In a similar vein, Blais et al. (1986) carried out a longitudinal study involving a group of consumers that were exposed to a 12 months low sodium diet and a control group. A drop in the preferred level of salt in soup was observed for individuals on the low-salt diet, who after a 24-week period showed preference for a soup with 54% less salt. Further evidence was provided by Elmer (1988). This author found that individuals on low salt diets during a 12-month period preferred lower salt levels in crackers from month 3 onwards. In addition, they gave higher saltiness intensity scores at the end of the study. In either of these studies, no significant changes were observed for the control group, suggesting that changes in individual preference for salt and saltiness perception were driven by the dietary context. A similar trend occurred in the opposite direction: exposure to higher salt diets led to preference for higher salt contents (Bertino et al. 1986; Huggins, Nicolantonio, & Morgan, 1992). This provides further confirmation that humans adapt to the salt level most often consumed in their diet, which shapes their preference patterns for salt in food. While there is not common agreement on the time needed for changes on salt preferences to occur, evidence suggests that at least several weeks are required (Bobowski, 2015).

LITERATURE REVIEW

More recent studies have explored how repeated exposure to a single low sodium food may affect consumer preference on the context of unrestricted sodium diets. Methven, Langrenney and Prescott (2012) assessed the effect of repeated exposure to low salt soup on consumers' perception. Consumers were assigned to one of three groups: a control group (received a commercially available soup) and two consumer groups that received a different serving size of a no-added salt soup. Consumers were exposed to the product in eight different occasions, separated by daily intervals. Results showed that consumers exposed to the no-added salt soup, regardless of the serving size, gave significantly higher liking scores to this soup from the third exposure onwards. In addition, at the end of the exposure period no significant differences were found between this sample and the initially preferred saltier soup in terms of overall liking. Findings pointed out that the mere exposure to low sodium (no added salt) soup sufficed for shifting consumers liking towards lower salt contents in this product category, even in the context of unrestricted sodium diets. Bobowski, Rendahl and Vickers (2015b) provided further evidence on this regard. Consumers were assigned to either a straight or gradual salt reduction approach and exposed to low-sodium tomato juice over a 16 weeks period. Over the exposure period, liking for tomato juice showed a significant increase relative to the baseline (before exposure period). In addition, the percentage of consumers who showed preference for the salt-reduced tomato juice significantly increased after the exposure period. Taken all together these studies suggest it may be feasible to downshift preference for salt in a single food through repeated exposure to the low-salt version of the product, even in the context of unrestricted salt diets. Under the assumption that preference for the salt content of food is shaped by the salt level most often consumed, the exposure to a salt-reduced version may lead to relearn which the appropriate salt content is (Methven, Langrenney, & Prescott, 2012). However, in the context of unrestricted salt diets, the increase on liking for a single low-salt product did not translate into a decrease on liking for higher salt levels in food. This puts forward that maintaining unrestricted (high) sodium diets may hinder progress in altering salt preferences towards lower concentrations (Bobowski et al., 2015b). Directing sodium reduction efforts towards one or a few foods at a time seems promising to accomplish population-wide changes in dietary sodium intake, even if consumers do not modify their food choices.

LITERATURE REVIEW

Strategies for reducing the sodium content of food

Different salt reduction strategies have been developed to accomplish sizeable salt declines without negatively affecting consumer perception and acceptance of the reformulated product. A critical issue in achieving successful salt reductions is to establish how much salt could be removed from the product without causing a negative impact on consumer perception (Robinson, 2007).

Gradual reduction

The implementation of small but cumulative changes has been regarded as a promising approach to modify the composition of food products without affecting consumer perception (Bobowski et al., 2015a). In fact, based on the current body of evidence, the Institute of Medicine (IOM, 2010) has recommended gradual salt reduction in processed foods as an effective and feasible alternative to obtain a sustained drop in dietary salt intake at the population level (Institute of Medicine, 2010).

The core idea behind gradual salt reduction is to perform stepwise reductions through steps that are small enough to ensure that changes remain unnoticeable while consumers get accustomed to the new (lower) salt content. The gradual nature of this approach provides a means to cope with the negative effect that sodium removal has on sensory and hedonic perception. In this regard, the time elapsed between two consecutive reduction steps is paramount in ensuring that consumers get adapted to the lower salt level before further steps are taken (Dötsch et al., 2009; Wyness, Buttriss & Stanner, 2011, Zanstra et al., 2015).

Several studies have provided evidence on the convenience of gradual salt reduction over abrupt approaches. Girgis et al. (2003) found that 25% salt reduction in white bread was feasible through a cumulative series of undetectable salt reduction steps over a six weeks period. Consumers were randomly assigned to either a control group (that received the same bread sample throughout the study period) or an intervention group that week-to-week received a bread with 5% less salt. Results from this study revealed that a weekly 5% salt reduction remained unnoticed by consumers, reinforcing the potential of gradual strategies to reduce sodium intake at the population level. More recently, Boboswki, Rendahl and Vickers (2015a) compared the gradual and abrupt salt reduction approach in tomato juice over a 16 weeks period. Consumers were divided into two groups: one was exposed to the abrupt salt reduction (received the tomato juice with the target salt reduction from week 4 onwards) and the other was exposed to a gradual reduction condition. This latter group received, on a week-to-week basis, tomato juice samples that were reduced in salt via cumulative difference

thresholds (12% salt reduction each). Although the two consumer groups gave similar overall liking scores to the low salt juice sample at the end of the study, significant differences were found between groups in the trajectory of liking scores. Gradual reduction proved to be more effective in maintaining consumer acceptability throughout the salt reduction process, which is critical for food reformulation to success. On the contrary, an abrupt drop in acceptability may lead to consumer switching products (i.e. consumer abandoning a product in favour of an alternative product) (Bobowski et al., 2015a).

Food companies have also successfully implemented the gradual approach to reduce the salt content of their products. Campbell's Soup Company reached a 32% reduction in the salt content of V8 100% Vegetable Juice without affecting sales, through the implementation of a series of undetectable steps over an eight years period (Bratt & Tamman, 2010). In contrast, when this company followed a straight approach to reach a similar percentage of salt reduction in Campbell's Selected Harvest soups, the product was rejected by consumers and salt was added back to avoid further falls in sales (Geller, 2011). In a similar vein, ConAgra reported a 35% salt reduction in Chef Boyardee canned pastas over a five years period (Bratt & Tamman, 2010). Heinz has also used a gradual approach for reducing the salt content in baked beans. They carried out a study involving their current product and five salt reduction levels (9%, 15%, 20%, 26% and 37% reduction) and found that the sample with 15% salt reduction was the preferred one. The control sample (current product) and those with up to 20% salt reduction showed similar liking ratings whereas, higher salt reductions (26% and 37%) showed significantly lower scores. Based on these results, the company decided to follow a salt reduction programme that encompassed two steps: a first step involving a 15% salt reduction and a second step in which an additional 14% salt reduction was done to meet the reduction target (approximately 30%). A number of months elapsed before the second reduction step was taken, to allow consumers to adapt to the new product (Robinson, 2007).

While a plethora of evidence has shown the gradual approach prove effective for reducing salt content in food, its main disadvantage is the time needed to meet the target level. This becomes particularly critical in the current food environment, characterized by the wide availability of foods with a salt content that exceeds nutritional recommendations. To remedy this apparent shortcoming, combining this strategy with other product reformulation approaches has been proposed (Busch et al., 2013).

LITERATURE REVIEW

Partial replacement of sodium by potassium

The partial replacement of sodium chloride (salt) by other salts that do not contain sodium has been proposed as a promising strategy to reduce sodium in food. In this context, the replacement of the sodium cation by potassium, ammonium, calcium and lithium has received special attention. Salts from anions other than chloride, such as phosphate and glutamates, have been also explored as potential salt replacers (Kilcast & den Ridder, 2007). Albeit lithium chloride and ammonium chloride elicit salty taste, they have proved unsuitable as salt replacers due to their poor stability, smell and, in the case of lithium, toxicity.

Potassium salts are particularly appealing salt replacers given the well-known health benefits of potassium, mainly related to its potential to decrease the risk of hypertension (Aaron & Sanders, 2013; He & MacGregor, 2007; van Buren, Dötsch-Klerk, Seewi, & Newson, 2016). In particular, potassium chloride (KCl) is by far the most extensively used salt replacer (Busch et al., 2013; Kilcast & den Ridder, 2007; Paulsen, Nys, Kvarberg & Hersleth, 2014; van Buren et al., 2016). Yet, one of the major barriers to the widespread use of potassium chloride is related to the fact that it does not only elicit salty taste but also undesirable flavors, such as bitterness, chemical and metallic flavour as well as aftertaste, when used above a given concentration (Feltrin, Rios de Souza, Goncalves Saraiva, Nunes, & Marques Pinheiro, 2015; Gelabert, Gou, Guerrero, & Arnau, 2003; Gou, Guerrero, Gelabert & Arnau, 1996; Kilcast & den Ridder, 2007). For this reason, partial rather than total substitutions of NaCl by KCl has been used to minimize undesirable flavours. The effect of partially replacing a proportion of NaCl with KCl has received great attention in the last decades and several studies, encompassing a wide range of product categories, have explored the potential of this alternative for removing salt from food (Armenteros, Aristoy, Barat, & Toldrá, 2012; Gomes et al., 2011; Bernklau et al., 2017; Braschi, Gill, & Naismith 2009; Charlton, Macgregor, Vorster, Levilt, & Steyn 2007; Grummer, Bobowski, Karalus, Vickers, & Schoenfuss, 2013; Horita, Messias, Morgano, Hayakawa, & Pollonio, 2014; Paulsen, et al., 2014; Soglia et al., 2014). The amount of sodium chloride to be replaced by potassium chloride without affecting the sensory profile of products markedly depends on the product category, its sensory characteristics and complexity. Globally, a 30% replacement of NaCl by KCl may be feasible, although, up to 50% replacement has been reported in feta cheese (Katsiari, Voutsinas, Alichanidis, & Roussis, 1997).

Masking the undesirable flavours elicited by potassium chloride remains a major challenge to heighten the percentage of NaCl substitution by KCl. One promising alternative to circumvent this problem is to combine potassium chloride with salt

enhancers such as monosodium glutamate (MSG), ammonium chloride, magnesium sulphate and amino acids. Yet, the current trend towards 'clean labels' goes on the opposite direction and does not welcome the use of salt enhancers or replacers that contravene this idea. In this sense, the use of MSG and other glutamates has been reported to be related to different health conditions, such as hyperactivity, sickness and migraine. While evidence supporting the effect of MSG on health remains scarce, restrictions against glutamate use have been stated by a number of food industries and retailers. Moreover, the use of specific food ingredients, such as peptides imply substantial additional cost which may limit their widespread use (Kilcast & den Ridder, 2007).

It is worth noting that, different from sugar substitutes, sodium-free salt substitutes are not yet available. This may respond to the intrinsic differences in the transduction mechanism behind saltiness and sweetness perception. The great specificity of the mechanism behind salty taste transduction makes it extremely challenging to find sodium replacers (McCaughey, 2007).

Non-homogeneous spatial distribution of salt in solid food products

Non-homogeneous spatial distribution of tastants in food has been reported to enhance taste perception and appears promising for salt reduction (Emorine, Septier, Thomas-Danguin, & Salles, 2013; Fan, 1991; Mosca et al., 2013; Noort et al., 2012; Noort et al., 2010). Under normal chewing conditions, taste receptors are exposed to constant (prolonged and uninterrupted) stimulation which is thought to cause a gradual decrease in taste responsiveness as a result of adaptation processes (Busch et al., 2013; Mosca, van de Velde, Bult, van Boekel, & Stieger, 2010). Adaptation is an intrinsic property of sensory systems, that seeks to alert changes on the ambient level of stimulation (Lawless & Heymann, 2010).

The mechanism underlying taste enhancement in non-homogeneous systems is thought to be related to the discontinuous stimulation of taste receptors in a desynchronized fashion (i.e. some receptors are exposed to high tastant concentration whereas other are exposed to low concentrations) (Mosca et al., 2010). Albeit the chewing process ultimately leads to a homogeneous bolus, the contrast effect in mouth during mastication seems to be enough to enhance tastant intensity (Mosca et al., 2010). The overall receptor response when it is exposed to high salt "spots/zones" in a non-homogeneous food is expected to be heightened relative to that elicited from the continuous stimulation that occurs when the tastant is homogeneously distributed in the food matrix (Mosca et al., 2010). Both the partial recovery from taste receptor adaptation

LITERATURE REVIEW

and the higher activation frequency of the afferent fibers that innervate the receptor cells are thought to mediate taste enhancement (Busch et al., 2013; Mosca et al., 2010).

Expectations may also contribute to the taste enhancing effect of non-homogeneous distribution. The fact that the product is expected to be homogeneous may contribute to maintain an individual unaware of the sensory contrast (Woods, Poliakoff, Lloyd, Dijksterhuis, & Thomas, 2010). Up to date many technological aspects related to the industrial implementation of such an approach remains unresolved and the principles of non-homogeneous distribution are less readily translated for liquid products (Busch et al, 2013).

Additional strategies

Other strategies have also been proposed to reduce salt in foods over the last years, including the addition of salt enhancers and use of salt-congruent aromas. Diverse studies have explored these approaches, providing evidence on their potential to reduce up to 50% of salt (Charlton et al. 2007; Kremer, Mojet, & Shimojo, 2009; Hooge & Chambers, 2010; Batenburg & van der Velden, 2011; Goh et al. 2011; Lawrence et al. 2011).

A less explored but also promising alternative for reducing sodium in food is the use of salt from different origins. Crystal morphology and mineral content may influence saltiness intensity and the time intensity profiles of salty taste (Drake & Drake, 2011). The rationale behind this strategy is that the use of sea salt instead of conventional salt may lead to the same salty taste at lower concentrations. Yet, this approach remains little explored and the few studies on the topic have reported incongruent results. Some studies suggest this strategy poses potential (Drake & Drake, 2011; Quilaqueo, Duizer, & Aguilera, 2015). However, other studies did not find correlation between salt crystal size and maximum perceived saltiness intensity, casting doubts over the feasibility of this strategy (Vella, Marcone, & Duizer, 2012). Further research is warranted to bring fundamental understanding to this area.

Inclusion of front-of-pack (FOP) nutritional labelling on processed foods products

The inclusion of nutritional information has been regarded a key pillar in empowering consumers to make informed food choices and nudge them towards low sodium diets (Campbell et al., 2012; WHO, 2017). However, several studies have shown that conventional nutrition information is difficult to find and understand for consumers, who are not often willing to invest too much time and cognitive effort for making their food choices (Cowburn & Stockley, 2005; Grunert, Fernández-Celemín, Wills, Genannt Bonsmann, & Nureeva, 2010). In this context, the inclusion of front-of-pack (FOP)

nutritional labelling has gained increasing popularity worldwide as a means to cope with some of the apparent shortcomings of conventional nutritional information (Cecchini & Warin, 2016). The use of front-of-pack nutritional labelling has been reported to be successful in making nutritional information more accessible by providing simplified information that is easy to find and understand (Cioffi, Levitsky, Pacanowski, & Bertz, 2015; Guthrie, Mancino, & Lin, 2015).

Different FOP nutrition labelling schemes differing in the extent to which they assist consumers to evaluate product healthfulness have been developed and implemented worldwide (Hodgkins et al., 2012). In particular, nutritional warnings have emerged in recent years as a promising alternative to discourage consumption of foods with excessive content of nutrients associated with non-communicable diseases, including sodium (Khandpur, Swinburn, et al., 2018). There is now growing evidence supporting that nutritional warnings facilitate the identification of foods with excessive content of sodium and effectively discourage consumers from choosing them (e.g. Ares et al., 2018; Machín, Curutchet, Giménez, Aschemann-Witzel, & Ares, 2019; Khandpur, de Moraes Sato, et al., 2018; Taillie, Hall, Popkin, Ng, & Murukatla, 2020; Deliza, de Alcantara, Pereira, & Ares, 2020). This has motivated several countries worldwide to compulsorily introduce this scheme, including Chile (Ministerio de Salud, 2015), Israel (Ministry of Health of Israel, 2019), Peru (Congreso de la República, 2013), Uruguay (Ministerio de Salud Pública, 2018), Mexico (Secretaría de Economía, 2020) and Colombia (Minsalud, 2020). Yet, research on the impact of nutritional warnings on consumer food choices, and particularly how they influence consumer perception after tasting, remains scarce.

While nutritional warnings mainly seek to assist consumers during product selection, they may also nudge the food industry to engage in product reformulation towards lower sodium contents (Kanter, Reyes, Vandevijvere, Swinburn, & Corvalán, 2019). This effect is expected to increase the availability of low-sodium products in the marketplace, which may further contribute to reduce sodium intake (Cecchini & Warin, 2015).

LITERATURE REVIEW

Sensory tools for reducing the salt content in processed foods

In the following, the most challenging topics in sensory science in relation to salt reduction are discussed. The description of the sensory characteristics of products is a core activity in sensory and consumer science (Lawless & Heymann, 2010; Varela & Ares, 2014). In the context of product reformulation towards lower salt contents, it represents a valuable tool to gain an in depth understanding of how salt removal affects the sensory characteristics of products.

The traditional approach for obtaining a detailed description of the sensory profile has been descriptive analysis and its variants (Stone & Sidel, 2004; Lawless & Heymann, 2010). However, consumer-based approaches have gained growing interest in the last decades (Ares & Varela, 2017). Given that information is directly obtained from consumers, these methodologies enable to more fully integrate consumers' perception into the reformulation process (Ares, 2015). In addition, consumer-based approaches do not require to train and maintain a sensory panel, which heavily reduces the time and resources needed to obtain sensory product characterizations (Hopfer & Heymann, 2013).

Difference thresholds for salt in food

The design and implementation of gradual salt reduction programmes requires the definition of the sodium reduction target to be met, the size of salt reduction in each step, the number of steps to be taken, as well as the time period between two consecutive reduction steps (Bobowski & Vickers, 2012). Difference thresholds represent a useful tool for this purpose.

Conceptually, difference thresholds are defined as the minimum physical change needed for the change to be sensed 50% of the time (Lawless & Heymann, 2010). Difficulties arise when applying this concept in practice due to the high variability among individuals and within a single individual across time. This has led to the development of practical rules to determine an arbitrary value (within a range of physical intensity levels) which ultimately describes a probability function for change detection (Lawless & Heymann, 2010). Difference thresholds can be experimentally estimated as the smallest change in the stimuli needed for 50% of the individuals to perceive the change (Boring, 1942). Different sensory approaches have been used to find thresholds or have employed the threshold concept (Lawless & Heymann, 2010).

Weber's law states that the ratio between the difference thresholds, or just noticeable difference and the original stimulus value is a constant ratio. This ratio is sometimes referred as to Weber fraction and represents an index that is specific to the

stimulus (Lawless & Heymann 2010). Assuming that Weber's law applies, the difference threshold for a particular stimulus (e.g. salt content) represents a valuable tool for predicting how much this stimulus could be changed in a product without altering perception (Bobowski & Vickers, 2012).

Several studies have used difference thresholds to define gradual changes in order to alter different ingredients without affecting consumer perception. Dubow and Childs (1998) determined a series of undetectable steps to gradually change the classic Coca-Cola formula without consumers awareness. Using triangle tests (discriminative test), these authors found that replacing 12.5% of Coca-Cola classic by the new Coca-Cola formulation did not cause distinguishable changes. Although this strategy was not put into practice by the company, the implementation of a series of eight undetectable steps (12.5% replacement each) proved to be effective in transitioning between two product formulations without raising consumer awareness (Dubow & Childs, 1998). Similarly, Delk and Vickers (2007) proposed to gradually increment the proportion of whole wheat in a bread rolls recipe targeted to children, as a way to improve their liking for whole wheat by stealth. Using forced-choice tests (discriminative test), the authors set a series of 14 difference thresholds (steps) for whole-wheat flour in bread rolls. Based on these results they proposed a stepwise reduction programme to increase whole-wheat flour content in bread roll from 0 to 91%, without compromising acceptability (Delk & Vickers, 2007).

More recently, Bobowski and Vickers (2012) set a series of sequential difference thresholds for salt in two matrices: plain water and an aqueous solution containing different tastants (including salt), that sought to mimic a more complex-flavoured broth. By means of paired-comparison tests (discriminative test), the authors determined a series of 26 and 12 salt difference thresholds in plain water and the aqueous solution, respectively, providing guidelines for the implementation of gradual sodium reduction via unnoticed steps. The fact that salt reduction in plain water requires more than twice the number of steps set for the aqueous solution (containing not only salt but also other taste stimuli) pointed out that salt reduction programmes are largely product specific (Bobowski & Vickers, 2012).

Single and multiple intake evaluations

Common practice in sensory and consumer science involves performing single sip/bite evaluations. Yet, eating is a dynamic process that generally involves multiple ingestions of the food product (i.e. repeated ingestions occurring one immediately after the other) (Appelqvist, Poelman, Cochet-Broch, & Delahunty, 2016). Food perception

LITERATURE REVIEW

may change across repeated food ingestions as a consequence of adaptation processes. Sensory adaptation does not operate exactly in the same way for all products as it may be more rapid and/or long-lasting for certain sensory characteristics than for others. Moreover, in products with complex sensory profiles not all the sensory characteristics may be perceived at once (Köster, 2003).

Building on the aforementioned reasons, the question arises whether the evaluation of a single intake properly reflects the sensory experience that occurs during product consumption. This issue has particular relevance for the evaluation of complex products and samples with small differences among them (Appelqvist, et al., 2016; Köster, 2003; Rocha-Parra, García-Burgos, Munsch, Chirife, & Zamora, 2016; Stein, Nagai, Nakagawa, & Beauchamp, 2003; Zorn, Alcaire, Vidal, Giménez, & Ares, 2014).

To the authors knowledge, multiple intake evaluations have remained scarcely explored in the field and, to date, only few studies have addressed the topic (Appelqvist, et al., 2016; Rocha-Parra et al., 2016; Stein et al., 2003; Zorn et al., 2014). However, available evidence suggests that differences between products become more apparent after repeated ingestions. Thus, care must be taken when drawing conclusions on consumers' sensory perception of food products based on a single intake evaluation as it may not accurately reflect the sensory experience with the product in a real-life consumption situation.

Static and dynamic methods for sensory characterization

One of the most often used methods for sensory product characterisation with consumers is check-all-that-apply (CATA) questions (Adams, Williams, Lancaster, & Foley, 2007; Ares & Jaeger, 2015). CATA questions basically consist of asking consumers to select all the attributes that they consider applicable to describe the product from a predefined list (Ares et al., 2014; Ares et al., 2013; Jaeger & Ares, 2014). This methodology has been reported to provide accurate results, similar to those provided by trained assessors (Ares, Antúnez, et al., 2015).

Sensory perception of food is a dynamic phenomenon, as the perceived sensory characteristics of the products change during consumption (Lawless & Heymann, 2010). The dynamic nature of food perception is closely related to the series of in-mouth transformations that food undergoes during chewing, salivation, tongue movements and swallowing processes (Lawless & Heymann 2010; Sudre, Pineau, Loret, & Martin, 2012). This has prompted the need to develop and use dynamic sensory approaches to better understand how products are perceived in mouth during consumption.

Temporal-check all-that-apply (TCATA) questions have been introduced as an extension of CATA questions to account for the dynamic nature of sensory perception (Castura, Antúnez, Giménez, & Ares, 2016). Similarly to CATA, TCATA requires assessors to select, from a predefined list, all the attributes they consider applicable to describe a sample at each moment of the evaluation, i.e. they should answer a CATA question continuously as consumption takes place. As soon as they perceive a sensory attribute, the corresponding term has to be checked. On the contrary, when they no longer perceive the attribute, the corresponding term should be immediately unchecked. TCATA has been already used to obtain the temporal sensory profile of a wide range of products (e.g., Ares et al., 2016; Ares, Jaeger, et al., 2015; Baker, Castura, & Ross, 2016; Boinbaser, Parente, Castura, & Ares, 2015; Castura et al., 2016; Jaeger, Beresford, et al., 2017; Jaeger et al., 2018; Oliveira et al., 2015).

The effect of information on consumer perception

Consumer perception is a complex process that depends upon several factors related to the characteristics of the product (either intrinsic or extrinsic), the individual and the context (Asioli et al., 2017; Köster, 2003; Oliveira, Ares, & Deliza, 2018; Piqueras-Fiszman & Spence, 2015). Getting insights on the intricate interplay of these factors is crucial in gaining a complete and more realistic picture of consumer behaviour under real-life settings (Asioli et al., 2017; Deliza & MacFie, 1996; Köster, 2009; Simeone & Marotta, 2010; Piqueras-Fiszman & Spence, 2015).

It is now widely accepted that the interaction between sensory and extrinsic product characteristics (such as brand, product description, nutritional information, graphic design and price) plays a major role in shaping consumers' perception (Asioli et al., 2017; Deliza, 2018; Grunert, 2015). Moreover, in situations where the consumer does not have previous experience with the product, extrinsic cues, which anticipate product characteristics and generate expectations, are thought to be key determinants of food choices (Olson & Jacoby, 1972).

Among extrinsic cues influencing consumer perception, nutritional and health-related information has received special attention in the last decades. However, the impact of this information on consumer perception still deserves further research. Besides, in response to the growing demand for healthy products in the food supply many food companies are including health related information on the package as part of their marketing strategies. To date, experimental evidence suggests the provision of health-related information (mainly calories, fat and sodium content) may shape consumer expectations and beliefs about product healthiness (Piqueras-Fiszman &

LITERATURE REVIEW

Spence, 2015). In addition, several studies have shown that health-related information affects consumer hedonic perception of products (Fernqvist & Ekelund, 2014).

When the focus is put on sodium reduction, there is no conclusive evidence on how information may affect consumer expectation. Some studies have found that information regarding sodium reduction can cause a negative effect on consumers expectations of biscuits (Vázquez et al., 2009) and a downshift on perceived saltiness intensity of soups (Liem, Aydin, & Zandstra, 2012). Yet, the opposite trend has also been reported (Kahkonen, Tuorila, & Rita, 1996; Willems, van Hout, Zijlstra, & Zandstra., 2014). The available evidence reflects that the impact, either positive or negative, that sodium information has on consumer perception is to a large extent driven by the way the information is conveyed (i.e. the precise message formulation) (Zandstra et al., 2015). Further research is needed to gain an in-depth understanding of how best to inform consumers of healthier options.

Individual characteristics also play a major role in shaping expectations. The impact of different external cues on consumers expectations and perception is shaped by their beliefs, attitudes and personality traits. Previous studies have reported that health-conscious consumers gave higher overall liking scores to product featuring a fat-reduced-label compared to unlabeled products (Aaron, Mela, & Evans, 1994; Kähkönen, Tuorila, & Lawless, 1997; Kähkönen et al., 1996; Westcombe & Wardle, 1997). Besides, Wansink and Park (2002) explored the effect of soy labels and health claims on consumers perception. The influence of labelling on product taste perception was explored considering two consumers segments: taste-conscious and health-conscious. Different response patterns were identified between the two consumer segments. While taste-conscious consumers showed high sensitivity to the soy labelling, this label did not significantly affect health-conscious consumers, who, on the other hand, considered nutritional bars featuring a health claim to taste better than expected. The study by Wansink and Park (2002) provided further evidence that nutrition and health-related information generates expectations that influence consumer preference and taste perception. In addition, they found that not all consumers are equally influenced by information. In a similar vein, informing low sodium content to health-conscious consumers, who are keen on this type of products, may cause a positive effect on their hedonic perception and lead to an increased willingness to buy the product (Levis & Chambers IV, 1997). However, the opposite effect may occur among those who do prioritize taste and are not concerned regarding their actual sodium intake (Vázquez et al., 2009).

OBJECTIVES

The general aim of this thesis was to study different strategies for salt reduction in foods from a sensory perspective. The main focus was placed on product reformulation but the impact of communicating excessive sodium content on consumer perception was also addressed. To accomplish this aim, the following specific objectives were set:

- To estimate difference thresholds for salt in bread and propose a consumer-based approach for the implementation of gradual salt reduction without affecting consumer sensory and hedonic perception of bread.
- To determine the sodium content of bread samples formulated with varying added salt levels.
- To explore the effect of two bite evaluations (vs. a single bite evaluation) on consumer sensory and hedonic perception of salt-reduced breads.
- To assess the effect of salt reduction on consumers' hedonic perception of white rice with special emphasis on exploring individual differences in consumers' reaction towards salt reduction.
- To assess the effect of partially replacing NaCl with KCl on the sensory characteristics and consumer perception of white bread.
- To explore the non-homogeneous spatial distribution of salt using fatty coatings for sodium reduction in white bread.
- To assess consumers' reaction towards sodium reduction in bread in the context of the implementation of warnings in three scenarios: package evaluation, tasting and intention to re-purchase the product after tasting.

STRUCTURE OF THE THESIS

The thesis is structured in seven chapters. CHAPTER 1 and CHAPTER 4 were dedicated to the gradual salt reduction approach considering white bread and white rice as focal product, respectively. In CHAPTER 1, the focus was put on estimating difference thresholds for salt in bread and then evaluating consumer sensory and hedonic reaction towards salt-reduced breads. CHAPTER 2 involved the analytical determination of the sodium content in the white bread samples formulated according to the sequential difference thresholds for salt determined in CHAPTER 1. CHAPTER 3 focused on comparing single and two bite evaluations of consumers sensory and hedonic perception of salt-reduced breads. In CHAPTER 4 the gradual salt reduction approach was addressed with special focus on consumers' heterogeneity towards sodium reduction.

While the gradual reduction approach has proven effective for reducing salt in food, long time periods are needed to achieve the target sodium content. To overcome this limitation the combination of gradual salt reduction with other salt reduction strategies have been proposed. CHAPTER 5 focused on partial replacement of sodium chloride by potassium chloride as a sodium reduction strategy. Three NaCl:KCl blends with different NaCl:KCl ratios were assessed to find a compromise between sodium reduction and the onset of undesirable flavours. CHAPTER 6 is dedicated to addressing a more recent, and scarcely explored, sodium reduction strategy: the non-homogeneous spatial distribution of salt using fatty coatings. This chapter is exploratory in nature and provides a first approach to the topic rather than a comprehensive overview.

CHAPTER 7 was dedicated to approaching consumers' reaction towards sodium reduction in the context of the recent implementation of sodium warnings. The effect of sodium warnings on consumer choices, perception and intention to re-purchase the product after tasting was explored. At the end of the thesis an overview and synthesis of the general conclusion from all the experimental chapters is provided.

CHAPTER 1

A consumer-based approach to salt reduction: Case study with bread

A CONSUMER-BASED APPROACH TO SALT REDUCTION

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Author contributions:

- Antúnez, L: Conceived and designed the work, collected the data, analysed and interpreted the data, wrote the paper.
- Giménez, A: Contributed to the conception and design of the study, as well as to data interpretation, performed a critical revision of the article.
- Ares, G: Contributed to the conception and design of the study, as well as to data interpretation, performed a critical revision of the article.

Abstract

In recent years high sodium intake has raised growing concern worldwide. A widespread reduction of salt concentration in processed foods has been claimed as one of the most effective strategies to achieve a short-term impact on global health. However, one of the major challenges in reducing salt in food products is its potential negative impact on consumer perception. For this reason, gradual salt reduction has been recommended. In this context, the aim of the present work was to present a consumer-based approach to salt reduction, using bread as case study. Two consumer studies with a total of 303 consumers were carried out. In the first study, four sequential difference thresholds were determined through paired-comparison tests, starting at a salt concentration of 2.00%. In the second study, 99 consumers performed a two-bite evaluation of their sensory and hedonic perception of five bread samples: a control bread containing 2.00% salt and four samples with reduced salt content according to the difference thresholds determined in the first study. Survival analysis was used to determine average difference thresholds, which ranged from 9.42% to 14.29% of the salt concentration of the control bread. Results showed that salt concentration significantly influenced consumer overall liking of the bread samples. However, large heterogeneity was found in consumer hedonic reaction towards salt reduction: two groups of consumers with different preference and hedonic sensitivity to salt reduction were found. Results from the present work confirm that cumulative series of small salt reductions may be a feasible strategy for reducing the sodium content of bread without affecting consumer hedonic perception and stress the importance of considering consumer perception in the design of gradual salt reduction programmes.

A CONSUMER APPROACH TO SALT REDUCTION

1.1. Introduction

In recent years, high sodium intake has raised growing concern all over the world (Downs et al., 2015). It has been widely reported that high daily sodium intake is closely related to increased risk of high blood pressure and cardiovascular disease and that even relatively small changes in dietary sodium intake would cause major impact on global health (He & MacGregor, 2009; WHO, 2007).

Processed foods have been identified as one of the main contributors to the daily sodium intake of consumers in developed countries, accounting for an average of 75%-80% of the total sodium intake (Appel & Anderson, 2010; Campbell et al., 2012; Grimes, Campbell, Riddell, & Nowson, 2011). Hence, the reduction of salt concentration in processed foods has been proposed as one of the most realistic strategies to reduce the sodium intake levels in short time frames (Downs et al., 2015).

In particular, cereal products have been reported to be responsible for 30% of the overall salt intake (Lynch, Bello, Sheehan, Cashman, & Arent, 2009). Thus, reducing salt content in these products could have an important positive health impact in society. Given the widespread consumption of bread several studies have explored the impact of sodium reduction in this product (Rødbotten et al., 2015; La Croix et al., 2014; Spina et al., 2015; Pflaum et al., 2013). However, to the best of the authors' knowledge, there is little research on how gradual salt reduction programmes in bread should be implemented.

