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Impact on sensory preference and physicochemical parameters of salt reduction on bread

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Impacto na avaliação sensorial e nos parâmetros físico-químicos da redução de sal no pão

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Resumo

O pão é um alimento basilar na alimentação e, por isso, é também um grande contribuidor para a ingestão de sal. Vários países, nas suas iniciativas para a redução de sal, escolheram o pão como um dos alimentos prioritários a intervir, incluindo Portugal. O sal tem impacto nas propriedades sensoriais do pão, atuando como modificador do *flavor* e influenciando o desenvolvimento da crosta e a estrutura do miolo. Sendo a redução do teor de sal prioritária, essa redução pode comprometer a qualidade do pão e as suas caraterísticas organoléticas, que poderão afetar a aceitação por parte dos consumidores. Quatro tipos de formulações de pão foram testados: pão d'água, carcaça, pão de mistura e regueifa, que foram produzidos com diferentes concentrações de sal (0.0%, 0.8%, 1.0%, 1.1%, 1.3%, e 1.4% de sal por farinha). Foi avaliado o impacto da redução de sal nos parâmetros físico-químicos (peso, volume específico, humidade e teor de sódio), cor e estrutura do miolo, assim como nas características sensoriais, e foram selecionados os níveis de redução de sal com melhor aceitação pelos consumidores. Foram ainda avaliadas as associações entre os atributos sensoriais, os parâmetros físico-químicos, a cor e a estrutura do miolo.

Para o parâmetro peso do pão, não foram encontrados resultados significativos, exceto para a carcaça ($p = 0.002$), no entanto, sem relação linear ($R^2 = 0.492$, $p = 0.120$). O parâmetro volume específico pareceu aumentar com o nível de sal adicionado, embora sem apresentar uma relação linear para todos os tipos de pão, exceto para a carcaça ($R^2 = 0.953$, $p = 0.001$). Relativamente ao teor de humidade, este parece diminuir à medida que o nível de sal aumenta ($p < 0.050$), mas sem relação linear. No que diz respeito à morfologia e cor do miolo, não foi observada nenhuma relação linear, exceto para as *células de tamanho pequeno* (pão d'água R^2 = 0.277, p = 0.001), *células de tamanho grande* (pão d'água, R^2 = 0.240, p = 0.002), *a** (carcaça, R^2 = 0.686, p < 0.001), e *b** (carcaça, R^2 = 0.562, p < 001; mistura, R^2 = 0.166, p = 0.014) para os diferentes níveis de adição de sal.

A redução de sal teve um impacto limitado na avaliação sensorial. *A apreciação global* apresentou diferenças para todos os tipos de pão (pão d'água, p = 0.005; carcaça, pão de mistura, e regueifa, p < 0.001). Os resultados obtidos no teste de consumidor apenas demonstraram diferenças significativas para os atributos *preferência de sabor* (pão d'água, carcaça, pão de mistura, e regueifa, p < 0.001), *preferência de textura* (carcaça, p < 0.001) e *preferência global* (pão d'água, p = 0.002; carcaça, p = 0.001; pão de mistura, p < 0.010; e regueifa, p = 0.005), onde a redução de sal apresentou um efeito negativo.

O mapa de preferências externo demonstrou as preferências do consumidor e permitiu conhecer a menor concentração de sal com melhor aceitação, nomeadamente, 0.8% para o pão d'água; 0.8% para a carcaça; 1.0% para o pão de mistura; e 1.1% para a regueifa.

Os resultados sugerem que é possível reduzir, em certa medida, a concentração de sal em todos os tipos de pão sem grande impacto nas características do pão e sem comprometer a aceitação do consumidor.

Palavras-Chave: pão, sal, análise sensorial, análise físico-química, análise de imagem, mapa de preferências, consumidor.

Abstract

Bread is a staple food and one of the major contributors to dietary salt intake. Many countries have chosen bread as one of the priority foods for reducing salt content in their national salt reduction initiatives, including Portugal. Salt has impact on bread sensory properties as it acts as *flavor* modifier, and influences crust development and crumb structure. While reduction of salt content in bread is paramount, it may compromise bread quality and organoleptic characteristics that will affect sensory acceptance by consumers. Four types of bread formulations were tested: “D’água”, “Carcaça”, “Mistura”, and “Regueifa”, produced with different salt concentrations (0.0%, 0.8%, 1.0%, 1.1%, 1.3%, and 1.4% of salt per bread). The impact of salt reduction was evaluated on bread physicochemical parameters, crumb color and structure, as well as on sensory evaluation, and select the reduction levels with best consumers’ acceptance. Relationships between sensory attributes and physicochemical parameters, color and crumb structure were further evaluated.

For bread weight, no significant results were found, except for “Carcaça” bread (p = 0.002), but without a linear pattern (R^2 = 0.492, p = 0.120). Specific volume seemed to increase with increasing added salt, although no linear pattern (p > 0.050) was observed, except for “Carcaça” bread (R^2 = 0.953, p = 0.001). Moisture content seemed to decrease as the level of added salt increased (p < 0.050). Furthermore, no linear relationship with salt addition levels was observed for any of the crumb morphology or colour parameters studied except for *small size cells* (“D’água” bread, R^2 = 0.277, p = 0.001), *large size cells* (“D’água” bread, R^2 = 0.240, p = 0.002), a^* (“Carcaça” bread, R^2 = 0.686, p < 0.001), and b^* (“Carcaça” bread, R^2 = 0.562, p < 0.001; “Mistura” bread, R^2 = 0.166, p = 0.014).

Overall, salt reduction had limited impact on sensory evaluation. *Overall assessment* presented significant differences for all bread types (“D’água”, *p* = 0.005, “Carcaça”, “Mistura”, and “Regueifa”, *p* < 0.001). Results obtained from the consumer test only showed significant differences for salt reduction with *taste liking* (“D’água”, “Carcaça”, “Mistura”, and “Regueifa”, *p* < 0.001), *texture liking* (“Carcaça”, *p* < 0.001) and *overall linking* (“D’água”, *p* = 0.002; “Carcaça”, *p* = 0.001; “Mistura”, *p* < 0.010; and “Regueifa”, *p* = 0.005) attributes, where salt reduction had a negative effect.

External preference mapping indicated consumer preferences and enabled selection of the lowest salt concentration with best acceptance, namely 0.8% for “D’Água” bread; 0.8% for “Carcaça” bread; 1.0% for “Mistura” bread; and 1.1% for “Regueifa” bread.

The results suggest that it is possible to reduce, to some extent, the salt concentration in all bread types without major impact on bread characteristics and without compromising consumers’ acceptance.

Keywords: Bread; salt reduction; sensory analysis; physicochemical analysis, image analysis; external preference mapping; consumer.

List of abbreviations

BP - Blood Pressure

CVD - Cardiovascular disease

NCD - Noncommunicable diseases

WHO - World Health Organization

DGS - Direção Geral da Saúde (Directorate-General of Health)

SBP - Systolic blood pressure

DBP - Diastolic blood pressure

NaCl - Sodium Chloride

IOM - Institute of Medicine

DRI - Dietary Reference Intake

AI - Adequate Intake

UL - Tolerable Upper Intake

DGA - Dietary guidelines for Americans

UK - United Kingdom

EU - European Union

FOV - Field of view

BI - Browning index

HNO_3 - Nitric acid

QDA - Quantitative descriptive analysis

PCA - Principal component analysis

ANOVA - One-way analysis of variance

PLS - Partial least square

Chapter I

Introduction and Aims

1. Salt, the “white gold"

For millions of years, humans as well as other mammals lived with reduced amounts of salt, which were naturally present in food (1). The use of salt by man had an important role in the evolution of culture and history of human civilization, and this link can be found in documents since 2700 BC in China (2). Some historians defend the hypothesis that the discovery of salt was the necessary impulse for man to evolve from simple hunter to the era of agriculture (3). The need to keep food in safety conditions showed that salt could be used as a method for food conservation, thus allowing the establishment of communities (4).

There are records of salt use by ancestral Egyptian who used it for mummification, drug production, and food preservation, including fish. The social impact of salt was also referenced in other civilizations history and has been the cause of wars and achievements. Expressions such as "*salarium argentum*", originally from the Roman empire (2), and "Salt March" from colonial India (5), mark the importance of salt in world history.

Portugal, as a maritime country, presents a set of geographic, morphological/ physical, climatic, and technical factors that allowed, since medieval times, the cultivation of marine saliculture as an economic activity. Consequently, salt production in quantity and quality is fundamental for the history of Portuguese commerce (6).

The monopolies and the economic dynamics created around salt were important factors for markets’ structuring that surpassed population mere food needs. Besides using it mainly as a substitute for spices, bread production, meat and fish preservation, population also used it for other purposes, such as in tanneries (7).

Salt also left its mark on maritime commerce, being a currency trading within the alliance of the British Empire (8).

In 2015, 117 thousand tons of salt were produced in the Portuguese saltworks (9). Over time, salt has become a mineral intrinsic to Portuguese culture, and therefore also to national gastronomy. Salted cod, for example, has become an important landmark in traditional Portuguese cuisine. The hedonic preference for the salty taste developed by the population would have been acquired by the long exposure to salt, resulting in resistance to sensitization to reduce salt intake (10).

Worldwide, the economic valorization of salt and its respective consumption suffered a decline with technological development. The emergence of cold conservation took salt down from being widely used for food preservation (4).

Lifestyles changed with industrialization and, to a certain extent, eating habits became similar among populations. Consequently, there was an exponential growth in the consumption of processed and ultra-processed foodstuffs, generally characterized by a high energy density and low nutritional density (11). The increase in the consumption of these convenience products, which also combine economic accessibility with sensorial pleasant characteristics, have been promoting a rise in salt intake by the population that cannot be dissociated from the simultaneous increase in fat and sugar intake.

There are several physiological changes demonstrated as a consequence of changes in salt intake and its excessive consumption is currently recognized as a serious public health problem (12).

1.1. Evidence of the effects of salt on health

Throughout the history of human evolution, the scarce amount of salt present in food has led to the development of physiological mechanisms for sodium preservation, preparing the organism to survive periods of severe deficiencies of this mineral. However, this genotype presents a risk factor for human life when conditions change (13).

Evidence clearly indicates that high salt intake is the most important factor in the raise of blood pressure (BP) and consequently increasing the prevalence of cardiovascular diseases (CVD) (4, 14, 15). In addition, high salt intake can directly promote, altogether with increased BP, the occurrence of stroke (16), left ventricular hypertrophy (17), progression of chronic kidney disease and proteinuria (18). Excessive salt intake has also been associated with other diseases, such as osteoporosis (19), increased risk of neoplasia (20), and obesity (21).

1.1.1. Hypertension and Cardiovascular Diseases

Noncommunicable diseases (NCDs) are the leading cause of death worldwide. In 2015, NCDs were responsible for 40 million deaths, accounting for 70% of the overall total of 56 million deaths. From which, 17.7 million (accounting for 45% of all NCDs deaths and 31% of all global deaths) were attributed to CVDs (22). Additionally, 37% of the premature deaths, under the age of 70, due to NCDs, are caused by CVDs (23), being still referred to as the main cause of disability in the world (24). According a report by World Health Organization (WHO), Ischemic heart disease and stroke remained the biggest killers globally in the last 15 years (25).

In Portugal, in 2015, CVDs also lead the causes of mortality, representing 29.8% of the overall deaths in the country. Cerebrovascular disease, namely stroke, was responsible for 10.8% of these deaths (26). Directorate-General of Health (DGS) reported that the CVDs mortality rate in Portugal has a tendency to decrease, being the present value was the first below 30% (27). However, due to unknown reasons, the ratio of cerebrovascular diseases to ischemic heart disease is inverse to most countries in Europe (27).

Hypertension is the most prevalent and important risk factor for cerebrovascular and cardiovascular diseases worldwide (16, 27). Hypertension is defined as a clinical condition of persistent elevation of systolic blood pressure (SBP) equal to or greater than 140 mmHg and/or diastolic blood pressure (DBP) equal to or greater than 90 mmHg (28).

Different types of studies support the theory that salt intake is primarily responsible for the increase in BP in the human subjects, as well as its role in regulating it (29-32). In 1904, Ambard and Beaujard (33) were the first to show experimentally that noticeable salt reductions in diet decreased BP. However, in 1960, the classic works of Dahl (34) demonstrated the linear correlation between salt intake from various populations and the development of essential hypertension. Designed to answer the question posed by Dahl et al. in 1984, the INTERSALT international study of the National Heart, Lung and Blood Institute, demonstrated a positive relationship between 24 h urinary sodium excretion and systolic blood pressure in a sample of 10,079 people recruited from 52 communities according to a standardized method (29, 35). Since then, other studies have been developed to study the effects of salt on blood pressure (Table 1) (30, 32, 36-40).

Table 1. “Classic” studies on the effect of salt in blood pressure.

Study/ Author (Year)	Aim	Sampling	Sample Size	Age range (Years)	Methodology	Salt intake	Principal results
Ambard and Beaujard[33] (1904)	Study the effect of salt intake on BP	Hypertensives pacients	6	--	Experimental	3 diets type: very little salt; little salt; very salt (10.5 g)	Positive relationship between salt balance and BP
Dahl[34] (1960)	Review the sum total of the evidence, which we have been gathering since 1954	Eskimos (Alaska) Marshall Islanders Americans (Northern U.S.) Japanese (Southern Japan) Japanese (Northern Japan)	20 231 1124 452 5301	38 41 36 43 45		4 (1 – 10) 7 (1.5 – 13) 10 (4 – 24) 14 (4 – 29) 26 (5 – 55) salt (g)	Linear relation between daily salt intakes with prevalence of hypertension in different geographic areas and among different races. Prevalence of hypertensives (%) Eskimos: 0; Marshall Islanders: 6.9; Americans: 8.6; Southern Japanese: 21; Northern Japanese:39.
INTERSALT study[29, 35] (1988)	Test in a large standardized high quality international epidemiologic study prior hypotheses on the relation of dietary sodium (also potassium), to SBP and DBP	52 communities from 32 countries worldwide	10074	20-59	Cooperative cross-sectional epidemiologic study 24 h urinary Na excretion	Salt intake (estimated by 24 h urinary Na excretion) from 0.05g per day in Yanomamo Indians in Brazil to 16g per day in Tianjin in China	Significant independent relation between 24 h urinary Na excretion. Median systolic BP: 95.4 – 132.4 mmHg Median diastolic BP: 61.4 - 82.1 mmHg
INTERMAP study[36] (1996 – 1999)	Clarify unanswered questions on the role of multiple dietary factors in the aetiology of unfavourable BP patterns prevailing for most middle-aged and older individuals.	Age-sex stratified random samples from 17 populations from Japan (four samples north to south), Republic of China (three sample north to south), UK (two samples) and United States of America (eight samples)	4680 men and women	40 - 59	Epidemiological study -four 24 h dietary recalls -two 24 h urinary Na collections -eight measured SBP/DBP	Japan: Men: 210.5±56.6 Women: 186.0±53.1 China: Northern men: 293.2±91.8 Northern women: 250.2±77.3 Southern man: 149.7±58.9 Southern women:128.1±52.9 UK Men: 160.9±51.3 Women: 125.0±42.0 USA Men: 182.7±62.4 Women: 142.3±48.3	Consistent results with the role of salt in determining blood pressure levels in the population. SBP/ DBP (mmHg): mean±SD Japan Men: 120.4±12.9/ 76.8±10.0 Women: 114.1±13.9/ 70.5±9.6 China Men: 120.9±17.2/ 73.7±10.3 Women: 121.6±17.7/ 72.7±10.1 UK Men: 123.7±14.4/ 80.7±9.2 Women: 116.6±13.8/ 73.4±9.3 USA Men: 120.4±12.7/ 75.7±9.6 Women:116.8±14.8/ 71.1±9.2

Table 1. (continued) “Classic” studies on the effect of salt in blood pressure

Study/ Author (Year)	Aim	Sampling	Sample Size	Age range (Years)	Methodology	Salt intake	Principal results
TOHP I study(38) (1992)	Test the short-term feasibility and efficacy of seven nonpharmacologic interventions in persons with normal BP	men and women’s, with DBP from 80 through 89 mm Hg	2182	30 - 54	Randomized control multicenter trials.	Intervention group: salt reduction of 25 to 30%	Reduction urinary sodium excretion by 44 mmol/24, DBP by 0.9 mm Hg, and SBP by 1.7 mm Hg (P less than .01).
TONE study(37) (1998)	Determine whether weight loss or reduced sodium intake is effective in the treatment of older persons with hypertension	Men and women with SBP lower than 145 mmHg and DBP lower than 85mmHg while receiving treatment with a single antihypertensive medication.	975	60 - 80	Randomized controlled trial Two groups: reduced sodium intake and usual care 24 h24 h urine collection	Group of reduced sodium intake: 24 h24 h dietary sodium intake of 80 mmol (measured by 24 h24 h urine collection)	Importance of salt intake role in BP levels and that salt intake reduction is an additive point to antihypertensive drugs and to other non-pharmacological approaches used in the treatment of hypertension
DASH study(30, 39) (2001)	Compare the feeding study impact of 3 dietary patterns on blood pressure		412		multicenter, randomized feeding study. Control group: Occidental diet; Intervention: DASH diet with high, intermediate, and low levels of sodium in a random order		Reducing the sodium intake from the high to the intermediate level reduced the systolic blood pressure by 2.1 mm Hg during the control diet and by 1.3 mm Hg during the DASH diet. Reducing the sodium intake from the intermediate to the low level caused additional reductions of 4.6 mm Hg during the control diet and 1.7 mm Hg during the DASH diet.
Cook et al.(32, 40) (TOHP I and TOHP II studies) 2007	Examine effects of reduction in dietary sodium intake on cardiovascular events using data from two completed randomized trials, TOHP I and TOHP II	Individuals with prehypertension	744 TOHP I and 2382 TOHP II	30-54	Long term follow-up assessed 10-15 years after the original trial: randomized controlled trial salt-reduced intervention groups (for 18 months in TOHP I and 36 to 48 months in TOHP II) or in the control group.	Dietary sodium reduction, including comprehensive education and counselling on reducing intake, for 18 months (TOHP I) or 36-48 months (TOHP II).	A salt reduction of 25 to 30% resulted in a decrease in BP by 1.7/0.9 mmHg in TOHP I and 1.2/0.7 mmHg in TOHP II. Additionally, 10 to 15 years after the tests, the study subjects had a 25% lower incidence of CVD, after adjusting for confounders

A study carried out in the Finnish population showed that the decrease in salt intake over several years led to a decrease in diastolic blood pressure and lower cardiovascular mortality (41).

He et al. (42) developed a controlled trial with 1906 subjects, to study the role of salt and potassium intake in BP. The results showed that salt reduction is one of the most effective methods in the treatment of hypertension.

In a more recent meta-analysis, He et al. (43), described that a reduction of 100 mmol sodium in a 24 h urinary excretion analysis promoted a decrease in systolic and diastolic BP of 5.4/2.8 mmHg between hypertensive individuals and 2.4/1.0 mmHg in normotensive individuals. Thus, it was concluded that a modest reduction in the ingested salt content causes important effects in the decrease of the blood pressure levels not only in hypertensive individuals, but also in normotensive individuals (43).

The evidence regarding the role of blood pressure in the occurrence of stroke is clear, and it is known that there is a direct and progressive relationship, and it is estimated that hypertension contributes in approximately 54% of strokes and 47% of events by coronary disease (44). However, epidemiological studies have shown that a diet high in salt may have a direct effect on the risk of stroke, regardless of BP (45, 46).

Cross-sectional studies have also shown that there is a positive correlation between sodium intake and left ventricular hypertrophy, an important predictor of cardiovascular mortality and morbidity in both hypertensive and normotensive individuals (47).

However, the relationship between salt consumption and the cardiovascular systems seems not be unclear as thought. Mente et al. (48), in a recent pooled analysis of data coming from four observational studies with 133,118 individuals hypertensives and normotensives, find that high salt consumption (≥ 7 g/day) was associated with increased cardiovascular events and mortality in hypertensive persons (without association in normotensive), while low salt consumption (≤ 3 g/day) was associated with increased cardiovascular events and mortality in both hypertensive and normotensive individuals. These results suggest that, a too low salt intake may be prejudicial in hypertensive patients and general population (49).

Several studies have supported the theory that potassium is an ion with a crucial role in preventing the development of hypertensive pathology (29, 50-58). Thus, potassium can attenuate the prejudicial effects of excessive sodium intake (29, 50, 56). In addition, it has been demonstrated that the sodium-to-potassium ratio have a superior determinant role in determining the relation to blood pressure and cardiovascular disease risk, as compared to separate sodium and potassium values (29, 53, 55, 56). The Dash Diet (30, 39) and the Mediterranean Diet (59) are good examples scientifically recognized as promoters of cardiovascular health, and characterized by the abundance of foods rich in potassium such as fruits and vegetables.