One of the major challenges for the implementation of salt reduction programmes on food products is human beings' preference for salty taste (Mennella, 2014). In this sense, Beauchamp et al. (1982) reported that large reductions in the salt content of food products markedly affect its palatability. Similarly, Rødbotten et al. (2015) reported that reducing the sodium content of bread had a negative impact on consumer preference, even when they were moderately positive to a salt reduction in this product. Therefore, changes on consumer food choices towards a lower sodium intake are expected to occur only when the reduced-sodium products match the characteristics of the regular product (Zandstra et al., 2015).

The Food Standards Agency (FSA, 2003) and the Institute of Medicine (IOM, 2010) have recommended gradual salt reductions as an effective strategy to achieve lower sodium intake levels (Food Standards Agency [FSA], 2003; IOM, 2010). This strategy is based on the idea of making salt reductions without causing any change in consumer sensory and hedonic perception (Busch et al., 2013, MacGregor & Hashem, 2014). Once

A CONSUMER APPROACH TO SALT REDUCTION

consumers are adapted to the new taste without being aware of the change, further reduction steps are taken (Zandstra et al., 2015).

Implementing gradual salt reduction programmes require the estimation of difference thresholds for salt as well as establishing the time necessary to shift from one reduction step to the other and the number of sequential reductions necessary to achieve the target salt content in the product (Bobowski & Vickers, 2012). Difference thresholds for salt can be experimentally estimated as the smallest change in salt concentration that causes a change in saltiness intensity that is perceived by 50% of the individuals (Boring, 1942). According to Weber's law, difference thresholds are a constant proportion of the stimulus (Lawless & Heymann, 2010). Therefore, for a given product the percentage of salt content that can be reduced without consumer awareness is constant. Bobowski and Vickers (2012) have recently established sequential difference thresholds for sodium chloride reduction in water with and without added taste stimuli. However, as sensory perception is a multimodal perception (Verhagen & Engelen, 2006), different thresholds for salty taste vary from product to product, which makes it necessary to design product-specific gradual salt reduction recommendations.

Difference thresholds have been traditionally determined with trained assessors, as consumers have been deemed not capable of accurately and objectively evaluating specific characteristics of the products (Stone & Sidel, 2004). Trained assessors outperform consumers in their ability to identify differences among samples (Ishii, Kawaguchi, O'Mahony, & Rousseau, 2007). This implies they may be able to identify differences among samples that are not relevant for consumers and therefore difference thresholds estimated with trained assessors may be too conservative. For this reason, consumer-based difference thresholds may provide more realistic recommendations for gradual salt reduction programmes.

Consumer hedonic perception of salt reduced products is also relevant. Research has shown that small changes in the sensory characteristics of products, even if perceived, do not significantly affect their hedonic perception (Oliveira et al., 2015). This implies that several salt-reduction steps may be performed without affecting consumer hedonic perception, which suggests that gradual salt-reduction programmes should be designed based on consumer sensory and hedonic perception.

Most sensory perception research is performed on single sips/bites (Appelqvist et al., 2016). However, it has been reported that repeated food ingestions may cause changes in food perception as a consequence of adaptation processes (Köster, 2003). Therefore, the evaluation of a single bite/sip of the food product could not properly reflect

the sensory experience that occurs when consuming the product, especially when considering complex products. Previous studies have shown that small differences among products only became noticeable after repeated ingestions (Köster, 2009; Köster, Couronne, Léon, Lévy, & Marcelino, 2002; Stein et al., 2003; Zandstra, Weegels, van Spronsen, & Klerk, 2004).

In this context, the present work is aimed at exploring a consumer-based approach to salt reduction, using bread as case study. Difference thresholds for salt in bread were determined based on consumer perception and their sensory and hedonic perception of reduced-salt products was assessed. Results from the present work are expected to provide valuable information on how to design consumer-based gradual salt reduction programmes in bread, so that informed decisions in this regard can be taken.

1.2. Materials and methods

1.2.1. Bread formulation

The breads were produced using 58% Uruguayan commercial wheat flour (Molino Americano S.A, Montevideo, Uruguay), 37% tap water, 2.4% sugar (Alcoholes del Uruguay S.A., Bella Unión, Uruguay), 1.6% high oleic sunflower oil (Compañía Oleaginosa Uruguay S. A, Montevideo, Uruguay), 2.0% to 1.25% salt/ kg flour (Antil S.A, Montevideo, Uruguay) and 1% powered yeast (Fleischmann, Argentina). The breads were prepared in a bread-making machine (Phillips® model HD9015/30) one day prior to testing and were stored in plastic bags overnight.

The morning of the test, breads were cut in 10 mm slices and the crust was removed. Each assessor received a piece of bread, corresponding to approximately 5 g, served on plastic plates, coded using three-digit numbers.

1.2.2. Participants

Two consumer studies involving 303 naïve assessors were carried out. All participants were recruited from the data base of the Sensometrics & Consumer Science group of Universidad de la República (Uruguay), based on their bread consumption and willingness to take part in the study. Participants signed an informed consent form and received a small gift for their participation.

A total of 204 consumers, aged between 18 and 29 years (57% female), took part in the first study, that involved the estimation of four sequential difference thresholds. Four groups of 50-52 consumers participated in each of the four difference threshold estimations.

A CONSUMER APPROACH TO SALT REDUCTION

The second study, in which consumers evaluated their sensory and hedonic perception of salt reduced breads, involved 99 consumers. Participants aged from 18 to 66 years and were 55% female.

1.2.3. Estimation of difference thresholds

Four sequential difference thresholds for salt in bread were determined. The first difference threshold was determined in a bread with a salt content comparable with the average salt concentration of commercial breads available in the Uruguayan marketplace (2.00% salt). The second threshold was determined in a bread that was reduced in salt according to the previously determined difference threshold. This pattern was repeated until 4 difference thresholds were determined.

In each study, consumers completed six paired-comparison tests. Each pair was composed of a control bread (that remained constant in each study) and a sample that was reduced in salt from the control. The salt concentrations considered in each of the 4 studies were selected by pilot testing. Table 1.1 shows the salt concentration of the control and the salt-reduced samples in each of the studies.

Consumers were requested to taste each of the samples in a pair and to indicate which was the tastier one by selecting the corresponding code number. Assessors were asked to indicate the tastier rather than the saltier sample based on results from preliminary studies that showed that consumers did not use the term salty to describe bread and that higher discriminative ability was achieved when consumers were asked to evaluate tastiness instead of saltiness. Samples in each pair were presented following a balanced design. Assessors were instructed to cleanse their palate with water after each trial.

All the studies were carried out using Compusense-Cloud (Compusense Inc., Guelph, Ontario, Canada). Testing took place in a sensory laboratory designed in accordance with International Organization for Standardization [ISO] 8589 (ISO, 2007), under artificial daylight and temperature control (22 °C).

Table 1.1 Salt concentration and salt-reduction percentage (between brackets) of bread samples considered in the 4 studies in which sequential difference thresholds for salt were determined.

Study	Control sample	Salt reduced samples					
		1	2	3	4	5	6
1	2.00	1.96	1.92	1.88	1.84	1.80	1.76
		(2.0)	(4.0)	(6.0)	(8.0)	(10.0)	(12.0)
2	1.80	1.73	1.66	1.59	1.52	1.45	1.38
		(3.9)	(7.8)	(11.7)	(15.5)	(19.4)	(23.3)
3	1.61	1.56	1.51	1.46	1.41	1.36	1.31
		(3.1)	(6.2)	(9.3)	(12.4)	(15.5)	(18.6)
4	1.38	1.36	1.34	1.32	1.30	1.28	1.26
		(1.4)	(2.9)	(4.3)	(5.8)	(7.2)	(8.7)

1.2.4. Consumers' sensory and hedonic perception of breads with different salt concentration

Consumers' sensory and hedonic perception of bread samples with different salt concentration was assessed in a separate study. Five bread samples were considered: a control bread containing 2.00% salt and four samples reduced in salt according to the difference thresholds determined in the first study.

Consumers performed a multiple bite evaluation. They were asked to try a first bite of the sample and to indicate their overall liking using a 9-point hedonic scale (1 = dislike very much, 9 = like very much) as well as to answer a check-all-that-apply (CATA) question composed of 16 sensory characteristics: *salty, hard, soft, spongy, dry, light, dense, tasteless, barely salty, off-flavour, crumbly, sticky, smooth, gummy, tasty, characteristic bread flavour*. The order in which terms were listed was balanced across consumers, following a Williams' Latin square design. Once they have completed the CATA task for the first bite they were requested to try a second bite of the sample and to evaluate it following the same procedure. Assessors were instructed to cleanse their palate with water between samples but not between bites. Only data from the evaluation of the second bite is shown in the present study.

Samples were presented according to an experimental design that was balanced for order and carry-over effects (Williams' Latin Square design). Data were collected using Compusense-Cloud (Compusense Inc., Guelph, Ontario, Canada). Testing took place in a sensory laboratory, as described in the previous section.

A CONSUMER APPROACH TO SALT REDUCTION

1.2.5. Data analysis

1.2.5.1. Estimation of difference thresholds

Difference thresholds were estimated by means of survival analysis using the modified procedure proposed by Alcaire et al. (2014) for the estimation of equivalent sweetness in orange juice.

For each of the six paired comparisons, consumers' responses were coded with the word "No" if the control was identified as tastier than the reduced-salt sample and with the word "Yes" otherwise. It was argued that if the salt reduction was higher than the difference threshold, consumers would always give a "No" answer when performing the paired comparison. The difference threshold was estimated as the concentration at which a consumer starts to consistently perceive the salt-reduced sample as less tasty than the control and therefore to answer "No".

The advantage of using survival analysis to estimate the difference thresholds is that this approach acknowledges the existence of censored data (Hough, Langohr, Gómez, & Curia, 2003). Because of the discrete nature of the salt-reduction percentages evaluated by assessors, the exact salt-reduction percentage at which each consumer starts perceiving the salt-reduced sample as less tasty than the control cannot be exactly observed. Difference thresholds can only be estimated as an interval defined between the immediate lower salt-reduction percentage at which the consumer perceives the salt-reduced sample as less tasty than the control for the first time (i.e. gives the first "No" answer) and the first salt-reduction percentage at which he/she starts consistently answering "No".

Survival analysis methodology was used to determine the difference threshold in each of the four studies. A random variable R can be defined as the salt reduction percentage at which an assessor starts consistently perceiving the salt-reduced sample as less tasty than the control, which corresponds to the difference threshold. The rejection function $F(r)$ can be defined as the probability of a consumer having his/her difference threshold at a salt-reduction percentage lower or equal than r , that is $F(r) = P(R \leq r)$. Choosing a lognormal distribution for the rejection function (Hough et al., 2003), F can be expressed as:

$$F(r) = \Phi \left(\frac{\ln(r) - \mu}{\sigma} \right)$$

where $\Phi(\bullet)$ is the standard normal cumulative distribution function and μ and σ are the model's parameters.

The likelihood function, which is used to estimate the parameters of the rejection function, corresponds to the joint probability of the given observations of the n assessors (Klein & Moeschberger, 1997). The parameters of the lognormal distribution (μ and σ) for $F(r)$ were obtained by maximizing the likelihood function for the given experimental data using the R scripts provided by Hough (2010). Goodness of fit of the lognormal distribution was visually compared to other parametric models (Logistic, Weibull, Log-logistic), as recommended by Hough et al. (2003).

After the parameters were calculated, the percentage of consumers having their difference threshold lower than salt reduction percentage was graphed. Difference thresholds for each sample were determined as the salt-reduction percentage at which 50% of the consumers had their difference thresholds.

1.2.5.2. Overall liking and CATA data

A linear mixed model was carried out on data from the evaluation of the second bite to assess the existence of significant differences in overall liking scores among samples. Sample was specified as a fixed effect, whereas consumer was specified as a random effect. A significance level of 5% was considered in the analysis. When the effects were significant, honestly significant differences were calculated using Tukey's test.

Groups of consumers with different overall liking of the samples were identified using hierarchical cluster analysis. Euclidean distances and Ward's clustering method were used in the clustering procedure (Næs, Brockhoff, & Tomic, 2010). A linear mixed model was performed separately on data from each consumer group.

The frequency of use of each CATA term was determined by counting the number of consumers who used it to describe each sample after the second bite. Cochran's Q test (Manoukian, 1986) followed by the sign test for each pair of products (Meyners, Castura, & Carr, 2013) was carried out to identify significant differences among samples in each of the sensory terms. A correspondence analysis (CA) was performed on the frequency table. Confidence ellipses around the projected coordinates of the samples were obtained using partial truncated bootstrapping considering the first two dimensions of the configurations. CATA questions data was analysed separately for each of the consumer groups identified in the hierarchical cluster analysis following the procedure described for the original dataset.

All data analyses were carried out using R software version 3.1.1 (R Core Team, 2014).

A CONSUMER APPROACH TO SALT REDUCTION

1.3. Results

1.3.1. Difference thresholds

Survival analysis was used to model the percentage of consumers having difference thresholds lower than each salt-reduction percentage. In all the studies, the lognormal distribution showed the best fit to the experimental data. The average difference thresholds were determined as the salt-reduction percentage at which 50% of the consumers had their difference threshold. The difference thresholds for each of the studies ranged from 9.42% to 14.29% of the salt concentration of the control bread (Table 1.2). In the specific case of the bread with the maximum salt concentration, salt reductions lower than 10.00% would not be noticeable for consumers.

Table 1.2 Difference thresholds and 95% confidence intervals for added salt in bread with respect to references with different added salt concentration in four consumer studies.

Study	Added-salt concentration of the control (%)	Difference threshold (%)	Difference threshold respect to the control sample (%)	95% confidence interval
1	2.00	1.80	10.00	7.50 -13.00
2	1.80	1.61	10.56	6.67 -16.67
3	1.61	1.38	14.29	9.94 -21.12
4	1.38	1.25	9.42	4.35 -10.87

Given that the confidence intervals of the estimations overlapped, it can be concluded that difference thresholds were a constant proportion of the original salt concentration of the breads. Considering an average threshold, the Weber fraction for salt reduction in bread corresponded to 11.01%.

1.3.2. Sensory and hedonic perception of salt-reduced bread

1.3.2.1. Hedonic perception

Salt concentration had a significant influence on consumer overall liking of the breads after the second bite ($p < 0.001$). Significant differences were found between bread samples containing 2.00% -1.80% added salt and those containing 1.38% -1.25% salt. As shown in Table 1.3, the bread sample formulated with 1.61% salt did not significantly differ from the bread sample containing 1.25% salt nor from those containing 1.80% - 2.00% salt. Therefore, considering data from all consumers, salt reductions up to 19.5% could be achieved without affecting consumers' hedonic perception, even if they perceived saltiness differences (Table 1.2).

Consumer segmentation was explored and large heterogeneity in how consumers perceived the bread samples was identified. Two groups of consumers differing in their hedonic reaction towards salt reduction in bread were identified by means of hierarchical cluster analysis on overall liking data from the evaluation of the second bite. Group 1 (n=57, relative size 58%), comprised those consumers who gave higher overall liking scores for samples with higher salt content. The bread samples formulated with 2.00% and 1.80% salt showed the highest liking scores, whereas the bread containing 1.25% showed the lowest score (Table 1.3). On the other hand, Group 2 (n=42, relative size 42%) showed preference for the bread sample with the lowest salt content and did not discriminate samples containing 2.00% -1.38% salt. No significant differences on the basis of gender (p=0.61) and age (p=0.53) were found between the two identified clusters.

Table 1.3 Average overall liking scores of bread samples with different salt concentration for all consumers and for the two groups identified in the hierarchical cluster analysis, for the evaluation of the second bite.

Salt concentration (% of flour weight)	All consumers (n=99)	Group 1 (n=57)	Group 2 (n=42)
2.00	6.1 ^c	6.2 ^{c,d}	5.9 ^a
1.80	6.0 ^c	6.4 ^d	5.5 ^a
1.61	5.8 ^{b,c}	5.9 ^c	5.6 ^a
1.38	5.2 ^a	5.2 ^b	5.4 ^a
1.25	5.5 ^{a,b}	4.4 ^a	7.0 ^b

Values within a column with different letters are significantly different according to Tukey's test (p<0.05).

1.3.2.2. Sensory perception

1.3.2.2.1. Sensory characterization considering all the consumers

Significant differences among samples were identified in the frequency of use of the terms *salty*, *dry*, *tasteless*, *crumbly*, *smooth* and *tasty* (Table 1.4). In particular, salt reduction caused a significant decrease in the frequency of selection of the terms *salty* and *tasty*, as well as an increase in the frequency of use of the terms *dry* and *tasteless*.

A CONSUMER APPROACH TO SALT REDUCTION

Table 1.4 Percentage of consumers who used each of the terms of the CATA question when evaluating the second bite of 5 bread samples with different added salt concentrations.

Term	Salt concentration (% of flour weight)				
	2.00	1.80	1.61	1.38	1.25
Salty *	14 ^b	13 ^b	7 ^{a,b}	4 ^a	5 ^a
Hard ^{ns}	10 ^a	13 ^a	19 ^a	20 ^a	9 ^a
Soft ^{ns}	31 ^a	27 ^a	23 ^a	18 ^a	24 ^a
Spongy ^{ns}	35 ^a	35 ^a	34 ^a	23 ^a	39 ^a
Dry *	20 ^a	23 ^{a,b}	34 ^{b,c}	36 ^c	27 ^{b,c}
Light ^{ns}	29 ^a	21 ^a	22 ^a	27 ^a	28 ^a
Dense ^{ns}	31 ^a	27 ^a	27 ^a	21 ^a	22 ^a
Tasteless *	10 ^a	16 ^{a,b}	15 ^a	33 ^c	27 ^{b,c}
Barely salty ^{ns}	26 ^a	27 ^a	28 ^a	38 ^a	36 ^a
Off-flavour ^{ns}	6 ^a	8 ^a	9 ^a	13 ^a	14 ^a
Crumbly*	13 ^{b,c}	18 ^c	6 ^{a,b}	14 ^{b,c}	4 ^a
Sticky ^{ns}	24 ^a	25 ^a	16 ^a	16 ^a	19 ^a
Smooth*	33 ^{b,c}	31 ^{b,c}	22 ^{a,b,c}	16 ^a	40 ^c
Gummy ^{ns}	18 ^a	28 ^a	25 ^a	21 ^a	22 ^a
Tasty*	30 ^b	26 ^{a,b}	30 ^b	19 ^{a,b}	15 ^a
Characteristic bread flavour ^{ns}	29 ^a	27 ^a	22 ^a	15 ^a	25 ^a

The frequency of use of terms indicated by * significantly differed among samples for a significance level of 0.05, whereas frequency of use of terms indicated by ^{ns} did not significantly differ among samples. Values within a row with different letters are significantly different according to the Sign Test ($p < 0.05$).

The term *tasteless* showed a higher frequency of use for samples containing 1.25% and 1.38% salt compared to the rest of the samples. On the contrary, the frequency of use of the term *tasty* was lower for the bread samples formulated with 1.25% and 1.38% salt. Significant differences in the frequency of use of the term *salty* were found between samples containing 2.00% -1.80% salt and those formulated with 1.25%-1.38% salt. In despite of this, it is interesting to highlight that the attribute *salty* was almost not used for describing samples (Table 1.4). This points out that consumers may have used the term *tasty* rather than *salty* to describe samples with higher salt intensity. Regarding texture characteristics, no clear relationship between salt reduction and frequency of use of the terms *dry* and *crumbly* was found (Table 1.4).

The first two dimensions of the CA performed on data from the evaluation of the second bite of bread samples using CATA questions explained 82.9% of the inertia. The first dimension of the sensory map sorted samples according to their salt concentration (Figure 1.1). This dimension was positively associated with the term *tasteless* and negatively associated with the term *salty*. Samples containing 2.00% and 1.80% salt were sorted from those containing 1.38% to 1.25%.

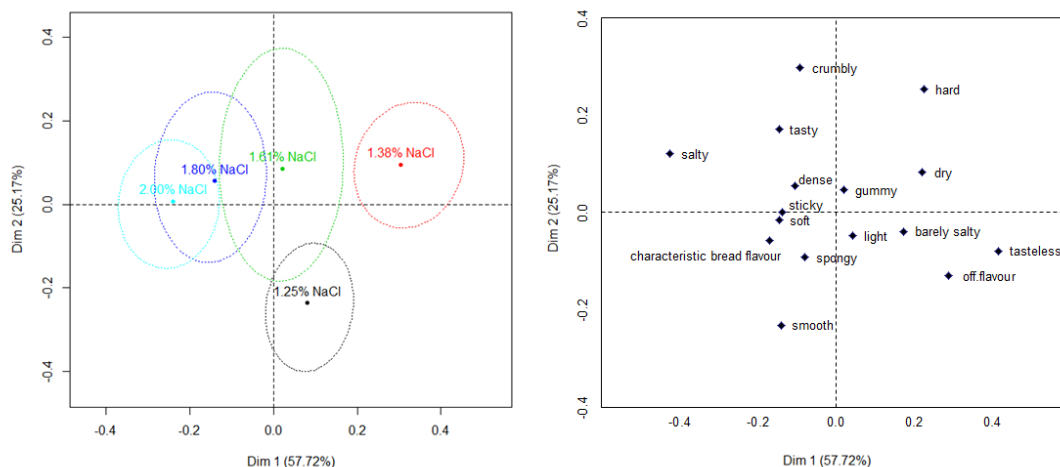


Figure 1.1 Representation of samples and terms in the first two dimensions of the CA performed on results from CATA questions data for the evaluation of the second bite of bread samples with different salt concentration (2.00% -1.25%).

1.3.2.2.2. Sensory characterization based on the perception of each consumer segment

Figure 1.2 shows the sensory maps obtained from the first two dimensions of the CA performed on CATA questions data from each of the consumer groups identified in the hierarchical cluster analysis performed on acceptance data from the second bite evaluation. As shown in Figure 1.2a, the sensory map from Group 1 (relative size 58%) was similar to that obtained from all the consumers (Figure 1.1). The first two dimensions of the CA performed on data from Group 1 explained 85.9% of the inertia. The first dimension of the sensory space was positively associated with the term *tasteless* and negatively associated with the terms *salty* and *tasty*. This dimension sorted samples according to their salt content: breads samples containing 2.00% -1.80% salt were located far apart from those formulated with 1.38% and 1.25% salt. Significant differences among samples were mainly identified in attributes related to flavour characteristics (*tasteless*, *tasty*, *characteristic bread flavour*, *off-flavour*, and *smooth*). On the other hand, 79.5% of the inertia was explained by the first two dimensions of the CA performed on CATA questions data from Group 2 (relative size 42%). For these consumers, the first dimension was positively correlated with the term *smooth* and

A CONSUMER APPROACH TO SALT REDUCTION

negatively associated with the terms *tasteless* and *off-flavour* (Figure 1.2b). This dimension sorted the bread sample formulated with 1.25% salt from those containing 1.80% -1.38% salt. Meanwhile, the second dimension was positively associated with the term *salty* and sorted samples with 2.00% and 1.80% salt apart from the rest. Significant differences among samples were mainly identified in attributes related to bread texture (*smooth, hard, spongy*), as well as on the flavour term *tasteless* (data not shown).

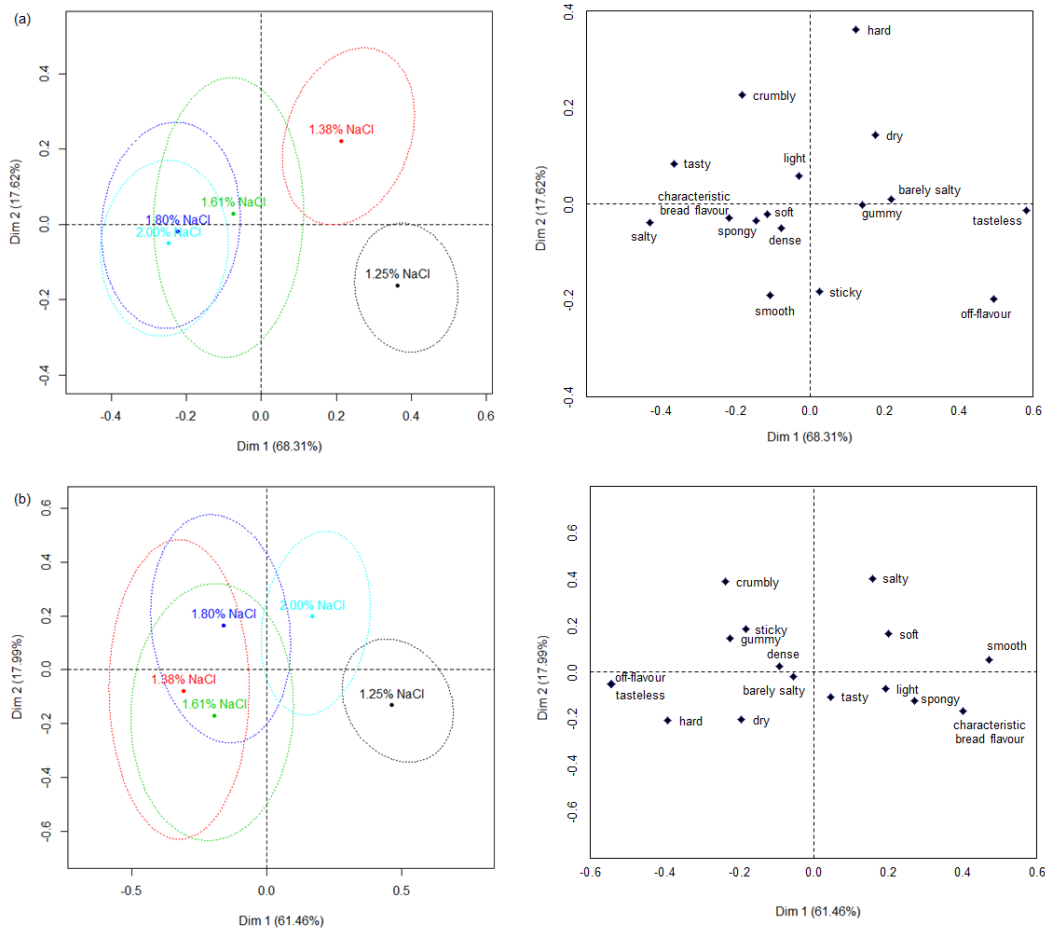


Figure 1.2 Representation of samples and terms in the first two dimensions of the CA performed on CATA questions data from the evaluation of the second bite of bread samples with different salt concentration (2.00% -1.25%) for each of the clusters identified in the hierarchical cluster analysis on overall liking data: (a) Group 1 (n=57) and (b) Group 2 (n=42).

1.4. Discussion

Four difference thresholds for salt in bread were determined based on consumer perception. Difference thresholds ranged between 9.20% and 14.59% and did not significantly differ among them, in agreement with Weber's law (Lawless & Heymann, 2010). In a similar vein, Bobowski and Vickers (2012) have previously estimated a series of sequential thresholds for salt in broth (6.3% -19.8%). However, it is interesting to highlight that these authors worked with trained assessors, whereas in the present work naïve consumers were used, which may provide more realistic recommendations.

The salt reduction of each reduction step can still be regarded as conservative considering that consumers in a real-life setting would not evaluate two bread samples one right after the other. Instead, they would compare reformulated products with their memory of the sensory characteristics of the regular product. Therefore, reductions close to 11% seem to be a safe criterion for the design of gradual salt-reduction programmes. Further research is necessary to extend the findings from the present work to other bread formulations. In this sense, La Croix et al. (2014) reported that a salt reduction of 10% in bread samples made with 50% whole-wheat flour and 50% white bread flour was not detected by consumers.

In the present work, salt reduction only caused relevant changes in the flavour characteristics of the breads, particularly when it was reduced below 1.61% (Figure 1.1). Salt reduction resulted in breads with lower intensity of saltiness and tastiness. Samples containing 2.00% and 1.80% salt were located close together, suggesting that a salt reduction of 10% in bread did not significantly affect its sensory characteristics. Lynch, Dal Bello, Sheehan, Cashman and Arendt (2009) explored the effect of sodium reductions from 1.2% to 0.6%, 0.3% and 0% (w/w) on dough and bread characteristics. Results from their work suggested that while the production of bread with lower sodium content is feasible from a technological perspective taste needs to be improved. These authors reported that reducing the salt content by 50% had a significant effect on saltiness perception. However, results from the present work showed that salt reductions higher than 10% are expected to modify saltiness perception.

Consumer hedonic perception of the breads was not affected in the first two salt reduction steps, corresponding to a reduction of 19.5% (Table 1.3), suggesting that a short time could be elapsed between them. However, further reductions cause changes in consumer hedonic perception. Previous research has reported that changes on consumer preference towards salt reduced products are expected to occur at least eight weeks after having started a salt reduced diet (Bobowski & Vickers, 2012; Bobowski,

A CONSUMER APPROACH TO SALT REDUCTION

2015a). Research should be carried out to evaluate the time period that should be elapsed between two successive reduction steps. La Croix et al. (2014) have also explored the sodium concentration at which American consumers detected differences between reduced-sodium breads and the regular product. These authors found that consumers detected salt reductions of 20% and 30% from the original salt content (2.00% of flour weight) but did not modify their liking or purchase intention.

Large heterogeneity was found in consumer hedonic reaction towards salt reduction. Two consumer groups differing in their overall liking of reduced salt breads were identified: Group 1, showing preference for bread samples containing higher salt content and Group 2, which gave higher overall liking scores to the bread sample with least salt content. These two groups also differed in their hedonic sensitivity to changes in the salt content of breads (Table 1.3). One of the groups tolerated a 19.5% salt reduction, whereas the other did not modify their hedonic perception with a 31% reduction in salt content. The sensory description of the two groups showed that they attached different relative importance to saltiness (Figure 1.2). This result points out the importance of considering consumer segmentation in order to better understand consumer reaction towards salt reduction. It is worth highlighting that different strategies may be used to successfully develop salt-reduced food products, depending on the target group.

According to the results from the present work salt in bread could be reduced by 37.5% following four sequential salt reduction steps. The first two steps could be implemented without much waiting time, as they are not expected to influence consumer hedonic perception (Table 1.3). Similar results have been reported by other studies with other product categories which have reported that total salt reductions from 20% to 30% could be achieved in small stepwise salt reduction over time (Zandstra et al., 2015). In particular, Girgis et al. (2003) assessed the effect of gradual salt reduction in bread. These authors found that salt reductions by 25% could be achieved on bread without consumer awareness through equal cumulative salt reductions over a 6 weeks period.

However, it is worth noting that when implementing stepwise salt reductions in food products long time frames are required to achieve the target sodium content. In order to overcome this limitation the combination of gradual salt reduction with other strategies that lead to sodium reductions without affecting consumer perception have been proposed (Busch et al., 2013). In the last few years, different sodium reduction strategies have been assessed, such as: non homogeneous spatial distribution of salt in food products (Noort et al., 2010; Noort et al., 2012; Mosca et al., 2013), the use of salts with different time intensity profiles, mineral contents and morphology (Quilareo et al.,

2015; Vella et al., 2012), as well as the replacement of NaCl with other salts, such as KCl (Phelps et al., 2006; Liem et al., 2011). Further research in this respect is needed to explore the influence of these strategies for sodium reduction on consumer perception of different product categories.

1.5. Conclusions

The present work proposed a consumer-based methodology to design gradual salt reduction programmes in processed products. Four difference thresholds for salt in bread were determined, providing valuable information for the implementation of gradual salt reduction in bread. Results from the present work suggest that the salt content of bread could be reduced by 10% without affecting consumer sensory perception of the products, whereas the first two cumulative reduction steps, that account for a 19.5% of salt reduction, could be rapidly implemented without causing changes in consumer hedonic perception. Further reductions should be implemented with an adequate waiting time to get consumers accustomed to the sensory characteristics of the salt-reduced breads. Research should be carried out to evaluate the time period that should be elapsed between two successive reduction steps.

Large heterogeneity on consumer hedonic reaction towards salt reduction, and particularly on consumer hedonic sensitivity, was found. This result stresses the need to consider consumer segmentation to improve insights when exploring consumer perception of salt reduction in processed products.

CHAPTER 2

Determination of sodium in bread

SODIUM DETERMINATION IN BREAD

Abstract

The sodium content of five bread samples formulated with varying added salt concentrations (2.00%, 1.80%, 1.61%, 1.38% and 1.25% salt, expressed on the basis of flour weight) were determined using low temperature flame photometry. Bread samples were previously ashed, using a microwave wet ashing method, and the moisture content was determined. According to the regulatory framework recently approved in Uruguay only the bread formulated with 2.00% salt (530 mg sodium/ 100 g bread) would feature a sodium warning.

2.1. Introduction

Excessive sodium intake represents a serious public health problem worldwide. While sodium is required for physiological needs, the current sodium intake is far in excess of nutritional requirements and is causing an adverse effect on health (He & MacGregor, 2007; WHO, 2012). In this sense, there is overwhelming evidence suggesting a close linkage between sodium intake and high blood pressure, the major risk factor for cardiovascular disease (He et al., 2019; He & MacGregor, 2007; WHO, 2012).

Salt and sodium are often used indistinctly but, only sodium contributes to the adverse health effect associated with excessive salt consumption (Capuano et al., 2013). Dietary sodium could be derived from different sources, for instance sodium occurring naturally in food. However, the greatest contribution to dietary sodium intake is provided from added salt. There are two main sources of added salt in food: discretionary salt (i.e. added by consumers during cooking or at the table) and salt added during the production process of prepared and processed foods (Purdy & Armstrong, 2007; Zandstra et al., 2015). In addition, sodium salts other than sodium chloride are in some cases added to food products for specific technological purposes (e.g. food additives) (Bussell & Hunt, 2007).

Since 2006, the inclusion of nutritional information on food packages is mandatory in our country. According to the regulatory framework, it is compulsory to declare sodium, energy, total carbohydrates, proteins, total fat, saturated fat, trans fat and total fiber content in the nutritional information provided on food packages (Ministerio de Salud Pública, 2006). In August 2018, Uruguay approved a presidential decree on front-of-pack (FOP) nutritional labelling (Decreto N° 272/018). The decree entered into force on March 1st, 2020 in order to grant the food industry an 18-month period to adapt to the new regulation (Ministerio de Salud Pública, 2018). On March 11th, 2020 a 120 days extension was granted to the food industry (Presidencia de la República Oriental del Uruguay, 2020). The decree applies to packaged processed food products that contain fat, sugar or salt as ingredients. Products falling within the scope of regulation that contain excessive amount of nutrients associated with non-communicable diseases (sugar, fat, saturated fat and sodium) should feature an octagonal black sign with the expression “Excessive in” on the FOP for each nutrient that exceeds the pre-established criteria (Ministerio de Salud Pública, 2018). In the case of sodium, products which sodium content exceeds 500 mg per 100 g product or 8 mg sodium per 1 kcal should feature a FOP sodium warning.

SODIUM DETERMINATION IN BREAD

The compulsory inclusion of nutritional information on food packages has led to a global growing demand for rapid and accurate methods for analysis of minerals (such as sodium) and other food components in the last couple of decades (Penner, 2010). Atomic spectroscopy is widely used for accurately measuring the mineral composition of foods. Atomic spectra consist of discrete lines that are characteristic of individual elements (i.e. every element has a unique spectrum). This makes it possible to accurately identify and quantify individual elements, even when atoms of other chemical elements are present (Penner, 2010). The basic principle behind these methods is that the energy content of matter is quantized and, therefore, matter may only absorb or emit energy if the energy associated with the photon of radiations corresponds to the energy difference for allowed transition levels. Spectroscopic methods encompass a wide range of techniques both for qualitative and quantitative analysis of the chemical composition of foods that could be based on either the absorption (AAS) or emission (AES) of radiation by the analyte (Penner, 2010).

Among AES techniques, flame emission spectroscopy and inductively coupled plasma-atomic emission spectroscopy are the most widespread used in food analysis. In particular, flame emission, in which the energy for excitation is produced by heat (from the flame), represents a reliable and convenient alternative for elements with relatively low excitation energy (i.e. 1500-2000°C), such as Na, K and Ca (Penner, 2010). In low temperature flame photometry, emissions with characteristics wavelengths, are produced when electrons from excited neutral atoms return to lower energy states. Then, emissions are isolated, by passing them through an optical filter and converted to an electrical signal by the photo detector. Sample atomization is required to produce usable spectra for quantitative analysis purposes. In addition, foods are typically ashed as a previous step for mineral determination in order to get minerals freed from the organic matrix of the foods (Penner, 2010).

In the present study, the sodium content of five bread samples formulated with varying added salt levels were determined and expressed in both dry and wet weight basis. The moisture content of breads was determined as a previous step to estimate the dry weight of samples.

2.2. Materials and methods

2.2.1 Bread samples

Five bread samples were analysed to determine their sodium content: a control bread (2.00% added salt, expressed on the basis of flour weight) and four salt-reduced breads (1.80%, 1.61%, 1.38% and 1.25% salt), which corresponded to the difference thresholds for salt in bread previously determined in Antúnez, Giménez and Ares (2016). The control sample represents the average sodium content of the breads available at the local marketplace at the moment the study was carried out. This information was provided by the Bromatological Laboratory of Intendencia de Montevideo based on data collected in 2015 on the sodium content of 124 French bread samples taken from bakeries in Montevideo city (*).

All the analyses were performed considering genuine run replicates (i.e. for each salt level two breads units were produced in independent batches). The breads were produced using 58% Uruguayan commercial wheat flour (Molino Americano S.A, Montevideo, Uruguay), 37% tap water, 2.4% sugar (Alcoholes del Uruguay S.A., Bella Unión, Uruguay), 1.6% high oleic sunflower oil (Compañía Oleaginosa Uruguay S. A, Montevideo, Uruguay), 2.0% to 1.25% g salt / kg flour (Antil S.A, Montevideo, Uruguay) and 1% powered yeast (Fleischmann, Argentina). The breads were prepared in a bread-making machine (Phillips® model HD9015/30) one day prior to the analysis and were stored in plastic bags overnight.

2.2.2 Moisture content of bread

The moisture content of bread was determined using an oven drying method. The day prior to moisture determination the capsules were oven treated (103 ± 2 °C overnight) and then cooled to room temperature in a functioning desiccator. Two 10 mm slices were sampled from each bread unit and grounded prior to analysis. Each capsule was assigned a code to identify the bread sample it would hold, and weight registered with a precision of ± 10 mg. Then, 5 g of the grounded bread were placed on the capsules and the final weight (capsule plus sample) was registered with a precision of ± 10 mg. The capsules holding the samples were covered and put into a desiccator until being disposed in the oven at 103 ± 2 °C overnight (completing a drying period of 13 hours).

(* Sodium determination was performed using atomic absorption spectroscopy. The sodium content in French bread ranges from 2079 ppm to 8199 ppm with an average sodium content of 5241 ppm (which corresponded to 1.97% salt, expressed on the basis of flour weight).

SODIUM DETERMINATION IN BREAD

The cover was slipped to one side during drying to allow for moisture evaporation. Samples were then removed from the oven, cooled to room temperature in a desiccator and weighted. The determination was performed in duplicate for each run replicate. The moisture content was estimated as follows (Robert & Bradley, 2010), and duplicated data were averaged:

$$\% \text{ Moisture (wt/wt)} = \frac{\text{wt of wet sample} - \text{wt of dry sample}}{\text{wt of wet sample}} * 100$$

2.2.3 Sodium determination in bread

2.2.3.1 Microwave wet ashing

As a previous step for sodium determination, an acid digestion of the bread samples was carried out in a closed vessel device using temperature control microwave heating. Bread samples were dried prior to analysis (following the procedure described in 2.2). A total of 350 mg \pm 10 mg homogenized sample was weighed and placed in a Mileston PTFE (TFM Fluoropolymer) vessel. Then, 7 mL nitric acid (65% m/m) and 1 mL hydrogen peroxide (30% m/m) were added. A blank was prepared by adding the same amount of nitric acid and hydrogen peroxide to the vessel but without sample addition. All the reagents were of analytical grade.

The vessel was put into the HTC safety shield, closed, introduced into the rotor segment (Milestone vent-and-reseal system) and then tighten with the torque wrench. The rotor segment was placed into the microwave with a PC controlled temperature program (model Ethos one; Milestone Inc., Shelton, US) and the microwave program was run until completion. Table 2.1 shows the steps of the microwave program used for the digestion of the bread samples.

Table 2.1. Microwave program used for the digestion of bread samples

Step	Operation	Time (min)	To (°C)	Ti (°C)
1	Heating until set temperature	15	20	200
2	Keeping set temperature	15	200	200
3	Cooling	20	200	80

Once the digestion has finished, the vessels were taken out of the rotor segment and cooled by air to room temperature. Subsequently the vessel was opened and the digested solution quantitatively transferred to a 25 mL marked flask, made up to volume with Milli Q water and mixed. For each bread sample, the resulting solutions were diluted using Milli Q water to obtain a final sodium concentration of 2-3 mg/L. Dilutions factors

were defined considering the expected sodium content in each bread, which was estimated based on the amount of salt added during bread production. Cesium chloride was added to the solutions (0.1%) as an auxiliary spectroscopic buffer reagent.

2.2.3.2 Sodium determination using a flame emission photometer

The sodium content was determined by low temperature flame photometry, using an emission flame photometer (Model 360 Flame Photometer; Sherwood scientific LTD, UK) with propane/butane/air flame at a wavelength of 589 nm. A series of standards of varying sodium concentrations were prepared to build a calibration curve in the linear working range of the flame photometer (0.00 -6.00 mg/L). A stock standard solution of sodium (1000mg/L) was used to prepare the calibration solutions in water (1.00, 3.00 and 6.00 mg/L).

Prior to analysis the spectrophotometer was stabilized for 20 min with demineralized water. The zero was set with Milli Q water and the top standard (6 mg sodium/L) was used to adjust the required reading on the display. The prepared standards were aspirated, in increasing concentrations, and their stable display readings were manually recorded (three display readings were performed). This data was used to build the calibration curve: display reading (signal intensity) against standard concentration. Then, sample solutions were aspirated and their stable display reading was recorded following the same procedure. A digestion blank, containing all the constituents of the sample solutions -except the sample- and submitted to the digestion process, was used. Sodium concentrations were then determined by interpolation from the calibration curve.

2.3 Results and discussion

2.3.1 Moisture content of bread

As shown in Table 2.2 the moisture content of the pan bread samples ranged between 39.9% (± 1.5) and 41.3% (± 1.2), providing evidence that this parameter was not affected by the sodium content in bread. The average moisture content for this product category in the local marketplace has been reported as 33.4% (Ministerio de Trabajo y Seguridad Social, Universidad de la República, Facultad de Química, 2002). Deviation from the average moisture content reported for the product category may be related to differences in the cooking conditions (time and temperature) between the bread-making machine and a real bakery industrial setting.

SODIUM DETERMINATION IN BREAD

Table 2.2. Moisture content of bread samples.

Sample	Percent moisture (% wt/wt)
2.00%	40.7 (± 0.6)
1.80%	40.5 (± 0.0)
1.61%	40.5 (± 0.9)
1.38%	41.3 (± 1.2)
1.25%	39.9 (± 1.5)

2.3.2 Sodium content of bread

Table 2.3 summarizes the sodium content of the five bread samples, expressed both in a wet and dry weight basis. According to the regulatory framework recently approved in our country (Ministerio de Salud Pública, 2018), food products with salt added during the production process which sodium content exceeds 500 mg per 100 g product, should feature a FOP nutritional warning indicating excessive sodium content. Thus, the bread containing 2.00% salt (530 mg sodium/ 100 g bread), but none of the salt reduced breads, would feature the sodium warning.

Table 2.3. Sodium content in pan bread samples with varying amount of added salt, expressed in both wet weight basis (wwb) and dry weight basis (dwb).

Sample	Na content wwb (mg Na 100 g ⁻¹)	Na content dwb (mg Na 100 g ⁻¹)
2.00%	530.1	893.5
1.80%	486.5	818.0
1.61%	424.2	712.9
1.38%	379.6	646.5
1.25%	347.9	578.8

As expected, the sodium content determined by means of low temperature flame photometry was systematically higher than expected if only sodium from the salt added during the bread production was taken into account (Table 2.4). This points out that sodium sources other than added salt occur among bread ingredients.

Table 2.4. Comparison between salt added during bread production and the amount of salt estimated based on sodium determination

Sample	Na(g)/ bread unit	NaCl (g)/ bread unit	NaCl (g)/ unit added to bread (recipe)	Difference (g/bread unit)
2.00%	4.7	11.9	11.2	0.7
1.80%	4.3	10.9	10.1	0.8
1.61%	3.7	9.5	9.0	0.5
1.38%	3.4	8.6	7.7	0.9
1.25%	3.1	7.8	7.0	0.8

The fact that 5-10% of sodium in bread does not come from added salt but from other ingredients, emphasizes the importance of the analytical determination of sodium to obtain an accurate estimation of the sodium content. In this sense, as the number and complexity of the ingredients increases, the contribution of salt from sources other than added salt is expected to increase.

CHAPTER 3

Consumer perception of salt-reduced breads: Comparison of single and two-bites evaluation

COMPARISON OF SINGLE AND TWO-BITES EVALUATIONS

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Author contributions:

- Antúñez, L: Conceived and designed the work, collected the data, analysed and interpreted the data, wrote the paper.
- Giménez, A: Contributed to the conception and design of the study, as well as to data interpretation.
- Alcaire, F: Contributed to data collection, performed a critical revision of the article.
- Vidal, L: Contributed to data collection, performed a critical revision of the article.
- Ares, G: Contributed to the conception and design of the study, as well as to data interpretation, performed a critical revision of the article.

Abstract

Salt-reduction in processed products has been proposed as a high-impact intervention for reducing the sodium intake at population level. A major limitation for this approach is its potential negative impact on the sensory characteristics of products. The current practice in sensory and consumer science involves single sip/bite evaluations, which may not properly reflect the sensory experience that occurs during product consumption. In this context, the aim of the present work was to compare single and two bite evaluations of consumer sensory and hedonic perception of salt-reduced breads. Five studies with a total of 499 consumers were carried out, in which overall-liking scores of five salt-reduced bread samples were collected after the first and the second bite evaluation. In one of the studies consumers also answered a CATA (check-all-that-apply) question after the first and the second bite. Neither bite nor the interaction between samples and bite had a significant effect on hedonic scores. However, when hedonic scores were analysed separately for each bite, the overall liking scores from the second bite evaluation better reflected differences among samples according to their salt content in two of the five studies. The sensory characterization of the samples did not largely vary between the first and the second bite. Results suggest that consumers perception of salt reduced bread samples did not largely vary between a single and a two bites evaluation. Further research is warranted in this regard, in particular considering more complex products.

3.1. Introduction

The burden of non-communicable diseases (NCDs) over the last decade has raised serious global public health concerns, being the major cause of mortality and morbidity all over the world (WHO, 2014). In terms of attributable deaths, high blood pressure has been identified to be the leading metabolic risk factor for the prevalence of NCDs, in particular of cardiovascular diseases. One of the factors that is most closely related to the growing prevalence of high blood pressure is excessive sodium intake (Elliott et al., 1996). In this sense, even relatively small changes in population dietary sodium intake are expected to cause a major impact on global health (He & MacGregor, 2009; WHO, 2007). For this reason, reducing sodium intake at population level has been proposed as a high-impact intervention to prevent and control the prevalence of high blood pressure (Campbell et al., 2012).

In developed countries, processed foods have been identified as the main source of dietary sodium, accounting for 75%-80% of the total sodium intake (Appel & Anderson, 2010; Campbell et al., 2012; Grimes et al., 2011). Hence, the reformulation of processed foods through a lower salt content has been proposed as a cost-effective strategy to reduce the sodium intake at the population level (Downs et al., 2015). For this purpose, either voluntary or mandatory targets for salt reduction in different food products categories are being established to encourage the food industry to engage in reformulation strategies (Downs et al., 2015; Legowski & Legetic, 2011).

Given the widespread consumption of bread and its major contribution to the daily sodium intake, this product has been established as a target product category for salt reduction in many countries (Girgis et al., 2003; Belz, Ryan, & Arendt, 2012; Kloss, Meyer, Graeve, & Vetter, 2015). Several studies have already explored the impact of sodium reduction in bread production (Antúnez, Giménez, & Ares, 2016; Beauchamp et al., 1982; La Croix et al., 2014; Lynch et al., 2009; Pflaum et al., 2013; Rødbotten et al., 2015; Spina et al., 2015). These studies have shown that despite of being technologically feasible, the production of salt reduced breads had a negative impact on the flavour characteristics of bread and, consequently on consumer's preferences. Therefore, a major limitation to salt reduction in bread and other processed foods is its potential negative impact on the sensory characteristics of the product. Understanding the influence of salt reduction on consumers' perception is a key aspect for the design and implementation of salt-reduction strategies.

Current practice in sensory and consumer science involves single sip/bite evaluations. However, eating is a dynamic process that generally involves multiple

COMPARISON OF SINGLE AND TWO-BITES EVALUATIONS

ingestions of the food product (Appelqvist et al., 2016). Thus, the evaluation of a single bite/sip may not properly reflect the sensory experience that occurs during product consumption, especially when considering complex products or samples with small differences among them (Appelqvist, et al., 2016; Köster, 2003; Rocha-Parra et al., 2016; Stein et al., 2003; Zorn et al., 2014). According to Köster (2003) repeated food ingestions may modify food perception as a consequence of adaptation processes. In this sense, several studies have suggested that single hedonic evaluations may not properly predict the future liking (Köster, 2003). In addition, it has been reported that small differences among products only become noticeable after repeated food ingestions (Köster, 2009; Köster et al., 2002; Stein, et al., 2003; Zandstra et al., 2004). Therefore, single bite evaluations may not accurately reflect how consumers perceive salt-reduced products in real-life consumption situations, which could lead to inaccurate recommendations for salt-reduction.

To the authors knowledge, few studies have considered multiple sip/bite evaluations when assessing the sensory and hedonic perception of food products. Zorn et al. (2014) used an extension of the Temporal Dominance of Sensations (TDS) method, that involves multiple sip/bite TDS evaluations (Vandeputte, Romans, Pineau, & Lenfant, 2011), to assess the dynamic sensory profile of orange juices samples. This approach has also been used by Schlich, Urbano and Visalli (2013) on chocolate samples. More recently Rocha-Parra, et al. (2016) proposed a multiple bite method to evaluate the temporal changes in consumers overall liking of a new healthy beverage and Appelqvist et al. (2016) used a multiple ingestion approach to assess the sensory characteristics of different oil in water emulsions.

In this context, the aim of the present work was to explore the effect of two bite evaluations (vs. a single bite evaluation) on consumer sensory and hedonic perception of food products, using salt reduced bread as focal product.

3.2. Materials and methods

Five consumer studies were carried out to compare the evaluation of the first and second bite on consumers' hedonic perception of salt-reduced breads. In addition, in Study 1 the effect of multi-bite evaluation on product sensory characterizations was explored by asking consumers to answer a CATA question after having tried the first and the second bite. Details regarding each of the studies are presented in Table 3.1.

Table 3.1 Overview of the studies used to compare single and multiple (two) bite evaluations.

Study ID	Number of consumers	Age range (average age)	Gender (%)	Product category	Number of samples
1	99	18 - 66 (average: 30.0)	F: 55% M:45%	Pan bread	5
2	101	18 - 60 (average: 23.9)	F:43% M:57%	French bread	5
3	99	18 - 67 (average:27.7)	F:71% M:29%	French bread	5
4	99	18 - 54 (average: 24.3)	F:66% M:34%	French bread with Danbo cheese ^a	5
5	100	18 - 67 (average:28.2)	F:73% M:27%	French bread samples presented with information about percentage of salt reduction	5

The following abbreviations were used to indicate consumers' gender: F for female and M for male.

^a 'Danbo cheese' is a semi-soft, cow's milk cheese that is usually consumed as an accompaniment to bread in Uruguay. The Danbo cheese used in the present study was obtained from the local marketplace; its salt content was 543 mg sodium/100 g of cheese.

3.2.1. Participants

The number of participants in each of the studies ranged from 99 to 101; their age and gender characteristics are summarized in Table 3.1. Participants from Study 2 and 4 belong to the same sample population. They were randomly divided into two groups and were assigned either to Study 2 or Study 4. The same occurred in relation to study 3 and 5. Participants were recruited from the data base of the Sensometrics & Consumer Science research group of Universidad de la República (Uruguay), based on their bread consumption and willingness to take part in the study. All participants signed an informed consent form and received a small gift for their participation.

COMPARISON OF SINGLE AND TWO-BITES EVALUATIONS

3.2.2. Samples

Bread samples with different salt content were considered. The salt concentrations used for bread formulation (2.00%, 1.80%, 1.61%, 1.38% and 1.25% salt) were defined according to gradual salt reduction steps established in a previous study involving the same product category (Antúñez et al., 2016). Two types of bread were considered: pan bread and French bread.

Study 1 involved pan breads samples. All samples were produced using 58% Uruguayan commercial wheat flour (Molino Americano S.A, Montevideo, Uruguay), 37% tap water, 2.4% sugar (Alcoholes del Uruguay S.A., Bella Unión, Uruguay), 1.6% high oleic sunflower oil (Compañía Oleaginosa Uruguay S. A, Montevideo, Uruguay), 1% of powered yeast (Fleischmann, Argentina) and salt -2.00% to 1.25% salt/ kg flour- (Antil S.A, Montevideo, Uruguay). The breads were prepared in a bread-making machine (Phillips® model HD9015/30) one day prior to testing and were stored in plastic bags overnight. The morning of the test, breads were cut in 10 mm slices and the crust was removed. Each assessor received two pieces of bread, corresponding to approximately five grams each.

On the other hand, studies 2 - 5 involved French bread samples that were specially produced for the purpose of the study by a local baking company. Bread samples were cut in 10 mm slices immediately before the test. Each assessor received two bread slices, corresponding to approximately five grams each. In Studies 2 and 3, consumers evaluated the salt reduced French breads. In Study 4 consumers received the same French bread samples with a slice of Danbo cheese, whereas in Study 5 samples were presented with information about the percentage of salt-reduction.

Samples were served on white plastic plates, coded using three-digit numbers, and were presented in a monadic sequence following an experimental design balanced for order and carry-over effects (Williams' Latin Square).

3.2.3. Single and two bite evaluations for assessing consumer hedonic and sensory perception of bread samples

In Study 1, consumers were asked to try a first bite of the sample, to rate their overall liking using a 9-point hedonic scale (1 = dislike very much, 9 = like very much) and to answer a check-all-that-apply (CATA) task. The CATA question comprised 16 sensory attributes related to texture and flavour characteristics of the bread samples: *salty, hard, soft, spongy, dry, light, dense, tasteless, barely salty, off-flavour, crumbly, sticky, smooth, gummy, tasty and characteristic bread flavour*. The terms of the CATA question were selected based on consumers' free description of similar products in

previous studies. After the evaluation of the first bite, consumers were asked to try a second bite of the sample and to evaluate it following the same procedure. Assessors were instructed to cleanse their palate with water between samples, but not between bites. The terms included in the CATA question were balanced across consumers, following a Williams' Latin square design.

In Studies 2 - 5, consumers were requested to try a first bite of the sample and to rate their overall liking using a 9-point hedonic scale. Then, they were asked to try a second bite of the sample and to rate their overall liking again.

In all the studies data were collected using Compusense-Cloud (Compusense Inc., Guelph, Ontario, Canada). Testing took place in a sensory laboratory designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22 °C).

3.2.4. Data analysis

3.2.4.1. Overall liking

For each study, a linear mixed model was carried out to assess the existence of significant differences in overall liking scores among samples across the first and the second bite evaluation. Sample, bite and their interaction were specified as fixed effects, whereas consumer was considered as a random effect. Besides, data from the evaluation of the first and the second bite were analysed separately by means of linear mixed modelling, considering samples as fixed effect and consumers as a random effect. A significance level of 5% was considered in the analysis. When the effects were significant, honestly significant differences were calculated using Tukey's test.

3.2.4.2. Sensory characterization

Data from the CATA question from the evaluation of the first and second bite in Study 1 were separately analysed. The frequency of use of each CATA term was determined by counting the number of consumers who used it to describe each sample. Cochran's Q test was carried out to identify significant differences among samples in each of the sensory terms (Manoukian, 1986). When differences among samples were significant, the sign test was used to identify significant differences between pairs of samples in each of the sensory terms (Meyners et al.,2013).

All data analyses were carried out using R software version 3.1.1 (R Core Team, 2014). Mixed linear models were run using `lmerTest` (Kuznetsova, Brockhoff, & Christensen, 2015).

COMPARISON OF SINGLE AND TWO-BITES EVALUATIONS

3.3. Results

3.3.1. Hedonic perception

As shown in Table 3.2, significant differences in overall liking were established in all the studies ($p < 0.001$), suggesting that salt concentration markedly influenced consumer hedonic perception of the bread samples. Neither bite nor the interaction between bite and sample had a significant effect on the overall liking scores. This result points out that eliciting hedonic scores after the second bite evaluation (vs. the first bite evaluation) did not largely affect consumers' perception.

Table 3.2 Results of linear mixed modelling of overall liking scores of Studies 1 - 5.

Study ID		F.value	p-value
Study 1	Sample	11.20	<0.001
	Bite	0.45	0.505
	Sample*Bite	0.43	0.789
Study 2	Sample	14.26	<0.001
	Bite	1.54	0.215
	Sample*Bite	0.09	0.986
Study 3	Sample	12.69	<0.001
	Bite	0.02	0.903
	Sample*Bite	0.12	0.975
Study 4	Sample	6.75	<0.001
	Bite	2.14	0.144
	Sample*Bite	0.25	0.911
Study 5	Sample	24.61	<0.001
	Bite	0.10	0.754
	Sample*Bite	0.12	0.976

When overall liking scores were analysed separately for each bite, the second bite evaluation led to slightly higher F-values for sample effect in three of the five studies (Table 3.3). The opposite trend was observed for the other two studies. However, overall liking scores from the second bite evaluation better reflected differences among samples according to their salt content in Studies 1 and 4. In addition, in those studies the second bite evaluation showed greater hedonic discrimination according to Tukey's test (Table 3.3). In this sense, results from the first bite evaluation in Study 1 only discriminated the bread sample containing 1.38% salt from the rest. However, results from the second bite evaluation revealed significant differences between the overall liking scores of bread samples containing 2.00% -1.80% added salt from those containing 1.38%-1.25% salt.

Table 3.3 Results from the linear mixed model performed separately on overall liking scores from the evaluation of the first and second bite in Studies 1- 5.

Study ID	Bite	F.value	p-value	Salt concentration (% of flour weight)				
				1.25%	1.38%	1.61%	1.80%	2.00%
1	Bite 1	4.51	0.001	5.8 ^b	5.3 ^a	5.7 ^{a,b}	6.1 ^b	6.1 ^b
	Bite 2	6.27	<0.001	5.5 ^{a,b}	5.2 ^a	5.8 ^{b,c}	6.0 ^c	6.1 ^c
2	Bite 1	7.30	<0.001	6.0 ^a	6.0 ^a	6.3 ^a	6.2 ^a	6.9 ^b
	Bite 2	5.86	<0.001	6.1 ^a	6.2 ^a	6.4 ^a	6.3 ^a	7.0 ^b
3	Bite 1	12.85	<0.001	6.3 ^{a,b}	6.1 ^a	6.4 ^{a,b}	6.6 ^{b,c}	6.9 ^c
	Bite 2	9.74	<0.001	6.2 ^{a,b}	6.0 ^a	6.4 ^b	6.6 ^{b,c}	7.0 ^c
4	Bite 1	3.04	0.017	6.6 ^{a,b}	6.6 ^{a,b}	6.5 ^a	6.9 ^{b,c}	7.1 ^c
	Bite 2	3.41	0.009	6.6 ^a	6.8 ^a	6.6 ^a	6.9 ^{a,b}	7.3 ^b
5	Bite 1	5.28	<0.001	6.0 ^a	6.0 ^a	6.5 ^b	6.9 ^{b,c}	7.3 ^c
	Bite 2	6.68	<0.001	6.0 ^a	6.1 ^a	6.5 ^{a,b}	7.0 ^{b,c}	7.2 ^c

Average values within a row for each study with different letters are significantly different according to Tukey's test ($p < 0.05$).

3.3.2 Sensory characterization

In Study 1, assessors answered the same CATA questions twice: for the first and for the second bite. Results showed that both the evaluation of the first and the second bite provided similar information regarding the sensory characteristics of the salt reduced bread samples (Table 3.4). The percentage of terms used for describing samples did not largely differ between bites. Moreover, according to the Cochran's Q Test, the percentage of CATA terms for which differences among samples were significant was slightly higher for the evaluation of the second bite (37%) relative to first bite (31%).

COMPARISON OF SINGLE AND TWO-BITES EVALUATIONS

Table 3.4 Frequency of use of the terms from the CATA question during the first and the second bite evaluation of five bread samples with different added salt concentration.

Attribute	Bite	p-value	Salt concentration (% of flour weight)				
			1.25%	1.38%	1.61%	1.80%	2.00%
Salty	Bite 1*	0.012	6 ^a	5 ^a	8 ^{a,b}	15 ^b	16 ^b
	Bite 2*	0.021	5 ^a	4 ^a	7 ^{a,b}	13 ^b	14 ^b
Hard	Bite 1 ^{ns}	0.119	9 ^a	15 ^a	15 ^a	6 ^a	14 ^a
	Bite 2 ^{ns}	0.051	9 ^a	20 ^a	19 ^a	13 ^a	10 ^a
Soft	Bite 1 ^{ns}	0.149	28 ^a	22 ^a	19 ^a	28 ^a	32 ^a
	Bite 2 ^{ns}	0.172	24 ^a	18 ^a	23 ^a	27 ^a	31 ^a
Spongy	Bite 1 ^{ns}	0.552	38 ^a	34 ^a	41 ^a	42 ^a	34 ^a
	Bite 2 ^{ns}	0.066	39 ^a	23 ^a	34 ^a	35 ^a	35 ^a
Dry	Bite 1*	0.001	28 ^{a,b}	38 ^b	32 ^b	19 ^a	18 ^a
	Bite 2*	0.010	27 ^{a,b,c}	36 ^c	34 ^{b,c}	23 ^{a,b}	20 ^a
Light	Bite 1 ^{ns}	0.628	31 ^a	27 ^a	27 ^a	22 ^a	28 ^a
	Bite 2 ^{ns}	0.492	28 ^a	27 ^a	22 ^a	21 ^a	29 ^a
Dense	Bite 1 ^{ns}	0.164	17 ^a	21 ^a	26 ^a	28 ^a	29 ^a
	Bite 2 ^{ns}	0.412	22 ^a	21 ^a	27 ^a	27 ^a	31 ^a
Tasteless	Bite 1*	0.001	25 ^{b,c}	30 ^c	16 ^b	14 ^b	12 ^a
	Bite 2*	<0.001	27 ^{b,c}	33 ^c	15 ^a	16 ^{a,b}	10 ^a
Barely salty	Bite 1 ^{ns}	0.306	38 ^a	33 ^a	27 ^a	31 ^a	27 ^a
	Bite 2 ^{ns}	0.095	36 ^a	38 ^a	28 ^a	27 ^a	26 ^a
Off-flavour	Bite 1 ^{ns}	0.098	12 ^a	14 ^a	5 ^a	9 ^a	6 ^a
	Bite 2 ^{ns}	0.224	14 ^a	13 ^a	9 ^a	8 ^a	6 ^a
Crumbly	Bite 1*	0.044	7 ^a	16 ^{a,b}	9 ^a	19 ^b	10 ^{a,b}
	Bite 2*	0.006	4 ^a	14 ^{b,c}	6 ^{a,b}	18 ^c	13 ^{b,c}
Sticky	Bite 1 ^{ns}	0.416	11 ^a	13 ^a	15 ^a	18 ^a	19 ^a
	Bite 2 ^{ns}	0.227	19 ^a	16 ^a	16 ^a	25 ^a	24 ^a
Smooth	Bite 1 ^{ns}	0.054	41 ^a	23 ^a	37 ^a	32 ^a	33 ^a
	Bite 2*	<0.001	40 ^c	16 ^a	22 ^{a,b}	31 ^{b,c}	33 ^{b,c}
Gummy	Bite 1 ^{ns}	0.983	20 ^a	18 ^a	20 ^a	18 ^a	20 ^a
	Bite 2 ^{ns}	0.402	22 ^a	21 ^a	25 ^a	28 ^a	18 ^a
Tasty	Bite 1 ^{ns}	0.868	19 ^a	14 ^a	18 ^a	17 ^a	17 ^a
	Bite 2*	0.009	15 ^a	19 ^{a,b}	30 ^b	26 ^{a,b}	30 ^b
Characteristic bread flavour	Bite 1*	<0.001	23 ^a	18 ^a	27 ^{a,b}	36 ^b	40 ^b
	Bite 2 ^{ns}	0.086	25 ^a	15 ^a	22 ^a	27 ^a	29 ^a

The frequency of use of terms from the CATA question indicated with * significantly differed among samples for a significance level of 0.05, whereas frequency of use of terms indicated by ^{ns} did not significantly differ among samples. Values within a row with different letters are significantly different according to the sign test ($p < 0.05$).

According to the evaluation of the first bite, significant differences among samples were found in the frequency of use of the terms *salty*, *dry*, *tasteless*, *crumbly* and *characteristic bread flavour*. Salt reduction was associated with a decrease in the frequency of use of the terms *salty* and *characteristic bread flavour*, as well as an increase in the frequency of mention of *tasteless*. Differences among samples in texture terms were minor and were not related to salt reduction. Similarly, results from the evaluation of the second bite evidenced significant differences among samples in the frequency of selection of the terms *salty*, *dry*, *tasteless*, *crumbly*, *smooth* and *tasty*. Salt reduction caused an increase in the frequency of use of *tasteless* while decreasing the frequency of use of the terms *tasty* and *salty* (Table 3.4). Regarding the comparison of the evaluation of the first and second bite, it is interesting to note that the frequency of use of the term *tasty* showed a marked increase between the first and second bite, particularly for bread samples formulated with 1.61%- 2.00% salt (Table 3.4). A clear relationship between salt reduction and frequency of use of texture attributes was not found for the evaluation of the first bite, nor for the evaluation of the second bite.