1.1.2. Other adverse effects of salt

There is increasing evidence that salt promotes other harmful effects that are independent or related to BP (4).

Several studies have shown that salt intake significantly influences the development of stomach cancer (20, 60, 61). A plausible explanation for this fact may be the increase in the number and severity of *Helicobacter Pylori* bacterial infections, promoted by the greater fragility of gastric tissue caused by the ingestion of salt and potentially salt-bearing foods.

Another effect of excessive salt intake is the worsening of proteinuria leading to a progressive deterioration of renal function and the consequent development of chronic kidney disease (62). Epidemiological studies have demonstrated a direct association between salt intake and urinary albumin excretion, independent of BP (18, 63). A randomized double-blind study involving individuals of various ethnicities, with an average BP of 147 ± 13/ 91 ± 8 mmHg, showed that even a small reduction in salt intake significantly reduced urinary excretion at 24 h of albumin (31).

Salt intake is the main determinant of urinary calcium excretion (19). Calcium is the major component in the formation of kidney stones. Therefore, salt intake can be an important cause of renal lithiasis. Randomized studies have shown that reducing salt intake decrease calcium excretion (64, 65) and consequently the occurrence of renal lithiasis (65). There is also evidence that with increased salt intake, a negative balance of calcium metabolism occurs, stimulating mechanisms of compensation involving the absorption of calcium at the intestinal level, but also a calcium mobilization of the bone tissue, which promotes a greater unbalance of bone metabolism, and as a consequence the development of osteoporosis (19, 66).

High salt intake has also been associated as an indirect factor in increasing the risk of obesity (67). The reason for this association is the stimulation of thirst sensation and increased fluid intake, thus increasing the intake of sugary drinks (21, 68). The association between salt and obesity can also be partly caused by the ingestion of processed foods, usually rich in salt and energy. However, a recent English study with a representative sample that included adults and children showed a significant association between salt intake and various degrees of adiposity after adjustment for total caloric intake and intake of sugary drinks. Although the mechanisms remain unclear, data suggest that salt intake appears to change body fat metabolism by increasing subcutaneous fat and plasma leptin (69).

Epidemiological and clinical studies suggest that salt may still be responsible for the exacerbation of asthma symptoms, and a poor salt diet may improve respiratory function and decrease bronchial reactivity (70-72). However, a systematic review in adults, found no evidence that dietary salt restriction had significant effects on asthma control (73).

1.2. Recommendations *versus* salt consumption

Chemically, sodium chloride (NaCl) is the result of conjugation of the sodium cation with the chloride anion, in the proportion of 40 and 60%, respectively. The word “salt” is commonly associated a NaCl, used in cooking.

Sodium is the main extracellular fluid cation, critical for fluid balance and cellular homeostasis. It is still involved in other physiological functions such as muscle contraction and conduction of nerve impulses (74). In a healthy individual, maintenance of sodium balance is achieved through urinary excretion, where about 98% of the ingested salt is absorbed into the small intestine, remaining in extracellular compartments. When sweat losses are minimal, the amount of sodium ingested is approximately equal to the amount excreted in urine (75).

Minimal physiological sodium requirements are not yet well established but are estimated to range from 200-500 mg/day. Thus, the nutritional deficiency in sodium is not usual, since the minimum needs of this mineral are found naturally in several foods (76).

In 2005, the Institute of Medicine (IOM) launched the Dietary Reference Intakes (DRI’s) for sodium, establishing the needs based on Adequate Intake (AI’s). These values were established for the majority of the individuals according to the age. It was also determinate the Tolerable Upper Intake Levels (UL), based on the impact of sodium on BP. For the adult and adolescent population, IOM recommendations for sodium ingestion should not exceed 2300 mg/day (75) (Table 1), which corresponds to 6 g/day of salt.

In 2012, the WHO, based on worldwide scientific evidence on sodium intake and its effects, developed a guideline to reduce the number of NCDs. The recommendations refer to a reduction of sodium intake to <2000 mg/day, approximately 5 g/day of salt, with adjusted values for energy needs in children (76) (Table 2).

Recently, US Department of Agriculture and US Department of Health and Human Services published the Dietary Guidelines for Americans (DGA), where the daily nutritional goal for sodium intake is less than 2300 mg for adolescents and adults, corresponding approximately to 6 g of salt (77).

Table 2. Dietary recommendations for sodium (mg per day) (Adapted from Gonçalves C. (10))

Life Stage Group	IOM[1]		OMS[5]	DGA[6]	DGS[7]
	AI[2]	UL[3]			
0-6 months	120	ND[4]			
6-12 months	370	ND[4]			
1-3 years	1000	1500	<2000 adjusted downward based on energy requirments	<1500	<2000 adjusted downward based on energy requirments
4-8 years	1200	1900		<1900	
9-13 years	1500	2200		<2200	
14-18 years	1500	2300		<2300	
19-50 years	1500	2300	<2000	<2300	<2000
51-70 years	1300	2300		<2300	
>70 years	1200	2300		<2300	
Pregnancy					
≤18 years	1500	2300	<2000	--	<2000
19-50 years	1500	2300		--	
Lactating					
≤18 years	1500	2300	<2000	--	<2000
19-50 years	1500	2300		--	
Persons with diabetes, hypertension or chronic kidney disease	--	--	--	<1500	--

[1]IOM – Institute of Medicine; [2]AI – Adequate Intake: [3]UL – Tolerable Upper Intake Level; [4]ND – not determinable; [5]OMS – Organização Mundial de Saúde; DGA2010-Dietary Guidelines for Americans 2010 [7]DGS – Direção-Geral da Saúde.

In Portugal, as in other Member States of the European Union, the Directorate-General of Health (DGS) has adopted the recommendations endorsed by WHO (78).

Global estimates of salt intake point to values that far exceed the recommendations. According to the results of the INTERSALT study, published in 1989, salt intake, evaluated in 52 different populations by 24 h urinary excretion ranged from 0.05 g/day in an indigenous population of Brazil to 16 g/day in Tianjin, China (35). More recent studies have shown that salt intake in general population, remains too excessive (79, 80).

An analysis conducted worldwide in 2010 showed that the average adult sodium intake was 3950 mg, equivalent to a salt intake of 10.06 g/day. In this study, the countries of Central Asia present the highest values of sodium intake, while sub-Saharan Africa has the lowest values, being 5510 mg (14.02 g of salt) and 2180 mg of sodium/day (5.47 g of salt) respectively. For Western Europe, the estimated sodium intake values ranging from 3.28 g/day (Denmark) to 4.43 g/day Italy (79).

In Portugal, a 2006 study carried out by Polónia et al. (81) evaluated sodium intake through 24 h urinary excretion and concluded that there was a general pattern of high salt intake in the Portuguese population, with an average intake of 11.9 g/day. In the same study, a positive correlation was found between salt intake and BP, suggesting that the high salt intake is related to the high prevalence of hypertension and mortality due to stroke in our country. More recently, data from the PHYSA study showed an average intake of 10.7 g/day of salt in the Portuguese population (82).

According to the Portuguese Food Balance Sheet (83), in 2016, the available quantities of sodium were 1194.5 mg per person per day, considering only the estimated mineral intrinsic to foods (naturally present). This value represents more than half of the 2000 mg per day, the recommended daily limit.

The results from the National Food, Nutrition and Physical Activity Survey published in 2017, showed that on average, sodium intake is 2848 mg/ day (equivalent to 7.3 g of salt), higher in males (3221 mg/ day) than females (2510 mg/ day). It was also reported that on average, 75.9% of the Portuguese population exceeded daily reference values for sodium intake, with an incidence of ingestion of 85.9% and 65.5% in males and females, respectively (84).

1.3. Salt reduction initiatives

Considering worldwide epidemiological evidence on the association between high salt intake and population mortality and morbidity, in 2004 the World Health Assembly, endorsed WHO Global Strategy on Diet, Physical Activity and Health in view to implement measures for salt reduction (85). Table 3, shows a timeline of some important reduction salt initiatives in the Europe and Portugal.

Table 3. Timeline of some important dates of reduction salt in Europe and Portugal (adapted from European Commission (86))

Year	Important events
2004	Adoption of the WHO Global Strategy on Diet, Physical Activity and Health
2006	Adoption by WHO European Member States of the European Charter on Counteracting Obesity
2006	Paris Technical Meeting to develop recommendations for WHO member states on interventions to reduce salt intake
2006	Portuguese Society of Hypertension set up Portuguese Action against Salt and Hypertension
2007	Adoption of the EU White Paper on a strategy for Europe on nutrition, overweight and obesity related health issues and of the WHO European Action Plan for Food and Nutrition Policy
2007	Creation of the National Platform against Obesity by Portuguese Ministries of Health, Education, the Economy and Agriculture
2007-2012	Implementation of the WHO European Action Plan for Food and Nutrition Policy
2008-2013	Adoption and implementation of the WHO 2008–2013 Action Plan for the Global Strategy for the Prevention and Control of Noncommunicable Diseases
2008	Creation of the EU framework for national salt initiatives to describe a common vision for a general European approach towards salt reduction
2009	Seminar on salt reduction in bread inviting national and EU level bakery associations
2009	Portuguese legislation fixed an upper limit 550mg/100g bread
2010	Discussion of the United States Institute of Medicine's draft standards for salt content of food by the United Kingdom Food Standards Agency and WHO in London
2011	Holding of the First Global Ministerial Conference on Healthy Lifestyles and Noncommunicable Diseases Control, Moscow
2011	Adoption of the Political Declaration of the High-level Meeting of the United Nations General Assembly on the Prevention and Control of Non-communicable Diseases
2012-2016	Endorsement of the Action Plan for Implementation of the European Strategy to Prevent and Control Noncommunicable Diseases
2012	Discussion of the global monitoring framework for noncommunicable diseases
2013	All WHO member states signed up to the target to reduce salt intake in population by 30% by 2015 with a target 5g per day
2013	Report the situation and actions taken in EU and its Member States since 2008
2013	Portuguese Ministry of Health reports the strategy to reduce salt consumption in Portugal
2015	Creation of the interministeral Portuguese Government Working Group. Working Group presented 14 proposals that summarize the priorities and strategies adopted by consensus among food industry, government and researches
2017	Portuguese Government signed a protocol with Bakery and Pastry Portuguese Industry, which determines a gradual reduction of the salt content in bread between 2018 and 2019 (0.1 g per year)
2017	Order no. 11418/ 2017 by Portuguese Government approves the Integrated Strategy for the Promotion of Healthy Food, which defines monitor the salt content in larger food categories

In 2006, WHO organized a technical meeting entitled “Reducing salt intake in populations”, with an overall objective to develop a recommendation to reduce population-wide salt intake. These recommendations focused essentially on three points: i) product reformulation, including industry engagement to reduce the salt content of products; ii) consumer awareness and education; and iii) environmental changes, with regard to healthy food choices access, easy and affordable for everyone (87).

In response, in 2008, European Union (EU) developed a Framework for National Salt Initiatives (88) describing a common vision for a general European approach towards salt reduction. The Framework set a benchmark of a 16% salt reduction over 4 years for all food products, also encompassing salt consumed in restaurants and catering.

However, in order to effectively reduce salt intake in general population, EU salt reduction activities have concentrated on 12 specific food categories: bread, meat products, cheese, ready meals, soup, breakfast cereals, fish products, crisps, savory snacks, restaurant meals, sauces, condiments and spices, and potato products (88).

Several countries have developed operational salt reduction programs. Finland and the United Kingdom (UK) have been identified as good examples of successful salt reduction strategies. Finland started, in 1970, a set of initiatives for the reduction of salt intake in the population, including media campaigns, cooperation with the food industry and mass catering, public and health care sector education campaigns, and implementation of salt labeling legislation (89). According to surveys on representative samples of adults, this program resulted in a reduction of salt intake by 12 g/day to less than 9 g/day between 1979 and 2002, measured by 24 h urinary sodium excretion (90).

In UK, salt reduction program lasted 7 years and since it started, significant progress has been made, as demonstrated by the reduction in salt content of processed food and a 15% reduction in 24 h urinary sodium from 9.5 to 8.1g/day. This was possible using a strategy that was based on a voluntary inclusion of the industry and also a greater public health campaign effort that gave the consumer awareness to the reduction of salt (91, 92).

In Portugal, a national legislation concerning salt content in bread was the most popular national salt reduction initiative. In 2009, Law no. 75/2009, August 12, set a maximum of 550 mg sodium/100 g bread, corresponding at 1.4 g salt/100 g serving of bread (93). It should be noted that although Portugal was a pioneer in this area, most of the EU member states participating in Framework for National Salt Initiatives chose bread as one of four priority intervention products (86). In 2009, EU organized a seminar on salt reduction in bread, including technical, taste, and other parameters for healthy eating, inviting national and EU level bakery associations (94).

In 2013, a strategy to reduce salt consumption in Portugal was reported by the DGS, which defined four strategic objectives: i) provide labeling capable of assisting consumer decision-making; ii) modify availability, creating conditions for foods with a higher salt content to be more inaccessible; iii) raise awareness and empower citizens to a reduced salt intake; and iv) implement evaluation systems of intake and monitor the supply of salt in food (78).

In 2015, the Portuguese Government through Order no. 8272/2015 of July 29, promoted the creation of the interministerial Working Group with the aim to set measures quantifiable and monitored, for the reduction of population salt consumption, especially by reducing the supply in food products with high salt content, namely in catering (95).

Working Group presented 14 proposals summarizing the priorities and strategies approved by the majority, consensus in the strategies of consumer education and, mainly, the availability of food with a focus on its nutritional reformulation (12).

More recently, the Portuguese Government approved the Integrated Strategy for the Promotion of Healthy Food proposed by the interministerial Working Group, through Order no. 11418/2017 of December 29. This strategy aims to encourage adequate food consumption and consequently, improving the nutritional status of the population, with a direct impact on the prevention and control of chronic diseases (96).

One of the measures of this strategy is to monitor the salt content in the following categories foods: bread and breakfast cereals, meat and meat products, ready meals, potato products and others snacks, sauces, ready soups, cheese and fish products, and restaurant meals. The selection of this food categories is based on the recommendations provided by High Level Group on Nutrition and Physical Activity of European Commission and WHO guidelines (96).

2. Bread: history and cultural importance

"Give us this day our daily bread."

Mateus 6:9-13

Bread is one of the oldest foods in the world. Being a millenarian food, bread has a significant historical burden associated with its cultural and religious symbolism (97). Its discovery goes back to Ancient Egypt, where the first food similar to bread appeared, most likely randomly (98). In Europe, bread arrived through the Greeks that have been responsible for developing fermentation techniques through hops and fresh must. However, it was in Ancient Rome that the bakery was established as a commercial commodity, contributing to the technological development of agriculture fields, grinding and manufacturing techniques of the Roman legions (99). Bread was the main food available for the Roman army during the battles. Therefore, the scarcity of wheat and consequently of bread was considered one of the factors responsible for the fall of the Roman Empire. With this, bread was restricted to the elites and the Clergy, only returning to the table of people many years later with the French Revolution. In the XIX century, Pasteur uncovered the fermentation phenomena and today bread is a fundamental food in most cultures of the planet (98).

Bread permeates the whole history of Man, especially by his religious side. In the Christian Religion, bread is the symbol of life, food of body and soul, symbol of sharing. It was sublimated in the multiplication of the loaves, in the Last Supper, and to this day it symbolizes faith in the Catholic events through the Host that representing the body of Christ (100).

In Portuguese gastronomy, the roots and histories left by the traditional Mediterranean Diet, make cereals and particularly bread, still primordial elements (99).

According to Portuguese Legislation, bread is defined, in Law no 425/98 of July 25, as “the product obtained from the mixing, fermentation and cooking of various grades of flour (wheat, rye, triticale or maize, on its own or in a mixture) under suitable conditions, the use of drinking water, yeast or yeast, as well as the possible use of salt and other ingredients, including additives, as well as technological auxiliaries, under legally established conditions” (101).

Depending on the raw materials used and the way in which the various stages of the manufacturing process are performed, the nutritional characteristics of the bread are variable, but it is undoubtedly a food of high nutritional value and a basic component in human food (97).

The nutritional value of bread is intrinsic to its richness in complex carbohydrates (in the form of starch), proteins, fibers (in the form of cellulose and lignin), vitamins (thiamine, niacin, E and B complex), and some minerals (calcium, potassium, phosphorus and magnesium) (97).

The wide variety of bread types, and their associated raw materials and manufacturing processes, also have broadly variable impacts on the glycemic index (102). The degree of flour extraction, which ranges from refined to whole-meal, is an important characteristic. In this field, the complex carbohydrates and short chain organic acids produced during fermentation, mostly present in bread types with whole flours, play an important role in reducing the glycemic response. Additionally, consumption of whole grain foods, including whole bread, has been linked to reduced risk of coronary disease, type II diabetes and certain cancers (103-105).

2.1. Bread and Salt

Bread is a staple component of the diet and is one the most widely consumed foods (97, 106). Recommendations of bread consumption, according to WHO dietary guidelines, in European countries, is around 250 g of bread per person per day (107). Processed foods have a significant level of sodium added during the manufacturing. Therefore, processed foods are the main source of salt in the diet, representing about 70-75% of the total intake (108, 109). Cereal and cereal products, in which bread is included, contributes to 30% of overall salt intake (109). Due to its high consumption, bread is one of the major contributors to dietary sodium intake.

According to results from the National Food, Nutrition and Physical Activity Survey, the mean consumption of the subgroup “bread and toasts” by Portuguese population is 102.71 g per person per day (84). Interestingly, it also showed that the elderly consume more bread with a daily average of 123.36 g. Moreover, this study also showed that the "bread and toasts" subgroup is the second largest provider to the total salt intake of the Portuguese, with a contribution of 18%, just from the addition salt (84).

In a recent study evaluating sodium intake in Portuguese adolescents, Gonçalves C et al. (110), showed that major food group of “cereals and derivates”, including bread, contribute on average 41% of the total sodium intake of this population.

Additionally, according Portuguese Food Balance Sheet (83), the variation in an average of food availability in “Cereals, roots and tubers” group between quinquennium 2008-2011 and 2012-2016 was -1.5%. In 2016, an average of 339.4 g per day of cereals were available to every inhabitant for consumption. This value was below that of 2012, 348.0 g/day, and also from the period 2008-2011, 344.1 g per inhabitant per day.

Wheat is the most available cereal, representing an average of 69.4% of total cereal production. However, the apparent wheat consumption in 2016 was below that recorded in 2012 with average values of 234.8 g and 246 g per inhabitant per day, respectively.

The trend of lower bread consumption can be justified by changes in dietary patterns and greater supply of alternatives such as breakfast cereals (111). Nevertheless, bread remains one of the most sought-after foods today. This fact leads to a concern about the reduction of salt levels in bread by authorities and health organizations (112).

Salt reduction in foods influences many quality characteristics that are important for consumer acceptance and industrial suitability (109). Salt has specific properties, essential for the physical processing and production of the final product. Additionally, it acts as flavor modifier, conferring a salty taste in food as well as the enhancing effect on other flavor constituents. Moreover, salt works as a preservative, directly by inhibiting microbial growth or indirectly by promoting other changes, namely reduction of water activity (113).

For bread production, salt has specific properties, essential for processing and exert a significant influence on rheological characteristics of the dough and quality of the final product (114). Therefore, salt reduction in bread is a concern for the bakery industry.

2.1.1. Technological impact of salt on bread production

The functions of salt in bread can be summarized as: control of yeast growth and fermentation rate; improvement of product texture; sensory effect when conferring flavor; and reduction of spoilage, particularity mould spoilage (115).

The technological process of every stages of bread production, namely mixing, fermentation and baking was positively influence by salt, as well as on final quality characteristics (109).

2.1.1. a) Dough characteristics and gluten network

During the mixing phase, ingredients are incorporated and dispersed to form dough with specific viscoelastic properties. The dough quality has a direct influence on the quality of the final product, which is due to the structural development and gas incorporation (109).

Salt promotes the development of wheat gluten-structures, making it more stable and less extensible. The overall result is a less sticky dough which leads to better processability (115). The ionic nature of NaCl (salt) seems to be responsible for these effects (113). When salt is absent, the positive charge at the protein surface in the dough makes protein molecules repel each other. This way, protein-protein interactions are reduced, resulting in a weaker dough network (116). In the presence of salt, the extensive interactions between NaCl and water or macromolecules occurs, promoting a reduced water availability in the dough. As consequence, changes in bread moisture content and water activity occur, modifying the development of the gluten network (117). Additionally, sodium ions present in salt compete for hydrogen bonding sites that in turn shields the charge on the gluten protein, reducing the electrostatic repulsion between protein molecules. Thus, stronger inter-protein hydrophobic interactions occur, promoting an increased aggregation and producing a stronger protein network. Consequently, longer time is required to reduce these aggregations in order to obtain an adequate dough development (109, 116).

Therefore, in the mixing phase, salt influences physical requirements for dough development. The ratio of salt and water to flour influences the conditions throughout this step that have to be adjusted, namely mixing time, mixing intensity and relaxion time. Additionally, in order to obtain a viscoelastic network, other processing conditions also have to be adjusted, such a temperature and amount of energy required for adequate mixing (113, 117).

In the cereal industry, fundamental and, specially, empirical rheology assume a great importance to evaluate bread making performance and quality control, and have been used to characterize the observed phenomena during low salt bread production (109).

Farinograph studies have demonstrated that salt decreases water absorption and lengthens the mixing time and dough development time. Addition of salt results in increased dough consistency and softer dough (118, 119). Lynch et al. (108), trough extensograph, found that an increase in dough resistance correlated with an increasing amount of salt.

2.1.1. *b) Yeast activity and fermentation rate*

During the fermentation process, dough development continues through stretching of the gas cell membranes and CO_2 production, and gluten network development is completed. In this stage, an increase the specific volume of the dough occurs and is required to obtain the optimal conditions for the baking process (120).

Salt influence in fermentation results from the increase in osmotic pressure, but mainly due to specific actions on the semipermeable membrane of yeast cells. The most important effect of salt in bread making is a reduced rate of gas production with increased levels of salt. Consequently, an extended proof time in necessary to maintain final loaf volume (113).

In laboratorial studies, rheofermentometer can be used to determine the retention coefficient, indicating the capability of dough to retain gas as a percentage of the total amount of gas produced. Lynch et al. (108), reported that when salt level decreased, significant increase in the maximum dough height and decrease in retention coefficient was observed. This suggests an impairment in the gluten network that cannot support all gas produced, with a higher amount of CO_2 escaping from the dough. Additionally, dough with an inadequate amount of salt promotes an excessive fermentation and consequently a final product with poor texture and open grain (121).

2.1.1. c) Crumb structure and crust color

During baking, the final stage of the bread making process, fermented dough is heated up at temperatures between 200°C and 250°C for 20 to 45 min, depending on the bread type (109). This exposure to high temperatures induces physical modifications, including expansion of CO_2 and water vapor, starch gelatinization and protein denaturation (120). These modifications result in the formation of two bread sections: crumb and crust. Crumb results from the change on gas dispersion into a sponge structure, where the gas cells are interconnected (109). Salt is an indirect determinant of crumb formation, once it influences gluten network quality which determine the limit of gas cells expansion. Therefore, salt is responsible for the formation of an uniform crumb (112).

Even during baking, reactions between proteins or amino acids and reducing sugars, the Maillard reactions, occur, where compound such as melanoidins are formed. These compounds have an important role in development of the crust and crust color once producing a darker colored crust. The presence of salt improves this process indirectly, as it reduces yeast activity and, therefore, more sugar are available for the formation of browning products (109, 122).

Crust color and crumb structure are important bread sensory attributes that influences in consumer preference (123).

2.1.2. Salt influence on bread quality

Bread quality is usually defined according to sensory parameters, volume, texture, color, and flavor (124, 125), where salt seems to have an influence. Additionally, salt is determinant in microbial shelf-life, which is important in bread storage (108, 120).

2.1.2. a) Specific bread volume

Bake-loss and bread volume are some of the factors that are commonly used to predict bread quality. Bread specific volume is obtained by the ratio of bread volume to bread weight, and depend on the amount of dough and weight loss during the baking process. Salt can influence bread specific volume, once it controls gas production and consequently, bread volume (108, 109).

2.1.2. b) Shelf-life and microbial safety

Relatively to shelf-life, it is known that bread is a high moisture product with water activity values between 0.96-0.98 (126). Therefore, salt can interfere with microbial shelf-life, reducing water activity through increasing osmotic pressure. Thus, salt control the growth of molds and bacteria, acting as a preservative agent and preventing staling and increasing bread shelf life (109, 112).

2.1.2. c) Sensory profile

As aforementioned, bread quality is defined by assessment of sensory parameters as a volume, texture, color and flavor (124, 125, 127). In bread, more than 300 volatile compounds have been described, that contribute to bread aroma, and assuming an important role in consumer acceptance (125, 127).

During mixing and fermentation process, texture is mostly determined by the development of the gluten network and by the crumb grain characteristics, where salt exerts an important influence (109, 112, 121), as previously mentioned.

During baking, the exposure to high temperatures results in changes on sensorial characteristics like taste, flavor, and texture (120). Flavor is a result of complex interactions between oral, nasal and texture sensations perceived while eating (128). With regard to flavor, several studies have demonstrated that salt has an important impact in bread (129-135). According Belz et al. (109), bread without salt was described as “insipid” taste. However, salt seems to also exert an effect on the flavor conferred by others ingredients. For example, salt enhances the sweetness of some amino acids and reduces the sensation of bitterness. In addition, during crust formation, melanoidins formed by Maillard reaction contribute to bread flavor. Therefore, with salt reduction, light crust is formed and flavor composition is significantly reduced.

During storage, sensory characteristics of bread are influenced by salt addiction. In this phase, physical and chemical modifications are described, namely reduction of freshness, crispy proprieties, and aroma, due to pectin and amylopectin retrogradation, associated with a change on moisture content. This process, known as staling, causes changes in some sensorial characteristics, especially in texture and flavor (122).

Sensory evaluation is defined as a “scientific method that evokes, measures, analyzes, and interprets responses to products, as perceived through the senses of sight, smell, touch, taste, and sound”. These senses interact with sensorial proprieties in foods, namely appearance, texture, flavor and aroma (136).

In bread technology, as well as in general food industry, sensory analysis is typically applied to test consumer acceptance. Descriptive analysis is a methodology that provides a complete description of all the sensorial properties of a product, allowing a sensorial profile characterization. It has several applications, such as development of new products, track a product’s sensory changes over time to understanding shelf-life and packaging, control of the quality of industrialized products, making product matching, product mapping and study the relationship between sensorial and instrumental tests (137).

In bread, a staple food for human consumption, the knowledge of its sensory profile assumes high significance, allowing product quality improvement and product reformulation, both in nutritional and technological terms.

2.2. Reduction of salt in bread – projects and studies

Since the WHO launched the Global Strategy on Diet, Physical Activity and Health to limit the levels of trans-fatty acids, saturated fatty acids, salt and sugar in foods, many companies in the food and beverage industry have reformulated their products (94).

With the deployment of European Union Framework for national salt initiatives, many countries have chosen bread as one of the priority foods for reducing salt content. At the policy level, goals and partnerships with industry, including bakery associations, have been established. In 2009, EU organized a seminar on salt in bread, technical, taste and other parameters for healthy eating that provided a forum to discuss issues of salt content of bread, looking on health and technological issues and inform about experiences from some countries that have actively reduced the salt content in bread (94). Table 4 shown examples of specific reduction benchmarks or benchmarks that are under discussion for bread from some countries in Europe, resulting of Seminar on Salt bread.

Table 4. Examples of reduction benchmarks for bread (adapted from European Commission (94))

Country	Benchmark
Austria	Reduction by 15% in 4 years
Denmark	Reduction to a maximum of 1.1 to 1.2 g salt in 200 g bread, depending on the kind of bread
Germany	working on a reduction to a maximum of 1.6 % salt in 100 g fresh bread, depending on the kind of bread (wheat, rye, etc.), timeline not yet defined
Ireland	reduction by 16 % starting from 2004
Italy	reduction by 15 % in 4 years
Lithuania	reduction by 5 % until 2013
Spain	18 % reduction starting in 2004; this was over-achieved
Slovenia	reduction to a maximum of 1.0 g salt in 100 g bread over 10 years, reduction by 4% per year
UK	2010 targets: reduction to a sales weighted average of 1.1 g salt in 100 g bread and rolls without additions reduction to a sales weighted average of 1.3 g salt in 100 g bread and rolls with additions 2012 targets: reduction to a sales weighted average of 1.0 g salt in 100 g bread and rolls without additions reduction to a sales weighted average of 1.2 g salt in 100 g bread and rolls with additions

On a voluntary basis and encouraged by UK Food Standards Agency, UK reduce 20% salt content in bread from 2001 to 2011, from 1.23 to 0.98 g/100 g. A strong point in this campaign was the education and awareness of the population (138).

In Finland, salt reduction in bread is part of the overall nutrition and reformulation strategy. The “Heart Symbol” is a campaign labelling created tell the consumer at a glance that the product marked is a better choice in its product group regarding fat and sodium. In bread, a sodium criteria for deserving this symbol is a maximum content of 280 mg/100g (139).

Portugal instituted the Law nº 75/2009, August 12 (93), that limits the maximum salt content to 1.4g per 100g of bread, but recent projects and studies shown that it is possible to more reduce salt content without affecting its quality. Considering that bread is an important food, any further reduction of salt content will have a significant impact on health.

In 2017, on World Food Day, the Portuguese Government signed a protocol with Bakery and Pastry Portuguese Industry, which determines a gradual reduction of the salt content in bread between 2018 and 2019. Thus, the salt content added to bread should suffer a reduction of 0.1 g per year, reaching a value of 1.0 g per 100 g of bread in 2021 (140).

Girgis et al. (133), showed that a gradual sodium reduction of 5% per week to the final goal of 25% reduction, was not perceived by consumers. However, Lynch et al. (108) showed that 50% of salt reduction modify the bread flavor.

Additionally, different approaches have been studied for the reduction of salt in bread. Partial replacement of the NaCl by others salts, namely potassium chloride, magnesium chloride (141) or calcium chloride (142) have been proposed. An inhomogeneous distribution of salt in bread (143), use encapsulated salt (144) and use of sourdough and organics acids such as acetic and lactic acid have also been studied as an alternative to conventional salt (145).

In the Central Region of Portugal, "pão.come" project was developed by Regional Health Administration (146). This community intervention that initially determined that the salt content used in bread production was 1.58 g/100 g. This value served as the basis for the implementation of a gradual methodology of salt reduction, with four goals, with the final target of 0.80 g of salt per 100 g of bread. In 2013, 1139 bakeries in 73 municipalities were involved in the project, covering 93% of the region's population (146).

Another Portuguese project in this area, developed a formulation called “Pão Vida" that can be purchased by Portuguese bakeries. This optimized product is a mixture of whole grain flour with the addition of salt to obtain a loaf bread with 1.2 g of salt per 100 g of bread, ensuring the sensory characteristics (147).

“Carcaça” bread, “Regueifa” bread, “D’água” bread and “Mistura” bread are traditional Portuguese breads, characterized by typical and unique recipes.

Therefore, the development of a guide for help bakery professionals in the reduction of added salt without changing original recipe and keeping the quality of the final product can be an important auxiliary for salt reduction in bread.

3. Aims

The main objective of this study was to assist the baking industry in the gradual reduction of the salt content added during bread production. Secondary objectives of this investigation were to:

- Evaluate the impact of 10%, 20%, 30% and 40% reductions in the bread salt content in the texture and organoleptic characteristics of the final product, based on 1.4 g of salt per 100 g of bread, according to current legislation;
- Evaluate the impact on sensory evaluation of bread with reductions of 10%, 20%, 30% and 40% of the salt content;
- Develop a guide for bakery professionals that facilitates the production of different types of bread with lower salt content.

It is important to emphasize that although several strategies could be used for salt reduction in bread, the gradual reduction of sodium chloride was the most suitable for an immediate application.

Chapter II

Material and Methods

4. Sampling

Bread samples for analysis were produced in an experimental laboratory of Moagem Ceres (Porto, Portugal). Samples of four types of bread were produced under controlled conditions (time/temperature/moisture), according to a standardized recipe: “Carcaça” bread, “Regueifa” bread, “Mistura” bread and “D’água” bread. In the production of bread samples, the ingredients used were flour, water, fresh yeast, commercial powder improver and salt, supplied by Ceres (Porto, Portugal). Depending on the type of bread, different types of flour were used (101). The flour type numbers indicate the ash content per 10g flour. Fresh yeast was obtained from a pure culture of yeast species *Saccharomyces Cerevisiae,* from FALA AZUL (Lasafree Ibérica S.A., Valladolid, Spain), with a fermentative power of the 135 cm^3 CO_2 /2h. The powder improver used was Cerpan (CERES, Portugal), contained acidity regulator (E170i, E341), emulsifier (E472), wheat flour, antioxidant (E300) and enzymes.

The original recipes are described in the Table 5 and the chemical and nutritional characterization of flours used for each type bread is described in the Table 6.

Table 5. Original recipes of the different bread types in study.

Type of bread	Flour type	Water (%)*	Yeast (%)*	Powder improver (%)*	Salt (%)*
“D’água” bread	Mixture 70:25:5 of type 65 wheat flour, type 80 wheat flour and 70 rye flour	80%	3%	1%	1.5%
“Carcaça” bread	Type 65 wheat flour	60%	5%	1%	1.5%
“Mistura” bread	Mixture 79:20:1 of type 65 wheat flour, type 70 rye flour and barley flour	75%	3%	1%	1.5%
“Regueifa” Bread	Mixture of wheat flour, vegetable oil palm powder and milk protein	50%	3%	1%	1.5%

* Percentages applied to the total flour used

Table 6. Chemical and nutritional characterization of flours per bread type.

Chemical/ Nutritional Characteristic	“D’água” bread flour	“Carcaça” bread flour	“Mistura” bread flour	“Regueifa” bread flour
Moisture	14.50 max	14.50 max	14.50 max	14.50 max
Protein content	--	9.00% min	10.00% min	--
Falling number	--	220s min	220s min	--
Gluten	--	8.00% min	--	--
Energy (100 g)	1576 KJ 370 Kcal	1435 KJ 343 Kcal	1577 KJ 370 Kcal	1491 KJ 352 Kcal
Lipids (g/ 100g)	1.5 g	1.6 g	1.3 g	2.4 g
Carbohydrates (g/ 100g)	75 g	73 g	73 g	75 g
Sugars (g/ 100g)	6.9 g	8.2 g	8.7 g	5.4 g
Fiber (g/ 100g)	5.1 g	3.5 g	3.0 g	1.3 g
Protein (g/ 100g)	12 g	11 g	13 g	9.5 g
Salt (g/ 100g)	<0.1 g	<0.1 g	<0.1 g	<0.01 g

4.1. Level of salt in original recipes – preliminary study

In a first phase, a preliminary study was carried out to determine the amount of salt that each type of bread has using in the original recipe of bakeries.

This preliminary study consisted in produce samples of the four types of bread, using the usual quantity of salt that was 1.5% (per flour). Then sodium content of these samples of bread were analyzed by flame photometry. These values (Table 7) were used to estimate the amount of salt to be added for each type of bread, to obtain a concentration of 1.4 g of salt per 100 g of bread.

In a second phase, samples with gradual reductions in salt content of 10%, 20%, 30%, 40% and 100% were produced for each type of bread.

Table 7. Results from preliminary study - Salt concentration values (g/100 g) for each bread type, with 1.5% of salt addition level (% wheat flour) according to original recipe.

Bread type	**Salt (g/100 g)***
“D’Água”	0.997 ± 0.047
“Carcaça”	1.225 ± 0.039
“Mistura”	0.896 ± 0.031
“Regueifa”	1.345 ± 0.013

*Data expressed as mean ± standard deviation, (n=10).

4.2. Bread making

All of the samples of each type of bread were produced in the same conditions of humidity and temperature, using industrial machines: mixer, fermentation chambers and ovens (Sopaco, Rio Tinto, Portugal). Before packaging or any evaluation the bread was left to cool dawn at room temperature.

4.2.1. “D’água” bread

The ingredients were mixed and kneaded in a spiral mixer for 25 min a “2” speed; dough was leaving to ferment at room temperature during 30 a 90 min. The dough was then mechanically shaped into portions of the approximate 65 g in the bun divider rounder, manually molded in balls and placed in a refrigeration chamber for 60 min. Finally, breads were baked for 30 min at 200 ºC.

4.2.2. “Carcaça” bread

Ingredients were mixed and kneaded for 20 min in a spiral mixer; dough was mechanically molded in portions of a 65 g in a bun divider rounder (Sopaco, Rio Tinto, Portugal) and posteriorly shaped manually into a ball and leaving to ferment at 30 ºC for 60 min; posteriorly, breads were baked for 10 min at 200 ºC.

4.2.3. “Mistura” bread

Dough was mixed for 25 min in a spiral mixer and leaving to ferment at room temperature for 90 min. After fermentation, the dough was mechanically molded in portions of approximately 70 g and then manually molded in balls. Before to baking, dough was leaving at stove at 30 ºC for 30 min. Posteriorly, the dough balls was baked in an oven at 220 ºC for 30 min.

4.2.4. “Regueifa” bread

Bread ingredients were mixed and kneading for 13 min in a spiral mixer, 3 min at slower speed and 10 min at faster speed; dough was pressed in a dough sheeter (Sopaco, Rio Tinto, Portugal) and molded manually in portions of 500 g. Posteriorly, dough was leaving to ferment 60 min at 30 ºC. Baking was performed for 10 min at 200 ºC.

5. Dough analysis

5.1. Rheofermentometer evaluation

The growth of the dough, CO_2 production and loss, was measured by the Rheofermentometer (Chopin, Villeneuve-La-Garenne, France), allowing the calculation of the retention capabilities. Dough of “Carcaça” bread was prepared with varying salt content, according to a protocol adapted and usually used by the bakery company: 250 g of wheat flour and 7 g of yeast; after kneading, 315 g of dough sample was placed in the fermentation basket and covered with the optical sensor. The proofing chamber was closed hermetically and gas production and dough height were measured at 33 ºC, over a 90 min period. From this analysis, the following values were obtained: H_m = maximum dough height; H = dough height at the end of measurement; H'_m = maximum height of gaseous release; T_1 = time to reach H_m; T'_1 = time of H'_m; and T_x = time of gas release.

The measurement was carried out only in “Carcaça” bread due to equipment limitations that did not support more complex doughs.

6. Bread analysis

6.1. Physical Characteristics

6.1.1. Bread weight evaluation

After 90 min of cooling, samples of each type of bread and salt content were individually evaluated for weight in Ceres laboratory in a digital scale METO (Esselte Meto International GmbH, Hirschhorn, Germany). Ten breads of each type were evaluated (n=10), except for “Regueifa” bread, where four replicates were used (n=4).

6.1.2. Bread volume evaluation

The bread-specific volume (SV) was measured using a seed displaced method and the following formula

$$\mathrm{SV}\ (\mathrm{cm}^3\ \mathrm{g}^{-1}) = \frac{\mathrm{S}\ (\mathrm{g}) \times 1.35\ (\mathrm{cm}^3\ \mathrm{g}^{-1})}{\mathrm{P}\ (\mathrm{g})},$$

where P is the bread weight, S is the weight of displaced seeds, and 1.35 is the specific volume (125). Measurements of 10 samples were made for each type of bread (n=10).

6.1.3. Bread moisture evaluation

Determination of bread moisture was performed according to the AACC International Method - Moisture-Air-Oven Methods (148).

A representative part of each bread sample was conveniently comminuted with a Moulinex shredder, obtaining an aliquot as homogeneous as possible. A 6 g portion of the sample was placed in an aluminum dish for moisture balance (KERN DLB 160-3A, Ziegelei, Germany). The sample was tested at 130 °C, and the moisture percentage calculated automatically. Sample readings were made in triplicates.

6.2. Crumb structure and color image analysis

For this analysis, three breads of every formulations and different levels of salt addition were cut in slices of 1.6 cm thickness and analyzed. Each slice was analyzed in pre-standardized conditions: positioned on the flatbed scanner and a black cardboard was placed over the slice in order enhance contrast (149). Images were captured in the RGB (24 bit) standard format with a resolution of 300 dpi and saved in JPG format. Each image was processed and analyzed using Matlab R2015a (MathWorks) as described by Martins et al. (123). Briefly, a single 300x300 pixel (51x51mm) field of view (FOV) was cropped, converted to a 256 level grey scale and segmented. Cell morphological parameters were analyzed and recorded values for crumb structure analyses included: number of cells, mean cell area (mm^2), and cell density (cells/mm^2). Additionally, cells were divided into different classes as a function of their area: *very small size* (cell area ≤ 0.2 mm^2); *small size* (0.2 mm^2 ≤ cell area ≤ 3.0mm^2); *medium size* (3.0 mm^2 ≤ cell area ≤ 10.0mm^2); *large size* (cell area > 10.0mm^2).

To study the crumb color, for the second approach, each single 300x300 pixel FOV obtained from bread image analysis was converted from RGB to CIElab system – lightness (L^*), redness (a^*) and yellowness (b^*) using code written in Matlab R2015a (MathWorks). Furthermore, crumb L^*, a^* and b^* values were combined in the browning index (*BI*) parameter (150) according to equations

$$BI=\frac{100(X-0.31)}{0.172}$$

$$X=\frac{a^*+1.75\,L^*}{5.645\,L^*+a^*-3.012\,b^*}$$

6.3. Sodium analysis

Bread samples were duly identified, transported and stored in a plastic bag at 4 ºC for the laboratory of the Faculty of Nutrition Sciences of the University of Porto, where, they were prepared and analyzed by flame photometry, according to the method validated by Vieira et al. (151).