3.4. Discussion

Consumer sensory and hedonic perception is one of the main challenges for the implementation of salt-reduction strategies. Across the five studies, the salt content of samples significantly affected their overall liking scores, providing evidence that consumers preferred bread samples with higher salt contents (Table 3.3). Except for Study 2, consumers did not distinguish between the bread samples containing 2% salt and those formulated with 1.8% salt, pointing out that a 10% salt reduction could be a safe criterion for this product category. These results are in agreement with results reported in previous studies. La Croix et al. (2014) assessed American consumers' perception of salt-reduced breads formulated with 50% whole-wheat flour and 50% white bread flour and reported that salt reductions of 10% did not modify consumer's perception. According to these authors, even when detected, salt reductions of 20% and 30% did not affect the liking or the purchase intent of bread samples. Rødbotten et al. (2015) evaluated the effect of salt reduction (regular salt content vs. 50% salt reduction from the original salt content) on European consumers' perception of barley breads. Results from their work also suggested that consumers preferred the breads with higher salt content.

Studies 3 and 5 enabled the assessment of the effect of providing information about salt reduction on consumers' hedonic perception. Results suggested that although hedonic perception was not influenced by information, consumers were more discriminative in the informed condition (Study 5) compared to the blind evaluation

COMPARISON OF SINGLE AND TWO-BITES EVALUATIONS

(Study 3) (Table 3.3). Therefore, information about salt-reduction seemed to make consumers more critical about the samples. Similar results were reported by Vázquez, Curia and Hough (2009) when evaluating the influence of information on consumers' perception of salt-reduced crackers. Information about salt-reduction seem to create negative expectations about the products, which can influence consumers' sensory and hedonic perception (Köster, 2003; Theunissen, Polet, Kroeze, & Schifferstein, 2000). Therefore, communicating salt reduction during the implementation of programmes to reduce salt intake at population level would not be recommended. Further research in this respect should be conducted.

Considering that bread is usually not eaten alone, the assessment of salt-reduced breads with an additional food component could be expected to provide more realistic information on the effect of salt reduction in a real-life setting. Although the traditional way of consuming bread can vary greatly within countries, bread is typically eaten spread with butter, jam or other bread-spreads and is also a common accompaniment to every lunch or dinner (Rødbotten et al., 2015). In this regard, comparison of results from Studies 2 and 4 showed that the evaluation of salt-reduced bread samples with a slice of Danbo cheese make consumers more tolerant regarding salt reduction. In this sense, when the bread samples were assessed alone, consumers showed clear preference for the regular bread. However, when bread was assessed with a slice of Danbo cheese consumers did not distinguish between the regular bread and the bread produced with 1.80% salt, suggesting that a 10% salt reduction may not affect consumers' perception. Moreover, the average overall liking of salt reduced samples was higher when the bread samples were evaluated with Danbo cheese. This result suggests that the evaluation of consumer perception of salt-reduced samples provides conservative results for the design of salt-reduction programmes.

In terms of the sensory characteristics of bread, salt reduction markedly affected the flavour characteristics, particularly when considering salt reductions of up to 20%. A decrease in *saltiness*, *characteristic bread flavour* and *tastiness* perception were the most relevant changes in the sensory characteristics of samples due to salt reduction. Lynch et al. (2009) found that a 50% salt reduction significantly modified the saltiness perception of bread samples, suggesting that the main challenge for successful salt reduction in food is to improve their taste.

The assessment of consumer hedonic perception based on single vs. two-bites evaluation was explored. Overall liking scores collected after the evaluation of the first and second bite did not significantly differ. However, when hedonic scores were analysed separately for each bite, the overall liking scores obtained from the second bite

evaluation better reflected differences among samples according to their salt content in two of the five studies. In those studies, greater discrimination among samples in terms of Tukey's test was observed. Similar results have been reported by Zandstra et al. (2004). These authors found no significant differences in the overall liking scores of instant chicken soups assessed after having tried three sips and the whole cup consumption. The authors highlighted that the portion size of soup (175 ml) considered in the study may have been too small to generate sensory specific satiety with the product. It is interesting to note that the portion size may have also affected results from the present work in which assessors only tried two bites, corresponding to approximately five grams each. In this sense, it would be interesting to consider bigger sample portions for future studies, as they may be more representative of real product consumption. However, it should be taken into account that bigger serving sizes may limit the number of samples to be included in the study in order to avoid consumer's fatigue.

Regarding the sensory characterization of salt reduced breads using CATA questions, highly similar sample descriptions were obtained from the evaluation of the first and the second bite in Study 1. However, after having tried a second bite, consumers found larger differences among samples in terms of their *tastiness* distinguishing between breads formulated with 2.00% - 1.61% salt and those containing 1.38% - 1.25% salt. Although exploratory in nature, results suggest that considering multiple bites evaluations could provide more comprehensive insights of consumer product experiences with salt reduced products. Similar results have been reported by other studies with other product categories. Appelqvist et al. (2016) assessed the sensory characteristics of different oil in water emulsions through a multiple ingestion procedure in which each assessor tried 12 spoons of the sample. Results from their work show that after repeated ingestions differences become more apparent, suggesting that care must be taken when drawing conclusions regarding consumers' sensory perception of food products based on a single sip/bite evaluation. Zorn et al. (2014) reported differences in the temporal sensory profile of samples of orange juices sweetened with different sweeteners from the first to the third sip. These authors found that larger differences between samples were observed when considering data from the third sip evaluation.

Further research is needed to deeply explore the effect of multiple bite/sip evaluations on consumer's perception of food products. In particular, it would be interesting to consider multiple bite/sip evaluations on complex products, as the effect could be less likely to occur on simple products categories. Besides, research on the number of sip/bites to be considered during sample evaluation is hardly recommended. Research on this topic is expected to provide valuable information for developing

COMPARISON OF SINGLE AND TWO-BITES EVALUATIONS

recommendations for best practice in the field, particularly when dealing with small changes in the sensory characteristics of products.

3.5. Conclusions

Results from the present work showed that the number of bites had no significant effect on consumers' hedonic perception of salt reduced bread samples. However, the second bite evaluation provided greater hedonic discrimination and tended to better reflect differences among samples according to their salt content in two of the five studies. Similarly, although the sensory characterization of salt reduced samples did not largely vary between bites, samples tended to be better discriminated according to their salt content in the evaluation of the second bite. These results suggest multi-bite evaluations hold potential to more accurately evaluate the influence of salt reduction on sensory and hedonic perception.

CHAPTER 4

Consumers' heterogeneity towards salt reduction: Insights from a case study with white rice

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

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- Antúnez, L: Conceived and designed the work, collected the data, analysed and interpreted the data, wrote the paper.
- Giménez, A: Contributed to the conception and design of the study, as well as to data interpretation, performed a critical revision of the article.
- Alcaire, F: Contributed to data collection, performed a critical revision of the article.
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Abstract

In the current context of increasing worldwide concern about the prevalence of health problems related to high sodium intake, reducing the sodium content of food products has been identified as a cost-effective strategy to improve public health. The present work was aimed to assess the effect of salt reduction on consumers' perception, using white rice as case study. Particular emphasis was put on exploring individual differences in consumers' reaction towards salt reduction. A preliminary study, using a trained panel, was conducted to determine the difference threshold for salt in rice. The consumer study involved 156 consumers and encompassed three parts. First, consumers were presented with a 150 grams portion of cooked rice prepared without added salt and were asked to indicate how much salt they would add to it (without tasting). Then, they were presented with six white rice samples differing in their salt content and were asked to indicate their overall liking, using a 9-point hedonic scale, to assess adequacy of saltiness intensity using a 5-point just-about-right scale, and to indicate whether they would add salt to the rice. Finally, consumers completed a short survey about their salt consumption habits, interest in health and socio-demographic profile. As expected, results revealed large heterogeneity in consumers' hedonic reaction towards salt reduction: two consumer segments with different hedonic reaction were identified. Both consumer segments also differed in their hedonic sensitivity and tolerance to salt reduction in rice. Significant differences between consumer segments were found in their salt consumption habits, in particular related to salt addition to food, and also in their interest in reducing salt intake. These results suggest that eating habits might play a major role in shaping our preferences, highlighting the potential of gradual salt reduction as a strategy for reducing sodium intake

4.1. Introduction

A robust body of evidence has shown a close linkage between excessive sodium intake and raised blood pressure (Chobanian et al., 2003; WHO, 2014), which is the leading cause of cardiovascular diseases (WHO, 2014). Public interventions aimed at reducing sodium intake at the population level have been proposed as a cost-effective strategy to cope with the growing burden of cardiovascular diseases (He & MacGregor, 2009; WHO, 2007).

In this context, the reformulation of processed food products towards lower sodium chloride (hereinafter, indistinctly referred to as salt) contents has emerged as a potential opportunity to tackle excessive sodium consumption (Campbell et al., 2012; Downs et al., 2015). Reducing the salt content of widely consumed food products is expected to accomplish population-wide changes in dietary sodium intake, even if consumers do not modify their food choices, which could have an important positive health impact at the societal level (He & MacGregor, 2009; WHO, 2007).

Pleasure has been identified as a main driver of food consumption in the developed world (Lowe & Butryn, 2007). In addition, it has been widely reported that consumers are not willing to compromise on their hedonic experience with food for potential health benefits (Civille & Oftedal, 2012; Tuorila & Cardello, 2002; Verbeke, 2006). Thus, one of the major barriers for reducing salt in food is our preference for salty taste (Mennella, 2014). Large salt reductions have been reported to cause negative changes in consumer perception of a wide variety of food products (Beauchamp et al., 1982; Kilcast, & den Ridder, 2007; Phelps et al., 2006; Rødbotten et al., 2015).

Gradual salt reductions have been proposed to deal with the negative changes on consumers' hedonic perception (FSA, 2003; IOM, 2010). The advantages of this strategy over abrupt salt reduction has been extensively reported (Bobowski et al., 2015a; Girgis et al., 2003). The core idea behind gradual salt reduction is to implement a series of unnoticeable salt reduction steps to get consumers gradually used to lower salt levels without being aware of the change. An adaptation period, in which consumers get used to the new salt level, must be elapsed before further reduction steps could be taken. This process continues until the target salt level is reached. In practice, the size of salt reduction steps could be estimated by means of difference threshold for salt. Difference thresholds for salt can be experimentally estimated as the smallest change in salt concentration that causes a change in saltiness intensity that is perceived by 50% of the individuals (Boring, 1942). Considering that saltiness perception depends on the

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

food matrix in which salt is added (Kilcast & den Ridder, 2007), recommendations for salt reduction should be made on a product specific basis.

The development of food preferences begins at conception and involves a complex interplay of biological and environmental factors that continues through the life course (Ventura & Worobey, 2013). While innate components are thought to be determinants of our liking for salty taste at early ages, food preferences in adulthood have been suggested to be markedly affected by dietary patterns (Bobowski, 2015). Previous research has shown that people exposed to high-sodium diets tend to prefer high sodium concentrations (Bertino et al. 1986; Huggins et al. 1992), whereas the opposite trend has been observed for those who follow low-sodium diets (Bertino et al. 1982; Blais et al. 1986; Elmer 1988). Therefore, large individual differences in consumer reaction towards salt reduction is expected.

The relationship between liking and saltiness intensity has been described as an inverted U shaped curve, i.e. there is an optimum salt intensity above and below which overall liking decreases (Zandstra, De Graaf, Mela, & van Staveren, 2000). However, according to Pangborn (1970) consumers can be divided into three groups based on their salty preferences: those who prefer higher levels of saltiness, those showing preference for no-salted foods and consumers for which the liking-saltiness curve has the classic inverted U shape.

Individual differences among consumers regarding salt preferences in food might be related to innate components, prior food experiences, customary eating habits, as well as attitudes toward health (De Kock, Zandstra, Sayed, & Wentzel-Viljoen, 2016; Zandstra et al., 2000). Such heterogeneity among consumers introduces additional complexity to the implementation of salt reduction programmes at the population level (Onwezen, 2018). Being unaware of these individual differences entails considerable risk of information loss (Jaeger, Hort et al., 2017). Yet, to the best of the authors' knowledge, a limited number of studies have explored individual differences to better understand the impact of salt reduction on consumers' perception (Bobowski et al., 2015a).

In this context, the present work aimed at assessing the effect of salt reduction on consumers' hedonic perception, using white rice as case study. Rice represents a widespread consumed foodstuff in Uruguay across socio-economic levels (Bove & Cerruti, 2008). Although there are plenty of food preparations with rice it is widely consumed as a side dish and almost all classic Uruguayan cookbooks recommend adding salt to water when cooking rice (Instituto Crandon, 2017). While it is still most

often prepared at home, the availability of precooked dried rice in the local marketplace has increased in the last years, following the current trend towards consumption of ready-to-eat processed food products (WHO, 2014). The main contribution of this work is to provide insights on individual differences in consumers' reaction towards salt reduction, which might add valuable information for the design and implementation of salt reduction strategies.

4.2. Materials and methods

The present work involved two studies: a preliminary study with a trained sensory panel, and a consumer study. The preliminary study aimed at determining difference thresholds for salt in white rice, which represents a valuable tool for establishing the size of the salt reduction steps to avoid consumers awareness of the change. The consumer study sought to explore the impact of salt reduction on consumer hedonic perception of white rice. Special focus was put on individual differences among consumers in terms of their reaction towards salt reduction.

4.2.1. Preliminary study: difference threshold estimation

4.2.1.1 Trained assessors

The trained panel consisted of sixteen assessors, ages ranging from 22 to 56 years old (13 female). Assessors were recruited among workers of Universidad de la República (Uruguay) and went through a selection and training process according to the guidelines of the ISO 8586:2012 standard (ISO, 2012). The sensory panel had been previously trained to evaluate saltiness intensity in bread, as reported in Antúnez, Giménez, Vidal and Ares (2018).

Eight 15 minutes training sections were carried out, on separated days, to get assessors familiarized with saltiness evaluation in the focal product. During these sessions, the panel performed paired comparisons involving rice with different salt concentrations.

4.2.1.2 Rice samples

Rice samples were prepared the day of the test using an automatic rice cooker (Panasonic SR-W18AG) with varying amounts of salt 0-3.6 g salt/ 100 g raw rice (Table 4.1). The cooked rice samples were stored at room temperature in glass containers until being served. Samples were served at room temperature (25°C) on white plastic plates coded with three-digit numbers.

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

4.2.1.3 Methodology

Paired comparisons were used to determine difference thresholds for salt in white rice. A rice sample with a salt concentration comparable to the average commercial precooked dried rice available in the Uruguayan marketplace (3.6% salt, expressed on the basis of raw rice weight) was considered as starting point for difference threshold determination.

The trained panel performed a series of six paired-comparison tests. Each pair involved a control rice (that remained constant in each study) and a sample that was reduced in salt from the control (Table 4.1). The salt concentrations considered in this study were selected by pilot testing.

Table 4.1. Salt concentration and percentage salt-reduction (between brackets) of the rice samples considered in the determination of the difference threshold for salt.

Pair comparison	Control rice (gram of salt/100 of raw rice)	Salt reduced sample (gram of salt/100 of raw rice)
1	3.60	2.40 (33.3)
2	3.60	2.60 (27.8)
3	3.60	2.80 (22.2)
4	3.60	3.00 (16.7)
5	3.60	3.20 (11.1)
6	3.60	3.40 (5.6)

Trained panellists were requested to try each of the samples in a pair and to indicate the saltier one by selecting the corresponding code number. Samples in each pair were presented following a balanced design in order to avoid order and carry-over effects. Water was used as palate cleaner between pairs. The threshold determination was performed in duplicate.

Testing took place in a sensory laboratory designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22°C). Data were collected using Compusense-Cloud (Compusense Inc., Guelph, Canada).

4.2.1.4. Data analysis

Difference thresholds was estimated by means of survival analysis, using the procedure proposed by Reis, de Andrade, Deliza and Ares (2016). For each of the six paired comparisons, assessors' responses were coded using a binary code-system ("No", "Yes"). If the control was identified as saltier than the reduced-salt sample the response was coded with "No", otherwise it was coded with "Yes". It was argued that if

the salt reduction was higher than the difference threshold, then the trained panelists would always give a “No” answer when performing the paired comparison. Thus, the difference threshold was estimated as the salt-reduction percentage at which a panelist started to consistently perceive the salt-reduced sample as less salty than the control, i.e. to systematically answer “No”. This salt-reduction percentage (i.e. the difference threshold) was defined as a random variable R .

The rejection function $F(r)$ was defined as the probability of a trained panelist having the difference threshold at a salt-reduction percentage lower or equal than r , that is $F(r) = P(R \leq r)$. Different parametric models (logistic, Weibull, lognormal) were evaluated for the rejection function. The parameters of the model were estimated by maximizing the likelihood function for the given experimental data using the scripts for R statistical software provided by Hough (2010). Goodness of fit was visually compared among models (Hough et al., 2003). Difference thresholds were determined as the salt-reduction percentage at which 50% of the judgements had their difference threshold (i.e. perceived the salt-reduced sample as less salty than the control).

4.2.2. Consumer study

The consumer study encompassed three parts. In the first task consumers were asked to indicate how much salt they would add to a rice portion cooked without added salt. The second task aimed at assessing consumers' hedonic perception towards rice samples with different salt content. Consumers were requested to rate their overall liking of the samples, and to assess the adequacy of saltiness intensity using a 5-point Just-about-right (JAR) scale. In addition, they were asked to indicate whether or not they would add salt to the rice sample. Finally, they completed a short survey about their socio-demographic profile and behaviour related to health and salt consumption.

4.2.2.1. Participants

A total of 156 consumers (65% female, ranging in age between 18 and 75 years old –mean=32.3, SD=13.8) participated in the study. They were recruited at a local food market in Montevideo (Uruguay) according to their rice consumption and their willingness to take part in the study. However, no criterion related to consumption frequency of rice was enforced. All participants signed a consent form and received a small gift for their participation. Table 4.2 summarizes the socio-demographic characteristics of the participants.

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

Table 4.2. Socio-demographic characteristics of participants (n=156).

Socio-demographic characteristic	Percentage (%)
Gender	
Female	65
Male	35
Age	
18-24	44
25-34	20
35-49	18
50 and more	18
Educational level	
Primary education	1
Secondary education (incomplete)	11
Secondary education (complete)	16
Technical education	12
University degree (unfinished)	38
University degree (finished)	15
Postgraduate studies	7
Number of adults in the household	
1	13
2	41
3	22
4	16
5 or more	8
Number of children younger than 10 years old	
0	85
1	13
2 or more	2

4.2.2.2 Rice samples

Six rice samples differing in their salt content were considered in this study: a control rice containing 3.60% added salt (expressed on the basis of raw rice weight) and five samples with different percentage of salt reduction relative to the control: 10%, 25%, 50%, 75% and 100%. The salt concentration of the salt-reduced rice samples was: 3.24%, 2.70%, 1.80%, 0.90% and 0.00%, respectively. The control rice sample represents the average sodium content of precooked dried rice available in the Uruguayan marketplace. The smallest salt-reduction (10%) was established to be slightly below the difference threshold (11.1%), estimated in the preliminary study conducted with trained assessors.

Samples were served on black plastic containers, coded using three-digit numbers, and were presented in a monadic sequence following an experimental design that was balanced for order and carry-over effects (Williams' Latin Square).

4.2.2.3. Experimental procedure

First, consumers were presented with a 150 g cooked rice portion served in a white ceramic plate and were asked to imagine they were having a meal that included that portion of rice as a side dish. They were told that no salt was added to the rice during cooking and were requested to indicate how much salt they would add to it (without tasting). For this purpose, they were presented with a set of five plastics containers, each holding a different amount of salt. The plastic containers were labelled from 1 to 5, corresponding to 0.71 g salt (75% salt reduction relative to the control), 1.42 g salt (50% salt reduction), 2.14 g salt (25% salt reduction), 2.57 g salt (10% salt reduction) and 2.85 g salt (control rice). The latter represents the average sodium content of precooked dried rice available in the Uruguayan marketplace (3.6 g salt/ 100g raw rice). Using the plastic containers 1 to 5 as reference, consumers were asked to indicate how much salt they would add to that portion of rice on the scale shown in Figure 4.1.

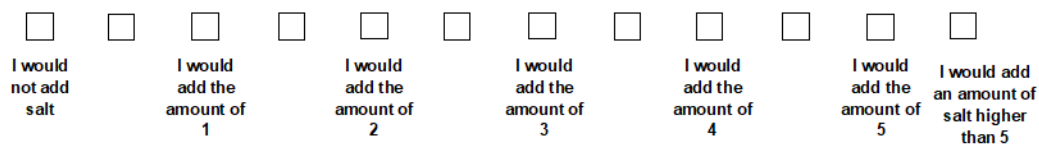


Figure 4.1. Semi-structured scale used for the assessment of how much salt consumers would add to a 150 grams cooked rice portion without added salt. Consumers received the rice portion and a set of five plastics containers, labelled from 1 to 5, each holding a different amount of salt: 0.71 g salt (75% salt reduction relative to the control), 1.42 g salt (50% salt reduction), 2.14 g salt (25% salt reduction), 2.57 g salt (10% salt reduction) and 2.85 g salt (control rice), respectively.

Then, consumers received six samples of rice with different salt content. They were asked to rate their overall liking, using a 9-point structured hedonic scale, and to assess the adequacy of saltiness intensity, using a 5-point JAR scale with end anchors “not salty enough” and “much too salty”. They were also asked to indicate whether they would add more salt to the rice to consume it or not, using the response options “yes” and “no”. Consumers were instructed to cleanse their palate with water between samples.

The last part of the study consisted on a set of questions related to their socio-demographic characteristics, their salt consumption habits and attitudes toward health. The questions included in the questionnaire, as well as the scales used in each question are shown in Table 4.3. Data were collected using paper ballots.

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

Table 4.3. Questions included in the questionnaire about salt consumption habits and attitudes toward health.

Question	Response options
How often are you responsible for preparing food at home?	Never, hardly ever, sometimes, regularly, always
How is salt used when preparing food in your home?	During cooking, after food has been cooked, no salt is used when preparing food at home
Do you usually add salt to food before trying it?	Never, sometimes, always
Do you usually add salt to home-cooked food after having tried it?	Never, sometimes, always
Do you usually add salt to food when you are eating at a restaurant?	Never, sometimes, always
Do you usually put the salt shaker on the table at home?	Never, sometimes, always
Do you usually request for the salt shaker in a restaurant?	Never, sometimes, always
How concerned are you about your salt intake?	1=Not concerned at all, 3=indifferent, 5=very concerned
Currently, do you look for information on the salt content of food products that you usually eat?	Never, sometimes, always
Are you interested in consuming low-salt food products?	1=Not interested at all, 3=indifferent, 5=very interested
Is eating healthy important to you?	1=Not important at all, 3=indifferent, 5=very important
Thinking about your eating habits, how healthy is your overall diet?	1=not healthy enough, 3=regular, 5=very healthy

4.2.2.4. Data analysis

All data analyses were carried out using R statistical software version 3.5.1 (R Development Core Team, 2017).

4.2.2.4.1. Overall liking

A linear mixed model was used to assess the existence of significant differences in overall liking scores among rice samples. The analysis was conducted specifying sample as fixed effect and consumer as random effect. A significance level of 5% was considered in the analysis. When the effects were significant, honestly significant differences were calculated using Tukey's test.

Consumer segmentation based on overall liking data was performed by means of hierarchical cluster analysis. Euclidean distances and Ward's clustering method were used in the clustering procedure (Næs et al., 2010). Data from each consumer segment identified in the hierarchical cluster analysis was analysed separately using the linear mixed model specified above.

4.2.2.4.2. *JAR scales*

JAR data were collapsed into three categories: below just-right, just-about-right and above just-right. Data from each consumer segment identified in the hierarchical cluster analysis were analysed separately. Stack charts summarizing the percentage of responses on each JAR category were constructed (Popper, 2014).

As a previous step for the statistical analysis, JAR data were recoded using a binary number system: JAR responses (i.e. a score of 3 in the 5-point JAR scale) were coded as "1" and all other responses as "0". A generalized linear model (glm) was used to identify significant differences between samples in the proportion of consumers who assessed the saltiness of rice as just-about-right. A significance level of 5% was considered in the analysis.

4.2.2.4.3. *Willingness to add salt to the rice samples*

4.2.2.4.3.1 *Willingness to add salt to rice samples with different salt reduction percentages*

Survival analysis was used to estimate the percentage of consumers that would add salt to the rice (i.e. percentage of consumers considering salt addition to rice is needed) as a function of salt reduction. Data from each consumer segment identified in the hierarchical cluster analysis was analysed separately. A random variable R was defined as the salt reduction percentage at which a consumer starts consistently answering that he/she would add salt to rice. Then, the rejection function $F(r)$ was defined as the probability of a consumer adding salt to rice at a salt reduction percentage lower or equal than r , that is $F(r) = P(R \leq r)$. The model selection and the estimation of model parameters was carried out as described in section 4.2.1.4.

Once the model was selected and the parameters were calculated, the percentage of consumers in each consumer segment adding salt to rice as a function of salt reduction was graphed. Besides, a model with covariates was applied to compare the consumer segments in terms of their willingness to add salt to the rice (Hough, 2010).

In addition, Fisher's exact test was used to compare the frequency with which consumers assessed the sample as not salty enough using the JAR scale and the

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

frequency with which they indicated that they would add salt to it. For this purpose, JAR data was divided into two categories: JAR scores <3 and JAR scores ≥ 3 . A global and a per sample Fisher's exact test was carried out separately on data from each consumer group.

4.2.2.4.3.2. *Willingness to add salt to rice prepared without added salt*

In order to facilitate the comparison of results throughout the paper, the 12-points scale used by consumers to indicate how much salt they would add to a 150 g cooked rice portion prepared without added salt was collapsed into 7 intervals: no salt added (100% salt reduction); (0g -0.71g salt addition], which corresponds to (100%-75% salt reduction); (0.71g-1.42 g salt addition], which corresponds to (75%-50% salt reduction]; (1.42 g- 2.14 g salt addition], corresponding to (50%-25% salt reduction]; (2.14 g- 2.57 g salt addition], corresponding to (25%- 10% salt reduction]; (2.57 g-2.85 g salt addition], which corresponds to (10%-0% salt reduction] and salt addition higher than 2.85 g (the amount of salt added is higher than the salt content in the control rice). The round brackets indicate the extreme value does not belong to the interval while the square brackets indicate it does belong the interval. Frequency procedures were used to analyse consumer responses.

4.2.2.4.4. *Questionnaire on socio-demographic characteristics*

Frequency procedures were used to analyse data from the questionnaire on salt consumption habits and attitudes toward health. The chi-square test per cell was carried out to assess the existence of significant differences between consumer segments (Symoneaux, Galmarini, & Mehinagic, 2012). A significance level of 5% was considered in the analysis.

4.3. Results

4.3.1. Difference threshold estimation

Survival analysis was used to estimate the difference threshold for salt in white rice. The Lognormal model showed the best fit to the experimental data. The difference threshold was estimated as the salt-reduction percentage at which 50% of the judgements assessed the salt reduced rice as less salty than the control. Results revealed that the difference threshold for salt in white rice corresponded to 11.1% of the salt added to the control rice, suggesting that salt reductions smaller than this percentage would be unnoticed by the majority of consumers.

4.3.2. Consumer study

4.3.2.1 Overall liking

Salt reduction significantly affected the overall liking scores of white rice ($p < 0.001$). As expected, as salt reduction increased, the average overall liking scores of the rice samples significantly decreased (Table 4.4). However, at the aggregate level, salt reductions up to 50% did not significantly affect consumer overall liking.

Two consumer segments were identified through hierarchical cluster analysis. Group 1 ($N=90$, relative size=57%) gave higher overall liking scores to rice samples with intermediate salt content (25%-50% salt reduction relative to the control). This group gave the lowest overall liking scores to the sample with the highest salt content (control) and to that prepared with no salt added. These samples did not significantly differ in their average overall liking score. Besides, no significant differences were found between the control rice and the rice with 10% salt reduction, which was to be expected as the salt reduction was below the difference threshold for salt in rice (11.1%). Consumers in Group 2 ($N=68$, relative size= 43%) gave the highest overall liking scores to the samples with the highest salt content and decreased their scores as the salt content decreased. Even a 10% salt reduction, which is below the difference threshold for salt in rice, had a significant negative effect on hedonic perception of consumers in Group 2. However, only when considering salt reductions higher than 25% the overall liking scores from this consumer group reached the dislike part of the hedonic scale (Table 4.4).

The two consumer segments also differed in their hedonic sensitivity to salt reduction. As shown in Table 4.4, significant differences between all pairs of samples were found for consumers in Group 2 but not for those in Group 1. In this sense, it is worth noting that consumers in Group 1 did not significantly distinguish between samples with 25%-50% sodium reduction, which in turn received higher overall liking scores than the rest of the samples (Table 4.4). Similarly, no significant differences were found in the overall liking score of rice samples with 10% and 75% salt reduction, nor between the sample without added salt (100% salt reduction) and the control rice (0% salt reduction).

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

Table 4.4. Overall liking of salt-reduced rice samples for all consumers and for the two consumer groups identified in the hierarchical cluster analysis on overall liking data. Data was collected using a 9-point structured hedonic scale with anchors “Dislike extremely”, “Neither like nor dislike” and “Like extremely”.

Sample	Salt content (% of raw rice weight)	All consumers (n=158)	Group 1 (n=90)	Group 2 (n=68)
Control	3.60	5.9 ^c	4.5 ^{a,b}	7.6 ^f
10 % red	3.24	5.9 ^c	5.0 ^{b,c}	7.0 ^e
25 % red	2.70	6.2 ^c	6.2 ^d	6.2 ^d
50 % red	1.80	5.8 ^c	6.5 ^d	4.9 ^c
75 % red	0.90	4.6 ^b	5.5 ^c	3.4 ^b
100 % red	0.00	3.2 ^a	4.0 ^a	2.2 ^a

Values within a column with different letters are significantly different according to Tukey's test ($p < .05$).

4.3.2.2 Saltiness assessment of salt-reduced rice using JAR scales

Consumers assessed the adequacy of saltiness intensity of the rice samples using a JAR scale. As shown in Figure 4.2, both consumer groups markedly differed in terms of their saltiness assessment. On the one hand, 56.7% of consumers from Group 1 considered the saltiness intensity of the rice sample with 50% salt reduction as optimum (just-about-right). On the other hand, most consumers from Group 2 (61.8%) considered the saltiness intensity of the control rice sample (without salt reduction) to be just-about-right, while 26.5% assessed it as too salty. In addition, within each consumer group, significant differences were found among samples in the percentage of consumers that assessed the saltiness intensity as just-about-right ($p < 0.001$ for both groups). In particular, it is worth highlighting that samples with more than 25% salt reduction were perceived as not salty enough by the great majority of consumers in Group 2, showing a significantly lower percentage of JAR responses than those with lower salt reductions (all p -values < 0.001). This result may explain the marked decrease in the overall liking scores of those samples relative to the control for consumers in Group 2 (Table 4.4).

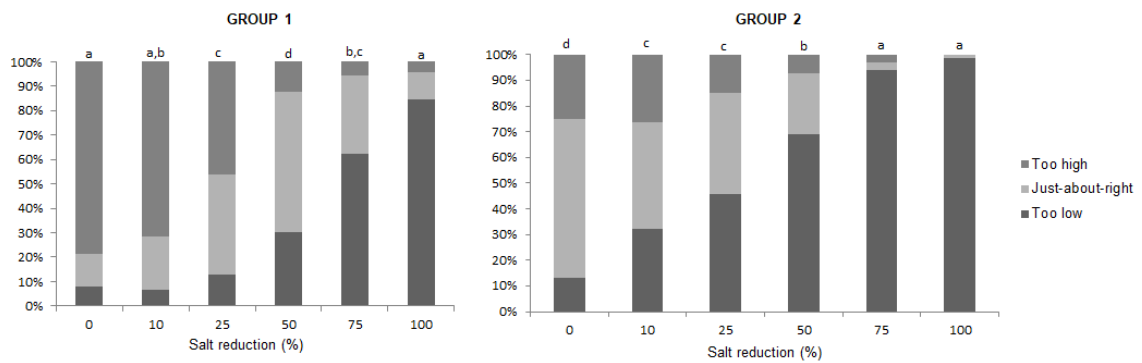


Figure 4.2. Stack charts summarizing results of the saltiness assessment, using a 5-point just-about-right scale, for the two consumer segments identified in the hierarchical cluster analysis on overall liking data (Group 1: n=90, Group 2: n=68). Data were collapsed into three categories (too high, just-about-right, too low). Sodium reduction percentages with different letters are significantly different in the percentage of just-about-right responses according to the Fisher's exact text ($p < 0.05$).

A clear relationship between sodium content and adequacy of saltiness intensity was found for the two consumer segments. For Group 1 the relationship between the salt reduction and the JAR scores showed an inverted U-shaped tendency, with an optimum around 50% salt reduction, as reflected by the significantly higher percentage of JAR response for this sample. On the other hand, for consumers in Group 2 the higher the salt-reduction the lower the score in the JAR scale (Figure 4.3).