6.3.1. Chemicals and Samples

From standard solutions of sodium (1,000 mg/ L) (Fluka, France), were prepared standard solutions with concentrations of 0.2, 0.5, 1.0, 1.5 and 5.0 µg/ ml and were established daily respective calibration curves.

To prevent sample contamination, all laboratory material used during the analyzes was immersed in a solution of 15% nitric acid (HNO_3) (Fluka, France) for at least 24 hours and then washed in deionized water and dried at room temperature.

All reagents and solutions were prepared with deionized water, obtained from Seralpur PRO 90 CN and Seradest LMF 20 Water Purification System.

6.3.2. Sample Preparation

The samples were properly ground and homogenized in a classical mincer (Moulinex mincer A320 R1); a portion of 2 g of sample was weighed accurately (Kern ALS 120-4 balance, Ziegelei, Germany) and placed in a 50 ml tube. Three extractions of each sample were performed. 4 ml of HNO_3 was pipetted into the tube, and the acid was allowed to act for 60 min, shaking the tube every 10 min. The volume was completed up to 45 ml with deionized water and a preparation was homogenized using a Ultra Turrax blender (Ultra Turrax blender T25, Sotel, Germany). The samples were vigorously shaken every 5 min for 30 min to allow sodium to dissolve. Afterwards 12 ml of the solution was pipetted into a test tube and centrifuged for 30 min at 4,000 rpm (Centrifuge 5810 R Eppendorf). Finally, 1.0 ml of supernatant was recovered and diluted up to 50 ml. This procedure was done in duplicate. These samples were shaken and immediately frozen at -18ºC. The samples were thawed and kept at room temperature 24 h before to reading. Sodium content was determinate with a flame photometer (Model PFP7, JenWay, England) and using the average of three readings for each extraction (Figure 1). Propane gas and air were supplied as the source of flame. The flow rate of the fuel was adjusted to obtain a maximum sensitivity.

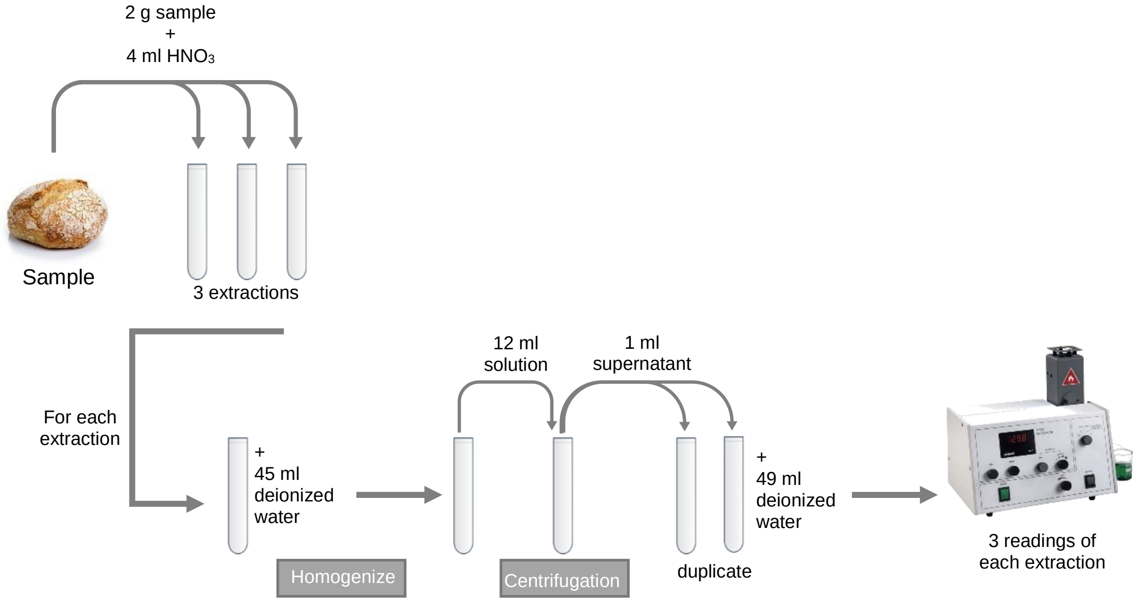

Figure 1. Process of sample preparation for flame photometry analysis

6.4. Bread Sensory Analysis

Sensory analysis of every bread types with different levels of salt under study was performed with a trained panel and a convenience sample.

In order to describe the sensory properties of the different samples of breads, the Quantitative Descriptive Analysis (QDA) was applied with a trained panel. For this method, two phases were used, an initial phase to select and train, and a subsequent phase focusing on the evaluation of samples.

6.4.1. Trained panel: selection and training

The recruitment of tasters was held at the University of Porto campus, through an invitation to participate, with an explicit explanation of the objective under study. All participants signed an informed consent. A questionnaire was applied to gather information related to availability, health conditions, intolerances or allergies that constituted an impediment to continue the study (Annex 1). Exclusion criteria considered were unavailability for tests, food allergies and smoking. The assessors were volunteer and did not receive remuneration for participating in the panel.

Eight candidates demonstrated interest to participate and all were considered for the study. Those assessors were part of the selection and training phase where several sessions were held with different types of tests. Firstly, assessors were expected to become familiar with the role of senses and sensory evaluation *per se*, as well as assess the aptitude for recognition, learning, and memorization of stimuli and intensities. In addition, it was intended that the assessors thoroughly knew the product to be evaluated in sensory terms.

6.4.1. a) Basic tastes test

The assessors were subjected to a basic taste test to identify sensory sensibility, memorizing and recognizing stimuli caused by these flavors. For this, two sessions were done and aqueous solutions (Table 8) were used for acid, sweet and salty flavors according to ISO 3972:2011 (152) (Annex 2). In the first session, four dilutions were presented with the basic sweet and salty tastes (BT1, Table 9). In the second session seven dilutions were presented simultaneously with acid, sweet and salty tastes with different concentration thresholds (BT2, Table 9).

To perform this test 25 ml plastic cups randomly coded, mineral water and answer sheet were used. The solutions were prepared immediately before the test by dissolving the compounds associated with the various flavors, and presented at room temperature. In addition, simple mineral water was always available to the assessors.

Table 8. Compounds and dilutions used in basic tastes test

Basic Tastes	Compounds and dilutions (%)
Acid taste	Crystallized citric acid 0.15%
Salty taste	Sodium Chloride (NaCl) 0.5%
Sweet taste	Sucrose 5%
Sweet + acid tastes	Sucrose 5% + Crystallized citric acid 0.2%
Sweet + Salty tastes	Sucrose 5% + Sodium Chloride 0.3%
Acid + Salty tastes	Crystallized citric acid 0.2% + Sodium Chloride (NaCl) 0.3%
Sweet + Acid + Salty tastes	Sucrose 10% + Crystallized citric acid 0.10% + Sodium Chloride (NaCl) 0.3%

6.4.1. b) Ranking test

The purpose of this test was to evaluate and train the assessors for the ability to discriminate sensorial characteristics, particularly of the final product and also the familiarization with the flavor to be tested. Three sessions were carried out according to ISO 8587:2006 (153), for training the assessors to discriminate different amounts of salt.

In the first session, five aqueous solutions with different concentrations of salt were presented, being asked to classify them according to the intensity of salty taste (R1, Table 9).

In the second session, three samples of "Carcaça" bread with different salt contents were presented, and the samples were also ordered according to the intensity of salty taste (R2, Table 9).

In the third session three ranking tests were performed, corresponding to three different bread types: “D’água” bread, “Mistura” bread, and “Regueifa” bread (Annex 3). For each type of bread/test, four samples with different salt contents were presented. The assessors were asked to order the samples by salty flavor intensity (R3.1, R3.2, R3.3; Table 9).

In all tests, the samples presented were duly coded and simple mineral water was offered. Dilutions were prepared in 25 ml plastic cups with mineral water and NaCl. The bread samples were individually displayed on plastic dishes, in similar portions of approximately 10 g.

Table 9. Sensorial evaluation: methodology, objectives and material.

Phase	Methodology	Objectives	Test code	Material
Selection and Training	Basic Tastes test (ISO 3972:2011 (152))	Recognizing basic tastes	BT1/ Tastes	50 ml plastic cups with dilutions of 2 basic tastes. Sucrose (24 g/ l); Sodium chloride (24 g/ L); Mineral water.
		Familiarization with different threshold types	BT2/ Tastes	50 ml of each sample for each dilution in plastic cups; Sucrose; Sodium chloride; Crystallized citric acid; Mineral Water.
	Ranking (ISO 8587:2006 (153))	Ordering samples according salt intensity	R1/ Salty taste (dilutions)	Five dilutions presented in 25 ml plastic cups with NaCl concentrations: 5 g/ L; 4 g/ L; 3g /L; 2 g/L; 1 g/ L; Mineral water
		Ordering bread samples according salty intensity	R2/ Salty taste (bread)	Three bread samples, presented in plastic dishes, prepared with approximately 10 g of “Carcaça” bread (provided preliminary laboratorial test) with different salt content: 0.5%, 1.0% and 1.5% NaCl
		Ordering bread samples according salty intensity	R3/ Salty taste (bread) R3.1 R3.2 R3.3	Three tests were performed with four samples each, for different concentrations of salt for “Mistura” bread, “D’água” bread and “Regueifa” bread: “Mistura” bread: 0.5%, 1.0%, 1.5%, 2.0% NaCl “D’água” bread: 0.5%, 1.0%, 1.5%, 2.0% NaCl “Regueifa” bread: 0.5%, 1.0%, 1.5%, 2.0% NaCl
	Triangle test (ISO 4120,2004)	Identify different bread samples	T1/ Salty taste (bread)	“Carcaça” bread samples with different salt content A: 1.0% NaCl; B:1.5% NaCl Test AAB
Validation	Triangle test (ISO 4120:2004 (154))	Evaluation the discriminating capacity, repeatability and reproductivity for assessors	V1/ Salty taste (bread) V1.1 V1.2 V1.3	Three tests were performed with “D’água” bread samples, of approximately 10 g, for different salt contents: A: 0,0 g NaCl/ 100 g of bread; B: 0,8 g NaCl/ 100 g of bread; C: 1,0 g NaCl/ 100 g of bread. Test AAC Test ACA Test CBC
Sensory evaluation of bread samples	Descriptive sensory analysis	Evaluate sensory attributes of bread	E1 “Carcaça” bread; “Regueifa” bread E2 “D’água” bread, “Mistura bread E3 “Carcaça” bread; “Regueifa” bread E4 “D’água” bread, “Mistura bread E5 “Carcaça” bread; “Regueifa” bread E6 “D’água” bread, “Mistura bread	Bread Samples were presented in similar slices of approximately 10 g, in dishes glass with different salt content: 0.0 g, 0.8 g, 1.0 g, 1.1 g, 1.3 g and 1.4 g of salt/ 100 g bread

6.4.1. c) Triangular test

Triangular test was performed according to ISO 4120:2004 (154) to train the capacity of the assessors for sensorial different samples.

In the follow-up line of the previous tests, contact and familiarization with the final product to be analyzed were provided, and samples of bread with different percentage of salt contents were used.

During the session, three number-codded samples of “Carcaça” bread was presented simultaneously (Annex 4). The assessors were asked to indicate which one was odd sample (different from other two) (T1, Table 9). All samples were presented in a similar way and mineral water was offered to the assessors between tests.

6.4.2. Trained panel: validation

With the aim to validate the panel, evaluating the ability of discrimination and repeatability of the assessors, three triangular tests were carried out (Annex 4). In order to increase the difficulty of the test, the different samples differed little from the similar samples relative to the salt content. In these tests, samples of “D’água” bread were used. Following the same principle, the assessors were asked to identify the different sample (V1.1, V1.2, V1.3, Table 9).

6.4.3. Descriptive Analysis for bread samples

In order to train and familiarize assessors with the use of QDA and to begin the development of vocabulary of description and understanding of scales, a session was held where the assessors performed the descriptive sensory analysis for a toast (Annex 5). For this session, the toasts were presented in plastic dishes and a QDA response sheet with an unstructured scale with preexisting list attributes was used. Mineral water was provided to the assessors. The test was performed individually and then the results were discussed in a group.

6.4.3. a) Descriptive sensory attributes

Prior to start of the study, assessors partook in the development of a descriptive vocabulary, compiling a list of attributes associated with the breads (Annex 5). Two sessions were performed to facilitate the acquisition of an accurate concept and refined terms in association with detection and recognition of smells, tastes, textures etc. Redundant descriptive terms were removed. Sensory attributes were classified based on four characteristics: appearance (visual perception), odor (olfactory perception), texture (tactile and oral texture) and flavor (oral and retronasal). Seventeen attributes were defined for a descriptive sensory analysis of the bread: c*rust color intensity*, c*rumb color intensity*, n*umber of large cells*, n*umber of small cells*, c*ell circularity*, c*ell homogeneity*, o*dor intensity*, c*runchy crust*, c*ohesiveness*, a*dhesiveness*, c*rumb elasticity*, s*hape recovery*, s*alty*, sw*ee*t, b*read aroma*, a*ftertast*e and o*verall assessment* (Table 10).

Table 10. Descriptive sensory attributes developed by trained sensory panel

Sensory attributes	Definitions
Appearance	
Crust color intensity	Degree of color darkness in the crust – light to dark
Crumb color intensity	Degree of color darkness in the crumb – light to dark
Number of Large Cells	Amount of large cells – low to high
Number of Small Cells	Amount of small cells – low to high
Cell circularity	Level of perfection of the circular shape of the crumb cells/ number of circular crumb cells
Cell homogeneity	Homogeneity of the size of the crumb cells
Odor	
Odor intensity	Degree of intensity of odor of the sample – low to high
Texture	
Crunchy crust	Degree of perceived noise when chewing the crust sample
Cohesiveness	Level of mass formation in the mouth before breaking
Adhesiveness	Degree in which the material adheres to the palate
Crumb elasticity	Ability to return to initial shape after being pressed
Shape recovery	Resistance to the crumb pressure on the finger
Aroma	
Salty	Perception of taste sensation for sodium chloride
Sweet	Perception of taste sensation for sugars
Bread aroma	Degree of perception of the intensity of the characteristic bread flavor
Aftertaste	Flavor remaining after tasting

6.4.3. b) Sensory evaluation of taste control

Based on the QDA using a 1 – 7 unstructured scale, with 1 representing the lowest intensity and 7 the highest intensity, assessors established ballot anchors for each selected attribute that were fitted on scale and the descriptive sensory evaluation of the control sample for each type of bread was carried out. The control sample select was bread with the addition of 1.1 g/ salt per 100 g of bread, because it represented the midpoint of salt concentrations range used in this study.

There were two sessions for classification/ scoring of the attributes for the control sample for each type of bread until it was unanimous and memorized by all the assessors (Annex 5).

6.4.4. Sensory profile of bread: Evaluation procedure

Once the panel was trained and validated, the assessors performed the sensory analysis of the four breads and their six salt concentrations evaluated in this study (including sensory control sample).

“D’agua”, “Carcaça”, “Mistura”, and “Regueifa” breads with the addiction of 0.0, 0.8, 1.0, 1.1, 1.3 and 1.4 g of salt per 100 g of the bread were evaluated through the same QDA previously developed and by comparison to the score obtained for sensory control sample (1.1 g/ 100 g for each bread type).

In total, during six sessions, 24 bread samples were evaluated by the assessors and the tests were carried out in triplicate to increase the validity of the responses (E1, E2, E3, E4, E5, E6; Table 9).

All samples were presented, in the similar conditions: at room temperature and similar size with approximately 7 g (a slice with 1.5 cm of thickness) including the crust and crumb in a random three-digit coded covered glass dish. Evaluation sessions occurred individually under white light at room temperature. Before the assessment, assessors were provided with mineral water and instructed to cleanse their palate between tastings.

6.5. Consumer test

To evaluate consumer acceptability for bread with different salt levels, the sensorial acceptance hedonic test was applied to students, professors and employees of the University of Porto Campus. Eighty consumers participated in the trial. Every 20 consumers evaluated one type of bread and its respective salt concentrations. In this way, six samples of bread in three-coded in plastic dishes were presented to each consumer in a randomized manner. Acceptability tests were conducted using a hedonic scale of 7 points, where 1 corresponds to "dislike extremely” and 7 to “like extremely” to assess the five following attributes: *appearance liking*, a*roma liking*, t*aste liking*, t*exture liking* and *overall liking* (Annex 6).

6.6. Preference Mapping

The consumer test data was submitted to cluster analysis and then external preference mapping was done to find a regression between consumer acceptability variables (dependent) and the main dimensions obtained by Principal Component Analysis (PCA) carried out on sensory data by trained panel (explanatory variables). Thus, mapping the consumer data in the assessor’s space and to obtaining the sensory properties that influence consumer acceptability. Four preference models were used: vector, circular, elliptical, and quadratic (155).

7. Statistical Analysis

All dependent variables from bread parameters analyzed were tested for distribution with Shapiro-Wilk test. Different samples were studied using a one-way analysis of variance (ANOVA), if normal distribution was confirmed. Welch correction was applied when homogeneity of variances was not verified. Whenever statistical significance was found, Tukey's test was applied for mean comparison on equal variance assumption, and Dunnett's T3 post hoc when not equal variance assumption.

If normal distribution was not found, different samples were studied using a Kruskal Wallis test. Whenever statistical significance was found, Mann-Whitney post hoc test was applied for median comparison.

Linear regression was used to identify physicochemical and image parameters that were related with salt reduction.

Partial least square (PLS) regression was used to identify attributes that were related positively with product acceptance. The overall liking was coded on a binomial scale (0/1), with 0 representing rejection (for overall liking values between 1 and 4), and 1 for acceptance (overall liking values higher than 5). The variables were not subject to data any pre-processing, in order to identify natural variation among consumers.

Overall acceptability from consumers was also used to select the lowest salt concentration with best sensory performance for each bread type studied. Sensory data collected was treated using External Preference Mapping technique (156-158).

PLS regression was also used to study the relationships between sensory attributes (Y-matrix) and physicochemical parameters, color and crumb structure (X-matrix) in terms of prediction of Y-variables from X-variables. Random validation was also applied to identify relevant X-variables. Scores and loading plots were analyzed, as well as, calibration and validation coefficients. Scores and loading plots were analyzed, as well as, calibration and validation coefficients. Auto scaling was used for data pretreatment i.e., data was converted to fluctuations around zero, subtracting the mean of a variable to the original values and divided by the square root of the standard deviation.

All statistical analyses were conducted with the XLSTAT for Windows version 2016.02 (Addinsoft, Paris, France) at 10% (Preference mapping) and 5% (ANOVA, Kruskal–Wallis, and PLS regression) significance level.

Chapter III

Results and Discussion

8. Dough evaluation: Rheofermentometer evaluation

The effect of salt reduction on “Carcaça” dough properties and fermentation was measured by the Rheofermentometer, was obtained information on gas expansion and retention capabilities.

The results of the time to reach the maximum dough height (T_1) and the maximum dough height (H_m) can be indicators to help the definition of the optimal conditions for the fermentation, specifically time conditions, depending on the formulation to be used (108).

According to Figure 2, it was possible to verify that T_1 decreases slightly between the concentrations of 0.0% and 0.8% of salt, while H_m remains relatively constant, with values close to 60 mm. Increasing the salt concentration to 1.0% increased T_1 for 90 min and of H_m to values close to 70 mm, reaching its maximum value. At higher salt concentrations, 1.1%, 1.3% and 1.4%, T_1 remains constant while H_m decreases gradually as the salt increases.

As for dough development, it was possible to observe the same tendency for gas production. The time to reach the maximum gas formation rate (T'_1) decreases between the concentrations of 0.0% and 0.8% and increases between the salt concentrations of 0.8% and 1.1%, remaining constant in the 90 min when increasing the salt concentrations to 1.3% and 1.4%. The maximum height of gaseous production (H'_m) in turn increases between 0.0% and 1.0%, then gradually decreases with the increase in salt content between the concentrations of 1.0% and 1.4%. It is still important to consider the time of porosity (T_X), which represents the time at which the dough ceases to be able to retain the gas. Here, we verified that T_X increased slightly between the concentrations of 0.0% and 0.8%, reaching this point in less time at 1.0%, indicating that the dough is weaker at this concentration.

At 1.1% of salt, T_X is reached later, remaining relatively stable with increasing salt content to 1.3% and 1.4%, indicating that the mass has a stronger structure at these salt concentrations.

These results are in agreement with the results reported by Lunch et al. (108), Beck et al. (159), and Belz et al. (160), who also studied the development of the dough and the behavior of the gas in different concentrations of salt. The explanation for these results may be based on the effects of salt on the metabolic activity of yeast, reducing its yeast leavening ability. Thus, when the salt concentration increases, it promotes an increase in the osmotic pressure in the yeast cells, inhibiting yeast growth and its metabolic activity, which translates into lower CO_2 production (108, 109, 112). On the other hand, the increase of T_X with the increase of salt can be explained by the strengthening influence of the salt in the gluten network, making the dough stronger and with greater capacity of retention of CO_2 (108). In addition, with lower CO_2 production rates, less pressure will be exerted on the membranes of the gluten network which will retard the loss of gas (161).

It is also important to mention that the protocol used in this measurement was adapted from the protocol usual to the company Ceres, and that the defined time (90 min) would be the time supposedly necessary to obtain the essential information.

Based on these assumptions and the results obtained, we can verify that, with respect to the development of the dough, the dough with concentration of 1.0% of salt was the dough that obtained better performance, taking about 90 min to obtain its maximum growth, with values close to 80 mm. However, by assessing the gas retention capacity, the dough with salt content of 1.1% will be stronger.

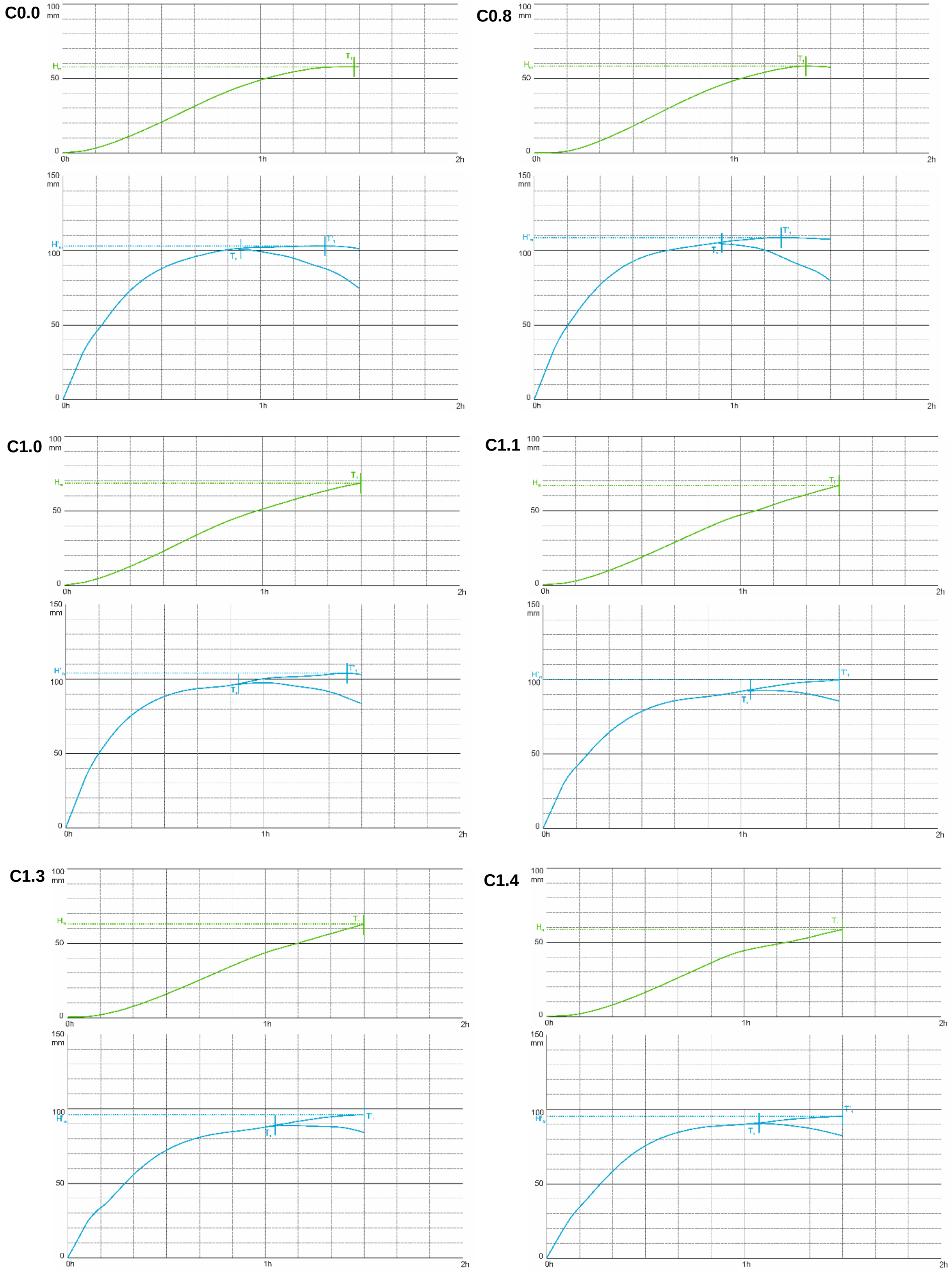

Figure 2. Curves for dough development (—) and gas production (—) throughout fermentation of “Carcaça” bread dough with different salt level.

C0.0, “Carcaça” dough with 0.0% salt; C0.8, “Carcaça” dough with 0.8% salt; C1.0, “Carcaça” dough with 1.0% salt; C1.1, “Carcaça” dough with 1.1% salt; C1.3, “Carcaça” dough with 1.3% salt; C1.4, “Carcaça” dough with 1.4% salt.

T_1 time to reach the maximum dough height (min), T'_1 time to reach the maximum gas formation rate (min), T_x time of porosity (gas starts to escape the dough matrix), H_m maximum dough height (mm), H'_m maximum height of gaseous production (mm)

9. Bread physicochemical characteristics: bread weight, volume, moisture and sodium

The parameters determined to evaluate physicochemical characteristics of each bread type with different salt addition levels are presented in Table 11.

Considering bread physical characteristics, the impact of salt addition at different levels had differed between bread types. Moreover, no linear pattern ($R^2 < 0.700$, $p > 0.050$) was observed, except for specific volume ($R^2 = 0.953$, $p = 0.001$) in “Carcaça” bread, where specific volume values increased as the level of added salt increases. Comparing to the control (1.4%), significant differences were found for specific volume in “D’Água” ($p = 0.033$), “Carcaça” ($p < 0.001$), and “Mistura” ($p < 0.001$) breads; and moisture in “D’Água”, “Carcaça”, and “Regueifa” ($p < 0.001$) breads.

Regarding specific volume, comparing to control bread, it was higher than 0.0% in “Carcaça” bread and 0.8% in both “D’Água” and “Carcaça” breads, whereas it was lower than 1.1% and 1.3% in “Mistura” bread. The results are in accordance with McCann et al. (162), where volume increased with increasing salt concentration, for breads prepared with 0%, 1% and 2% sodium chloride (w/w, flour base). Moreover, studies carried out by Czuchajowska et al. (163), Miller et al. (164), and He et al. (165) are also in agreement with these findings. However, other research studies point out to a possible absence of effect on volume with a decrease in the salt concentration (108, 159). Therefore, it is difficult to establish the impact of salt on bread volume. At some extent, technological variability, such as dough mixing time, formulation, proofing and baking time, might explain the difference in results reported.

Moisture content seemed to decrease as the level of added salt increased. Control bread had lower moisture than 0.0%, 0.8%, and 1.0% for “D’Água” and “Carcaça” breads; 1.1% for “Carcaça” and “Regueifa” breads; and 1.3% for “D’Água” bread. Results obtained are not in agreement with those reported by Lynch et al. (108), where breads with salt reduction, from 1.2% to 0.6%, 0.3% and 0% addition did not present significant differences in moisture content.

Regarding to bread weight, significant results were found only for “Carcaça” bread ($p = 0.002$), however without linear pattern linear ($R^2 = 0.492$, $p = 0.120$). In addition, when comparing these results to the control (1.4%), no differences were observed.

Concerning chemical characteristics, i.e. salt concentration, in general, salt addition at different levels had significant impact on salt concentration for every bread type ($p < 0.001$). As it would be expected, salt concentration increased with increasing salt addition levels, following a linear trend for every bread type (“D’água”, $R^2 = 0.727$, $p < 0.001$; “Carcaça”, $R^2 = 0.770$, $p < 0.001$; “Mistura”, $R^2 = 0.808$, $p < 0.001$; “Regueifa”, $R^2 = 0.996$, $p < 0.001$).

Table 11. Physicochemical parameters values for each bread type, with different salt addition levels (% bread).

Salt (%)	**Weight (g)**	**Specific volume (cm^3/g)**	**Moisture (%)**	**Salt concentration (g/100 g)**
"D'Água"				
0.0	52.23 ± 3.91	7.36 ± 1.01^{ab}	35.71 ± 0.21^{a}	0.000^{a} (0.000 – 0.000)
0.8	52.41 ± 3.45	6.64 ±0.40^{a}	34.98 ± 0.24^{a}	0.685^{ab} (0.656 – 0.692)
1.0	53.89 ± 6.65	6.88 ± 0.45^{ab}	40.51 ± 5.23^{a}	0.834^{ab} (0.813 – 0.864)
1.1	52.19 ± 6.31	7.36 ± 1.01^{ab}	33.00 ± 1.03^{b}	0.891^{abc} (0.875 – 0.899)
1.3	51.48 ± 4.91	7.00 ± 0.50^{ab}	34.26 ± 0.36^{c}	1.024^{bc} (1.003 – 1.050)
1.4	52.07 ± 7.40	7.67 ± 0.88^{b}	31.88 ± 0.65^{b}	1.146^{c} (1.094 – 1.159)
p	ns	0.033**	< 0.001**	<0.001***
"Carcaça"				
0.0	48.46^{a} (44.19 – 52.11)	3.54 ± 0.88^{a}	32.47 ± 0.19^{a}	0.000^{a} (0.000 – 0.012)
0.8	47.43^{ab} (44.82 – 49.96)	6.27 ± 0.26^{b}	33.46 ± 0.29^{b}	0.420^{ab} (0.223 – 0.530)
1.0	43.05^{b} (37.89 – 47.81)	6.65 ± 0.59^{bc}	33.01 ± 0.17^{ab}	0.693^{ab} (0.281 – 0.843)
1.1	43.24^{b} (38.55 – 47.88)	7.15 ± 0.29^{c}	32.71 ± 0.22^{a}	0.788^{b} (0.559 – 1.000)
1.3	44.94^{ab} (35.35 – 48.17)	7.15 ± 0.86^{c}	30.66 ±0.26^{c}	0.843^{b} (0.799 – 0.938)
1.4	46.48^{ab} (39.14 – 48.13)	7.24 ± 0.56^{c}	31.22 ± 0.37^{c}	0.893^{b} (0.625 – 1.075)
p	0.002***	< 0.001**	< 0.001*	<0.001***
"Mistura"				
0.0	52.41 ± 5.44	4.68 ± 0.23^{a}	29.89^{a} (27.16 – 29.91)	0.000^{a} (0.000 – 0.000)
0.8	52.05 ± 0.35	5.07 ± 0.23^{b}	32.37^{b} (32.14 – 32.22)	0.508^{ab} (0.376 – 0.575)
1.0	53.00 ± 4.88	5.27 ± 0.40^{bc}	31.61^{ab} (31.61 – 32.22)	0.659^{ab} (0.299 – 0.659)
1.1	50.94 ± 7.99	5.56 ± 0.40^{cd}	31.32^{ab} (30.88 – 31.74)	0.804^{b} (0.557 – 0.864)
1.3	51.80 ± 4.38	6.14 ± 0.34^{d}	31.09^{ab} (30.13 – 31.15)	0.850^{b} (0.585 – 1.051)
1.4	52.70 ± 4.76	4.91 ± 0.60^{ab}	30.16^{ab} (30.12 – 30.74)	0.851^{b} (0.442 – 0.955)
p	ns	< 0.001**	0.010***	<0.001***
"Regueifa"				
0.0	404.50 (383.00 – 440.00)	4.69 ± 0.34	35.08 ± 0.57^{ab}	0.000^{a} (0.000 – 0.000)
0.8	433.00 (395.00 – 481.00)	4.91 ± 0.40	33.59 ± 0.29^{c}	0.367^{ab} (0.149 – 0.509)
1.0	420.00 (415.00 – 470.00)	4.84 ± 0.12	34.54 ± 0.16^{ad}	0.449^{abc} (0.418 – 0.459)
1.1	470.00 (410.00 – 485.00)	4.28 ± 0.23	35.68 ± 0.13^{b}	0.528^{abc} (0.469 – 0.654)
1.3	412.50 (385.00 – 450.00)	4.62 ± 0.31	33.70 ± 0.36^{cd}	0.945^{bc} (0.764 – 1.312)
1.4	432.50 (365.00 – 460.00)	4.84 ± 0.14	33.60 ± 0.23^{c}	1.160^{c} (0.914 – 1.535)
p	ns	ns	< 0.001*	<0.001***

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=10).

ns, not significant.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey's, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn's test.

10. Crumb structure and color image analysis

Crumb structure and color are essential bread quality parameters along with taste and crumb texture (166, 167). Bread color is influenced by factors such as formulation or baking conditions, while brown color (measured using the browning index, BI) results from Maillard reactions and crust caramelization (168-171).

Figure 3 shows the scanned images of crumb structure of all bread types and salt concentration samples in study. From a qualitative comparison of the images, it was possible observe the differences between bread types and different salt concentrations. The different bread types are easily distinguished and it is also possible to identify some differences in alveolar structure as the salt content increases. In general, in all bread types, higher number of larger cells appear in the samples with higher salt concentration.

The “D’água” bread is the type of bread which presented the larger cells and less uniformity in its distribution. The “Carcaça” bread has smaller and generally more uniform cells and, as the salt content increases it is possible to visualize larger cells. In relation to “Mistura” bread, there is no uniform distribution of cells, and large and small cells are observed, regardless of the salt content. Finally, “Regueifa” bread is the type of bread that has a more compact structure with smaller cells.

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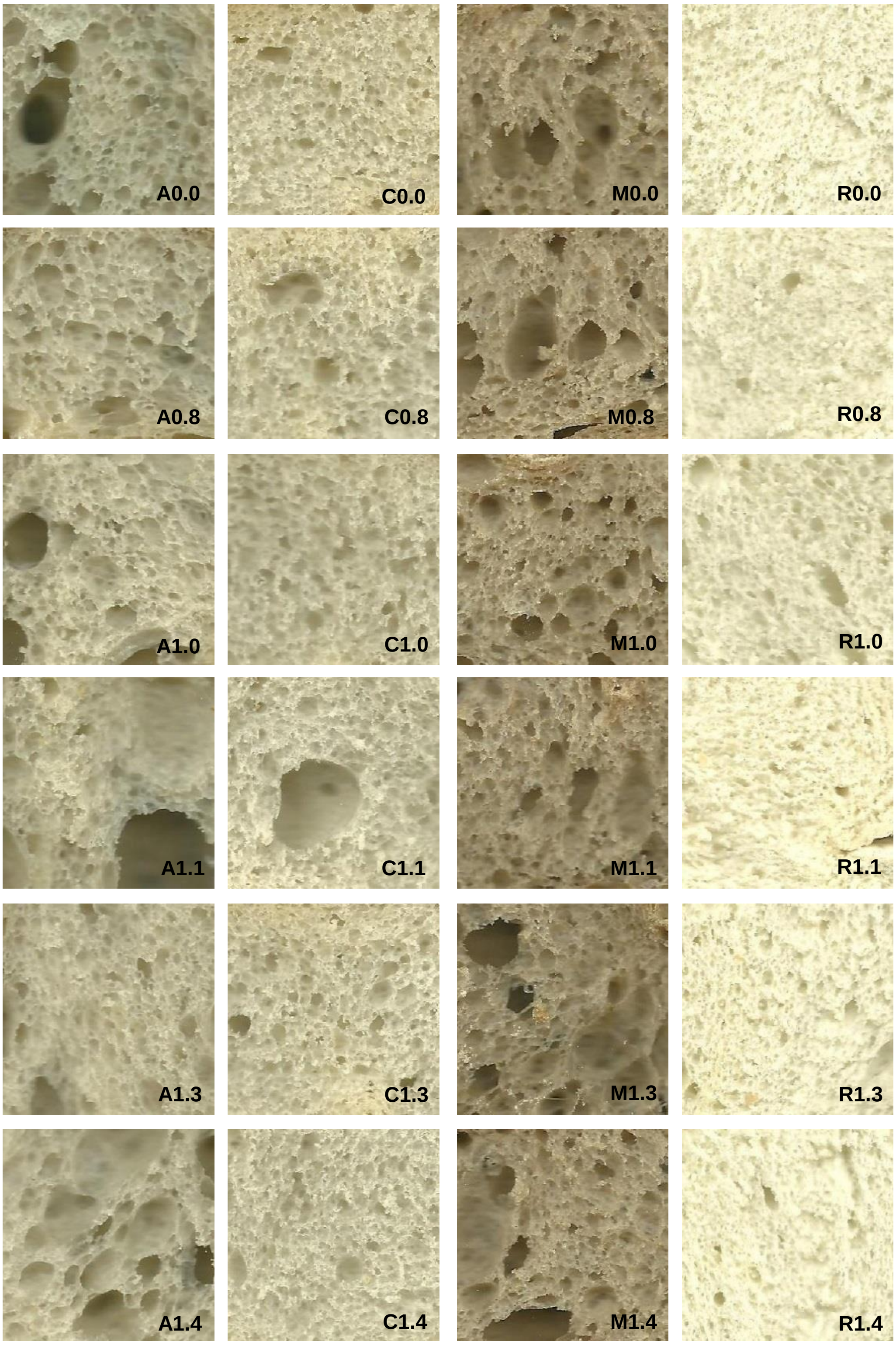


Figure 3. Scanned images of bread slices of each bread type, with different salt addition levels. A single 300x300 pixel (51x51mm) field of view (FOV) was considered.

A0.0, “D’Água” bread with 0.0% salt.; A0.8, “D’Água” bread with 0.8% salt; A1.0, “D’Água” bread with 1.0% salt; A1.1, “D’Água” bread with 1.1% salt; A1.3, “D’Água” bread with 1.3% salt; A1.4, “D’Água” bread with 1.4% salt; C0.0, “Carcaça” bread with 0.0% salt; C0.8, “Carcaça” bread with 0.8% salt; C1.0, “Carcaça” bread with 1.0% salt; C1.1, “Carcaça”” bread with 1.1% salt; C1.3, “Carcaça” bread with 1.3% salt; C1.4, “Carcaça” bread with 1.4% salt. M0.0, “Mistura” bread with 0.0% salt; M0.8, “Mistura” bread with 0.8% salt; M1.0, “Mistura” bread with 1.0% salt; M1.1, “Mistura” bread with 1.1% salt; M1.3, “Mistura” bread with 1.3% salt; M1.4, “Mistura” bread with 1.4% salt. R0.0, “Regueifa” bread with 0.0% salt; R0.8, “Regueifa” bread with 0.8% salt; R1.0, “Regueifa” bread with 1.0% salt; R1.1, “Regueifa” bread with 1.1% salt; R1.3, “Regueifa” bread with 1.3% salt; R1.4, “Regueifa” bread with 1.4% salt.

Overall, salt addition at different concentrations had limited impact on crumb morphology, but more influence on color parameters (Table 12). Furthermore, no linear relationship with salt addition levels was observed for any of the crumb morphology or colour parameters studied except for small size cells ("D'água" bread, $R^2 = 0.277$, $p = 0.001$), large size cells ("D'água" bread, $R^2 = 0.240$, $p = 0.002$), a* ("Carcaça" bread, $R^2 = 0.686$, $p < 0.001$), and b* ("Carcaça" bread, $R^2 = 0.562$, $p < 0.001$; "Mistura" bread, $R^2 = 0.166$, $p = 0.014$). Nevertheless, these relationships should be carefully interpreted, as R^2 values are low, which in turn could affect the quality of the model.

Regarding cell morphology, salt has been described as having a fundamental role on the formation of an even crumb (172). However, significant differences comparing to the control breads were only found for cell distribution as a function of their area, namely *very small size cells* in "Carcaça" bread ($p = 0.047$) and *small size cells* in "D'Água" bread ($p = 0.003$). Control bread had higher percentage of *very small size cells* than 1.0%, in "Carcaça" bread, and *small size cells* than 0.0% and 0.8% in "D'Água" bread. Results obtained are in agreement with Yovchev et al. (173) that found no significant differences on total number of cells in breads with salt reduction. However, the absence of significant differences on the percentage of large cells is not in agreement with what is described by Lynch et al. (108), where bread without salt resulted in a smaller number of larger cells when compared to bread containing salt.

Concerning color, salt influences Maillard reactions that occur throughout baking (112). Although salt impact is more described for bread crust, it would also be expected, at some extent, for bread crumb. Salt reduction resulted in significant differences for every parameters when comparing to respective controls, i.e. *L** for "D'Água" (*p* = 0.006) and "Regueifa" (*p* = 0.027); *a** for "Carcaça" (*p* < 0.001) and "Mistura" breads (*p* = 0.047); *b** for "Carcaça" (*p* < 0.001); *BI* for "D'Água" (*p* = 0.002), "Regueifa" (*p* < 0.001), and "Carcaça" (*p* = 0.029) breads. Control bread was lighter than 0.8% for "D'Água" bread, 1.0% and 1.1% for "Regueifa". While control bread was greener than 0.0%, 0.8%, and 1.3% in "Carcaça" bread, it was redder in "Regueifa" bread.