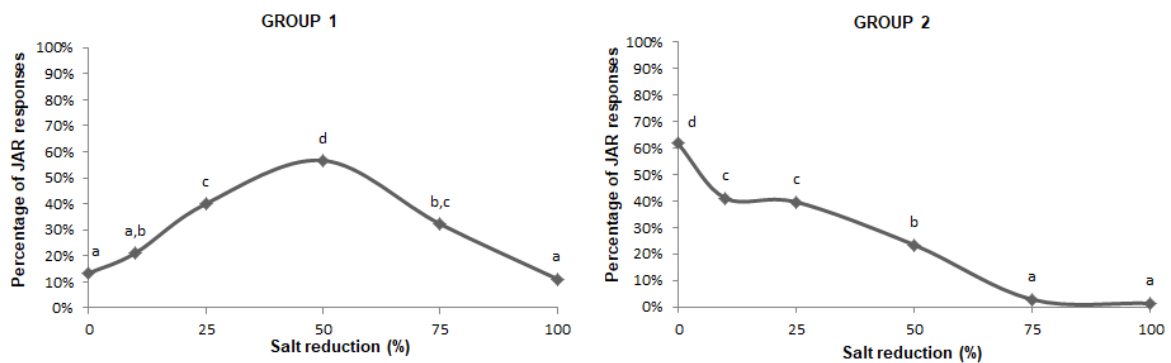


Figure 4.3. Percentage of consumers assessing the samples as just-about-right as a function of the sodium reduction percentage based on data from (a) Group 1 (n=90) and (b) Group 2 (n=68). Sodium reduction percentages with different letters are significantly different in the percentage of JAR responses according to the Fisher's exact text ($p < 0.05$).

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

4.3.2.3 Willingness to add salt to the rice samples

Survival analysis was used to model the percentage of consumers in each consumer segment that would add salt to rice as a function of salt reduction. The Weibull model showed the best fit to the experimental data. The covariate of the model was significant ($p < 0.001$), suggesting that the two consumer segments reacted differently to salt reduction. Figure 4.4 shows the curves describing the percentage of consumers who would add salt to the rice for the both groups. As shown, the salt reduction at which 50% of consumers would add salt to rice was 78.4% for Group 1 and 44.3% for Group 2, reflecting differences in terms of their tolerance for salt reduction.

Consumers' responses to whether they would add salt to rice samples with different salt-reduction percentages were contrasted against their assessment of adequacy of saltiness using the JAR scale. Results revealed that both approaches led to similar conclusions in terms of differences among samples. However, the percentage of consumers assessing the sample as not salty enough tended to be systematically higher than the percentage of consumers adding salt to rice ($p = 0.004$ for Group 1, and $p = 0.008$ for Group 2). For instance, 31% of consumers in Group 1 assessed the rice with 50% salt reduction as not salty enough, while only 23% of the consumers in this group reported that they would add salt to it. The same trend was observed when considering data from Group 2: even though 69% of consumers perceived the rice with 50% salt reduction as not salty enough, the percentage of consumers who stated that they would add salt to it was 49%. It is worth mentioning that when the analysis was performed separately at the sample level statistical significance was not reached, which might be related to the fact that sample size was not large enough to detect the difference (the statistical power depends on sample size). The exception was for Group 2 when considering the rice with 50% salt reduction, for which significant differences were found between the two approaches ($p = 0.023$).

Both consumer groups also significantly differed in the amount of salt they would add to a portion of rice prepared without added salt ($p < 0.001$). As shown in Table 4.5, half of consumers in Group 1 would add an amount of salt lower or equal than 0.71 g, which corresponds to salt reductions equal or higher than 75%. However, the percentage of consumers that would add this amount of salt was significantly lower in Group 2 (25%). On the other hand, the percentage of consumers in Group 1 reporting that they would add between 1.80 g and 2.70 g salt (which corresponds to salt reductions between 50% and 25%, respectively) was significantly lower when compared to Group 2 (4.4% vs. 23.5%, respectively). This result provides further evidence that consumers in Group 1 were more willing to consume rice with lower salt content.

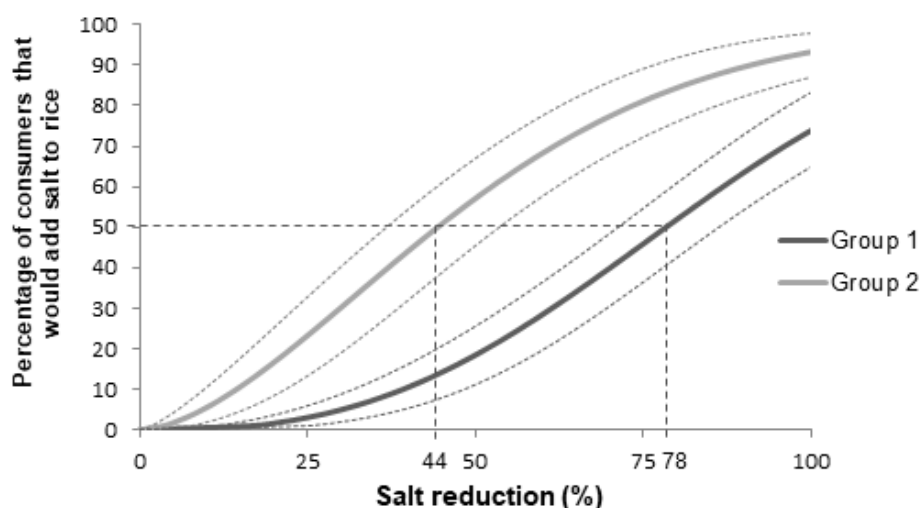


Figure 4.4. Percentage of consumers who would add salt to consume the rice as a function of sodium reduction for the two consumer segments identified in the hierarchical cluster analysis: Group 1 (n=90) and Group 2 (n=68).

Table 4.5. Percentage of consumers who reported they would add salt to a 150 g rice portion cooked without added salt. The amount of salt consumers were willing to add was also expressed as grams of salt/ 100 grams of raw rice to simplify the comparison with results from other sections.

Percentage salt reduction *	Salt added (g salt/100g raw rice)	Salt added to the 150 g cooked rice portion (g)	Group 1 (n=90)	Group 2 (n=68)
100	0	0	16.7	7.4
(100%-75%]	(0-0.90]	(0-0.71]	50.0 (+) **	25.0 (-) **
(75%-50%]	(0.90-1.80]	(0.71-1.42]	26.7	32.4
(50%-25%]	(1.80-2.70]	(1.42-2.14]	4.4 (-) ***	23.5 (+) ***
(25%-10%]	(2.70-3.24]	(2.14-2.57]	2.2	7.4
(10%-0%]	(3.24-3.60]	(2.57-2.85]	0	2.9
No salt reduction. Additional salt added	>3.60	>2.85	0	1.5

(+) or (-) indicate that the observed value is higher or lower than the expected theoretical value. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$; effect of the chi square per cell

*The scale used by consumers to indicate how much salt they would add to the 150 g cooked rice portion prepared without salt was collapsed into seven intervals to facilitate result comparison through the paper. When defining the intervals, round brackets were used to indicate that the extreme value does not belong to the interval while the square brackets indicate it does belong.

While the great majority of consumers would add salt to the 150 g cooked rice portion when informed that no salt was used during cooking, 12.6% considered that salt addition was not needed. These consumers accounted for 16.7% of Group 1 and 7.4% of Group 2. When focus was directed to how these consumers assessed the rice sample

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

with 0% salt when no information was provided (blind condition), it was found that almost half of them (55%) would not add salt to it, suggesting no changes between the “informed” and “blind” condition. On the contrary, the other half would add salt to this sample when no information was provided (blind condition). Interestingly, the opposite scenario did also occur and 11.6% of consumers who would add salt when information was provided, answered that salt addition was not needed when the sample with 0% salt was assessed under the blind condition.

4.3.2.4 Sociodemographic characteristics and sodium consumption habit

Table 4.6. Summary of results of the chi-square test per cell carried out to compare the two consumer groups identified by means of hierarchical cluster analysis in terms of their sociodemographic characteristics, salt consumption habits and attitudes toward health.

Sociodemographic characteristics	p-value
Gender	0.954
Age	0.487
Educational level	0.791
Number of adults living at home	0.814
Number of children younger than 10 years old living at home	0.225
Salt consumption habits/attitudes towards health	
Responsible for preparing food at home	0.499
Ways of using salt when preparing food at home	0.081
Salt addition before having tried food ***	0.008
Salt addition after trying food ***	0.007
Salt addition when eating at a restaurant	0.127
Salt shaker on the table at home	0.139
Salt shaker in a restaurant	0.281
Concern about self salt intake	0.249
Use of information on the salt content of food products	0.192
Interest in salt-reduced food ***	0.002
Importance of healthy eating	0.141
Healthiness of overall diet	0.158

Significant differences according to the global Chi-square test are indicated with *** ($p < 0.001$)

No significant differences between the groups were found in terms of gender ($p=0.954$), age ($p=0.487$), educational level ($p=0.791$), and number of adults or children younger than 10 years old living in the home ($p=0.814$ and $p=0.225$, respectively). Nevertheless, differences in terms of sodium consumption habits were found between the segments, particularly in relation to the addition of salt to food products (Table 4.6).

As shown in Table 4.7, the percentage of consumers reporting adding salt before trying food (either always or sometimes) was higher for Group 2 than for Group 1 ($p=0.008$). The same trend was observed regarding salt addition after trying food ($p=0.007$). No significant differences between consumer segments were identified regarding how often they were responsible for preparing food at home ($p=0.499$), the way in which they use salt when preparing food at home ($p=0.081$), how often they add table salt to food when eating at a restaurant ($p=0.127$), how often they put the salt shaker on the table at home ($p=0.139$) or how often they request for the salt shaker in a restaurant ($p=0.281$).

Table 4.7. Consumers' responses regarding frequency of salt addition before and after having tried food and interest in salt-reduced food consumption.

	Group 1 (%)	Group 2 (%)
<i>Salt addition before having tried food ($p=0.008$)</i>		
Never	60 (+) **	38 (-) **
Sometimes	32	40
Always	8 (-) *	22 (+) *
<i>Salt addition after trying food ($p=0.007$)</i>		
Never	45 (+) **	24 (-) **
Sometimes	53	66
Always	2 (-) *	10 (+) *
<i>Interest in salt-reduced food ($p=0.002$)</i>		
Not interested	10	21
Indifferent	16 (-) *	32 (+) *
Very interested	74 (+) ***	47(-) ***

(+) or (-) indicate that the observed value is higher or lower than the expected theoretical value.

*** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$; effect of the chi square per cell

Regarding attitudes towards health and sodium consumption, no significant differences were found between consumer groups in their concern about sodium intake ($p=0.249$), frequency of use of information on the sodium content of the food products they usually eat ($p=0.192$), perceived importance of healthy eating ($p=0.141$) and healthiness perception of their overall diet ($p=0.158$). However, as shown in Table 4.7, consumers in Group 1 showed higher interest in consuming low-salt food products than those in Group 2 ($p=0.002$).

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

4.4. Discussion

In the context of increasing concerns regarding excessive salt intake, the present work focused on studying the impact of salt reduction on consumer perception, using white rice as case study. Particular emphasis was placed on how consumer segmentation may contribute to better understand consumers' reaction towards sodium reduction.

The difference threshold for salt in white rice was estimated, providing actionable insights for the reformulation of this product towards lower salt contents. In this sense, the implementation of salt reductions close to 11% seems to be a safe criterion for reducing the salt content in rice without affecting consumer perception. Similar threshold estimations have been previously reported for broth (Bobowski & Vickers, 2012) and bread (Antúnez et al., 2016). As expected, consumer hedonic perception of white rice was significantly affected by salt reduction. At the aggregate level, the rice samples with the highest salt reduction percentages received the lowest hedonic scores, which suggest that the negative impact of salt reduction on consumer hedonic perception is a major challenge for the implementation of salt reduction programmes at the population level. This result is in line with those reported by previous studies involving a broad range of product categories (Kilcast & den Ridder, 2007).

Large heterogeneity was found in consumer hedonic reaction towards salt reduction in white rice, stressing the importance of consumer segmentation when designing and implementing salt-reduction programmes. In this sense, differences among samples may go unnoticed if only averaged data are considered. In the present study, average data from all consumers showed that salt can be reduced by 50% without causing significant changes in consumer affective response. However, for one of the consumer segments (Group 2) such reduction caused a significant decrease of 2.7 points of the hedonic scale.

Differences between the two consumer segments were also observed in terms of their hedonic sensitivity to changes in the salt content of white rice. Results from the present work suggest that higher hedonic sensitivity does not necessarily imply lower salt sensitivity. It could be hypothesized that consumers who gave higher overall liking scores to samples with higher salt content would be less sensitive to salt. However, as shown in Table 4.4, consumers in Group 2, significantly discriminated samples with different salt content, even at the point of identifying salt reductions below the difference threshold for salt in rice (11.1%). The latter result reflects that consumers from Group 2 are highly sensitive to salt, showing a difference threshold for salt below the average

difference threshold determined using trained assessors. On the other hand, at the aggregate level, consumers from Group 1 did not perceive significant differences in terms of overall liking between the control and the rice sample with 10% salt reduction (which is below the difference threshold for salt). This consumer segment gave higher overall liking scores to samples with intermediate salt content and tended to penalize both samples with low and high salt reductions. In a similar vein, Bobowski et al. (2015a) explored how consumers' hedonic sensitivity to salt affected their assessment of salt reduced samples. Consumers were classified as "low", "medium" or "high" hedonic sensitive based on their difference in liking between tomato juices with the lowest and the highest sodium concentration. These authors found that consumers with low and high hedonic sensitivity to salt differed in their hedonic perception of salt reduced products, being those with low hedonic sensitivity less affected by salt reductions and, therefore, more likely to adjust to the sodium reduced products.

Consumers' tolerance to salt reduction was also evaluated by asking consumers if they would add salt to each of the samples. As expected, the percentage of consumers adding salt to rice was higher for Group 2, providing further evidence on the fact that this consumer segment prefers higher salt contents in rice. It is worth mentioning that this issue was approached from different perspectives. Apart from indicating whether or not they would add salt to rice samples with different salt contents, consumers were asked to assess the adequacy of saltiness using a JAR scale. Both approaches provided similar information regarding the adequacy of saltiness. However, a general trend was observed that the percentage of consumers perceiving a sample as not salty enough (JAR responses), was higher than the percentage of consumers reporting they would add salt to it. This difference can be related to the fact that JAR scales have been reported to make consumers more critical about the product (Popper, Rosenstock, Schraidt, & Kroll, 2004). This finding suggests there may be a gap between perceiving the rice as not salty enough and adding salt back to it. Despite further research is needed on this regard, it seems that asking consumers whether they would add salt to food may provide more accurate information to assess potential compensatory effects.

In addition, consumers were presented with a 150 grams cooked rice portion without added salt and were asked to indicate how much salt they would add to it. Consumers were not allowed to try the sample, neither before nor after salt addition, they were just informed that no salt was added during cooking. As expected, the great majority of consumers reported they would add salt to rice. Special focus was put on those consumers who would not add salt to rice in spite of being informed that it was prepared without salt. Results revealed that, after having tried a sample with no added salt without

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

any information, about half of these consumers would not add salt to it, whereas the other half would. This result suggests that information regarding salt reduction may cause opposite effects on consumer perception. It is extensively acknowledged that expectations play a major role in food consumption, either improving or degrading our product perception (Deliza & MacFie, 1996). Vázquez et al. (2009) reported that information about salt reduction in biscuits markedly affected consumers' expectations. Results from their work showed that assessors who had only received the product package, featuring information about salt content, gave lower overall liking scores to the salt reduced biscuit relative to the regular one. This suggests that informing salt reduction generated negative expectations about the product, affecting consumers' sensory and hedonic perception (Vázquez, Curia, & Hough 2009). However, another scenario is possible and information about salt reduction may cause positive expectation for people that perceive the current product as too salty. Further research is required to shed light on the convenience of communicating salt reduction.

Data on how much salt consumers would add to each of the salt reduced rice samples were not gathered in this work. However, it would be interesting to get insights on the amount of salt added back to food to better assess possible compensatory effects. De Kock et al. (2016) explored consumer use of salt during salt-reduced chicken stew consumption. These authors found that regardless of the salt concentration, 81% of participants did not add salt to the chicken stews. However, those consumers who added salt back to chicken stews (19%) tended to add higher amounts of salt as the salt reduction percentage increases, even to the point of full compensation. Results from the present work support the idea that the addition of salt to the rice would be lower than salt reduction (Table 4.5).

Significant differences between consumer segments were found in terms of their salt consumption habits, in particular with regard to salt addition either before or after having tried food. At the aggregate level, consumers from Group 2 (who preferred higher salt contents and showed higher hedonic sensitivity to salt reduction) were more willing to add salt to foods. This result is in line with those from the survival analysis, which showed that both consumer segments differed in their willingness to add salt to rice (for a given salt reduction, the percentage of consumers adding salt was higher for Group 2). The differences between both consumer groups may, at least partly, explain differences in consumers' preferences for the salt content in rice. In particular, results from this study add further evidence on the relationship between customary salt eating habits and preferences for salt in food. Consumers in Group 2 who showed higher frequency of salt addition to food, and consequently may have been exposed to higher

salt levels in their everyday life, gave the highest overall liking score to the rice sample with the highest salt content, showing a pronounced decrease in their overall liking as the percentage salt reduction increased. On the other hand, consumers from Group 1, who reported lower frequency of salt addition to food, gave higher overall liking scores to rice with intermediate salt content (25%—50% salt reduction). This result reinforces the role of eating habits in shaping our preferences. Exposure to low salt diets have been widely reported to be effective to increase consumers overall liking of salt reduced products (Bertino et al. 1982; Blais et al. 1986; Elmer 1988). Moreover, repeated exposure to single food products with reduced salt content (soup and tomato juice) have proved to be effective in increasing consumers' overall liking of the low salt product (Bobowski et al., 2015b; Methven et al., 2012). Taking into consideration that both eating habits and repeated exposure may play a major role in the development of food preferences, gradual salt reduction has been proposed as a potential intervention to shift consumer preferences toward lower salt levels (FSA, 2003; IOM, 2010). The repeated exposure to lower salt contents, without being aware of the change, may lead consumers to get accustomed to the new (lower) salt content and this process should continue until the salt reduction target is reached (Zandstra et al., 2015). In this regard, results from the present work add to the evidence that abrupt salt reduction causes a negative impact in consumer hedonic perception and may encounter limited success in reducing the salt content of foods. This is particularly important considering that high salt reductions are needed to achieve target salt levels (5 g salt per day) (WHO, 2007).

Finally, it is worth mentioning that differences between consumer segments were found with regard to their interest in consuming low salt food products, being consumers from Group 1 more willing to shift down their sodium intake. Different results were reported by De Kock et al. (2016) who found no significant differences in the overall liking of chicken stew samples with different sodium content between consumers who were interested in reducing their sodium intake and those who were not.

CONSUMERS' HETEROGENEITY TOWARDS SALT REDUCTION

4.5. Conclusions

Consumer hedonic perception of white rice was significantly affected by salt reduction. Large heterogeneity in consumers' hedonic reaction towards salt reduction was found, providing further evidence on the importance of considering consumer segmentation to gain a more complete picture of the impact of salt reduction in foods. Two consumer groups differing in their preferences for salt in white rice, and particularly in their hedonic sensitivity and tolerance to changes in the salt content of rice were identified. Both consumer segments significantly differed in their habit of adding salt to food, either before or after having tried it, as well as in terms of their interest in reducing salt intake. Results from the present work suggest that eating habits might play a major role in shaping our preferences highlighting the potential of gradual salt reduction as a strategy for reducing sodium intake

CHAPTER 5

Partial replacement of NaCl with KCl in bread: Effect on sensory characteristics and consumer perception

PARTIAL REPLACEMENT OF NaCl with KCl

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- Antúñez, L: Conceived and designed the work, collected the data, analysed and interpreted the data, wrote the paper.
- Giménez, A: Contributed to the conception and design of the study, as well as to data interpretation.
- Vidal, L: Contributed to data collection.
- Ares, G: Contributed to the conception and design of the study, as well as to data interpretation, performed a critical revision of the article.

Abstract

Sodium reduction strategies that fully or partially compensate for the effect of reducing sodium on the sensory profile of foods have been proposed to decrease dietary sodium intake at the population level. The present work aimed at exploring the effect of partially replacing NaCl with KCl on the sensory characteristics and perception of white bread. Equivalent salty concentration of NaCl:KCl blends were estimated relative to different NaCl concentrations, using paired comparisons with a panel of trained assessors. The influence of partially replacing NaCl by KCl on the dynamic sensory profile of breads was assessed using the TCATA approach with a trained panel. Finally, a consumer study was carried out to evaluate consumers' sensory and hedonic perception of breads produced by partially replacing 30% NaCl with KCl. Results from the present work suggest that KCl can be deemed effective at compensating for saltiness intensity in sodium reduced breads. Partial replacement of 30% NaCl with KCl did not significantly affect the sensory characteristics of breads. However, NaCl replacement equal or higher than 40% caused an increase in bitterness, off-flavour and metallic flavour. Heterogeneity in consumer hedonic reaction towards partial replacement of NaCl with KCl was observed, but mainly associated to differences in texture perception.

5.1. Introduction

Excessive sodium intake, along with other dietary and lifestyle factors such as overweight, high alcohol consumption and physical inactivity (Chobanian et al., 2003), has been linked to the growing prevalence of high blood pressure, the leading metabolic risk factor for cardiovascular diseases worldwide (WHO, 2014). In response to growing concern about the global cardiovascular diseases burden, public policies aimed at reducing sodium intake at the population level have been proposed (He & MacGregor, 2009, WHO, 2014).

The main sources of dietary sodium markedly depend on the cultural context and the eating habits of the population (WHO, 2014). Nevertheless, the increasing availability and consumption of processed foods is causing a global shift in sodium sources towards these products (WHO, 2014). Therefore, the reformulation of processed foods has been identified as one of the most cost-effective strategies to reduce dietary sodium intake at the population level (Downs et al., 2015). However, successful implementation of sodium reduction programmes wrestles with the challenge of our preference for salty taste (Mennella, 2014). Sodium reduction has been widely reported to cause negative changes in consumer perception of different product categories (Beauchamp et al., 1982; Kilcast & den Ridder, 2007; Phelps et al., 2006; Rødbotten et al., 2015). Moreover, considering that salty taste preferences in adulthood are markedly affected by dietary patterns (Bobowski, 2015), the repeated exposure to high sodium products may have shifted consumers' preferences towards higher sodium contents (Bertino et al., 1986). This situation causes additional challenges for sodium reduction.

To overcome this limitation, the implementation of gradual sodium reductions has been proposed (FSA, 2003; Institute of Medicine, 2010). The idea behind this approach is to gradually modify the sodium content of food products without consumer awareness (Busch et al., 2013; MacGregor & Hashem, 2014). Once consumers become adapted to the new product, further reduction steps are taken following the same procedure (Zandstra et al., 2015). In such a way, consumer's preference for the sodium content of food is expected to gradually shift towards lower sodium contents. The main disadvantage of gradual sodium reductions is that a long time period is required to meet the sodium reduction target (Busch et al., 2013; Silow, Axel, Zannini & Arendt, 2016).

In this context, other sodium reduction strategies that fully or partially compensate for the effect of reducing sodium on the sensory characteristics of foods have been proposed (Busch et al., 2013; Kilcast & den Ridder, 2007; Silow et al., 2016). One of these approaches is the partial replacement of NaCl by either organic or inorganic salts

PARTIAL REPLACEMENT OF NaCl with KCl

that elicit saltiness but do not contain sodium (Sinopoli & Lawless, 2012; van Buren et al., 2016). Among the sodium chloride replacers, potassium chloride (KCl) is the most commonly used (Busch et al., 2013; Kilcast & den Ridder, 2007; Paulsen et al., 2014; van Buren et al., 2016). One of the additional advantages of replacing NaCl by KCl is that the body of evidence suggests a linkage between potassium intake and a decrease in the risk of hypertension (Aaron & Sanders, 2013; He & MacGregor, 2007; van Buren et al., 2016).

However, sodium chloride is the only substance that provides what is recognised as a pure salty taste (Kilcast & den Ridder, 2007), while most organic and inorganic salts show other taste qualities apart from salty taste (Kilcast & den Ridder, 2007; Silow et al., 2016; van der Klaauw & Smith, 1995). This represents the biggest drawback of using sodium replacers and explains why partial substitutions of NaCl by KCl are generally used to reduce the sodium content of foods. In this sense, there is general agreement on the fact that KCl does not only elicit salty taste but also undesirable flavours, such as bitterness and metallic flavour, when used above a given concentration (Feltrin et al., 2015; Gelabert et al., 2003; Gou et al., 1996). Nevertheless, the amount of KCl to be added without causing undesirable changes on the sensory profile of sodium reduced foods markedly depends on the product category, its sensory characteristics and complexity. It is well known that saltiness perception does not only depend on sodium concentration but also on the food matrix in which sodium is included. For instance, perceived saltiness might vary with food texture (solid vs. liquid foods) as well as due to physicochemical interactions between sodium and other taste components in foods (Bobowski & Vickers, 2012; Kilcast & den Ridder, 2007). Previous studies have explored the effect of replacing NaCl with KCl in a wide range of product categories: brown and white bread, pizza crust, cheddar cheese, sausages, dry-cured ham, rabbit meat, frankfurter sausages and Mina fresh cheese (Armenteros et al., 2012; Gomes et al. 2011; Bernklau, et al. 2017; Braschi et al., 2009; Charlton et al., 2007; Grummer et al., 2013; Horita et al., 2014; Paulsen, et al. 2014; Soglia et al. 2014). Results from those studies revealed that the maximum replacement of NaCl by KCl ranges from 20% in water solutions (Morris et al., 2010) to up to 50% in feta cheese (Katsiari et al., 1997), suggesting that recommendations on this regard should be made on a product specific basis.

Breads, cereals and bakery products have been identified as one of the main contributors to sodium intake at the population level across all major markets, with the exception of some Asian countries where the salt added during cooking and salt from sauces accounts for the majority of the dietary sodium intake (Campbell et al., 2014;

Centers for Disease Control and Prevention, 2011; European Commission, 2010; WHO, 2007). For this reason, special focus has been placed on reducing the sodium content of bread and it has been established as a target product category for sodium reduction in many countries (Girgis et al., 2003; Belz et al., 2012; Kloss et al., 2015; Cauvain 2007). Previous research has already addressed the effect of sodium reduction in bread, showing it has a negative impact on consumer overall liking of bread (Kilcast & den Ridder, 2007; Rødbotten et al., 2015; Spina et al., 2015). Lynch et al. (2009) suggested that sodium reduction in this product category is feasible from a technological perspective, although changes on flavour characteristics still remain a major challenge. The impact of reducing sodium concentration on bread does not only affect saltiness intensity but also overall flavour intensity (Kilcast & den Ridder, 2007). In addition, Charlton, Macgregor, Vorster, Levilt and Steyn (2007) and Braschi, Gill and Naismith (2009) have explored the partial replacement of NaCl with other salts in brown and white bread. Results from these studies have shown that 30-32% sodium reduction could be achieved by partially replacing sodium with either potassium or other cations. In addition, Spina et al. (2015) concluded that the addition of different levels of potassium chloride and yeast extract is a feasible strategy to produce 50% sodium-reduced durum wheat bread without affecting the quality parameters and bread shelf life. However, to the best of the authors' knowledge, research on the concentration of NaCl:KCl blends required to compensate for saltiness intensity as well as the impact of adding KCl on the sensory profile of sodium-reduced breads is still lacking.

In this context, the present work aimed at exploring the effect of partially replacing NaCl with KCl on the sensory characteristics and consumer perception of white bread. In particular, the specific objectives of the work were to: i) estimate equivalent salty concentrations of NaCl:KCl blends relative to NaCl in white bread, ii) evaluate the sensory dynamic profile of white breads formulated with NaCl:KCl blends, and iii) study consumers' sensory and hedonic perception of sodium-reduced white breads formulated with NaCl:KCl. Results from the present work are expected to contribute to the design of successful sodium-reduction strategies, which may encourage the food industry to engage in reformulation strategies.

PARTIAL REPLACEMENT OF NaCl with KCl

5.2. Materials and methods

The present work involved three studies. In Study 1 the equivalent salty concentration of NaCl:KCl blends (70:30, 60:40 and 50:50) were estimated using paired-comparison tests with a trained sensory panel. Study 2 involved the dynamic sensory characterization of white breads produced by partially replacing sodium with potassium, using the Temporal Check-all-that-apply (TCATA) approach with a trained panel. In Study 3, consumers' sensory and hedonic perception of sodium-reduced breads, formulated according to the equivalent concentrations of the 70:30 NaCl:KCl blend determined in Study 1, was assessed.

5.2.1. Bread formulation

Bread samples were produced using 58% Uruguayan commercial wheat flour (Molino Americano S.A, Montevideo, Uruguay), 37% tap water, 2.4% sugar (Alcoholes del Uruguay S.A., Bella Unión, Uruguay), 1.6% high oleic sunflower oil (Compañía Oleaginosa Uruguay S. A, Montevideo, Uruguay) and 1% powered yeast (Fleischmann, Argentina). Either NaCl or NaCl:KCl blends with different proportions of KCl were added to the bread dough (Antil S.A, Montevideo, Uruguay). Three NaCl:KCl blends with different NaCl:KCl ratios were considered in the present study to find a compromise between sodium reduction and the onset of undesirable flavours, which have been acknowledged as a side effect of adding potassium to foods. Based on previous research conducted with a wide range of food product categories, the following NaCl:KCl ratios were selected: 70:30, 60:40 and 50:50. The NaCl:KCl blends were exclusively produced by a local salt producer for the purpose of the study. The breads were prepared in a bread-making machine (Phillips® model HD9015/30) one day prior to testing and were stored in plastic bags overnight. The morning of the test, breads were cut in 10 mm slices and the crust was removed.

5.2.2. Study 1 - Estimation of equivalent salty concentrations of NaCl:KCl blends

5.2.2.1 Trained assessors

The trained panel consisted of sixteen assessors, ages ranging from 22 to 56 years old (13 female). Assessors were recruited among workers of Universidad de la República (Uruguay) and selected according to the guidelines of the ISO 8586:2012 standard (ISO, 2012). They all signed an informed consent form.

Sixteen 15 min training sessions, performed on separate days, were carried out to familiarize assessors with paired comparisons and to increase their ability to discriminate samples with different saltiness intensity. The first four sessions involved the assessment of water solutions with different concentrations of NaCl and NaCl:KCl

blends (70:30; 60:40 and 50:50 NaCl:KCl). Then, assessors performed paired comparisons involving bread samples with different concentrations of NaCl and NaCl:KCl blends.

5.2.2.2. Estimation of equivalent salty concentrations of NaCl:KCl blends using paired comparisons

Three NaCl:KCl blends with different NaCl:KCl ratios (70:30, 60:40 and 50:50) were considered in this study. For each NaCl:KCl blend, five equivalent salty concentrations were estimated, corresponding to five control samples with different NaCl concentrations: 2.00%, 1.80%, 1.61%, 1.38% and 1.25% NaCl. The control samples were produced according to sequential difference thresholds for salty taste, estimated in a previous study (Antúnez et al., 2016).

In each session, assessors completed series of six paired-comparisons, each of which comprised one control sample (2.00%, 1.80%, 1.61%, 1.38% or 1.25% NaCl) and one NaCl:KCl blend (70:30, 60:40 or 50:50). Within each series, each pair was composed of a control bread (that remained constant in the series) and a sodium-reduced sample produced by partially replacing NaCl with KCl. For each series of paired-comparison test, the concentration range of the NaCl:KCl blend used for bread production was selected by pilot testing. Table 5.1 summarizes the NaCl:KCl concentrations included in each test.

Assessors were requested to taste each of the samples in a pair and to indicate the saltier one by selecting the corresponding code number. They were instructed to cleanse their palate with water between trials. Samples in each pair were presented following a balanced design to avoid order effects. Each paired-comparison was performed by duplicate, accounting for a total of 30 sessions.

Data collection was performed using Compusense-Cloud (Compusense Inc., Guelph, Canada). Testing took place in a sensory laboratory designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22°C).

PARTIAL REPLACEMENT OF NaCl with KCl

Table 5.1 Series of paired-comparison test used for determining the equivalent salty concentration of NaCl:KCl blends relative to NaCl in white bread.

Control sample	NaCl:KCl blend	Bread samples formulated by partial replacement of NaCl with KCl					
		KCl					
		1	2	3	4	5	6
2.00% NaCl	70:30	2.00	2.20	2.40	2.60	2.80	3.00
	60:40	2.20	2.40	2.60	2.80	3.00	3.20
	50:50	2.20	2.40	2.60	2.80	3.00	3.20
1.80% NaCl	70:30	1.80	2.00	2.20	2.40	2.60	2.80
	60:40	1.80	2.00	2.20	2.40	2.60	2.80
	50:50	2.00	2.20	2.40	2.60	2.80	3.00
1.61% NaCl	70:30	1.80	2.00	2.20	2.40	2.60	2.80
	60:40	2.00	2.20	2.40	2.60	2.80	3.00
	50:50	2.00	2.20	2.40	2.60	2.80	3.00
1.38% NaCl	70:30	1.40	1.60	1.80	2.00	2.20	2.40
	60:40	1.60	1.80	2.00	2.20	2.40	2.60
	50:50	1.60	1.80	2.00	2.20	2.40	2.60
1.25% NaCl	70:30	1.40	1.60	1.80	2.00	2.20	2.40
	60:40	1.25	1.45	1.65	1.85	2.05	2.25
	50:50	1.60	1.80	2.00	2.20	2.40	2.60

5.2.2.3. Data analysis

The equivalent salty concentrations of each of the NaCl:KCl blends for each control sample were estimated using survival analysis, following the procedure proposed by Reis et al. (2016).