Salt reduction affected *L**, *a**, and *b** differently and, together with the inherent influence of factors such as formulation or baking conditions on bread color, the comparison with literature was not possible.

Table 12. Values for crumb color and structure parameters for each bread type, with different salt addition levels (% bread).

Bread type/ Salt (%)	**Number of cells**	**Mean area (mm^2)**	**Cell density (cells/mm^2)**	**Circularity**	**Cell area (% of total cells)**				**Crumb color**			
					Very small size	Small size	Medium size	Large size	L*	a*	b*	BI
“D’Água”												
0.0	190 (105 – 274)	17.21 (12.70 – 32.08)	11.71 (3.27 – 21.57)	0.69 ± 0.11	28.50 ± 7.65	40.00^{a} (36.70 – 40.51)	11.45 (8.39 – 18.35)	18.27 ± 5.35	61.20 ± 2.26^{ab}	-1.98^{a} (-2.64 – -1.65)	18.32 ± 0.70^{ab}	31.61 ± 1.59^{a}
0.8	120 (92 – 302)	29.58 (11.19 – 36.68)	4.52 (2.51 – 26.99)	0.67 ± 0.14	30.39 ± 8.82	38.07^{ab} (38.04 – 46.51)	12.59 (8.16 – 15.22)	17.19 ± 6.58	56.99 ± 5.59^{a}	-1.67^{ab} (-1.82 – -0.83)	19.03 ± 0.47^{b}	37.34 ± 3.14^{b}
1.0	243 (196 – 345)	14.37 (10.02 – 17.40)	16.93 (11.26 – 34.45)	0.75 ± 0.11	29.94 ± 4.35	46.90^{bc} (43.37 – 48.70)	12.20 (6.75 – 12.76)	12.58 ± 3.24	64.36 ± 2.84^{ab}	-1.42^{ab} (-2.00 – -0.92)	19.01 ± 0.99^{b}	31.98 ± 1.97^{a}
1.1	154 (86 – 288)	23.91 (12.29 – 39.71)	7.47 (2.17 – 23.43)	0.70 ± 0.09	27.66 ± 7.50	43.17^{abc} (34.88 – 47.22)	11.79 (8.14 – 18.61)	17.54 ± 7.91	58.69 ± 4.09^{ab}	-1.89^{ab} (-1.98 – -1.41)	18.00 ± 0.31^{a}	33.07 ± 3.04^{ab}
1.3	224 (121 – 441)	15.99 (7.57 – 28.05)	15.99 (4.31 – 58.29)	0.77 ± 0.04	33.01 ± 6.74	42.49^{abc} (40.00 – 51.10)	9.51 (6.35 – 13.94)	13.16 ± 4.39	66.00 ± 6.30^{b}	-1.39^{b} (-1.60 – -0.95)	18.74 ± 0.37^{b}	31.00 ± 3.73^{a}
1.4	120 (87 – 412)	28.61 (8.33 – 38.82)	4.22 (2.24 – 49.44)	0.69 ± 0.12	25.42 ± 7.26	47.18^{c} (40.71 – 52.87)	10.16 (5.83 – 13.27)	17.43 ± 6.02	64.90 ± 4.65^{b}	-1.58^{ab} (-1.74 – -0.95)	18.57 ± 0.49^{ab}	29.83 ± 1.49^{a}
p	ns	ns	ns	ns	ns	0.003***	ns	ns	0.006*	0.014***	0.034*	0.002*
“Carcaça”												
0.0	411 (149 – 605)	11.36 ± 6.57	56.07 ± 48.09^{a}	0.55 ± 0.05	37.12 ± 10.18^{a}	44.27 ± 3.46	8.64 ± 4.96	9.98 ± 6.29	71.84 (57.40 – 71.84)	-0.94 ± 0.28^{a}	20.74 ± 0.88^{a}	33.30 ± 2.92^{a}
0.8	231 (119 – 428)	17.14 ± 8.71	21.53± 16.66^{ab}	0.59 ± 0.08	32.80 ± 6.96^{ab}	45.92 ± 2.63	9.31 ± 3.27	11.97 ± 4.27	73.01 (67.95 – 73.01)	-1.77 ± 0.12^{bc}	19.08 ± 0.65^{b}	27.53 ± 1.23^{b}
1.0	169 (117 – 316)	19.52 ± 5.52	11.48 ± 9.45^{b}	0.54 ± 0.08	26.52 ± 5.74^{b}	45.36 ± 3.97	11.84 ± 3.22	16.30 ± 3.67	69.15 (61.88 – 69.15)	-1.96 ± 0.12^{bd}	18.06 ± 0.49^{c}	27.37 ± 1.55^{b}
1.1	154 (89 – 409)	23.92 ± 11.18	13.06 ± 17.62^{b}	0.57 ± 0.10	27.78 ± 9.06^{ab}	45.89 ± 4.12	10.93 ± 3.87	15.40 ± 4.90	69.31 (58.64 – 69.31)	-1.96 ± 0.11^{bd}	18.01 ± 0.31^{c}	27.33 ± 2.70^{b}
1.3	307 (129 – 523)	14.55 ± 8.45	35.00 ± 31.35^{ab}	0.63 ± 0.11	33.76 ± 9.06^{ab}	47.10 ± 3.11	9.11 ± 2.69	10.03 ± 4.98	73.89 (61.86 – 77.89)	-1.67 ± 0.17^{c}	19.08 ± 0.48^{b}	26.47 ± 0.75^{b}
1.4	354 (127 – 592)	13.94 ± 9.34	42.34 ± 38.15^{ab}	0.61 ± 0.08	37.19 ± 9.06^{a}	45.95 ± 3.74	6.91 ± 1.70	9.96 ± 5.64	75.63 (66.55 – 78.59)	-2.05 ± 0.15^{d}	18.04 ± 0.42^{c}	25.26 ± 2.38^{b}
p	ns	ns	0.004**	ns	0.047*	ns	ns	ns	ns	<0.001**	<0.001*	<0.001*
“Mistura”												
0.0	197 (133 – 380)	16.53 ± 6.53	11.38 (5.31 – 43.29)	0.65 ± 0.10	35.95 ± 7.79	43.78 ± 5.63	8.42 (5.98 – 11.28)	11.96 ± 4.21	53.28 (50.64 – 53.28)	1.50 ± 0.07^{a}	20.45 ± 0.56	47.89 ± 3.02
0.8	218 (109– 551)	16.88 ± 8.51	10.49 (3.62 – 26.84)	0.63 ± 0.07	34.27 ± 11.05	45.51 ± 5.50	8.92 (5.81 – 10.84)	9.69 ± 2.49	56.10 (46.62 – 58.41)	1.50 ± 0.25^{bc}	20.38 ± 0.63	47.81 ± 3.64
1.0	162 (126 – 505)	17.63 ± 8.90	7.97 (4.58 – 76.47)	0.67 ± 0.07	38.16 ± 10.70	41.04 ± 8.21	6.35 (5.40 – 15.87)	12.03 ± 5.80	49.95 (48.44 – 59.23)	1.59 ± 0.13^{bd}	20.47 ± 0.48	50.44 ± 4.58
1.1	154 (93 – 390)	21.52 ± 11.13	7.07 (2.54 – 45.71)	0.65 ± 0.06	33.04 ± 11.03	42.13 ± 4.94	9.10 (5.90 – 14.41)	15.28 ± 7.04	50.41 (44.74 – 56.35)	1.59 ± 0.20^{bd}	20.02 ± 0.69	50.02 ± 3.25
1.3	139 (93 – 383)	23.04 ± 9.29	4.77 (2.63 – 13.18)	0.71 ± 0.13	36.22 ± 5.55	38.48 ± 6.04	8.11 (4.96 – 13.28)	13.58 ± 2.82	47.18 (45.76 – 52.52)	1.11 ± 0.19^{c}	19.35 ± 0.42	50.80 ± 3.55
1.4	180 (121 – 219)	18.29 ± 7.28	9.67 (4.35 – 13.98)	0.75 ± 0.12	31.09 ± 7.42	46.54 ± 3.26	10.78 (5.42 – 13.70)	13.63 ± 2.73	52.98 (49.01 – 56.73)	1.58 ± 0.12^{d}	19.87 ± 0.57	46.93 ± 2.48
p	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.047*	ns	ns
“Regueifa”												
0.0	294 ± 112	10.14 (8.73 – 27.89)	28.40 ± 16.97	0.57 ± 0.13	34.94 ± 12.75	50.41 ± 6.45	6.50 ± 1.20^{a}	3.75 ± 0.89	90.55^{a} (81.05 – 92.19)	-2.85 ± 0.16^{ab}	19.41 ± 0.52	21.18^{ab} (19.44 – 24.35)
0.8	270 ± 106	14.26 (8.39 – 23.96)	24.23 ± 17.82	0.56 ± 0.14	32.52 ± 9.40	50.45 ± 3.07	9.66 ± 3.73^{ab}	7.37 ± 4.84	90.11^{ab} (84.36 – 92.30)	-2.69 ± 0.19^{a}	18.83 ± 0.36	20.79^{a} (19.35 – 21.41)
1.0	196 ± 63	16.18 (12.44 – 30.08)	12.31 ± 6.96	0.47 ± 0.09	27.33 ± 8.41	49.31 ± 3.70	11.16 ± 3.20^{ab}	12.21 ± 8.02	84.63^{b} (80.98 – 90.68)	-2.60 ± 0.11^{ab}	19.86 ± 0.22	23.52^{ab} (20.99 – 24.87)
1.1	247 ± 106	12.00 (9.36 – 38.46)	20.84 ± 13.93	0.62 ± 0.10	34.57 ± 4.18	50.82 ± 6.15	7.41 ± 1.45^{ab}	5.35 ± 1.50	91.54^{b} (79.44 – 92.79)	-2.62 ± 0.19^{b}	20.10 ± 0.88	22.51^{ab} (20.95 – 24.21)
1.3	218 ± 83	16.46 (10.25 – 26.19)	15.89 ± 11.07	0.53 ± 0.10	29.55 ± 7.31	49.79 ± 2.48	9.52 ± 3.29^{b}	9.15 ± 2.99	86.74^{ab} (56.54 – 90.97)	-2.69 ± 0.13^{b}	20.02 ± 1.01	23.61^{b} (21.38 – 33.82)
1.4	219 ± 121	18.98 (8.55 – 33.38)	17.57 ± 17.27	0.54 ± 0.08	29.91 ± 6.75	52.50 ± 5.92	8.82 ± 4.36^{ab}	8.77 ± 4.65	87.55^{a} (81.44 – 92.97)	-2.87 ± 0.18^{ab}	19.75 ± 0.49	22.37^{ab} (19.97 – 24.63)
p	ns	ns	ns	ns	ns	ns	0.006**	ns	0.027*	0.018*	ns	0.029***

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=36).

BI, Browning index; Large size, Cell area > 10.0 (mm^2); Medium size, 3.0 < Cell area ≤ 10.0 (mm^2); ns, not significant; Small size, 0.2 < Cell area ≤ 3.0 (mm^2); Very small size, Cell area ≤ 0.2 (mm^2).

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey’s, Fisher’s or Duncan’s test, since homogeneity of variances was confirmed by Levene’s test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene’s test ($p < 0.05$).

*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn’s test.

11. Bread Descriptive Sensory Analysis

Several studies $^{(108, 174\text{-}177)}$ have shown that salt reduction has a negative impact on bread characteristics, which can potentially affect its sensory characteristics and, consequently on consumer's preferences.

Values for sensory analysis scores for each bread type with different salt addition levels are presented in Table 13. Overall, salt addition at different concentrations had limited impact on sensory evaluation of the different bread types. This was even more perceptible when comparisons were made with respective controls (1.4%). Considering appearance attributes, significant differences were only found for *number of large cells* in “Mistura” bread ($p = 0.040$) and *cell homogeneity* in “D’Água” bread ($p = 0.028$). For “Mistura” bread, the control had more large cells the 1.1%, whereas for “D’Água” bread cells distribution was less homogenous in control than for 0.0% and 0.8%. As for odor attribute, no significant differences were observed. With texture attributes, significant differences were detected for *crunchy crust* in “Carcaça” ($p = 0.020$) and “Mistura” ($p = 0.010$) breads, and *cohesiveness* in “D’Água” ($p = 0.005$). While the crust of control bread was crunchier than 0.8% and 1.0% in “Carcaça” bread, it was less crunchy than 0.0% and 1.3% in “Mistura” bread. Although with the same median values, the mean of ranking of *cohesiveness* was significantly higher for control than for 0.8% in “D’Água” bread, therefore control was more cohesive than 0.8%. Aroma attributes was the category where salt reduction had more impact, with significant differences found for salty in all bread types ($p < 0.001$), *sweet* in “Mistura” bread ($p = 0.005$) and *bread aroma* in “D’Água” ($p = 0.002$), in “Mistura” ($p < 0.001$), and in “Regueifa” ($p < 0.001$) breads. However control bread was perceived as saltier than: 0.0% for all breads, 0.8% for all breads, except “Carcaça”, and 1.0% for “D’Água” bread; it was less sweet than 0.0% in “Mistura” bread. Moreover, control breads had more bread aroma than 0.0%, except for “Carcaça” bread. Finally, for *overall assessment*, significant differences were found for all bread types (*p* = 0.005, “D’Água” bread; *p* < 0.001, “Carcaça”, “Mistura”, and Regueifa” breads). Breads without salt addition (0.0%) were less preferable than control in “D’Água”, “Mistura”, and Regueifa” breads, while this was observed with 1.1% for “Carcaça” bread.

Globally, the effect of salt reduction was not consistent across sensory characteristics evaluated by the trained panel evaluation, which makes comparison with literature difficult and meaningless.

When sensory profile was compared with image analysis, the sensory panel was not able to identify differences between control and breads with salt reduction for some parameters, including cell distribution as a function of their area and crumb color. Thus, data gathered from image analysis provided relevant information that would not be possible to obtain from sensory data.

Table 13. Values for sensory analysis scores for each bread type, with different salt addition levels (% bread).

Bread type/ Salt (%)	**Appearance**						**Odor**	**Texture**					**Aroma**				**Overall assessment**
	Crust color intensity	**Crumb color intensity**	**Number of large cells**	**Number of small cells**	**Cell circularity**	**Cell homogeneity**	**Odor intensity**	**Crunchy crust**	**Cohesiveness**	**Adhesiveness**	**Crumb elasticity**	**Shape recovery**	**Salty**	**Sweet**	**Bread aroma**	**Aftertaste**	
“D’Água”																	
0.0	4.0^{ab} (2.0 – 5.0)	3.0 (2.0 – 3.0)	6.0 (1.0 – 7.0)	4.0 (2.0 – 7.0)	3.0 (2.0 – 5.0)	3.0^{a} (1.0 – 5.0)	4.0 (3.0 – 6.0)	2.0 (1.0 – 5.0)	4.0^{ab} (2.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 7.0)	6.0 (5.0 – 6.0)	2.0^{a} (1.0 – 4.0)	2.0 (1.0 – 3.0)	4.5^{a} (2.0 – 5.0)	4.0 (2.0 – 5.0)	4.0^{a} (1.0 – 6.0)
0.8	4.0^{a} (3.0 – 6.0)	3.0 (2.0 – 4.0)	5.0 (1.0 – 6.0)	4.0 (2.0 – 7.0)	3.0 (3.0 – 5.0)	3.0^{a} (2.0 – 6.0)	4.0 (3.0 – 6.0)	2.0 (2.0 – 5.0)	4.0^{a} (2.0 – 4.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 7.0)	3.0^{b} (2.0 – 4.0)	2.0 (1.0 – 2.0)	5.0^{ab} (4.0 – 5.0)	4.0 (2.0 – 6.0)	5.0^{b} (4.0 – 6.0)
1.0	4.0^{ab} (3.0 – 6.0)	3.0 (2.0 – 4.0)	5.0 (3.0 – 7.0)	4.0 (4.0 – 6.0)	3.0 (2.0 – 4.0)	2.0^{ab} (1.0 – 4.0)	4.0 (3.0 – 6.0)	2.0 (1.0 – 5.0)	4.0^{ab} (2.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 7.0)	3.0^{bc} (2.0 – 4.0)	2.0 (1.0 – 3.0)	5.0^{ab} (2.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (3.0 – 6.0)
1.1	4.0^{ab} (3.0 – 5.0)	3.0 (2.0 – 3.0)	6.0 (2.0 – 7.0)	4.0 (2.0 – 6.0)	3.0 (2.0 – 5.0)	2.0^{b} (1.0 – 5.0)	4.0 (2.0 – 5.0)	2.0 (2.0 – 4.0)	4.0^{b} (2.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 6.0)	6.0 (3.0 – 7.0)	4.0^{cd} (3.0 – 5.0)	2.0 (1.0 – 3.0)	5.0^{ab} (4.0 – 5.0)	4.0 (3.0 – 4.0)	5.0^{b} (4.0 – 6.0)
1.3	4.0^{b} (2.0 – 5.0)	3.0 (2.0 – 3.0)	5.5 (2.0 – 6.0)	4.0 (3.0 – 7.0)	3.0 (2.0 – 5.0)	2.5^{ab} (2.0 – 5.0)	4.0 (3.0 – 5.0)	2.0 (1.0 – 4.0)	4.0^{ab} (3.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 7.0)	4.0^{bcd} (1.0 – 5.0)	2.0 (1.0 – 5.0)	5.0^{b} (2.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (1.0 – 7.0)
1.4	4.0^{ab} (2.0 – 6.0)	3.0 (2.0 – 3.0)	6.0 (2.0 – 7.0)	4.0 (3.0 – 6.0)	3.0 (2.0 – 6.0)	2.0^{b} (1.0 – 5.0)	5.0 (2.0 – 6.0)	3.0 (1.0 – 5.0)	4.0^{b} (2.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 5.0)	6.0 (5.0 – 6.0)	3.0^{d} (3.0 – 6.0)	2.0 (1.0 – 3.0)	5.0^{b} (4.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 7.0)
p	0.005^{*}	ns	ns	ns	ns	0.028^{**}	ns	ns	0.005^{*}	ns	ns	ns	$<0.001^{*}$	ns	0.002^{*}	ns	0.005^{*}
“Carcaça”																	
0.0	3.0^{ab} (2.0 – 4.0)	2.0^{a} (2.0 – 4.0)	2.0 (1.0 – 4.0)	6.0 (4.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (3.0 – 7.0)	5.0 (3.0 – 7.0)	2.0^{ab} (1.0 – 5.0)	3.0 (2.0 – 4.0)	2.0 (2.0 – 4.0)	3.0 (1.0 – 4.0)	6.0 (4.0 – 6.0)	2.0^{a} (1.0 – 6.0)	2.0 (1.0 – 3.0)	4.0^{a} (1.0 – 6.0)	5.0 (3.0 – 6.0)	3.5^{a} (1.0 – 5.0)
0.8	3.0^{a} (2.0 – 5.0)	2.0^{ab} (2.0 – 3.0)	2.0 (1.0 – 3.0)	6.0 (5.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (1.0 – 6.0)	5.0 (3.0 – 6.0)	3.0^{a} (1.0 – 5.0)	3.0 (2.0 – 4.0)	2.0 (2.0 – 5.0)	3.0 (2.0 – 4.0)	6.0 (4.0 – 6.0)	4.0^{ab} (2.0 – 5.0)	2.0 (1.0 – 3.0)	5.0^{ab} (3.0 – 5.0)	5.0 (3.0 – 6.0)	5.0^{bc} (3.0 – 6.0)
1.0	3.0^{b} (1.0 – 4.0)	2.0^{b} (1.0 – 2.0)	2.0 (1.0 – 4.0)	6.0 (5.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (2.0 – 6.0)	5.0 (4.0 – 7.0)	3.0^{a} (1.0 – 5.0)	3.0 (2.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 5.0)	6.0 (4.0 – 6.0)	4.0^{ab} (2.0 – 5.0)	2.0 (1.0 – 2.0)	5.0^{ab} (4.0 – 6.0)	5.0 (4.0 – 7.0)	5.0^{bc} (3.0 – 5.0)
1.1	3.0^{ab} (2.0 – 4.0)	2.0^{b} (1.0 – 2.0)	2.0 (1.0 – 4.0)	6.0 (6.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (3.0 – 6.0)	5.0 (4.0 – 6.0)	2.0^{b} (1.0 – 5.0)	3.0 (2.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 5.0)	6.0 (4.0 – 6.0)	4.0^{b} (3.0 – 6.0)	2.0 (1.0 – 3.0)	5.0^{b} (4.0 – 5.0)	5.0 (4.0 – 5.0)	5.0^{c} (5.0 – 6.0)
1.3	3.0^{ab} (2.0 – 6.0)	2.0^{ab} (1.0 – 2.0)	2.0 (1.0 – 4.0)	6.0 (5.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (2.0 – 6.0)	5.0 (3.0 – 6.0)	2.0^{b} (1.0 – 4.0)	3.0 (2.0 – 4.0)	2.0 (2.0 – 5.0)	3.0 (2.0 – 4.0)	6.0 (4.0 – 6.0)	4.0^{b} (3.0 – 5.0)	2.0 (1.0 – 3.0)	5.0^{b} (4.0 – 6.0)	5.0 (4.0 – 6.0)	5.0^{bc} (4.0 – 6.0)
1.4	3.0^{ab} (2.0 – 4.0)	2.0^{ab} (2.0 – 2.0)	2.0 (1.0 – 5.0)	6.0 (5.0 – 5.0)	4.0 (3.0 – 5.0)	4.0 (3.0 – 6.0)	5.0 (3.0 – 6.0)	2.0^{b} (1.0 – 4.0)	3.0 (2.0 – 4.0)	2.0 (2.0 – 5.0)	3.0 (2.0 – 4.0)	6.0 (4.0 – 6.0)	4.0^{b} (1.0 – 6.0)	2.0 (1.0 – 5.0)	5.0^{ab} (3.0 – 6.0)	5.0 (4.0 – 6.0)	4.0^{ab} (2.0 – 6.0)
p	0.016^{*}	0.008^{*}	ns	ns	ns	ns	ns	0.020^{**}	ns	ns	ns	ns	$<0.001^{*}$	ns	0.002^{*}	ns	$<0.001^{*}$
“Mistura”																	
0.0	6.0 (3.0 – 7.0)	5.0 (3.0 – 6.0)	4.0^{ab} (2.0 – 5.0)	5.0^{ab} (4.0 – 6.0)	4.0 (1.0 – 5.0)	3.0 (2.0 – 6.0)	4.0 (3.0 – 6.0)	4.0^{a} (2.0 – 6.0)	4.0 (3.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 5.0)	6.0 (4.0 – 7.0)	2.0^{a} (1.0 – 3.0)	1.0^{a} (1.0 – 3.0)	3.5^{a} (2.0 – 4.0)	4.0 (2.0 – 5.0)	4.0^{a} (2.0 – 6.0)
0.8	6.0 (3.0 – 7.0)	5.0 (2.0 – 6.0)	4.0^{abc} (2.0 – 5.0)	5.0^{a} (4.0 – 6.0)	4.0 (2.0 – 6.0)	3.0 (2.0 – 5.0)	4.0 (3.0 – 5.0)	3.0^{ab} (2.0 – 5.0)	4.0 (3.0 – 6.0)	2.0 (2.0 – 5.0)	3.0 (2.0 – 6.0)	6.0 (5.0 – 7.0)	3.0^{b} (2.0 – 4.0)	2.0^{ab} (1.0 – 3.0)	4.0^{ab} (2.0 – 4.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 6.0)
1.0	6.0 (4.0 – 7.0)	5.0 (3.0 – 5.0)	4.0^{bc} (2.0 – 6.0)	5.0^{ab} (4.0 – 6.0)	4.0 (2.0 – 5.0)	3.0 (2.0 – 5.0)	4.0 (2.0 – 6.0)	3.0^{ab} (2.0 – 6.0)	4.0 (3.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 6.0)	4.0^{bc} (3.0 – 5.0)	2.0^{ab} (1.0 – 3.0)	4.0^{b} (2.0 – 6.0)	4.0 (3.0 – 5.0)	5.0^{b} (3.0 – 7.0)
1.1	6.0 (3.0 – 6.0)	5.0 (2.0 – 2.0)	4.0^{a} (2.0 – 4.0)	5.0^{ab} (3.0 – 6.0)	4.0 (2.0 – 5.0)	3.0 (2.0 – 4.0)	4.0 (3.0 – 6.0)	3.0^{b} (2.0 – 5.0)	4.0 (3.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 5.0)	6.0 (4.0 – 6.0)	4.0^{bc} (3.0 – 5.0)	2.0^{b} (1.0 – 3.0)	4.0^{b} (3.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 6.0)
1.3	6.0 (3.0 – 7.0)	5.0 (2.0 – 6.0)	4.0^{c} (3.0 – 6.0)	5.0^{b} (2.0 – 6.0)	4.0 (3.0 – 5.0)	3.0 (2.0 – 4.0)	4.0 (3.0 – 6.0)	4.0^{a} (2.0 – 5.0)	4.0 (2.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 6.0)	6.0 (3.0 – 6.0)	4.0^{bc} (3.0 – 5.0)	2.0^{b} (1.0 – 3.0)	4.0^{b} (4.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 6.0)
1.4	6.0 (4.0 – 7.0)	5.0 (2.0 – 5.0)	4.0^{bc} (2.0 – 5.0)	5.0^{ab} (4.0 – 6.0)	4.0 (2.0 – 6.0)	3.0 (2.0 – 5.0)	4.0 (3.0 – 6.0)	3.0^{b} (2.0 – 4.0)	4.0 (3.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 6.0)	4.0^{c} (3.0 – 6.0)	2.0^{b} (1.0 – 3.0)	4.0^{b} (4.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 6.0)
p	ns	ns	0.040^{*}	0.026^{*}	ns	ns	ns	0.010^{**}	ns	ns	ns	ns	$<0.001^{*}$	0.005^{*}	$<0.001^{*}$	ns	$<0.001^{*}$
“Regueifa”																	
0.0	4.0^{a} (2.0 – 6.0)	1.0 (1.0 – 1)	2.0^{ab} (1 – 2)	5.0 (4.0 – 7.0)	3.0^{a} (2.0 – 6.0)	4.0^{ab} (2.0 – 7.0)	4.5 (2.0 – 6.0)	4.0 (2.0 – 5.0)	6.0 (2.0 – 7.0)	5.0 (1.0 – 7.0)	5.0 (3.0 – 5.0)	3.0^{ab} (1.0 – 5.0)	1.0^{a} (1.0 – 2.0)	2.0 (1.0 – 5.0)	3.0^{a} (2.0 – 5.0)	3.0 (1.0 – 4.0)	3.0^{a} (2.0 – 6.0)
0.8	4.0^{a} (2.0 – 5.0)	1.0 (1.0 – 2)	1.5^{a} (1 – 2)	6.0 (5.0 – 7.0)	3.0^{ab} (1.0 – 6.0)	5.0^{a} (4.0 – 7.0)	4.0 (2.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (2.0 – 7.0)	5.0 (1.0 – 6.0)	5.0 (2.0 – 6.0)	3.0^{bc} (1.0 – 5.0)	3.0^{b} (2.0 – 4.0)	2.0 (1.0 – 6.0)	4.0^{ab} (2.0 – 6.0)	2.0 (2.0 – 5.0)	6.0^{b} (4.0 – 7.0)
1.0	4.0^{ab} (2.0 – 6.0)	1.0 (1.0 – 2)	2.0^{c} (2 – 3)	5.0 (4.0 – 7.0)	2.0^{ab} (2.0 – 5.0)	4.0^{b} (2.0 – 5.0)	4.0 (3.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (4.0 – 7.0)	5.0 (3.0 – 6.0)	5.0 (3.0 – 5.0)	3.0^{abc} (2.0 – 5.0)	3.0^{bc} (2.0 – 4.0)	3.0 (1.0 – 5.0)	4.0^{b} (3.0 – 5.0)	2.0 (2.0 – 4.0)	6.0^{b} (4.0 – 6.0)
1.1	4.0^{a} (1.0 – 5.0)	1.0 (1.0 – 2)	2.0^{abc} (1 – 2)	5.0 (4.0 – 7.0)	2.0^{b} (1.0 – 5.0)	4.0^{ab} (3.0 – 7.0)	4.0 (3.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (2.0 – 7.0)	5.0 (2.0 – 5.0)	5.0 (2.0 – 5.0)	3.0^{c} (2.0 – 6.0)	3.0^{bc} (2.0 – 5.0)	3.0 (2.0 – 5.0)	4.0^{b} (3.0 – 5.0)	2.0 (2.0 – 4.0)	6.0^{b} (5.0 – 7.0)
1.3	4.0^{b} (3.0 – 6.0)	1.0 (1.0 – 2)	2.0^{bc} (1 – 3)	5.0 (4.0 – 7.0)	2.0^{ab} (2.0 – 6.0)	4.0^{b} (2.0 – 6.0)	4.0 (2.0 – 6.0)	3.0 (2.0 – 5.0)	6.0 (3.0 – 6.0)	5.0 (3.0 – 6.0)	5.0 (3.0 – 7.0)	4.0^{a} (2.0 – 5.0)	3.0^{bc} (2.0 – 5.0)	4.0 (2.0 – 5.0)	4.0^{b} (4.0 – 5.0)	3.0 (2.0 – 4.0)	6.0^{b} (5.0 – 7.0)
1.4	4.0^{ab} (2.0 – 6.0)	1.0 (1.0 – 1)	2.0^{abc} (1 – 2)	5.0 (4.0 – 7.0)	2.5^{ab} (1.5 – 6.0)	4.0^{ab} (4.0 – 7.0)	4.0 (2.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (3.0 – 6.0)	5.0 (3.0 – 6.0)	5.0 (2.0 – 6.0)	3.0^{ab} (2.0 – 5.0)	4.0^{c} (2.0 – 6.0)	3.0 (2.0 – 5.0)	4.0^{b} (3.0 – 5.0)	2.5 (2.0 – 5.0)	6.0^{b} (2.0 – 6.0)
p	0.048^{*}	ns	0.001^{*}	ns	0.039^{*}	0.003^{**}	ns	ns	ns	ns	ns	0.028^{*}	$<0.001^{**}$	ns	$<0.001^{**}$	ns	$<0.001^{*}$

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=24).
ns, not significant.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.
* *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene’s test ($p < 0.05$).
** *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn’s test.

11.1. Principal Component Analysis (PCA)

The results of PCA analysis are showed in Figure 4. In “D’água” bread, the PCA analyses accounted for 67.30% variance, where that first factor explains 47.84% and the second factor explains 19.46% of the total variance, as shown in Figure 4a). PCA suggests that most breads are not similar, once breads characterized for this factor (D’Água breads with 0.0% of salt (A0), 0.8% of salt (A0.8), 1.1% of salt (A1.1) and 1.4% of salt (A1.4)) are clearly separated. A0 was characterized by the attribute *crumb elasticity*, while A0.8 was characterized by the *number of small cells*, *adhesiveness* and *cell homogeneity*. *Number of large cells* and *cohesiveness* characterized A1.1, and *salty*, *bread aroma* and *aftertaste* characterized A1.4.

In “Carcaça” bread, first factor explains 47.25% of the total variance whereas second factor account 26.69% for the 73.94% total variance observed in Figure 4b). “Carcaça” breads with 0.0% of salt (C0), 1.1% of salt (C1.1), and 1.3% of salt (C1.3) showed more variance and characterized the first component. For C0, the most important attributes were *cell homogeneity* and *cell circularity*. C1.1 was characterized by the attributes *crumb elasticity*, *number of small cells* and *sweet*, whereas C1.3 was characterized by the attributes *salty*, *aftertaste* and *bread aroma*.

In “Mistura” bread, according Figure 4c), first and second components explain 45.11% and 22.18% of total variance, respectively. For this type bread, breads with 0.0% (M0), 1.3% (M1.3), 1.4% (M1.4) and 1.1% of salt (M1.1) characterized the first factor. M1.4 was characterized by attributes *salty*, *sweet* and *bread aroma*. M1.1 was characterized by attributes *color intensity*, *cell homogeneity* and *shape recovery*, whereas M0 and M1.3 was characterized by *crumb color intensity* and by *aftertaste*, respectively.

Regarding to “Regueifa” bread, first factor explains 46.83% and second factor explains 23.74% to the total variance. As shown Figure 4d), samples with 0.0% of salt (R0), 1.3% of salt (R1.3) and 1.4% of salt (R1.4) characterized the first component. R0 was characterized by *crunchy cell circularity*. The attributes *number of large cells*, *crumb elasticity*, *crust color* i*ntensity* and *aftertaste* characterize sample R1.3, whereas R1.4 was characterized by attributes *sweet*, *bread aroma*, *salty*, *cohesiveness* and *crumb color intensity*.

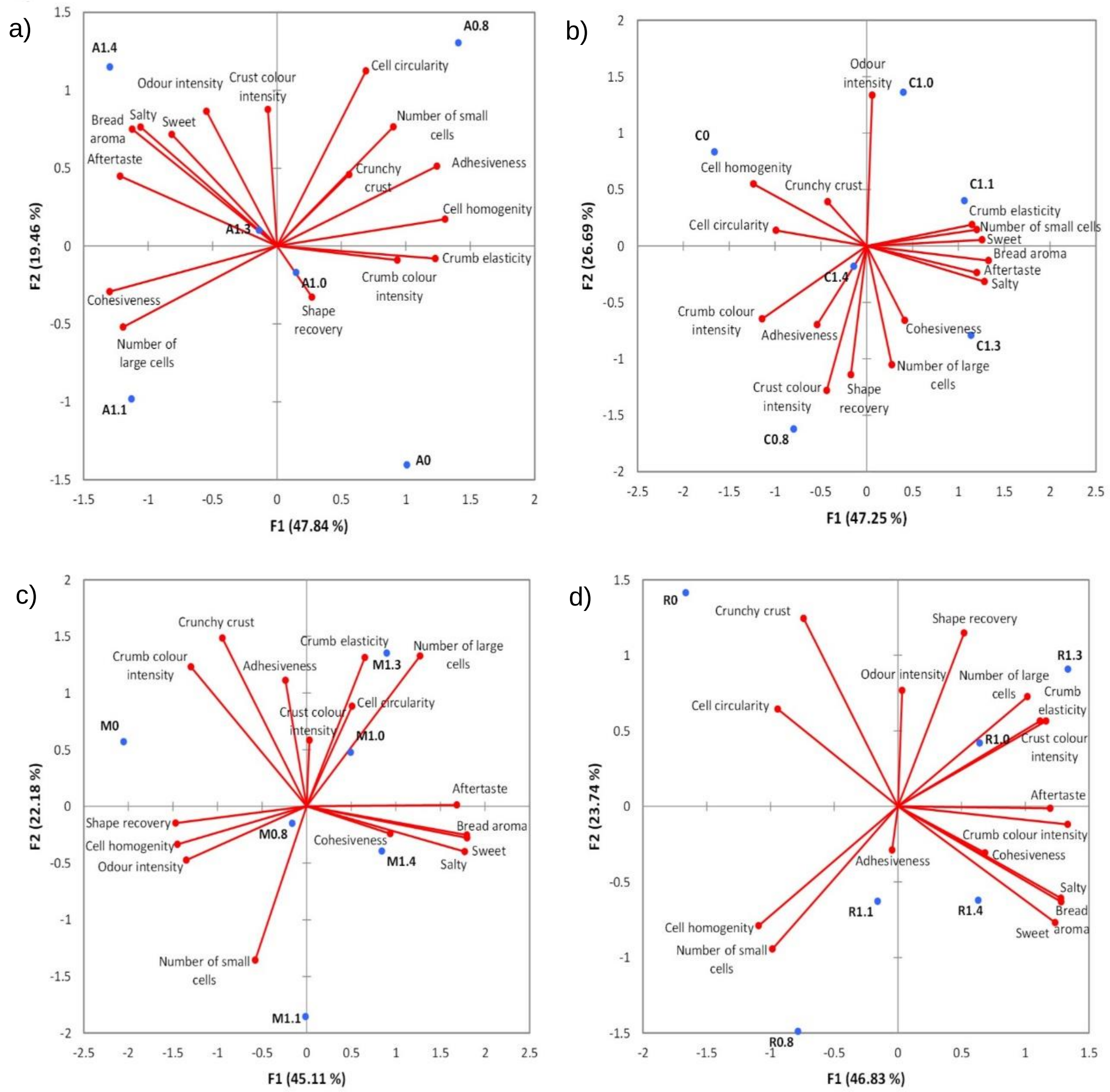


Figure 4. Principal component analysis of each bread type, with different salt addition levels (% bread), showing factors (F1 and F2), of attributes and samples. a) "D'Água" bread; b) "Carcaça" bread; c) "Mistura" bread; d) "Regueifa" bread.

A0, "D'Água" bread with 0.0% salt.; A0.8, "D'Água" bread with 0.8% salt; A1.0, "D'Água" bread with 1.0% salt; A1.1, "D'Água" bread with 1.1% salt; A1.3, "D'Água" bread with 1.3% salt; A1.4, "D'Água" bread with 1.4% salt; C0.0, "Carcaça" bread with 0.0% salt; C0.8, "Carcaça" bread with 0.8% salt; C1.0, "Carcaça" bread with 1.0% salt; C1.1, "Carcaça"" bread with 1.1% salt; C1.3, "Carcaça" bread with 1.3% salt; C1.4, "Carcaça" bread with 1.4% salt. M0.0, "Mistura" bread with 0.0% salt; M0.8, "Mistura" bread with 0.8% salt; M1.0, "Mistura" bread with 1.0% salt; M1.1, "Mistura" bread with 1.1% salt; M1.3, "Mistura" bread with 1.3% salt; M1.4, "Mistura" bread with 1.4% salt. R0.0, "Regueifa" bread with 0.0% salt; R0.8, "Regueifa" bread with 0.8% salt; R1.0, "Regueifa" bread with 1.0% salt; R1.1, "Regueifa" bread with 1.1% salt; R1.3, "Regueifa" bread with 1.3% salt; R1.4, "Regueifa" bread with 1.4% salt.

12. Bread Consumer Test

Consumer hedonic perception of salt reduced products is relevant. While the ability to identify differences among samples by trained assessors outperform consumers, they may be too conservative (178, 179). Thresholds estimated with trained assessors are based on differences that may not be relevant for consumers' liking preferences. The results obtained from the consumer test are shown in Table 14. Although significant differences were found between the different concentrations of salt for some liking attributes, they were not as evident when comparing to the control (1.4%). Apart from "Regueifa" bread, breads with 0.0% of salt were the only ones with significant lower scores, comparing to respective control. The lack of salt addition had a negative effect on the *overall liking*, for D'água" ($p = 0.002$), "Carcaça" ($p = 0.001$), and "Mistura" ($p < 0.001$) breads. This negative effect was also observable for other liking attributes, such as *appearance liking* ($p = 0.013$) and *texture liking* ($p < 0.001$) for "Carcaça" bread, and *taste liking* for both "Carcaça" ($p < 0.001$) and "Mistura" ($p < 0.001$). Overall, from consumer's point of view, only the lack of salt addition was relevant, which was more noticeable for "Carcaça" bread and less evident for "Regueifa" bread. These results are in agreement with what is described in literature that, even if perceived, small changes in the sensory characteristics of products do not significantly affect their hedonic perception (179).

Table 14. Overall liking attribute values from consumer acceptance testing for each bread type, with different salt addition levels (% bread) (n consumers=80).