The response of each assessor to each paired comparison was coded with a binary code system (“No”, “Yes”). If the control sample (bread produced with NaCl) was identified as saltier than the sample with partial replacement of NaCl with KCl the response was coded with “No”, otherwise, it was coded with “Yes”. A random variable C was defined as the concentration of the NaCl:KCl blend at which the assessor started to consistently perceive the bread with NaCl:KCl as saltier than the control, and therefore to consistently answer “Yes”. The proportion of assessors perceiving the sample produced with NaCl:KCl saltier than the control as a function of the concentration of NaCl:KCl blend was modelled using a lognormal distribution (Hough et al., 2003). The parameters of the model (σ and μ) were estimated using the R scripts provided by Hough (2010). Once the model was estimated, the equivalent salty concentration was determined as the concentration of the NaCl:KCl blend at which 50% of the judgments

indicated that the bread formulated with NaCl:KCl was saltier than the control (formulated with NaCl).

5.2.3. Study 2 - Dynamic sensory profile of sodium-reduced breads formulated by partial replacement of NaCl with KCl

5.2.3.1 Trained assessors

The trained panel consisted of sixteen assessors, ages ranging from 22 to 56 years old (13 female), as described in 2.2.1. Six training sessions, each lasting 15 minutes, were conducted to introduce assessors to Temporal Check-All-That-Apply (TCATA) and to familiarize them with the software used for data collection.

5.2.3.2 Temporal check-all-that-apply (TCATA)

Eight samples were considered in this study: a control bread formulated with 2.00% NaCl, a bread containing 1.80% NaCl (first difference threshold) and those produced according to the equivalent salty concentrations of the three NaCl:KCl blends estimated in Study 1. Table 5.2 shows a description of the samples included in the study.

The dynamic sensory profile of sodium-reduced bread samples was assessed using TCATA. Trained assessors were requested to select all the terms that applied for describing the bread at each moment of the evaluation. The list of terms comprised 10 sensory characteristics: *metallic*, *sticky*, *bitter*, *characteristic bread flavour*, *off-flavour*, *spongy*, *salty*, *soft*, *dry* and *smooth*. The sensory attributes were selected based on previous studies using the same product category. The order in which terms were listed was balanced across assessors, following a Williams' Latin square design.

Assessors were instructed to review the attributes before starting the TCATA evaluation. They were requested to click a Start button concurrently with placing the whole sample in the mouth and to immediately begin the term selection. Assessors were asked to track changes in the sample by checking and unchecking terms, so that all the terms that were selected described the sample in that moment. They were explained that multiple attributes can be selected simultaneously and were requested to uncheck an attribute when it was no longer applicable to describe the sample. The evaluation period was 50 seconds. Samples were evaluated in triplicate. Data were collected using Compusense-Cloud (Compusense Inc., Guelph, Ontario, Canada) and testing took place in a sensory laboratory, as described in the 5.2.2.2.

PARTIAL REPLACEMENT OF NaCl with KCl

Table 5.2 Characteristics of white bread samples included in Studies 2 and 3.

Study ID	Control sample (% NaCl)	NaCl:KCl blend ratios	Concentration of NaCl:KCl blend (% of flour weight)
Study 2	2.00%	70:30	2.46
		60:40	2.50
		50:50	2.68
	1.80%	70:30	2.34
		60:40	2.45
		50:50	2.53
Study 3	1.80%	70:30	2.34
	1.61%	70:30	2.25
	1.38%	70:30	1.76
	1.25%	70:30	1.64

5.2.3.3. Data analysis

TCATA data were analysed following the procedure proposed by Castura Antúnez, Giménez and Ares (2016). For each sample, aggregated data across all trained assessors were represented using line plots. The citation proportion of each attribute of the TCATA list was calculated as the proportion of judgments (assessors × replicates) for which the attribute was selected for describing a sample at a given time (every 1 s) of the evaluation. Smoothed TCATA curves were obtained using a spline type polynomial.

TCATA difference plots for selected pairs of samples were obtained by subtracting their citation proportions. A sign test was applied at each time point and for each attribute to assess the existence of significant differences in the citation proportions for each pair of samples (Castura et al. 2016). A significance level of 5% was considered in the analysis.

5.2.4. Study 3 – Consumers' sensory and hedonic perception of white breads formulated with partial substitution of NaCl by NaCl:KCl

5.2.4.1. Consumers

A total of 101 consumers, aged between 18 and 46 years (64% female), took part in Study 3. Participants were recruited from the database of the Sensometrics & Consumer Science research group of Universidad de la República (Uruguay), based on their bread consumption and their willingness to take part in the study. Participants signed an informed consent form and received a small gift for their participation.

5.2.4.2. *Experimental procedure*

Consumers' sensory and hedonic perception of sodium-reduced breads produced by straight sodium reduction or partial replacement of NaCl with KCl was assessed. Eight bread samples were considered in this study: four samples reduced in sodium according to a series of sequential difference thresholds determined in a previous work (Antúnez et al., 2016) and four breads reduced in sodium by partial replacement of NaCl with KCl. The latter samples were produced using the 70:30 NaCl:KCl blend according to the equivalent salty concentrations determined in Study 1 (Table 5.2).

Consumers performed a multiple bite evaluation. They were asked to try a first bite of the sample and to indicate their overall liking using a 9-point hedonic scale (1 = dislike very much, 9 = like very much). Then, they were asked to try a second bite of the sample, to indicate their overall liking and to answer a check-all-that-apply (CATA) question. The CATA question comprised 18 sensory characteristics: *salty, hard, soft, spongy, dry, light, dense, tasteless, barely salty, off-flavour, crumbly, sticky, smooth, gummy, tasty, characteristic bread flavour, metallic* and *bitter*. The order in which terms were listed was balanced across consumers, following a Williams' Latin square design. Assessors were instructed to cleanse their palate with water between samples but not between bites. The eight samples were presented according to an experimental design that was balanced for order and carry-over effects (Williams' Latin Square design).

All data were collected using Compusense-Cloud (Compusense Inc., Guelph, Ontario, Canada). Testing took place in a sensory laboratory, as described in the previous section.

5.2.4.3. *Data analysis*

Overall liking data from the second bite evaluation was analysed by means of a linear mixed model. Sample was specified as a fixed effect while consumer was specified as a random effect. A significance level of 5% was considered in the analysis and when the effects were significant, honestly significant differences were calculated using Tukey's test. In order to better understand the effect of partially replacing NaCl with KCl the variable "sample" was split into two variables: "type of salt used for bread production" (NaCl or NaCl:KCl blend) and "equivalent salty concentration". The latter refers to the amount of either NaCl or NaCl:KCl blend that elicit the same saltiness intensity. For instance, the bread produced with 1.80% NaCl and the one containing 2.34% NaCl:KCl (equivalent salty concentration determined in Study 1) corresponded to the same level of the variable equivalent salty concentration (Table 5.2).

PARTIAL REPLACEMENT OF NaCl with KCl

A hierarchical cluster analysis was performed on overall liking data to identify groups of consumers with different hedonic reaction towards partially replacing NaCl with KCl in bread. Euclidean distances and Ward's clustering method were used in the clustering procedure (Næs et al., 2010). A linear mixed model was performed separately on the hedonic data of each consumer group.

The frequency of use of each CATA term was determined by counting the number of consumers who used it to describe the sample. Generalized linear models (glm) were carried out to identify significant differences between samples for the frequency of use of each CATA term. For each term of the CATA list, the analysis was performed considering equivalent salty concentration, type of salt (NaCl or 70:30 NaCl:KCl blend) and their interaction as factors. A significance level of 5% was considered in the analysis and when the effects were significant pairwise comparisons were performed using the sign test (Meyners et al., 2013). CATA questions were also analysed separately for each of the consumer groups identified in the hierarchical cluster analysis.

All data analyses were carried out using R software version 3.2.2 (R Core Team 2015).

5.3. Results

5.3.1. Study 1 - Equivalent salty concentrations of NaCl:KCl blends in white bread

Survival analysis was used to estimate the equivalent salty concentration of three NaCl:KCl blends (70:30, 60:40 and 50:50 NaCl:KCl) relative to different NaCl concentrations (2.00%, 1.80%, 1.61%, 1.38% and 1.25%) in bread. The average equivalent concentrations were determined as the concentration of NaCl:KCl blend at which the bread produced by partial replacement of NaCl with KCl was perceived saltier than the control (produced with NaCl) in 50% of the judgments.

As shown in Table 5.3, when NaCl was replaced by the 70:30 NaCl:KCl blend, the concentration of sodium in bread was reduced from 9.7% to 20.5% (depending on the NaCl content of the control bread) without affecting saltiness perception. The reduction in sodium concentration increased to 25.2% - 32.4% when the 60:40 NaCl:KCl blend was considered, and from 28.6% to 41.1% when the blend was composed of 50% NaCl and 50% KCl. Regardless of the NaCl:KCl blend being taken into consideration, no clear relationship was found between the sodium reduction achieved and the NaCl concentration in the control bread.

As expected, the sodium reduction achieved with the partial substitution of NaCl by KCl was lower than the proportion of KCl added to the bread samples to compensate for sodium reduction, which indicates that KCl was perceived as less salty than NaCl. In order to compare results of the present work with those from other studies the saltiness potency of each NaCl:KCl blend was calculated as the ratio between the NaCl concentration in the control and the equivalent concentration of NaCl:KCl blend in bread. The saltiness potency of the 70:30 and 60:40 NaCl:KCl blends ranged from 0.72 to 0.82, whereas the NaCl:KCl blend with 50:50 NaCl:KCl showed a lower saltiness potency (0.62-0.75). In addition, the saltiness potency of KCl was estimated as the ratio between the NaCl reduction (difference between the NaCl concentration in the control and sodium-reduced bread) and the KCl added by partially replacing NaCl with NaCl:KCl (amount of KCl in the NaCl:KCl blend). According to this estimation, the saltiness potency of KCl ranged from 0.20 to 0.56, with an average of 0.42.

Table 5.3 Equivalent salty concentration of the NaCl:KCl blends (70:30; 60:40; 50:50 NaCl:KCl) and percentage of salt reduction achieved by partially replacing NaCl with KCl with their corresponding 95% confidence intervals.

NaCl concentration of the control sample (% of flour weight)	NaCl:KCl blend	Equivalent salty concentration (% of flour weight)	95% confidence interval	NaCl reduction respect to the control sample (%)	95% confidence interval
2.00%	70:30	2.46	2.28-2.63	20.5	15.0-26.3
	60:40	2.50	2.37-2.64	32.4	28.7-36.0
	50:50	2.68	2.42-2.98	41.1	34.5-46.8
1.80%	70:30	2.34	2.23-2.47	16.0	11.3-19.9
	60:40	2.45	2.27-2.65	26.4	20.4-31.8
	50:50	2.53	2.39-2.69	38.2	34.3-41.7
1.61%	70:30	2.25	2.01-2.52	9.7	0-19.4
	60:40	2.21	1.91-2.56	25.7	14.1-35.8
	50:50	2.56	2.22-2.94	30.2	19.6-39.4
1.38%	70:30	1.76	1.63-1.89	17.7	11.4-23.5
	60:40	1.91	1.64-2.23	25.2	12.7-35.8
	50:50	1.85	1.66-2.06	41.1	34.5-47.0
1.25%	70:30	1.64	1.54-1.76	14.9	9.1-20.4
	60:40	1.62	1.38-1.91	29.8	17.6-40.2
	50:50	2.03	1.92-2.15	28.6	24.4-32.5

PARTIAL REPLACEMENT OF NaCl with KCl

5.3.2. Dynamic sensory profile of sodium-reduced white breads formulated by partial replacement of NaCl with KCl

The effect of partially replacing NaCl with KCl on the sensory dynamic profile of bread was explored. In order to summarize the data and to facilitate comprehension, results from the dynamic sensory characterization are only shown for the bread containing 1.80% NaCl and those formulated according to the equivalent salty concentrations of each of the NaCl:KCl blends. However, it is worth noting that similar results were obtained from the dynamic sensory characterization of the bread with 2.00% NaCl and those formulated according to the respective equivalent salty concentrations (data not shown).

As shown in Figure 5.1, the bread produced with 1.80% NaCl was mainly characterized by the attributes *spongy* and *smooth* at the beginning of the evaluation period, and by its *characteristic bread flavour* from second 15 onwards. The bread formulated with the 70:30 NaCl:KCl blend showed a similar sensory profile, although differences were observed in relation to the citation proportion of some texture terms. As shown in Figure 5.2a, the bread produced by partially replacing 30% NaCl with KCl showed a higher citation proportion of the terms *spongy* and *smooth* in the central part of the evaluation period.

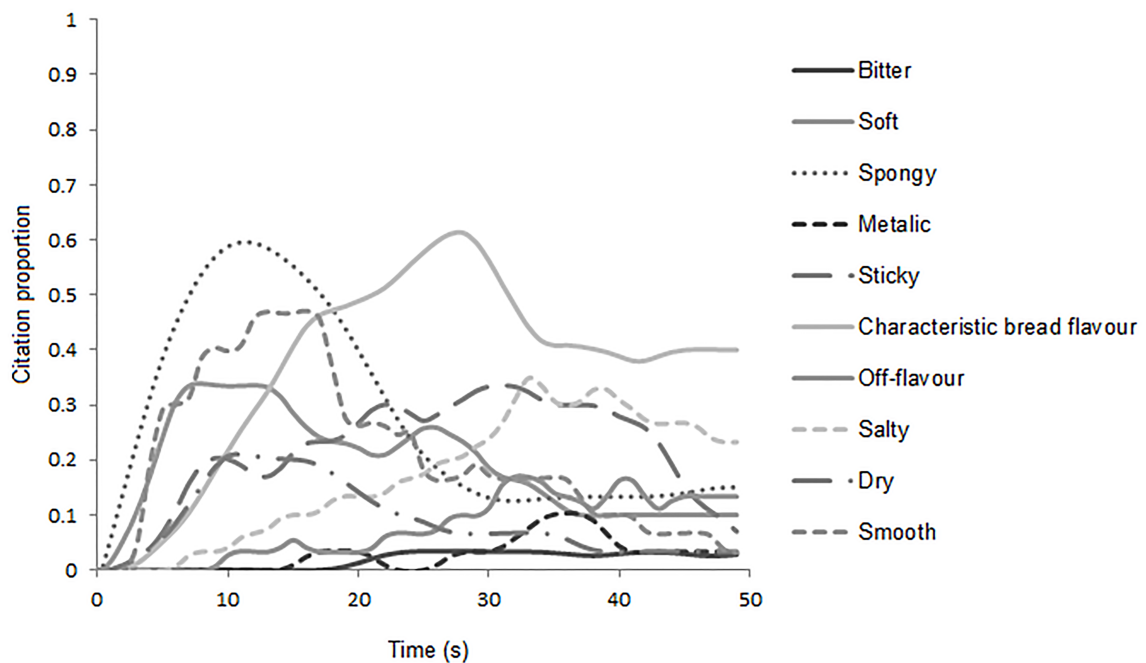


Figure 5.1 TCATA curve obtained from the evaluation of the white bread with 1.80% NaCl.

However, when the NaCl substitution raised to 40% a significant increase in the citation proportion of the term *metallic* was observed from second 25 onwards (Figure 5.2b). In addition, this sample showed a higher citation proportion of the term *salty* from second 10 to second 20. The replacement of 50% NaCl with KCl caused an increase in the citation proportion of the attributes *metallic* and *bitter* during the second half of the evaluation period, as reflected by the difference curve between this sample and the control (Figure 5.2c). The bread formulated with the 50:50 NaCl:KCl blend tended to show higher citation proportions for the attribute *off-flavour* compared to the control, although the difference was not statistically significant (data not shown). Regarding the texture characteristics of sodium-reduced breads, no clear effect of increasing the percentage replacement of NaCl with KCl was observed.

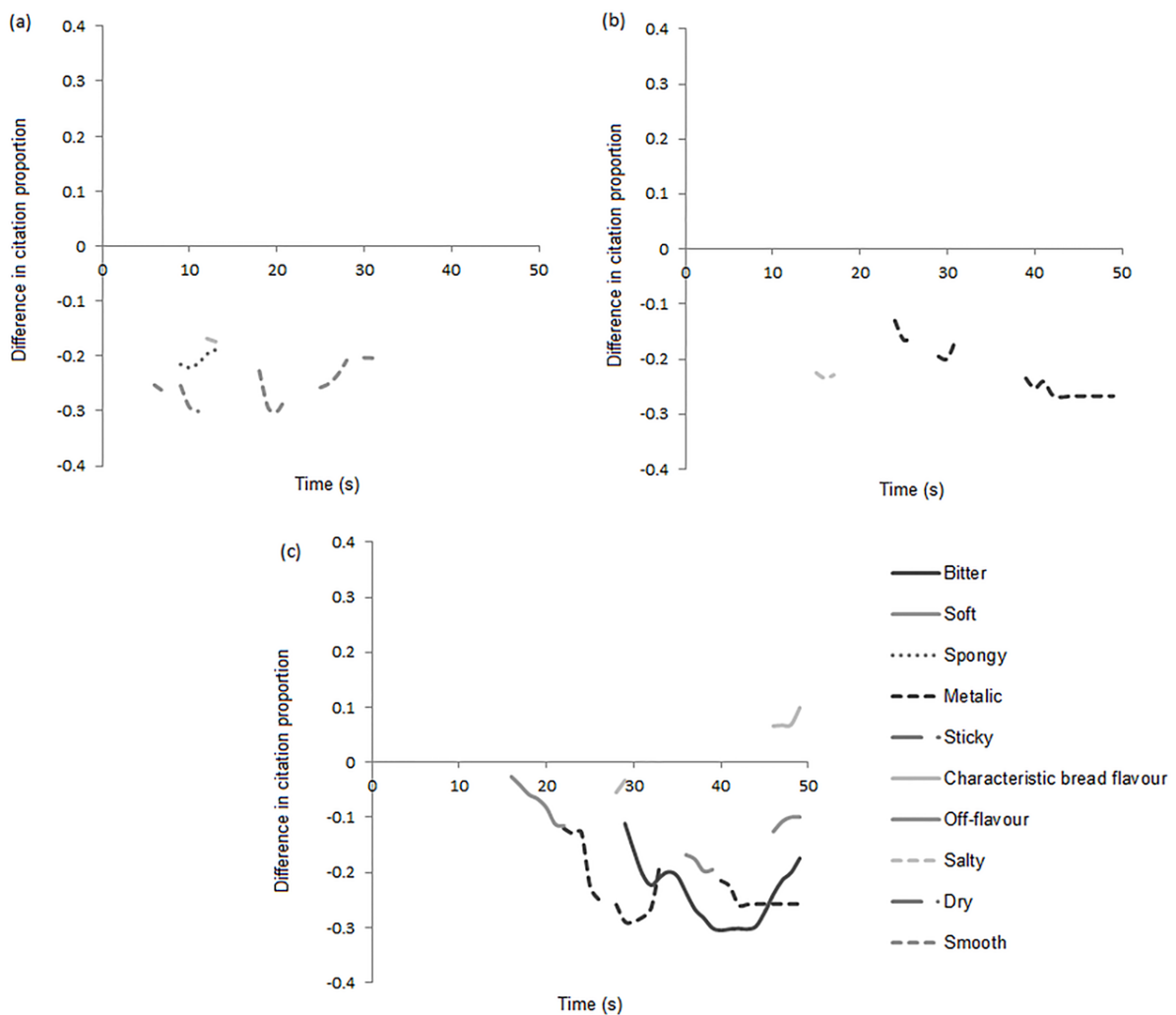


Figure 5.2 TCATA difference curves for the white bread sample containing 1.80% NaCl and those produced according to the equivalent salty concentrations of each of the NaCl:KCl blends. (a) 1.80% NaCl vs. 70:30 NaCl:KCl, (b) 1.80% NaCl vs. 60:40 NaCl:KCl; (c) 1.80% NaCl vs. 50:50 NaCl:KCl

PARTIAL REPLACEMENT OF NaCl with KCl

5.3.3. Consumers' sensory and hedonic perception of white breads formulated with partial substitution of NaCl by a NaCl:KCl blend

5.3.3.1 Hedonic perception

Significant differences in the overall liking of bread samples were found ($F=2.58$, $p=0.01$). As shown in Table 5.4, when considering data from all consumers overall liking scores ranged between 6.2 and 5.5. At the aggregate level, the samples with lower sodium content showed lower overall liking scores, regardless of the type of salt (NaCl or NaCl:KCl) used for bread production. The bread containing 1.25% NaCl and those formulated with 1.64% and 1.76% 70:30 NaCl:KCl blend showed the lowest overall liking scores. Significant differences were found between these samples and those formulated with 1.80% NaCl and 2.25% NaCl:KCl blend.

Table 5.4 Average overall liking scores of sodium-reduced white breads formulated with different NaCl or NaCl:KCl concentrations for all consumers and the two consumer groups identified in the hierarchical cluster analysis.

Type of Salt	Equivalent salty concentration	Concentration (% of flour weight)	Average overall liking score		
			All consumers (n=101)	Group 1 (n=54)	Group 2 (n=47)
NaCl	1.80	1.80	6.2 ^c	5.8 ^b	6.6 ^d
70:30 NaCl:KCl	1.80	2.34	5.9 ^{a,b,c}	5.4 ^a	6.4 ^{c,d}
NaCl	1.61	1.61	6.0 ^{b,c}	6.6 ^c	5.2 ^a
70:30 NaCl:KCl	1.61	2.25	6.2 ^c	6.3 ^{b,c}	6.0 ^{b,c,d}
NaCl	1.38	1.38	5.8 ^{a,b,c}	6.3 ^{b,c}	5.3 ^a
70:30 NaCl:KCl	1.38	1.76	5.6 ^{a,b}	5.5 ^a	5.7 ^{a,b}
NaCl	1.25	1.25	5.5 ^a	5.2 ^a	5.9 ^{b,c}
70:30 NaCl:KCl	1.25	1.64	5.6 ^{a,b}	5.0 ^a	6.3 ^{c,d}

Note: Average scores with different superscript letters within a column are significantly different according to Tukey's test ($p<0.05$).

Results from the linear mixed model considering equivalent salty concentration, type of salt and their interaction as fixed factors showed that the equivalent salty concentration significantly affected consumers' overall liking of samples ($F=4.89$, $p<0.01$). However, neither the type of salt ($F=0.34$, $p=0.56$), nor the interaction between these variables ($F=1.01$, $p=0.39$) had a significant effect. This suggests that at the aggregate level consumers' liking was only affected by salt concentration.

Two groups of consumers differing in their hedonic reaction towards partially replacing NaCl with KCl in white bread were identified by means of hierarchical cluster

analysis. No significant differences on the basis of gender ($p=0.76$) and age ($p=0.63$) were found between the two identified clusters. A linear mixed model was performed separately on data from each consumer group to explore the effect of using KCl to partially replace NaCl on consumers' hedonic perception of bread. A significant effect of equivalent salty concentration was found for both consumers groups (Group 1: $F=15.26$, $p<0.001$; Group 2: $F=8.34$, $p<0.001$). At the aggregate level, white breads with lower salt content (either NaCl or NaCl:KCl) showed lower overall liking scores. Type of salt also affected consumer hedonic perception (Group 1: $F=9.39$, $p=0.002$; Group 2: $F=5.74$, $p=0.017$). However, this variable had the opposite effect on the two consumer segments. For Group 1 ($n=54$), breads produced with NaCl showed higher overall liking scores than those produced using the 70:30 NaCl:KCl blend (6.0 vs. 5.5, respectively). On the other hand, consumers in Group 2 ($n=47$) gave, on average, higher overall liking scores to breads formulated with NaCl:KCl (6.1 vs. 5.8).

5.3.3.2 Sensory perception

Results from the glm performed on CATA data from all consumers revealed that the equivalent salty concentration had a significant effect on the frequency of use of the terms *salty* ($p<0.001$), *tasteless* ($p=0.009$), *barely salty* ($p=0.007$), *tasty* ($p=0.002$) and *dry* ($p=0.002$). As shown in Table 5.5, reducing the sodium content in white bread, regardless of the type of salt, caused a significant decrease in the frequency of use of the terms: *salty* and *tasty*. Despite the fact that samples significantly differed in the frequency of use of *salty*, this term was one of the least frequently used. The highest frequencies of use of the term *tasty* were observed for breads containing 1.38%-1.80% NaCl and 2.25% NaCl:KCl whereas, the opposite trend was observed in relation to the terms *tasteless* and *barely salty* (Table 5.5). It is worth highlighting that samples did not significantly differ in the frequency of use of the terms *bitter*, *metallic* and *off-flavour*, suggesting that partial replacement of NaCl with KCl, using a 70:30 NaCl:KCl blend, did not impart undesirable flavours to bread. In addition, the terms *bitter* and *metallic* were by far the least frequently used, reinforcing the idea that these sensory characteristics were not perceived in the bread samples (Table 5.5).

Type of salt (NaCl or NaCl:KCl blend) significantly affected the frequency of use of the term *spongy* ($p=0.037$). As shown in Table 5.5, white breads formulated with NaCl:KCl tended to show higher frequency of use of the term *spongy* than those formulated with NaCl. This result is in line with those from the dynamic sensory characterization of the white bread sample produced by partially replacing 30% NaCl by KCl (Figure 5.2). In addition, the interaction between equivalent salty concentration and type of salt significantly affected the frequency of use of the terms *hard* ($p=0.018$) and

PARTIAL REPLACEMENT OF NaCl with KCl

dry ($p=0.044$). However, no clear effect of the percentage of salt reduction and the type of salt on hardness and dryness was found (Table 5.5).

Table 5.5 Frequency of use of each of the terms of the CATA question when evaluating the second bite of 8 sodium-reduced white bread samples produced with two types of salts: NaCl (control breads) or 70:30 NaCl:KCl blend.

Term	Type of salt and concentration (% of flour weight)							
	NaCl 1.80%	NaCl:KCl 2.34%	NaCl 1.61%	NaCl:KCl 2.25%	NaCl 1.38%	NaCl:KCl 1.76%	NaCl 1.25%	NaCl:KCl 1.64%
Salty	18 ^c	7 ^{a,b}	9 ^{b,c}	10 ^{b,c}	5 ^{a,b}	4 ^{a,b}	4 ^{a,b}	2 ^a
Hard	11 ^{a,b,c}	13 ^{b,c}	11 ^{a,b,c}	4 ^a	7 ^{a,b}	19 ^c	15 ^{b,c}	13 ^{b,c}
Soft	31 ^a	29 ^a	29 ^a	39 ^a	33 ^a	29 ^a	33 ^a	32 ^a
Spongy	40 ^{a,b}	51 ^b	39 ^{a,b}	51 ^b	33 ^a	37 ^a	37 ^a	39 ^{a,b}
Dry	18 ^{a,b}	19 ^{a,b}	16 ^a	15 ^a	20 ^{a,b}	28 ^{b,c}	39 ^c	22 ^{a,b}
Light	27 ^a	24 ^a	22 ^a	32 ^a	26 ^a	25 ^a	23 ^a	28 ^a
Dense	36 ^a	30 ^a	40 ^a	29 ^a	33 ^a	38 ^a	34 ^a	24 ^a
Tasteless	18 ^a	19 ^a	18 ^a	19 ^a	25 ^{a,b}	23 ^{a,b}	29 ^{a,b}	33 ^b
Barely salty	25 ^a	31 ^{a,b,c}	30 ^{a,b}	33 ^{a,b,c,d}	40 ^{b,c,d}	44 ^{c,d}	35 ^{a,b,c,d}	46 ^d
Off-flavour	12 ^a	12 ^a	10 ^a	10 ^a	13 ^a	11 ^a	9 ^a	16 ^a
Crumbly	8 ^a	9 ^a	14 ^a	5 ^a	8 ^a	8 ^a	16 ^a	11 ^a
Sticky	15 ^a	15 ^a	16 ^a	14 ^a	18 ^a	16 ^a	13 ^a	17 ^a
Smooth	32 ^a	32 ^a	40 ^a	39 ^a	38 ^a	34 ^a	29 ^a	36 ^a
Gummy	19 ^a	22 ^a	15 ^a	19 ^a	16 ^a	23 ^a	17 ^a	16 ^a
Tasty	40 ^b	22 ^a	31 ^b	38 ^b	31 ^b	23 ^a	20 ^a	17 ^a
Characteristic bread flavour	27 ^a	24 ^a	16 ^a	21 ^a	17 ^a	21 ^a	17 ^a	21 ^a
Metallic	2 ^a	6 ^a	0 ^a	2 ^a	3 ^a	3 ^a	2 ^a	0 ^a
Bitter	3 ^a	3 ^a	2 ^a	1 ^a	2 ^a	1 ^a	4 ^a	1 ^a

Values of frequency of use of CATA terms with different letters within a row are significantly different according to the sign test ($p<0.05$)

Data from the sensory characterization of samples using CATA questions was analysed separately for the two consumer segments identified in the hierarchical cluster analysis. Both groups markedly differed in the number of terms for which significant differences were found among samples. For Group 2, the equivalent salty concentration significantly affected the frequency of use of the terms: *salty* ($p=0.001$), *spongy* ($p=0.006$) and *dense* ($p=0.024$). The effect of equivalent salty concentration on the frequency of use of the term *salty* was similar to that previously described for the whole data set: the higher the salt content, the higher the frequency of use of this term.

Regarding the texture attributes, white bread samples with an intermediate salt concentration (regardless of the type of salt) showed the highest frequency of use of the term *dense* and the lowest frequency of use of the term *spongy* (data not shown). Neither the type of salt nor the interaction between equivalent salty concentration and type of salt had a significant effect on the frequency of use of any of the terms of the CATA question.

On the other hand, for consumers in Group 1 equivalent salty concentration had a significant effect on the frequency of use of the terms: *hard* ($p=0.016$), *spongy* ($p=0.003$), *dry* (0.002), *smooth* (0.0012), *tasteless* ($p<0.001$) and *tasty* ($p<0.001$). In particular, white breads with an intermediate salt concentration (1.38% NaCl, 1.61% NaCl and 1.76% NaCl:KCl blend), showed the lowest frequency of use of the term *hard* and the highest frequency of use of the term *smooth*. On the other hand, no clear effect of salt reduction on the frequency of use of the terms *dry* and *spongy* was found (data not shown). A significant effect of the type of salt was observed on the frequency of use of the terms *salty* ($p=0.002$), *crumbly* ($p=0.005$) and *gummy* ($p=0.005$) whereas, the interaction between equivalent salty concentration and type of salt significantly affected the frequency of use of the term *tasty*. It is interesting to note that the frequency of use of the term *crumbly* tended to be higher for bread samples produced with NaCl compared with those produced with NaCl:KCl whereas, the opposite trend was observed in relation to the term *gummy*. This result suggests that for Group 1, type of salt mainly affected the texture characteristics of breads.

5.4. Discussion

The development of sodium reduction strategies is receiving increasing attention worldwide as a mean to reduce sodium intake at the population level and contribute to decreasing the prevalence of high blood pressure and cardiovascular diseases. In this context, the present work focused on studying the partial substitution of NaCl with KCl as strategy for sodium reduction in white bread. Different NaCl:KCl ratios (70:30, 40:60 and 50:50 NaCl:KCl) were considered to explore the impact of adding KCl to bread in terms of saltiness perception and sensory characteristics of the product.

PARTIAL REPLACEMENT OF NaCl with KCl

5.4.1. Equivalent salty concentrations of NaCl:KCl blends

The equivalent salty concentrations of each NaCl:KCl blend were estimated relative to different NaCl concentrations, which corresponded to a series of sequential salt reduction steps previously determined by Antúnez et al. (2016). The addition of KCl to sodium-reduced white breads has proved to be effective at compensating for saltiness intensity and proved promising in contributing to the development of low-salt foods. As expected, the magnitude of sodium reduction achieved without affecting saltiness perception depended on the percentage replacement of NaCl with KCl (Table 5.3). Compared to NaCl, higher concentrations of the NaCl:KCl blends were needed to obtain the same saltiness intensity. Similar results were reported by Guardia, Guerrero, Gou and Arnau (2006) who have suggested that compounds with higher molecular weight than NaCl may result in lower salty taste intensity. Although the mechanism behind saltiness perception in humans' beings has still not being completely depicted, it is suggested that it might be linked to the passage of sodium ions through a narrowly gated ionic channel. Given the specificity of this ionic channel, it seems not possible that other molecules pass through it (McCaughey, 2007). In this regard, other cations such as potassium, magnesium and calcium, have been reported to be more acidic and less salty than sodium (Mooster, 1980).

The saltiness potency of the NaCl:KCl blends in white bread ranged from 0.62 to 0.82 (depending on the NaCl:KCl ratio and the NaCl concentration used in the estimation). These values correspond to a saltiness potency of KCl in the range of 0.20-0.56 (average=0.42), which is lower to that reported by other authors. Feltrin et al. (2015) reported that the saltiness of a KCl aqueous solution was 75% the saltiness of a 0.75% aqueous solution of NaCl. Similarly, da Silva, de Souza, Pinheiro, Nunez and Freire (2014) estimated the saltiness potency of KCl in cream cheese as 0.83. The differences in the equivalent salty concentrations reported in the current work and those reported by Feltrin et al. (2015) and da Silva et al. (2014) may be related to the product category involved in the study, as well as to the use of complete vs. partial replacement of NaCl by KCl. In this sense, saltiness perception may be affected by psychophysical relationships between salt and other taste stimuli in the food matrix (Bobowski & Vickers, 2012). Results from the present study add evidence that recommendations regarding NaCl replacement should be tailored on a product by product basis.

5.4.2. Dynamic sensory profile of white breads formulated with NaCl:KCl blends

The present work added evidence that KCl used in large quantities modifies the sensory profile of food products leading to an increase in bitterness and metallic flavour intensity. Results suggested that when the percentage replacement of NaCl with KCl raises beyond 40% or higher, bitterness, metallic flavour and off-flavour are perceived. This limits the magnitude of sodium reduction to be achieved through this sodium-reduction strategy. Feltrin et al. (2015) used temporal dominance of sensations (TDS) to assess the dynamic sensory profile of aqueous solutions of NaCl and different sodium replacers. Results from their work showed that the sensory profile of a 0.75% NaCl solution and that from a KCl solution equivalent in saltiness to NaCl were similar. However, different from NaCl, KCl was characterized for being bitter during the last half of the evaluation period.

On the other hand, the sensory dynamic profile of white bread samples produced with the 70:30 NaCl:KCl blend did not significantly differ from the control bread (produced with NaCl). This result supports the feasibility of this sodium reduction strategy in white bread and reinforces the idea that partial rather than total substitutions should be used. In line with this, Mueller, Koehler and Scherf (2016) reported that it was possible to replace 30% of sodium chloride by KCl in pizza crust without noticeable changes in saltiness or bitterness perception.

5.4.3. Consumers' sensory and hedonic perception of white breads formulated with partial substitution of NaCl by a NaCl:KCl blend

At the aggregate level, the sodium content of white bread significantly affected consumers' hedonic perception. Regardless of the type of salt (NaCl or NaCl:KCl blend), consumers gave higher overall liking scores to breads containing higher sodium content. Results from the sensory characterization of samples using CATA questions, suggested that sodium reduction mainly caused relevant changes in the flavour characteristics of white breads. The breads with lower sodium content were described as less *salty* and *tasty* relative to those higher in sodium. The former samples were also characterized for being *tasteless* and *barely salty*. Therefore, the drop on overall liking scores may be explained by both changes in saltiness perception and a lower total flavour intensity as a consequence of the low sodium concentrations. In this sense, it is worth noticing that the attribute *salty* was almost not used for describing samples (Table 5.5), pointing out that consumers may have used the term *tasty* rather than *salty* to describe samples with higher sodium content. Similar results have been previously reported by Antúnez et al. (2016). This result adds to the body of evidence suggesting that salt reduction has a

PARTIAL REPLACEMENT OF NaCl with KCl

negative impact on liking (Beauchamp et al., 1982; Kilcast, & den Ridder, 2007; Phelps et al., 2006; Rødbotten et al., 2015).

At the aggregate level, the replacement of NaCl by KCl did not modify consumers' hedonic perception. In addition, the replacement of NaCl by 30% KCl did not cause any significant increase in the frequency of use of the terms *metallic*, *bitter* or *off-flavour*, which have been widely reported as side effects of using KCl. Similar results have been reported by Charlton et al. (2007), who concluded that it was possible to reduce 32% sodium in brown bread by partially replacing sodium with potassium, magnesium and calcium without causing a negative impact on the industrial production or the final product quality. Similarly, Braschi et al. (2009) showed that a 30% substitution of NaCl with potassium salts (1:1 mixture of potassium chloride and potassium bicarbonate) did not significantly affect consumers' liking.

In the present work heterogeneity in consumer hedonic reaction towards partial replacement of NaCl by KCl was found. Although both consumer groups decreased their overall liking scores as the salt concentration decreased, they differed in how they reacted to the replacement of NaCl by KCl. Group 1 showed preference for breads produced with NaCl, whereas Group 2 gave higher overall liking scores to those produced with the 70:30 NaCl:KCl blend. The main difference between the groups was related to texture terms. According to consumers in Group 1, type of salt had a significant impact on the frequency of use of the terms *gummy* and *crumbly*. The breads formulated with the NaCl:KCl blend tended to be gummier and less crumbly than those produced with NaCl. Although it has reported that potassium chloride and sodium chloride have similar effects on yeast activity and dough rheological properties (Cauvain, 2007; Salovaara 1982), results from this work suggest that the effect of partially replacing NaCl with KCl on the texture characteristics of bread still deserves further research.

5.5. Conclusions

Results from the present work showed that KCl can be deemed effective at compensating for saltiness intensity in sodium reduced breads. Partially replacing 30% NaCl with KCl did not cause negative changes on the sensory profile of white bread, providing support to the potential of this strategy for reducing the salt content in food products. Nevertheless, when the percentage replacement of NaCl with KCl rose to 40% or higher it was accompanied by undesirable flavours, in particular bitterness and metallic flavour.

CHAPTER 6

**Exploration of non-homogeneous spatial distribution of salt
using fatty coatings for sodium reduction in bread**

NON-HOMOGENEOUS SPATIAL DISTRIBUTION OF SALT

Abstract

The reformulation of processed food products towards lower sodium contents has been identified as a core intervention in the context of public policies geared towards sodium intake reduction at the population level. The aim of the present study was to explore a sodium reduction approach based on non-homogeneous spatial distribution of salt in a solid food-matrix structure, using white bread as case study. In a first stage, encapsulated salt granules were obtained using fatty coatings. Then, a bread with non-homogeneous spatial sodium distribution was produced using the encapsulated salt developed in the previous step. A paired comparison test was used to compare the saltiness intensity of two breads formulated with the same salt (NaCl) content but with different type of salt: regular or encapsulated salt crystals. No significant differences were found between homogeneous and non-homogeneous salt distribution in bread, giving rise to doubt as to the potential of this strategy. However, this result should be interpreted in the light of the limitations of the current study, mainly related to the obtention of the encapsulated salt grains. Further research is needed to get an in-depth understanding on the topic.

6.1. Introduction

Population based interventions to reduce sodium intake have proven to be highly cost-effective initiatives to tackle the continuous increase in NCDs, particularly cardiovascular diseases (He & MacGregor, 2009; WHO, 2007). Especial focus has been put on reducing the amount of salt added to processed foods, a major source of sodium in the current food environment (Campbell et al., 2012; Downs et al., 2015; He et al., 2019; Regan et al., 2017; Webster, 2015). However, one of the main challenges for this approach is obtaining products that are still accepted by consumers (Kilcast & den Ridder, 2007; Phelps et al., 2006; Rødbotten et al., 2015). To accomplish this goal, a solid body of evidence has recommended the gradual salt reduction approach over abrupt salt reductions (Bobowski et al., 2015a; Girgis, et al., 2003; FSA 2003; IOM, 2010; MacGregor & Hashem, 2014). Yet, combining a gradual strategy with other salt reduction approaches have been deemed necessary to strive for higher salt downshifts in shorter time periods (Busch et al., 2013).

In this context, the non-homogeneous spatial distribution of salt in the food matrix could be a promising strategy for removing salt from food without affecting consumer perception (Busch et al., 2013). Under normal eating conditions, taste receptors are exposed to a continuous and prolonged stimulation that cause a gradual drop in taste responses as a result of adaptation processes (Busch et al., 2013; Mosca et al., 2010). Tongue movements, salivation and swallowing reduce, at least partly, the sensory adaptation (Theunissen et al., 2000). The non-homogeneous spatial distribution of tastants in the food matrix is thought to cause a contrast effect in mouth during the mastication process, since some receptors are exposed to high tastant concentration while others are exposed to low concentrations. Meiselman and Halpern (1973) have shown that in liquid systems the pulsatile taste stimulation with a tastant solution can trigger a succession of high phasic taste receptor responses that may lead to a higher overall response relative to a continuous stimulation. The frequency with which taste receptors are exposed to different tastant concentrations, has been identified as a key determinant for taste enhancement (Burseg, Brattinga, de Kok, & Bult, 2010; Busch, Tournier, Knoop, Kooyman, & Smit, 2009; Meiselman & Halpern, 1973).

Based on the available evidence for liquid systems, it has been theorized that salty taste enhancement through a non-homogeneous salt distribution in the food matrix is mediated by the partial recovery from taste receptor adaptation and the higher burst frequency of the gustatory afferent nerve fibers. The latter occurs as a consequence of a discontinuous stimulation of taste receptors in a desynchronized fashion (Busch et al., 2013; Mosca et al., 2010). In this sense, Smith, Bealer, and van Buskirk (1978) found

NON-HOMOGENEOUS SPATIAL DISTRIBUTION OF SALT

that using water to rise between successive exposures to the taste stimulus heightened the overall burst frequency of the chorda tympani branches of the facial nerves in rats. A high amount of salt concentrated in a small food portion, may lead to high salt concentration in saliva which in turn may cause a burst of saltiness intensity, resulting in a higher overall saltiness perception (Emorine et al., 2013; Mosca et al., 2010).

Several studies have shown that the magnitude of the taste enhancement heavily depends on the concentration contrast in the stimuli and, indeed, large tastant concentrations differences are needed to cause a meaningful taste enhancement (Burseg et al., 2010; Holm, Wendin, & Hermansson, 2009; Mosca et al., 2010; Noort et al., 2010). Expectations may also contribute to the taste enhancing effect of non-homogeneous systems. The fact that consumers are expecting to face a homogeneous product may contribute to maintain the sensory contrast unnoticed (Woods et al., 2010).

Fan (1991) reported that a one third sodium reduction in cereals with non-homogeneous distribution of salt was feasible without causing significant changes on consumer overall flavour perception. The non-homogeneous salt distribution was reached through an internal portion with lower salt content and an external portion with higher salt content, in which salt was evenly distributed on the surface (Fan, 1991). It was hypothesized that salt on the surface provided instant flavour upon consumption of the cereal, while the salt in the interior portion provided continuous flavour during chewing (Fan, 1991). In a similar vein, more recent studies have pointed out that the modulation of the spatial distribution of tastant in the food matrix enhances taste perception and have proven efficient for salt reduction in different product categories without affecting neither saltiness perception nor hedonic perception (Emorine et al., 2013; Mosca et al., 2013; Noort et al., 2012; Noort et al., 2010). In all cases, results suggested the larger the concentration gradient of salt in the food matrix, the larger the taste enhancement.

Up to date, two strategies have been used to accomplish a non-homogeneous distribution of salt in solid foods: the generation of product layers with different salt concentrations and the generation of salt crystals encapsulated by fatty coatings (encapsulated salt) (Busch et al., 2013). The former approach has proved promising for reducing the salt content in food, albeit a series of difficulties arise in its implementation to an industrial level (Mosca et al., 2013; Noort et al., 2010). In practice, the coexistence of product layers arranged on top of each other trigger the diffusion from the higher to the lower salt content layers. This migration process reduces the difference in salt concentration between product layers and, ultimately leads the concentration gradient of salt to disappear. This represents a serious disadvantage of this approach and heavily

limits its feasibility at the industrial level. Moreover, its applicability is limited to product categories in which layering is possible (Noort et al., 2010) and requires additional manufacturing steps to prepare and combine layers with different salt concentrations (Busch et al., 2013). In spite of the above-mentioned limitations, this approach has been the most widely used for generating non-homogeneous spatial distribution of tastants in food (Holm et al., 2009; Mosca et al., 2010; Mosca et al., 2013; Noort et al., 2010).

The use of encapsulated salt may overcome the main shortcomings of the layered approach (Noort et al., 2012). The rationale behind salt encapsulation is to enclose the salt particle within a non-aqueous coating agent (such as fat) that provides a physical barrier between the salt grain and the food matrix, preventing its dissolution in an aqueous matrix (Özdemir & Gökmen, 2015). In the particular case of bread, salt encapsulation seeks to avoid salt dissolution during the dough structure formation. In the baking process the fat coating melts releasing the salt crystals and salt diffuses into a small surrounding area, leading to a non-homogeneous salt distribution in the final product matrix. A major advantage of encapsulated salt is that no major changes are required in the production process as it basically consists on replacing regular salt for encapsulated salt particles (Noort et al., 2012). However, many technological aspects related to the industrial implementation of such an approach remains unresolved (Busch et al., 2013).

In this context, the aim of the present work was to explore the non-homogeneous spatial distribution of salt using fatty coatings for sodium reduction in white bread. The objective was twofold: (i) to obtain encapsulated salt grains using a fatty coating material, and (ii) to explore the generation of a non-homogeneous spatial distribution of salt using fatty coatings as sodium reduction strategy. The study was focused on confirming and extending previous research on the topic, with especial emphasis on generating information adapted to the reality of the country that lead to actionable outputs for the local food industry.

NON-HOMOGENEOUS SPATIAL DISTRIBUTION OF SALT

6.2. Materials and methods

6.2.1. Preparation of encapsulated salt crystals

An adaptation of the methodology proposed by Noort et al. (2012) was used to prepare the encapsulated salt crystals. Commercial coarse salt (Antil S.A, Montevideo, Uruguay) was manually pan sieved using a 12-mesh stainless steel sieve to reduce the heterogeneity of particle sizes. Two fractions were obtained: one composed by the salt grains retained in the mesh (higher particle size) and another corresponding to those passing through the mesh (smaller particle size). For the purpose of the study only the retained fraction was used.

Palm stearin IV 34 (melting point (MP): 49.0-55.4°C) was used for generating fatty coatings. Among the fat materials available in the local marketplace at the moment the study was carried out, this material has the most similar melting point to the high melting fat used by Noort et al. (2012) (Revel™ C, Loders Croklaan, The Netherlands, MP: 56-61°C).

Salt grains (500 g) were placed in a planetary mixer with a heating jacket where they were kept moving while the melted fat material was sprayed using a manual spraying pump. Palm stearin IV 34 was previously melted in a water bath at 90°C and 0.1% β -carotene (a liposoluble colouring) was added to it. The addition of β -carotene sought to allow a better visualization on how encapsulated salt particles were distributed in the final food matrix. The temperature of the heating jacket was set at 30°C to keep salt particles warm and to avoid the fat material to immediately solidify when getting into contact with salt. After 10 minutes at medium rotation speed, the encapsulated salt particles were removed from the planetary mixer and disposed in a sieve mesh to cool them to room temperature.

6.2.2. Sensory evaluation

6.2.2.1 *Trained assessors*

The trained panel consisted of sixteen assessors, ages ranging from 22 to 56 years old (13 female). Assessors were recruited among workers of Universidad de la República (Uruguay) and went through a selection and training process according to the guidelines of the ISO 8586:2012 standard (ISO, 2012). The sensory panel had been previously trained to evaluate saltiness intensity in bread, as reported in Antúnez et al. (2018).

6.2.2.2 *Bread samples*

The breads were produced using 58% Uruguayan commercial wheat flour (Molino Americano S.A, Montevideo, Uruguay), 37% tap water, 2.4% sugar (Alcoholes del Uruguay S.A., Bella Unión, Uruguay), 1.6% high oleic sunflower oil (Compañía Oleaginosa Uruguaya S. A, Montevideo, Uruguay) and 1.0% powered yeast (Fleischmann, Argentina). Salt was added (1.8% salt/ kg flour) either as regular salt (Antil S.A, Montevideo, Uruguay) or encapsulated salt. In order to add the same amount of salt (NaCl) to both bread formulations, the proportion of fat in the encapsulated salt was estimated using a gravimetric method (the salt weight was registered prior to salt encapsulation and after having added the fatty coating). The amount of encapsulated salt to be added to the bread recipe to obtain a 1.80% NaCl concentration was then calculated based on this estimation.

The breads were prepared in a bread-making machine (Phillips® model HD9015/30) one day prior to testing and were stored in plastic bags overnight. The morning of the test, breads were cut in 10 mm slices and the crust was removed. For each paired-comparison test, assessors received two bread samples, each corresponding to approximately five grams, served on ceramic plates that were coded using three-digit numbers.

6.2.2.3. *Paired comparisons*

A paired comparison test was used to compare the saltiness intensity of a control bread (homogeneous salt distribution) and a bread produced with encapsulated salt (non-homogeneous distribution), both containing the same salt (NaCl) concentration.

Assessors received the samples presented in pairs and were requested to taste each of the samples in the pair and to indicate the saltier one by selecting the corresponding code number. They were forced to make a choice and were instructed to cleanse their palate with water between trials. Samples in each pair were presented following a balanced design to avoid order effects. The test was divided into two sessions, each involving a series of three identical paired-comparison tests (homogeneous vs. non-homogeneous salt distribution). Assessors performed replicated evaluations in order to increase the number of judgments and improve the statistical power of the test.

Data collection was performed using Compusense-Cloud (Compusense Inc., Guelph, Canada). Testing took place in a sensory laboratory designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22°C).

6.2.2.4. *Data analysis*

NON-HOMOGENEOUS SPATIAL DISTRIBUTION OF SALT

A chance-corrected beta-binomial model was used for data analysis as recommended by Naes, Brockhoff and Tomic (2010). This approach takes into account individual differences and allows replicates to be pooled, making adjustments in the binomial calculation towards more conservative criteria when non fully independent data is considered (Naes et al., 2010; Lawless & Heymann, 2010). Data analysis was carried out using R statistical software version 3.6.1 (R Development Core Team, 2019). Functions from the R package sensR were used to perform the analysis (Christensen & Brockhoff, 2020).

6.3. Results and Discussion

Encapsulated salt grains were obtained using Palm stearin IV 34, providing evidence on the feasibility of using this fat material to generate fatty coatings. Technical difficulties in the obtention process of encapsulated salt grains were faced, mainly related to the obtention of an evenly distributed fatty coating on the salt particle. This limitation was accentuated by the fact that the salt particles showed a heterogeneous particle size distribution. In this sense, it is hardly recommended to reduce, as much as possible, the heterogeneity of particle size for future research. Moreover, the thickness and integrity of the fatty coating was assessed by visual inspection. The use of more sophisticated techniques, such as scanning electron microscopy, may provide an in-depth analysis of the microstructure of the encapsulated salt grains. The physicochemical characteristics of the fat material have been deemed a key determinant of fatty coatings success and, in this sense, exploring different fat materials alternatives is warranted. In addition, particle size is thought to largely influence saltiness perception (Rama et al., 2013; Moncada et al., 2015; Shepherd, Wharf, & Farleigh, 1989; Galvão, Moura, Barretto, & Pollonio, 2014) but, this has not been well-understood yet. Future research is needed to shed light on the effect of particle size on saltiness perception.

A bread with a non-homogeneous salt distribution was produced using the salt encapsulated crystals obtained in the first stage and its saltiness intensity was compared to that elicited by a regular bread (homogeneous salt distribution). Results from the paired comparison test showed that 33 out of 66 judgements indicated the bread with non-homogeneous salt distribution was saltier than the regular one, revealing no significant differences in the saltiness intensity between the two bread samples ($p=1.00$). Although exploratory in nature, these findings point out the non-homogeneous salt distribution using fatty coating was not effective in rising saltiness perception. Yet, results should be interpreted in the light of the limitations of the present work and caution should be taken in generalizing the current findings.

Results from the present work go in the opposite direction to the up to date evidence, which have attached great potential to this sodium reduction strategy. In particular, Noort et al. (2012) used encapsulated salt particles to obtain a non-homogeneous salt distribution in bread and reported that sodium reduction up to 50% could be achieved without affecting saltiness intensity perception. Further research on this salt reduction strategy is needed to gain scientific evidence that allow to make recommendations on whether or not it represents a feasible operational window for salt reduction in food.

CHAPTER 7

**Can sodium warnings modify preferences? A case study with
white bread**

CAN SODIUM WARNINGS MODIFY PREFERENCES?

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- Antúnez, L: Conceived and designed the work, collected the data, analysed and interpreted the data, wrote the paper.
- Alcaire, F: Contributed to data collection.
- Giménez, A: Contributed to the conception and design of the study, as well as to data interpretation.
- Ares, G: Contributed to the conception and design of the study, to data collection as well as to data interpretation. Performed a critical revision of the article.

Abstract

Several studies have shown that the inclusion of front of pack nutritional warnings is a potentially effective strategy to encourage consumers to avoid products with high content of nutrients associated with non-communicable diseases. In this context, the present work aimed at exploring consumers' reaction towards sodium reduction in the context of the implementation of warnings in three scenarios: package evaluation, tasting and intention to re-purchase the product after tasting, using white bread as case study. A total of 171 consumers participated in the study that encompassed four parts. Firstly, consumers received two bread samples with different salt content (2.00% and 1.38% salt) under blind conditions and were asked to rate their overall liking and to indicate the one they preferred. Then, they were asked to look at four bread packages differing in terms of graphic design and the presence (or absence) of warnings. They had to rate their expected liking and to indicate the one they would purchase. Finally, consumers were asked to try the bread they had previously chosen, to assess their overall liking and to indicate their willingness to purchase it again. Salt content significantly affected consumer hedonic reaction towards the breads. Two consumer segments with different preference for the salt content in bread were identified: 58% of consumers preferred the bread with 2.00% salt while 42% preferred breads with 1.38% salt. However, when looking at the packages the majority of consumers in both groups selected bread packages that did not feature warnings. In addition, after having tried the bread they had previously chosen, most consumers were willing to buy it again, which is promising in terms of reaching sustainable changes towards lower salt levels. Results from the present work suggest that, in the case of bread, nutritional warnings have potential to shift consumers' preferences to lower sodium content, even after trying the products.

7.1. Introduction

Population-based interventions to reduce sodium intake have been deemed necessary to stall the continuous and alarming increase in hypertension and cardiovascular disease (He & MacGregor, 2009; WHO, 2007). In this sense, multi-faceted actions oriented towards improving the healthiness of the current food environment and raising consumer awareness about the sodium content of processed foods have been identified as public health priorities (Campbell et al., 2012; Downs et al., 2015; Regan, et al., 2017; Webster, 2015). Two core interventions to fulfil this goal are the reformulation of processed products towards lower salt contents and the inclusion of front-of-pack (FOP) nutritional labelling on food packages to empower consumers for selecting healthier food options (He et al., 2019).

A major challenge for the implementation of salt reduction programmes at the population level lies in the fact that large salt reductions in food cause negative changes in consumer sensory and hedonic perception (Beauchamp et al., 1982; Kilcast & den Ridder, 2007; Phelps et al., 2006; Rødbotten et al., 2015). However, repeated exposure to low sodium foods may be sufficient to shift consumers' salt preferences for those specific product categories even within the context of high salt diets (Bobowski et al., 2015b). Over time, consumers may get familiarized with the salt reduced version of specific products without modifying their preferences for salt in other food categories. Thus, a promising course of action may be to define a few target product categories on which to direct the sodium reduction. Given its widespread consumption and its relevant contribution to sodium daily intake, many countries have embarked on bread reformulation towards lower salt content, through either voluntarily or mandatory programmes (La Croix et al., 2014; Pflaum et al., 2013; Rødbotten et al., 2015; Spina et al., 2015).

However, the sensory profile of food products is not the only factor influencing the complex process of food choice. In particular, extrinsic product-related attributes, which are not inherent to the product and can be changed without modifying the product itself (e.g. brand, price, package design and claims), have been extensively reported to largely influence expectations and choices (Asioli et al., 2017; Jaeger, 2006; Oliveira, Ares & Deliza, 2018). Extrinsic characteristics do not require consumers to try the product as they are directly exposed to them when facing the product at the shelf. For this reason, extrinsic characteristics are usually more relevant than intrinsic characteristics in defining consumer choices when they have no previous experience with the product (Olson & Jacoby, 1972). Extrinsic attributes also generate expectations about the product that may strongly influence the way consumers perceive the intrinsic

CAN SODIUM WARNINGS MODIFY PREFERENCES?

characteristics and, ultimately, their global experience with the product (Deliza & MacFie., 1996). Therefore, a more holistic approach that combines intrinsic and extrinsic product characteristics to explore consumer food-related decisions is warranted (Asioli et al., 2017). In this sense, considering information on salt content (extrinsic characteristic) when addressing consumer perception of sodium reduced products may provide a complete and more realistic picture on how consumers react towards sodium reduction.

In recent years, the inclusion of front-of-pack (FOP) nutritional labelling has gained popularity and many countries are debating, developing or implementing new schemes to remedy some of the apparent shortcomings of conventional nutritional information (Cecchini & Warin, 2016). The idea behind FOP nutritional labels is to improve access to information by the provision of simplified formats that rapidly catch consumer attention and facilitate understanding (Hawley et al., 2013; Scrinis & Parker, 2016). In particular, nutritional warnings have emerged as a promising alternative to discourage unhealthy food choices. They were first implemented in Chile and since then have gained popularity in the Americas (Ministerio de Salud, 2015; Government of Canada, 2017; Ministerio de Salud de Perú, 2018; Ministerio de Salud Pública, 2018). Nutritional warnings highlight products with excessive content of nutrients associated with non-communicable diseases. Chile, Peru and Uruguay have implemented this scheme as octagonal black signs with the expression “Excessive in..” or “High in..”. Although the main objective of nutritional warnings is to assist consumers during product selection, they may also nudge the food industry to engage in product reformulation towards healthier nutritional profiles (Kanter et al., 2019). This effect may also contribute to shift consumer choices by increasing the availability of healthy food in the marketplace, thus avoiding people to settle for foods with unfavourable nutritional profiles (Cecchini & Warin, 2015). Previous studies have shown that nutritional warnings have potential to encourage healthier selections within a product category (Acton & Hammond, 2018; Arrúa et al., 2017; Dodds et al., 2014; Khandpur, de Morais Sato et al., 2018; Machín, Aschemann-Witzel, Curutchet, Giménez, & Ares, 2018; Schnettler et al. 2019; Talati et al., 2017; Tórtora, Machín, & Ares, 2019). However, research on the impact of nutritional warnings on consumer food choices under more realistic situations, and particularly how they influence consumer perception after tasting, is still scarce.

Consumers usually face a trade-off between health and hedonic considerations; healthfulness and taste are thought to be inversely related and consumers usually regard healthy products as less tasty than unhealthy products (Raghunathan, Naylor, & Hoyer, 2006). In addition, pleasure has been identified as a key driver of food consumption

(Lowe & Butryn, 2007) and consumers are not often willing to sacrifice the hedonic pleasure in pursuit of potential health benefits (Civille & Oftedal, 2012; Tuorila & Cardello, 2002; Verbeke, 2006).

Previous studies have shown that information on packages can affect consumer sensory (taste) and hedonic perception of food (Zandstra et al., 2015). Providing information regarding sodium reduction has been reported to cause a negative effect on consumer expectations of biscuits (Vázquez et al., 2009). Liem, Aydin and Zandstra (2012) reported a decrease in perceived saltiness intensity of soups when information about sodium reduction was included (Liem et al., 2012). However, the opposite effect has also been reported (Kahkonen et al., 1996; Willems et al., 2014). As yet, the available evidence suggests the way the message is conveyed is critical on the effect it causes on consumer perception (Zandstra et al., 2015) and it remains unclear how best to inform consumers of healthier options. Research on the impact of sodium warnings on consumer expectations and perception of salt-reduced products is highly relevant as this scheme gains popularity worldwide.

In this context, the present work aimed at exploring consumer reaction towards sodium reduction in bread, in the context of the implementation of warnings FOP nutritional labels in three scenarios: package evaluation, tasting and intention to re-purchase the product after tasting. A second objective of the present work was to evaluate if preferences for salt in bread moderate the influence of nutritional warnings on hedonic perception and choice.

7.2. Materials and methods

7.2.1. Participants

A total of 171 consumers participated in the study (60% female). The consumer sample comprised varied household compositions, income levels, and education levels, but could not be regarded as representative of the population of Montevideo (Uruguay). Consumers were recruited from two locations in Montevideo: a local food market and an open doors event in the Botanical Garden. Before the beginning of the study, the minimum number of participants was set in 110, based on recommendations for best practice in sensory and consumer science (Hough et al., 2006). Recruitment criteria was based on consumers bread consumption and their willingness to take part in the study, but no requirements related to consumption frequency of bread were established. All participants provided informed consent in an online form and received a small gift for their participation. The study was approved by the ethics committee of the School of

CAN SODIUM WARNINGS MODIFY PREFERENCES?

Chemistry of Universidad de la República (Uruguay). Table 7.1 summarizes the socio-demographic characteristics of the participants.

Table 7.1. Socio-demographic characteristics of the participants (n=171).

	Percentage (%)
Gender	
Women	60
Men	40
Age	
18-25	26
26-35	35
36-45	20
46-60	15
>60	4
Education level	
Primary school	1
Incomplete secondary school	9
Complete secondary school	9
Technical education	8
Incomplete University studies	34
University degree	22
Post-graduate studies	16
Socio-economic level (*)	
Low	8
Medium	44
High	48

(*) Socio-economic level was estimated using the criteria provided by Centro de Investigaciones Económicas (2016)

7.2.2. Bread samples

Two bread samples differing in their salt content were considered in the study: a control bread containing 2.00% added salt (expressed on the basis of flour weight) and a salt-reduced bread with 1.38% salt (30% reduction relative to the control). The control bread represents the average salt content in this food category in the Uruguayan marketplace at the moment the study was carried out. The salt concentration in the salt-reduced bread was established based on results from a previous study with the same product category (Antúnez et al., 2016) in order to include samples that were expected to significantly differ in terms of their sensory characteristics and consumer hedonic reaction. In addition, the selection sought to include one sample featuring a warning for excessive sodium content as determined by the Uruguayan regulatory framework (Ministerio de Salud Pública, 2018).

Bread samples were produced using 58% Uruguayan commercial wheat flour (Molino Americano S.A, Montevideo, Uruguay), 37% tap water, 2.4% sugar (Alcoholes

del Uruguay S.A., Bella Unión, Uruguay), 1.6% high oleic sunflower oil (Compañía Oleaginosa Uruguaya S. A, Montevideo, Uruguay), 1% of powered yeast (Fleischmann, Argentina) and 2.00% or 1.38% salt/kg flour (Antil S.A, Montevideo, Uruguay). The breads were prepared in a bread-making machine (Phillips® model HD9015/30) one day prior to testing and were stored in plastic bags overnight. The morning of the test, breads were cut in 10 mm slices and the crust was removed. In addition, whole breads were packaged, mimicking the way they are normally sold in the Uruguayan market, to generate the bread packages presented to consumers in the expected condition (Figure 7.1).

7.2.3. Bread packages

Four bread packages were designed following a 2x2 factorial design with the factors: graphic design and salt content. The two levels of the variable salt content corresponded to the salt concentration in the bread samples considered in the blind condition (i.e. 2.00% and 1.38% salt). Bread packages with different levels of salt differed in terms of featuring or not the sodium warning: packages of bread with 2.00% salt featured the sodium warning while those with 1.38% salt did not. In addition, differences between samples were reflected in the sodium content declared in the nutritional panel (see Figure 7.1). At the moment the study was carried out nutritional warnings have not yet been implemented in Uruguay thus, consumers were not familiar with them. The breads did not feature any other nutritional warning.

The graphic design of the packages was selected based on results from an online pre-test in which consumers were presented with 6 labels, specially designed for this study, and were asked to select the healthiest and the tastiest product. The labels differed in terms of brand name, background colour and composition of the image and did not correspond to commercial products available in the Uruguayan marketplace. Two graphic designs were selected, corresponding to the design most frequently associated with a healthy product (Package A, Figure 7.1a) and the design most frequently associated with a tasty product (Package B, Figure 7.1b). The labels were printed on matte white self-adhesive paper and adhered to a plastic package containing the sliced whole bread (Figure 7.1).

CAN SODIUM WARNINGS MODIFY PREFERENCES?

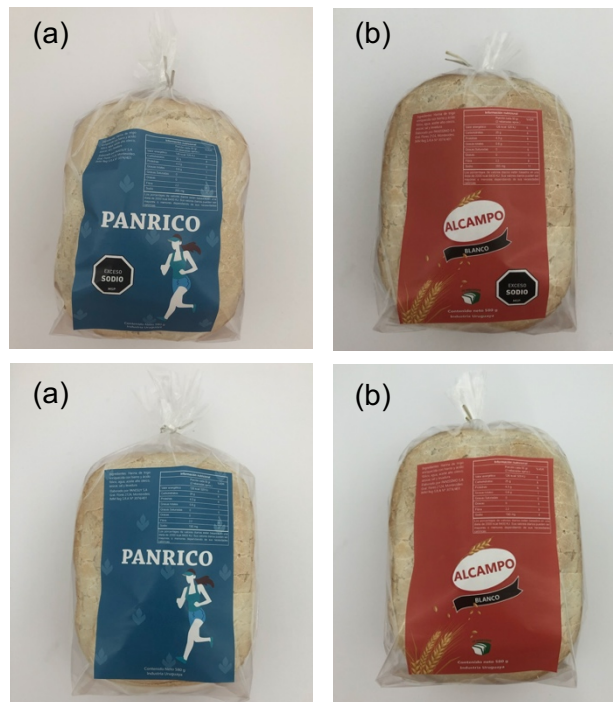


Figure 7.1. Bread packages presented to consumers in the expected condition. Packages corresponding to breads with 2.00% salt featured the sodium warning, while those corresponding to breads with 1.38% salt did not. For each salt level, two graphical designs, were considered: (a) “Package A” (associated with healthiness) and (b) “Package B” (associated with tastiness).

7.2.4. Experimental procedure

The study was divided into four sections. First, consumers received two bread samples with different salt content under blind conditions. Two pieces of bread, corresponding to approximately five grams each, were served on ceramic plates coded using three-digit numbers and were presented in a monadic sequence following an experimental design that was balanced for order and carry-over effects (Williams' Latin Square). Consumers were instructed to try a first bite of the sample, to rate their overall liking using a 9-point hedonic scale (1=dislike very much, 9=like very much) and to repeat the same procedure with a second bite of sample. After having completed the blind assessment of samples, consumers were asked to indicate which bread they preferred.

In the second task, consumers were presented with the four bread packages generated based on the factorial design described above. They were asked to imagine they were in a supermarket purchasing bread. They were told to hold the products, observe them, and to indicate how much do they think they would like the bread, using the classic 9-point hedonic scale. The bread packages were assessed in a monadic sequence following a Williams' Latin Square design. Once consumers have completed this task, they were requested to indicate which one they would buy (forced choice).

In the third task, consumers were asked to try the bread corresponding to the package they had previously chosen (informed condition), to rate their overall liking, using a 9-point structured scale, and to indicate their willingness to purchase it again using the options 'yes' and 'no'.

After finishing the third task, consumers answered a short survey about the use of discretionary salt to explore possible behavioural determinants of differences in salt preferences (Antúnez, Giménez, Alcaire, Vidal, & Ares., 2019). Socio-demographic data -including gender, age, educational level and a series of questions to determine their socio-economic status (Centro de Investigaciones Económicas, 2016)- were also gathered. All data were collected using Compusense Cloud software (Compusense Inc., Guelph, Canada) on I pads (Apple Inc., Cupertino, California, USA).

7.2.5. Data analysis

All data analyses were carried out using R statistical software version 3.6.1 (R Development Core Team, 2019). Functions from `FactoMiner` (Lê, Josse, & Husson, 2008), `lmerTest` (Kuznetsova et al., 2016) and `multcomp` (Hothorn et al., 2019) packages were used.

7.2.5.1. Evaluation of the samples under blind conditions

A linear mixed model was used to assess the existence of significant differences in the overall liking scores of the two bread samples. The analysis was conducted specifying sample and bite as fixed effect and consumer as random effect. Two-way interactions between fixed effects were also included in the model. A significance level of 5% was considered in the analysis and when the effects were significant, honestly significant differences were calculated using Tukey's test.

The percentage of consumers that preferred each bread sample was calculated and used to identify groups of consumers with different preferences for the two breads. Overall liking data was analysed separately for each of the two groups, using the linear mixed model specified above.

Frequency procedures were used to analyse data from the questionnaire on discretionary salt consumption habits. The chi-square test was used to assess the existence of significant differences between the two consumer segments, identified based on their reported preference for the salt content in bread, in terms of discretionary salt consumption habits as well as socio-demographic characteristics. A significance level of 5% was considered in the analysis.

CAN SODIUM WARNINGS MODIFY PREFERENCES?

7.2.5.2. Evaluation of the packages

A linear mixed model was carried out to assess the existence of significant differences in expected overall liking scores among bread packages for the two groups identified in the blind evaluation. Graphic design and salt content were specified as fixed effect whereas consumer was set as random effect. Two-way interactions between fixed effects were also considered in the model. A significance level of 5% was considered in the analysis. When the effects were significant, honestly significant differences were calculated using Tukey's test.

A generalized linear model (glm) was used to identify significant differences among product packages in the likelihood of selection. The analysis was performed considering consumer group, graphic design, salt content and their interaction as factors. A significance level of 5% was considered in the analysis and when the effects were significant, honestly significant differences were calculated using Tukey's test.

7.2.5.3. Evaluation of the selected sample under informed conditions

Overall liking scores under the blind and informed condition were compared using the Student's t-test. The analysis was separately performed on data from each consumer group. The chi-square test was used to compare the percentage of consumers who indicated that would re-purchase the bread they had previously selected between the two consumer groups.

7.3. Results

7.3.1. Evaluation of the bread samples under blind conditions

As shown in Table 7.2, salt content significantly affected the overall liking scores of the breads when evaluated under blind conditions ($p=0.016$). At the aggregate level consumers gave significantly higher overall liking scores to the bread with 2.00% salt compared to that containing 1.38% salt (6.6 and 6.3, respectively). Neither bite ($p=0.24$) nor the interaction between bite and salt content ($p=0.85$) had a significant effect on the overall liking scores. Hereinafter, data from the second bite evaluation is excluded from further analysis and results are presented based on data from the first bite evaluation. This decision was taken owing to facilitate the comparison between overall liking scores in the blind and informed condition (in which a single bite evaluation was performed).

Table 7.2. Results from the linear mixed model performed on overall liking data from all consumers under blind conditions.

Factor	F-value	p-value*
Salt content	5.84	0.016
Bite	1.39	0.240
Salt content*Bite	0.03	0.853

*Values in bold are significant at a significant level of 5%

Preference for salty products is expected to be one of the main barriers to choose salt-reduced products. In order to explore how preference for salt in bread influences the effect of nutritional warnings on consumer hedonic perception and choice, consumer segmentation was sought. Two consumer segments with different preference for the two breads under blind conditions were identified based on their response to the preference question. Group 1 (n=100, relative size=58%) comprised those consumers who preferred the bread with 2.00% salt (control) while Group 2 (n=71, relative size=42%) preferred the bread with 1.38% salt (30% salt reduction relative to the control). As expected, consumers in Group 1 gave significantly higher overall liking scores to the bread with 2.00% salt, while the opposite trend was found for Group 2 (Table 7.3).

Table 7.3. Overall liking (and standard deviation between brackets) of the control (2.00% salt) and salt-reduced (1.38% salt) breads under blind conditions for the two consumer groups identified based on their reported preference: Group 1 (n=100) and Group 2 (n=71).

Bread sample	Bite	Group 1 (n=100)	Group 2 (n=71)
2.00% salt	1	7.2 ^b (1.6)	5.5 ^a (1.5)
(control)	2	7.3 ^b (1.6)	5.6 ^a (1.5)
1.38 % salt	1	5.8 ^a (1.7)	6.9 ^b (1.6)
(30% reduction)	2	5.9 ^a (1.7)	7.2 ^b (1.6)

Notes: Data were collected using a 9-point structured hedonic scale with anchors “Dislike extremely”, “Neither like nor dislike” and “Like extremely”. Values within a column with different letters are significantly different according to Tukey’s test (p<0.05).

CAN SODIUM WARNINGS MODIFY PREFERENCES?

The two consumer groups were compared in terms of socio-demographic profiles and discretionary salt use habits to explore possible reasons underlying their preference for the salt content of bread. As shown in Table 7.4, no significant differences were found between them in terms of gender ($p=0.472$), age ($p=0.167$) or socio-economic status ($p=0.634$). As summarized in Table 7.5, consumers did not significantly differ in their habits related to the use of discretionary salt (salt addition to food -either before or after trying -, salt shaker usage, ways of using salt when preparing food at home), nor in terms of usage of information on salt content when shopping for food. This suggests differences between the two consumer groups on the preference pattern for the salt content of bread could not be explained through these variables.

Table 7.4. Socio-demographic characteristics of participants in the two groups with different preference for salt in bread, Group 1 ($n=100$) and Group 2 ($n=71$), and results from the chi-square tests (p -values).

Socio-demographic characteristic	Group 1 (%)	Group 2 (%)	p-value
Gender			0.472
Female	63	56	
Male	37	44	
Age			0.167
18-25	30	21	
26-35	36	32	
36-45	15	28	
46-60	14	17	
> 60	5	1	
Socio-economic level			0.634
Low	6	10	
Medium	46	42	
High	48	48	

Table 7.5. Distribution of the two groups of consumers with different preference for salt in bread, Group 1 (n=100) and Group 2 (n=71), in terms of their use of discretionary salt and results from the chi-square tests (p-values).

Characteristic	Group 1 (%)	Group 2 (%)	p-value
<i>Ways of using salt when preparing food at home</i>			0.088
While preparing food	78	65	
After preparing food	6	14	
Salt is not used when preparing food	16	21	
<i>Salt addition before having tried food</i>			0.206
Always	9	17	
Sometimes	42	94	
Never	49	132	
<i>Salt addition after trying food</i>			0.313
Always	0	3	
Sometimes	61	59	
Never	39	38	
<i>Salt addition when eating at a restaurant</i>			0.514
Always	4	1	
Sometimes	41	48	
Never	55	51	
<i>Salt shaker on the table at home</i>			0.497
Always	7	11	
Sometimes	23	25	
Never	71	63	
<i>Salt shaker in a restaurant</i>			0.852
Always	5	7	
Sometimes	46	44	
Never	49	49	
<i>Use of information on the salt content of food products</i>			0.133
Always	19	31	
Sometimes	36	35	
Never	45	34	

7.3.2. Evaluation of the packages

The two consumer groups elicited similar responses when they rated their expected overall liking based on the bread packages (without tasting). For both groups, overall liking scores significantly differed with salt content ($p < 0.001$). However, neither graphic design ($p = 0.378$ for Group 1, and $p = 0.395$ for Group 2) nor the interaction between graphic design and salt content ($p = 0.953$ for Group 1, and $p = 0.647$ for Group 2) had a significant effect on the expected liking (Table 7.6). Regardless of the graphic design, the sodium warning led to significantly lower expected overall liking scores compared to packages that did not feature the warning (Table 7.7). It is worth highlighting that even consumers who preferred the bread with 2.00% salt in the blind evaluation

CAN SODIUM WARNINGS MODIFY PREFERENCES?

(Group 1) gave higher scores to the bread packages without the sodium warning (1.38% salt) when they faced the bread packages.

Table 7.6. Results from the linear mixed model performed separately on expected overall liking data from the two groups identified in the blind evaluation based on consumers preferences for salt in bread: Group 1 (n=100) and Group 2 (n=71).

Group	Factor	F-value	p-value*
Group 1	Graphic design	0.78	0.378
	Salt content	74.91	<0.001
	Graphic design*Salt content	0.00	0.953
Group 2	Graphic design	0.73	0.395
	Salt content	38.77	<0.001
	Graphic design*Salt content	0.21	0.647

*Values in bold are significant at a significant level of 5%

Table 7.7. Expected overall liking scores (and standard deviation between brackets) for each of the four bread packages for the two consumer groups identified in the blind evaluation (Group 1 (n=100) preferred the bread with 2.00% salt and Group 2 (n=71) preferred the bread with 1.38% salt).

Sodium warning	Bread package	Group 1 (n=100)	Group 2 (n=71)
Absent	Package A 1.38% salt	6.4 ^b (1.6)	5.9 ^b (1.7)
	Package B 1.38% salt	6.2 ^b (1.8)	6.2 ^b (1.7)
Present	Package A 2.00% salt	5.0 ^a (2.3)	4.7 ^a (2.5)
	Package B 2.00% salt	4.8 ^a (2.1)	4.8 ^a (2.5)

Notes: Data were collected using a 9-point structured hedonic scale with anchors “Dislike extremely”, “Neither like nor dislike” and “Like extremely”. Values within a column with different letters are significantly different according to Tukey’s test (p<0.05).

As shown in Table 7.8, the consumer group did not significantly affect consumer choice when he/she face the bread packages (p=1.000). Moreover, none of the interactions between consumer group and the other factors (graphic design and salt content) were significant (all p≥0.069). This suggests that consumer preference for the salt content in bread under blind conditions did not significantly affect his/her choice pattern when the decision was made based on the bread packages. On the other hand, the salt content of bread, which determined the presence/absence of the sodium warning, had a significant effect on consumer choice (all p<0.001). At the aggregate level, 74% of consumers selected a bread package that did not feature the sodium

warning. When data from each consumer segment was separately analysed, the percentage of consumers in Group 1 and Group 2 selecting a bread package that did not feature the sodium warning was 79% and 68%, respectively. However, no significant differences between the two groups existed.

Table 7.8. Results of the generalized linear model used to evaluate the influence of the graphic design of the label, salt content, consumer group (differing in their preference for salt in bread), and their interactions on the likelihood of selection of bread packages.

Factor	p-value*
Graphic design	0.015
Salt content	<0.001
Consumer group	1.000
Graphic design:Salt content	0.050
Graphic design*Consumer group	0.919
Salt content*Consumer group	0.0687
Graphic design*Salt content*Consumer group	0.2163

*Values in bold are significant at a significant level of 5%

The graphic design also significantly affected choice ($p=0.015$): 58.0% of consumers selected the breads corresponding to “Package B” (associated with tastiness) while 42.0% selected those corresponding to “Package A” (associated with healthiness). In addition, the interaction between salt content and graphic design was significant ($p=0.05$). While consumer willingness to purchase breads featuring a sodium warning was similar for both packages designs, the absence of the sodium warning tipped the balance in favour of Package B. As shown in Figure 7.2, consumer willingness to purchase the bread featuring a sodium warning showed a higher downshift for Package B (45% and 13%, respectively) than for Package A (29% and 13%, respectively).

CAN SODIUM WARNINGS MODIFY PREFERENCES?

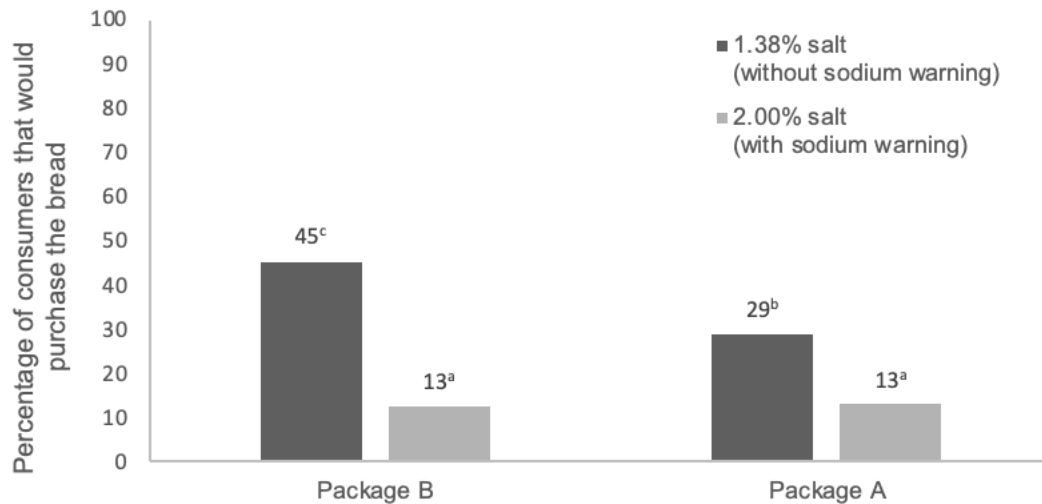


Figure 7.2. Interaction effect between salt content and graphical design on the percentage of consumers that would purchase the product when evaluating the bread packages (without tasting). Values with different letters are significantly different according to Tukey's test ($p < 0.05$).

7.3.3. Evaluation of the selected bread sample (Informed condition)

Consumers tasted the sample they had selected from the package evaluation. The scores provided by the two consumer groups in the blind and informed evaluations were compared. Significant differences in the average overall liking scores were found between the blind and informed condition when considering data from Group 1 ($p = 0.006$). As shown in Figure 7.3, the overall liking of salt-reduced breads (which did not feature the sodium warning) significantly increased from the blind to the informed assessment (5.9 and 6.9, respectively), in which consumers were aware that they were tasting the bread they had previously selected. The opposite trend was observed for the breads with 2.00% salt: the average overall liking scores tended to decrease from the blind to the informed condition, although significance was not reached. This might relate to the fact that the sample size was not large enough to detect the difference ($n = 22$), as most consumers in Group 1 selected the salt-reduced bread when facing the bread packages.

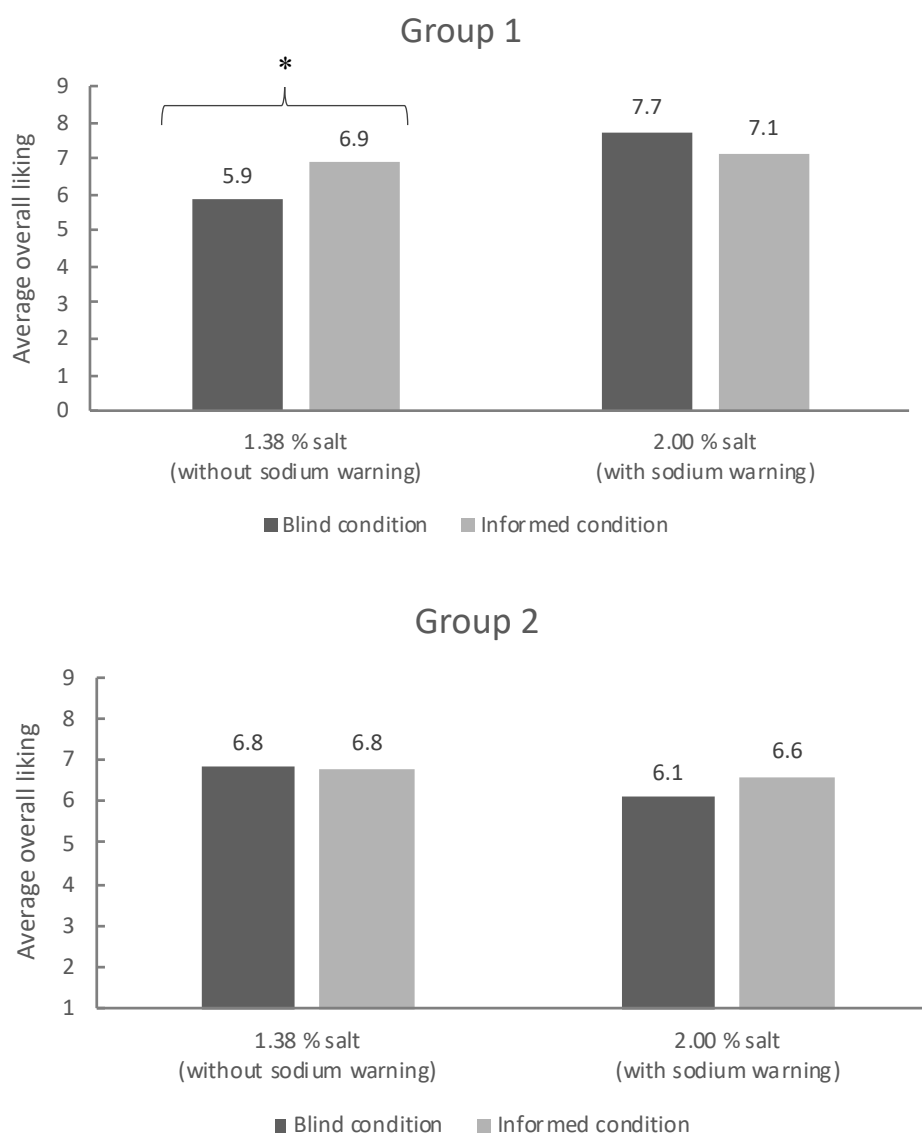


Figure 7.3. Overall liking scores of the bread samples with 1.38% salt (without sodium warning) and 2.00% salt (with sodium warning) elicited under blind and informed conditions. Data are presented separately for each of the two consumer segments identified based on their preferences for the salt content in bread in the blind condition: Group 1 (n=100) and Group 2 (n=71).

On the other hand, no significant changes on the overall liking scores between the blind and informed condition were observed for Group 2 ($p=0.585$). As shown in Figure 7.3, the average overall liking scores remained unchanged for the salt-reduced bread. Although statistical significance was not reached, the overall liking score of breads with 2.00% salt tended to increase from the blind to the informed condition. It should be noted, that similarly to Group 1, when facing bread packages the great majority of consumers in Group 2 (68%) selected breads that did not feature the sodium warning (1.38% salt) and therefore a small sample size ($n=23$) was used for the statistical

CAN SODIUM WARNINGS MODIFY PREFERENCES?

comparison. It is interesting to note that for both the groups, differences in the overall liking scores between the two bread samples (1.38% salt and 2.00% salt) were smaller in the informed compared to the blind condition.

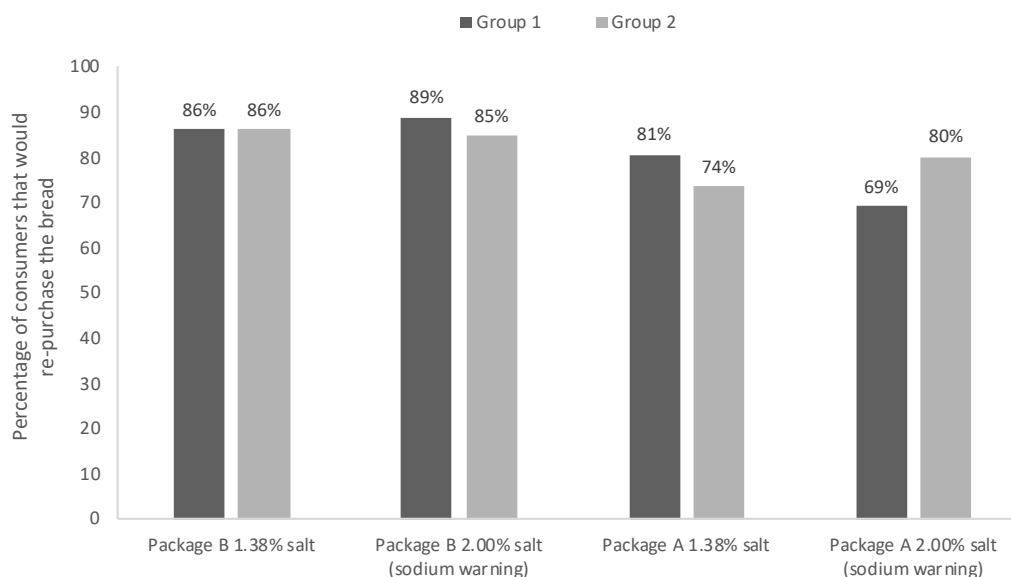


Figure 7.4. Percentage of consumers who would re-purchase the bread they had chosen when facing four bread packages differing in their salt content (presence/absence of the sodium warning) and graphic design (Package A or Package B). For each bread package, the percentages were calculated based on the total of consumer that have selected this option when they were asked to indicate which bread they would buy based on the bread packages.

The two consumer segments showed similar responses patterns with regard to their willingness to re-purchase the bread they had previously chosen. As shown in Figure 7.4, after having tried the bread, 69-89% of consumers in Group 1 and 74-86% of consumers in Group 2, indicated that they would re-purchase the bread they had chosen when facing the bread packages. The salt content of bread (which determines whether or not the bread package features the sodium warning) did not significantly affect consumers' willingness to re-purchase the bread.

7.4. Discussion

Product reformulation towards healthier nutritional profiles and the inclusion of FOP nutritional labelling are gaining widespread popularity in the context of growing efforts to deal with the continuous increase in NCDs (Cecchini & Warin, 2016). The present work contributes to get further insights on how consumers react towards salt-reduced products in the context of the implementation of sodium warnings.

Results from the blind assessment of samples provided further evidence that the salt content significantly affects consumer hedonic perception. These results are in line with a large body of evidence, comprising different food product categories, suggesting that sizable salt reductions in food negatively affect consumer perception (Antúnez et al., 2016; Beauchamp et al., 1982; Kilcast & den Ridder, 2007; Phelps et al., 2006; Rødbotten et al., 2015). However, heterogeneity in consumer preferences towards salt reduction was observed. Two consumer groups with different preference patterns for the salt content of bread were identified: 58% of the consumers preferred the bread with the highest salt content, whereas 42% preferred the bread with the lowest salt content. This result is in agreement with previous studies revealing large heterogeneity among consumers in terms of their salt preferences (Antúnez et al., 2016; Antúnez et al., 2019; Bobowski et al., 2015a) and reinforces the importance of exploring consumers segmentation. The existence of two groups with similar size showing opposite trends in their preference for salt in bread caused a compensation effect when overall liking data was averaged over groups. When considering data from all consumers the difference in overall liking scores between the two bread samples corresponded to 0.3 points (data not shown). However, this difference reached 1.4 and 1.6 points in the 9-point hedonic scale when data from Group 1 and Group 2 were separately analysed (Table 7.3). Thus, ignoring individual differences implies a meaningful loss of information which may lead to conclusions that do not properly reflect reality (Jaeger et al., 2017, Næs, Varela, & Berget., 2018). Besides, consumer segmentation has important implications for the design and implementation of tailor-made salt reduction programmes to address each of the groups. In particular, the current results suggest that almost half of consumers preferred a bread sample containing 30% less salt compared to the average salt content in the local marketplace, revealing a clear opportunity for reducing salt in this product category.

In the context of multi-faceted interventions owing to generate healthier food environments, understanding how intrinsic and extrinsic product attributes interact to drive changes in consumer behaviour is critical. In the present work, significantly higher expected overall liking scores were found for the bread packages that did not feature the sodium warning. In addition, the presence of sodium warnings encouraged the majority of consumers to select breads with low sodium content. This result provided additional evidence that nutritional warnings have proven effective to discourage unhealthy food choices (Acton & Hammond, 2018; Arrúa et al., 2017; Dodds et al., 2014; Khandpur, de Morais Sato et al., 2018; Lima, de Alcantara, Ares, & Deliza 2019; Machín et al., 2018; Schnettler et al. 2019; Talati et al., 2017; Tórtora et al., 2019). Interestingly, the effect of

CAN SODIUM WARNINGS MODIFY PREFERENCES?

sodium warnings on consumer expectations and choice were not affected by preference for salt. This result emphasizes the potential of sodium warnings as a key driver of consumer food choices.

After having tried the bread corresponding to the package they had previously selected, the great majority of consumers were willing to purchase it again, regardless of their initial preference. This suggests an assimilation of expectations, particularly for consumers that showed a preference for breads with high sodium content. This is perhaps the most revealing outcome of the study: sodium warnings may nudge consumers towards healthier food options and have great potential to cause shifts in their hedonic perception towards lower salt contents in bread. Consumers who, under blind conditions, preferred the bread with higher salt content (Group 1) gave significantly higher overall liking scores to the bread with 1.38% salt in the informed compared to the blind condition (an increase of 1 point of the 9-point hedonic scale was observed), suggesting that they accepted a less preferred product when faced with packages that did not feature sodium warnings.

Different results were reported by Lima, Alcantara, Ares and Deliza (2019) when exploring the effect of two FOP nutritional labelling schemes (traffic light system and nutritional warnings) on children and adults' perception of regular and sugar-reduced drinks under different evaluation conditions (blind, expected and informed). These authors found that nutritional warnings encouraged healthier choices under the expected conditions but, had almost no influence on consumer perception under informed conditions. Differences between results from this study and results from the present work may be related to the degree of difference between product samples, as well as the methodological approach used in the studies. Changes in consumer hedonic perception of the products are expected to be more likely when differences between expectations and the real characteristics of the product are small, which makes assimilation effects more likely (Deliza & MacFie, 1996). In the present work the difference between products may have been smaller than those considered by Lima et al. (2019). Regarding methodological differences, a core idea behind the methodological approach used in the current study was to mimic, as close as possible, real-life conditions to overcome some of the main disadvantages of the blind/informed testing. In this sense, Lima et al. (2019) have pointed out that presenting consumers with sugar-reduced drinks samples along with their packages lacks ecological validity and may prompt consumers to base their choices on the sensory characteristics of the products with little or no focus on the information available on the product package. This may not properly reflect consumer behaviour as in a real-life setting they would not have the opportunity to try products

when making their choices. Therefore, in order to gain more realistic insights, in the present work the informed condition was implemented so that consumers only received the bread sample corresponding to the package they had previously selected when evaluating their expectations based on package information. While promising in terms of gathering results that better reflect a real-life setting, a major drawback of this approach is that the impact of information (sodium warnings) on consumer perception is gathered only for the selected sample.

The message used to convey information on sodium content seems to play a critical role on how information impacts on consumer perception and choice. In this sense, Antúñez, Giménez, Alcaire, Vidal and Ares (2017) found that information about salt reduction did not affect consumer hedonic perception of French bread. However, consumers tended to be more discriminative in the informed condition relative to the blind evaluation, suggesting that they became more critical about the samples when they were aware of sodium reduction. Previous studies have pointed out that information about salt-reduction might create negative expectations about salt-reduced biscuits (Vázquez et al., 2009) and soups (Liem et al., 2012), affecting consumer sensory and hedonic perception. Similar results have been reported in studies involving other key nutrients. For instance, Norton, Fryer and Parkinson (2013) found that labelling chocolate as “reduced-fat” had a negative impact on consumer expectations about their liking of the product. While previous evidence suggests that information on sodium reduction would presumably lead to negative expectations on the product characteristics and negatively impact consumer perception, results from the present work pointed out that the inclusion of sodium warnings indicating “excess of sodium” can encourage consumers to select the healthier options even to the point of increasing their liking. Therefore, informing excess of sodium may have a larger and more sustained effect on consumer choice than highlighting sodium reduction. In this sense, Lima et al. (2019) had previously suggested that consumers may react differently to positive health-related information (e.g. nutrient reduction) compared to nutritional warnings which highlight products with excessive content of critical nutrients associated with NCDs. Further research in this respect is warranted to inform the implementation of FOP nutrition labelling schemes and the development of communication campaigns to encourage healthy eating.

CAN SODIUM WARNINGS MODIFY PREFERENCES?

7.5. Conclusions

Results from the present work provided additional evidence that salt content significantly affect consumer hedonic perception of food products. Two consumer segments with different pattern of preferences for salt in bread were identified, stressing the need to explore consumer segmentation when addressing consumer reaction towards salt reduction. The inclusion of sodium warnings encouraged consumers to select breads with lower salt content, regardless of their preference for salt in bread, which suggests that this FOP nutrition labelling scheme is a promising alternative to guide consumer towards healthier food choices. In addition, sodium warnings showed potential to shift consumer preferences to breads with 30% sodium reduction, even after trying the products. Further research to confirm and expand the current results is warranted. From a methodological perspective, the present work proposed a promising approach for evaluating the impact of information on consumer perception, which might better reflect consumer behaviour in a real-life setting.

CONCLUSIONS

CONCLUSIONS

CONCLUSIONS

The thesis explored different strategies for salt reduction in foods from a sensory perspective. Results provided additional evidence of the large influence of salt content on consumer sensory and hedonic perception of food products. This confirms that reducing salt while maintaining sensory and hedonic perception is one of the major challenges for the implementation of salt reduction programmes at the population level.

Abrupt salt reduction negatively affected consumer hedonic perception, suggesting that it may encounter limited success in achieving changes in salt intake. Results confirmed the potential of the gradual salt reduction strategy for reducing the sodium content in food while keeping consumers unaware of the change. Difference thresholds proved to be a valuable tool for the design and implementation of gradual salt reduction strategies in food, providing a guidance on how much salt can be removed from the product without causing consumer awareness of the change. Results from the thesis showed that salt reductions close to 11% in rice and with the bread would not be noticeable for consumers and could be regarded as a safe criterion for removing salt. However, larger salt reduction percentages can be made without modifying consumers' hedonic perception. In the case of bread, the first two salt reduction steps (19.5% salt reduction) did not cause significant changes in consumer hedonic perception, pointing out that a short time could be elapsed between them. Future research is needed to shed light on how long the adaptation period between two consecutive salt reduction steps should be in order to allow consumers to get accustomed to the new salt level.

Large heterogeneity in consumers' hedonic reaction towards salt reduction was found. Consumer segments with different preferences for the salt content in bread and rice were identified. In particular, individual differences were found in terms of hedonic sensitivity and tolerance to changes in the salt content of food. Results from this work reinforced the importance of considering consumer segmentation to gain a complete and more realistic picture on how salt reduction impacts on consumer perception. Further research is needed to investigate the factors underlying consumers' preferences for salt in food. Results revealed that preference patterns for salt in rice were related to the habit of adding salt to food, either before or after having tried it. This highlights the need to investigate the use of discretionary salt, an under-researched topic worldwide.

The partial replacement of NaCl with KCl was addressed as a complementary strategy to gradual reduction. The 70:30 NaCl:KCl blend showed the best compromise between sodium reduction and the onset of undesirable flavours and proved promising in contributing to the development of low-salt foods. However, replacements beyond 40% caused changes on the sensory dynamic profile of breads leading to an increase in bitterness, metallic and off-flavour. This limits the magnitude of sodium reduction to be

CONCLUSIONS

achieved through this strategy and reinforces the idea that partial rather than total substitutions should be used.

The non-homogeneous spatial distribution of salt in a solid food-matrix structure was explored as an alternative strategy for sodium reduction. Although exploratory in nature, results suggested this approach was not effective in rising saltiness perception. However, given the limitations encountered for the implementation of this strategy, further research is recommended.

While the main focus of the thesis was placed on reducing the salt content of processed products, the impact of communicating excessive sodium content on consumer perception was also addressed. Results showed that the inclusion of sodium warnings encouraged the majority of consumers to select breads that did not feature the warning, confirming their potential to discourage the selection of food products with excessive sodium content. Apart from nudging consumers towards lower salt options, sodium warnings contributed to shifting consumer hedonic perception towards lower salt contents.

Overall, the thesis provided valuable insights on the feasibility of different strategies to reduce salt intake. Results provide guidance for their implementation and are expected to contribute to their uptake, which may ultimately contribute to an improvement in public health.

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