Bread type/ Salt (%)	**Appearance liking**	**Aroma liking**	**Taste liking**	**Texture liking**	**Overall liking**
"D'Água" (n=20)					
0.0	5.0 (2.0 – 7.0)	4.0 (1.0 – 6.0)	2.0^{a} (1.0 – 6.0)	4.0 (1.0 – 7.0)	3.0^{a} (1.0 – 6.0)
0.8	5.0 (2.0 – 7.0)	5.0 (2.0 – 7.0)	4.5^{b} (2.0 – 7.0)	5.0 (2.0 – 7.0)	5.0^{b} (2.0 – 7.0)
1.0	5.0 (2.0 – 7.0)	5.0 (2.0 – 6.0)	4.5^{b} (1.0 – 7.0)	4.5 (1.0 – 7.0)	5.0^{b} (2.0 – 7.0)
1.1	5.0 (2.0 – 7.0)	4.5 (3.0 – 6.0)	5.0^{b} (2.0 – 6.0)	5.0 (4.0 – 7.0)	5.0^{b} (3.0 – 6.0)
1.3	5.0 (2.0 – 6.0)	4.5 (2.0 – 6.0)	5.0^{b} (2.0 – 7.0)	5.5 (2.0 – 7.0)	5.0^{b} (2.0 – 6.0)
1.4	5.0 (2.0 – 7.0)	4.5 (1.0 – 6.0)	4.0^{ab} (1.0 – 7.0)	5.0 (1.0 – 7.0)	5.0^{b} (1.0 – 7.0)
p	ns	ns	<0.001*	ns	0.002*
"Carcaça" (n=20)					
0.0	4.5^{a} (1.0 – 7.0)	4.0 (2.0 – 6.0)	3.0^{a} (1.0 – 6.0)	3.0^{a} (1.0 – 6.0)	3.0^{a} (1.0 – 7.0)
0.8	5.5^{ab} (2.0 – 7.0)	5.0 (3.0 – 7.0)	5.0^{b} (1.0 – 7.0)	5.0^{b} (3.0 – 7.0)	5.0^{b} (3.0 – 7.0)
1.0	5.5^{ab} (2.0 – 7.0)	5.0 (3.0 – 7.0)	5.0^{b} (2.0 – 7.0)	5.0^{b} (3.0 – 7.0)	5.0^{b} (3.0 – 7.0)
1.1	6.0^{b} (2.0 – 7.0)	5.0 (3.0 – 6.0)	5.5^{b} (3.0 – 7.0)	5.0^{b} (2.0 – 6.0)	5.0^{b} (2.0 – 6.0)
1.3	6.0^{b} (3.0 – 7.0)	5.0 (1.0 – 7.0)	5.0^{b} (1.0 – 7.0)	4.0^{b} (1.0 – 7.0)	5.0^{b} (1.0 – 7.0)
1.4	6.0^{b} (4.0 – 7.0)	5.5 (2.0 – 7.0)	5.0^{b} (1.0 – 7.0)	6.0^{b} (1.0 – 7.0)	5.5^{b} (3.0 – 7.0)
p	0.013*	ns	<0.001*	<0.001*	0.001*
"Mistura" (n=20)					
0.0	5.0 (2.0 – 7.0)	5.0 (2.0 – 6.0)	3.0^{a} (2.0 – 5.0)	4.5 (2.0 – 7.0)	4.0^{a} (2.0 – 6.0)
0.8	6.0 (4.0 – 7.0)	6.0 (4.0 – 7.0)	6.0^{b} (5.0 – 7.0)	6.0 (3.0 – 7.0)	6.0^{b} (4.0 – 7.0)
1.0	6.0 (4.0 – 7.0)	6.0 (4.0 – 7.0)	5.5^{b} (3.0 – 7.0)	6.0 (3.0 – 7.0)	5.5^{b} (4.0 – 7.0)
1.1	6.0 (4.0 – 7.0)	6.0 (4.0 – 7.0)	6.0^{b} (3.0 – 7.0)	6.0 (3.0 – 7.0)	6.0^{b} (4.0 – 7.0)
1.3	6.0 (2.0 – 7.0)	6.0 (4.0 – 7.0)	6.0^{b} (4.0 – 7.0)	5.5 (3.0 – 7.0)	5.5^{b} (4.0 – 7.0)
1.4	6.0 (4.0 – 7.0)	5.0 (4.0 – 7.0)	6.0^{b} (4.0 – 7.0)	6.0 (3.0 – 7.0)	6.0^{b} (4.0 – 7.0)
p	ns	ns	<0.001*	ns	<0.001*
"Regueifa" (n=20)					
0.0	6.0 (3.0 – 7.0)	5.0 (2.0 – 7.0)	3.0^{a} (1.0 – 6.0)	5.0 (2.0 – 7.0)	4.0^{a} (2.0 – 6.0)
0.8	5.0 (4.0 – 6.0)	5.0 (3.0 – 7.0)	6.0^{b} (3.0 – 7.0)	6.0 (4.0 – 7.0)	6.0^{b} (4.0 – 6.0)
1.0	5.5 (3.0 – 7.0)	5.0 (3.0 – 7.0)	5.5^{b} (4.0 – 7.0)	5.0 (3.0 – 7.0)	5.0^{ab} (3.0 – 7.0)
1.1	5.5 (3.0 – 7.0)	5.5 (3.0 – 7.0)	5.5^{b} (2.0 – 7.0)	5.5 (3.0 – 7.0)	5.0^{ab} (3.0 – 7.0)
1.3	5.5 (3.0 – 7.0)	5.0 (4.0 – 7.0)	5.0^{b} (2.0 – 7.0)	6.0 (3.0 – 7.0)	6.0^{b} (4.0 – 7.0)
1.4	6.0 (3.0 – 7.0)	5.0 (3.0 – 7.0)	4.5^{ab} (2.0 – 7.0)	5.0 (2.0 – 6.0)	5.0^{ab} (3.0 – 6.0)
p	ns	ns	<0.001*	ns	0.005*

Data expressed as median (minimum−maximum), (n=80).
ns, not significant.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.
* *p* Values from one-way ANOVA analysis. Means were compared by Tukey's, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).
** *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).
*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn's test.

13. Application of sensometric tools

13.1. Consumers: relationship liking attributes and overall liking

Multivariate PLS regression was performed in order to study the correlation between consumer liking attributes (x-variables) and overall liking (y-variables) for each bread type. For this regression, only liking attributes with significant differences were considered (according Table 14). Table 15 summarizes the prediction models of consumer liking attributes. Regression models were considered successful when R^2X and $R^2Y \geq 0.70$, and as presenting good ability to predict new samples when $Q^2 \geq 0.50$. According table 15, it was possible to observe that good regression models, with good predictive ability were found for all bread types. *Taste liking* appears to have a significant positive impact for *overall liking* for every bread type.

Table 15. Results of multivariate PLS regression, between consumer liking attributes (X-variables) and overall liking (Y-variables) for each bread type, with different salt addition levels (% bread) to identify attributes that were related positively with product acceptance.

Bread type	R^2X	R^2Y	Q^2	RSME	Latent variables[1]
"D'Água"	1.000	0.933	0.896	0.149	Taste liking (+)
"Carcaça"	0.995	0.993	0.987	0.053	Texture liking (+) Taste liking (+) Appearance liking (+)
"Mistura"	1.000	0.967	0.953	0.107	Taste liking (+)
"Regueifa"	1.000	0.913	0.901	0.136	Taste liking (+)

[1] Moderately and highly influential latent variables were only considered for good models; (+), positive correlation with Y-variable; (-), negative correlation with Y-variable.

Q^2, cumulative predictive variation from internal cross-validation; R^2X, cumulative explained variation of X explained in terms of sum of squares; R^2Y, cumulative explained variation of Y explained in terms of sum of squares; RMSE, Root mean square error.

13.2. Relationship between sensory parameters and overall liking

Table 16 shows the results obtained for multivariate PLS regression between sensory parameters from trained panel (X-variables) and *overall liking* from consumer test (Y-variables), for each bread type. Regression models were considered successful when R^2X and $R^2Y \geq 0.70$, and as presenting good ability to predict new samples when $Q^2 \geq 0.50$.

Different sensory attributes were related positively with *overall liking* by consumers and included aroma, appearance, taste and texture attributes.

Nevertheless, *bread aroma*, *salty* and *crumb color intensity* were important in every bread type. The regression models obtained for “D’água” bread, “Mistura” bread, and “Regueifa” bread, successfully fitted X-variables and Y-variables data ($R^2X \geq 0.70$ and $R^2Y \geq 0.70$), presented a good predictive ability ($Q^2 > 500$) and low RMSE values. Furthermore, “Mistura” bread was the bread type with lowest RMSE (0.005) and best regression models ($R^2X = 0{,}887$ and $R^2Y = 1{,}000$), which could indicate that sensory attributes have more impact on *overall linking* for consumers for this bread type.

Regarding “Carcaça” bread, regression model had a poor fitting for the X-variables ($R^2X < 0.70$), which could indicate that, for this type bread, the sensory attributes were not relevant for consumer acceptance.

Table 16. Results of multivariate PLS regression, between sensory parameters (X-variables) and overall liking (Y-variables) for each bread type, with different salt addition levels (% bread) to identify attributes that were related positively with product acceptance.

Bread type	R^2X	R^2Y	Q^2	RSME	Sensory attributes positively related with product acceptance
“D’Água”	0.884	0.995	0.926	0.028	Bread aroma Salty Sweet Shape recovery Crumb elasticity Crumb color intensity
“Carcaça”	0.520	0.820	0.575	0.170	Crumb color intensity Bread aroma Aftertaste Salty Sweet Cell homogeneity Number of small cells Crumb elasticity Cohesiveness
“Mistura”	0.887	1.000	0.995	0.005	Sweet Bread aroma Salty Shape recovery Crumb color intensity Cell homogeneity Aftertaste Crunchy crust Cohesiveness
“Regueifa”	0.885	1.000	0.996	0.001	Sweet Bread aroma Salty Cell circularity Crunchy crust Crumb color intensity

Q^2, cumulative predictive variation from internal cross-validation; R^2X, cumulative explained variation of X explained in terms of sum of squares; R^2Y, cumulative explained variation of Y explained in terms of sum of squares; RMSE, Root mean square error.

14. External Preference Mapping

External preference mapping includes three sequential steps: creates the sensory map, groups the consumers and creates the preference map using PREFMAP method. PCA was applied on sensory data of attributes evaluated by the trained panel for create the sensory map (as discussed in previous section). Considering *overall liking* attribute, consumers were grouped into homogeneous groups according to their preference, using Agglomerative Hierarchical Clustering (AHC). PREFMAP method was employed using the sensory attribute coordinates in the two-dimensional facto space, resulting from PCA, and average *overall liking* scores for each 3 clusters, obtained from AHC. As result, four different regression models were tested to predict each consumer group *overall liking*: vector model, circular model, elliptical model and quadratic surface method (180).

For each bread type, the resulting preference map (see Figure 5) shows the best fitting model for each cluster and consumers preference. For "D'Água" bread (Figure 5a), the vector model was the best fit for cluster 1 (C1) and cluster (3) but only significant (p = 0.097) for C1, while elliptical model was the best (p = 0.094) for clusters 2 (C2). In C1 and C3, the vector indicated the direction in the map where *overall liking* increased. In C1, the preference order was A1.4 > A0.8 > A1.3 > A1.0 > A1.1 > A0.0, while in C3 was A1.4 > A1.1 > A1.3 > A1.0 > A0.8 > A0.0. The elliptical model for C2 showed a saddle point, where the thicker lines indicated the direction in which *overall liking* increased, and the thinner ones to the direction in which it decreased. Here, the preference order was A1.1 > A0.8 > A1.0 > A1.3 > A1.4 > A0.0. The best fitting models for "Carcaça" bread (Figure 5b) were the elliptical for C1 (p = 0.089), circular for C2 (p = 0.069), and vector for C3 (p = 0.078).

In C1, the preference order was C1.4 > C1.0 > C1.3 > C1.1 > C0.8 > C0.0. The circular model for C2 showed a maximum in terms of preference, known as the ideal point, with circular lines of isopreference drawn around it. Here, the preference order was C1.4 > C0.8 > C1.1 > C1.3 > C1.0 > C0.0. For C3, preference order was C0.8 > C0.0 > C1.4 > C1.3 > C1.0 > C1.1. Considering “Mistura” bread (Figure 5a), the vector model was the best fit for C1 (*p* = 0.020) and C2 (*p* = 0.071), while circular was the best for C3 (*p* = 0.025). The preference order for this bread was M1.4 > M1.1 > M1.3 > M1.0 > M0.8 > M0.0 for C1 and C2, and M0.8 > M1.0 > M1.4 > M1.1 > M1.3 > M0.0 for C3. As for “Regueifa” bread, the vector model was the best fit for all clusters, but they were not significant (*p* > 0.100). The preference order for the different clusters was R1.4 > R1.3 > R0.8 > R1.1 > R1.0 > R0.0 for C1; R0.8 > R1.4 > R1.1 > R1.0 > R1.3 > R0.0 for C2; and R1.4 > R1.3 > R1.0 > R1.1 > R0.8 > R0.0 for C3. Finally, with information gathered from this analysis it was possible to establish the lowest salt concentration with better percentage of satisfied assessors (Figure 5) namely: 0.8% for “D’Água” bread (67% of satisfied assessors) 0.8% for “Carcaça” bread (100% satisfied assessors); 1.0% for “Mistura” bread (100% satisfied assessors); and 1.1% for “Regueifa” bread (100% of satisfied assessors).

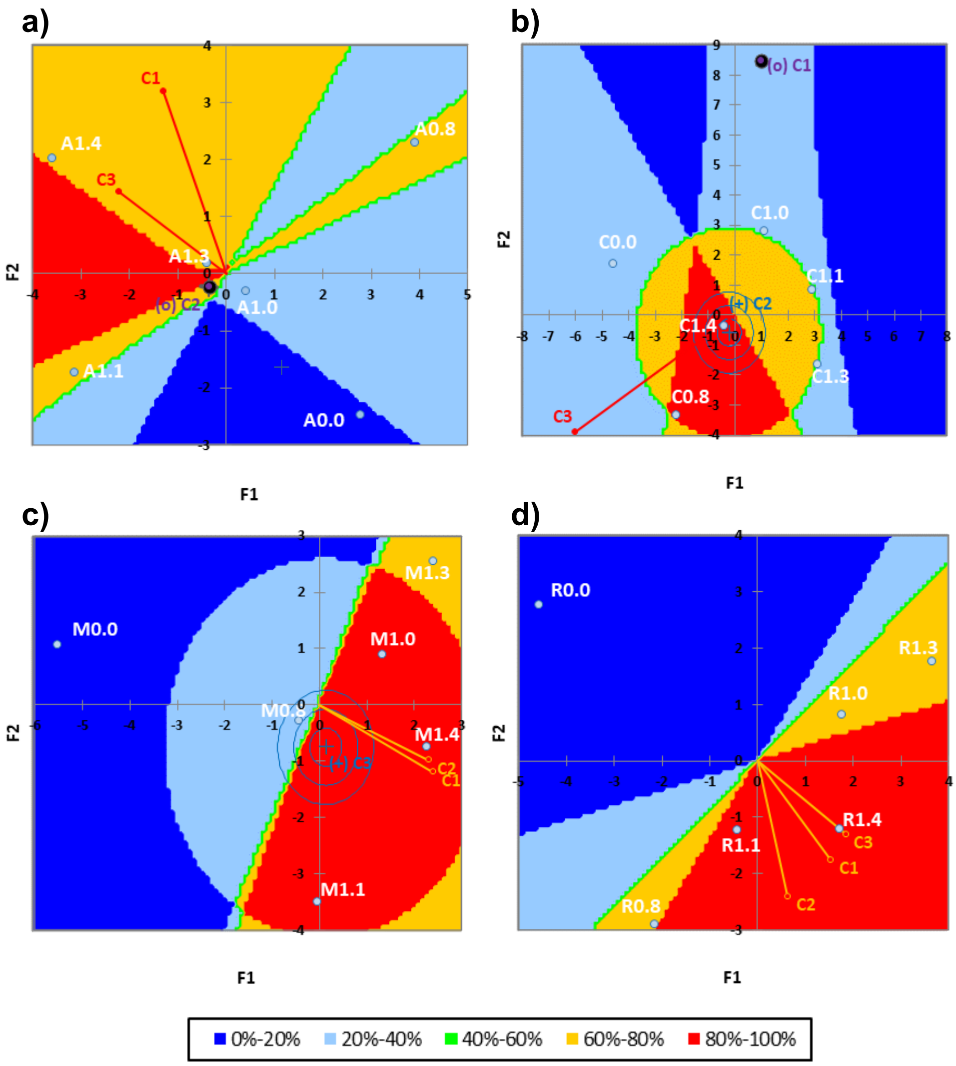


Figure 5. External preference mapping for a) “D’Água”, b) “Carcaça”, c) “Mistura”, and d) “Regueifa” breads. 3 clusters are illustrated: 1 and 3 (vector), and 2 (elliptical (○); where the circle indicates a point of low variability in preference, located immediately before a decrease or increase in preference area) and the 5 regions of the global average value of acceptance.

0.0, Breads with 0.0% salt; 0.8, Breads with 0.8% salt; 1.0, Breads with 1.0% salt; 1.1, Breads with 1.1% salt; 1.3, Breads with 1.3% salt; 1.4, Breads with 1.4% salt; A, “D’Água” bread; C, “Carcaça”; C1, Cluster 1; C2, Cluster 2; C3, Cluster 3; M, “Mistura”; R, “Regueifa”.

15. Correlation of sensory characteristics with physicochemical, crumb structure and color parameters

PLS regression model quality was performed to establish a simultaneous correlation between sensory attributes and analytical parameters: physicochemical parameters (weight, salt, moisture, and specific volume), crumb structure (*cell density*; *number of cells*; *mean area*; *circularity*; *very small size*, *small size*, *medium size*, and *large size cells*) and color (*L**, *a**, *b**, and *BI*). This model is based on sensory data prediction (Y-variables) from analytical parameters data (X-variables). For a successful regression model, the values obtained for R^2Y and R^2X must be equal or superior to 0.7 and the prediction ability is achieved by Q^2 values, which must be equal or superior to 0.5.

Table 17 summarizes individual sensory attributes prediction models from analytical parameters. Of the seventeen sensory attributes analyzed, nine were found to be correlated with analytical parameters. Overall, models with good predictive quality were obtained for *crust color intensity*, *crumb color intensity*, *cohesiveness*, *adhesiveness*, *crumb elasticity*, *shape recovery*, *salty*, *bread aroma*, and *aftertaste*. These results indicate that, at some extent, assessors were able to evaluate the parameters evenly, regardless of the type of bread analyzed. As for the attributes with lower quality values, they can be explained by a dispersion in the results, which may indicate that these parameters were considered in different ways for each type of bread. Consequently, the mathematical base cannot produce a model with good predictive quality.

The importance of X-variables (analytical parameters) in the projection and their correlation with Y-variables (sensory attributes) was also determined, and latent variables were identified (Table 17). Moreover, analytical parameters common in regression models with good performance for each group of sensory attributes were identified. Regarding appearance attributes, crust and crumb color were more intense for redder and browner breads, and less intense for darker breads. As for appearance attributes related to crumb structure, models were poorly fitted (R^2Y and $R^2X < 0.50$), and with poor ($0.00 < Q^2 < 0.50$) or lacking ($Q^2 < 0.00$) predictive ability. Odor and texture characteristics were not evaluated analytically, and therefore, it would be less likely to find correlations or successful predictive model for these parameters. Although models for *odor intensity* and *crunchy crust* models were poorly fitted ($R^2X < 0.50$) and with poor ($0.00 < Q^2 < 0.50$) predictive ability, it was possible to find good predictive models for other texture attributes, namely *cohesiveness*, *adhesiveness*, *crumb elasticity* and *shape recovery*. Lighter and heavier breads were the ones with higher *cohesiveness*, *adhesiveness*, and *crumb elasticity*, but also with lower *shape recovery*. Moreover, breads with higher percentage of large size cells were less cohesive, adhesive, and with lower *crumb elasticity*. Regarding aroma sensory attributes, breads with higher specific volume were saltier and with higher bread aroma and aftertaste. Furthermore, it was interesting to observe that saltier breads were the ones with higher salt concentration, as it would be expected. As regards to sensory attribute *overall assessment*, no association models with good fitting and predictive ability were found. This sensory attribute, unlike others, is more susceptible to a subjective evaluation and therefore, is a more difficult to standardize. Globally, these results could be expected at some extent; nevertheless, it is important to highlight that they show associations and not cause-effect relationships.

Table 17. Results of PLS regression between bread analytical parameters (X-variables) and sensory attributes (Y-variables) for all bread formulations.

Sensory attributes	Q^2	R^2Y	R^2X	**RSME**	**Latent variables[1]**
Appearance					
Crust color intensity	0.836	0.929	0.808	0.225	**L*(-)**; **a*(+)**; **b*(+)**; **BI (+)**; Weight (+); **Specific volume (-)**
Crumb color intensity	0.925	0.986	0.862	0.097	**Circularity (+)**; **L*(-)**; **a*(+)**; **BI (+)**
Number of large cells	0.415	0.579	0.406	0.597	-
Number of small cells	0.114	0.676	0.568	0.547	-
Cell circularity	0.465	0.593	0.332	0.558	-
Cell homogenity	-0.451	0.841	0.601	0.314	-
Odor					
Odor intensity	0.296	0.888	0.531	0.353	-
Texture					
Crunchy crust	0.212	0.773	0.121	0.441	-
Cohesiveness	0.733	0.951	0.795	0.337	**Number of cells (-)**; **Cell density (-)**; **Small size(+)**; **Large size (-)**; Moisture (+); **L*(+); Weight (+)**; **Specific volume (-)**
Adhesiveness	0.879	0.949	0.817	0.222	**Large size (-)**; **L*(+)**; a*(-); **BI (-)**; **Weight (+)**;
Crumb elasticity	0.753	0.958	0.789	0.198	**Number of cells (-)**; **Cell density (-)**; **Large size (-)**; **L*(+)**; **a*(-)**; **BI (-)**; **Weight (+)**;
Shape recovery	0.805	0.945	0.767	0.249	Small size (-); **L*(-)**; b*(-); BI (+); **Weight (-)**; **Specific volume (+)**
Aroma					
Salty	0.618	0.958	0.854	0.242	**Moisture (-)**; **Specific volume (+); Salt (+)**
Sweet	0.156	0.837	0.880	0.239	-
Bread aroma	0.698	0.898	0.916	0.341	**Moisture (+)**; **Weight (-)**; **Specific volume (+)**
Aftertaste	0.628	0.940	0.762	0.290	Number of cells(+); Cell density(+); **L*(-)**; **Weight (-)**; **Specific volume (+)**
Overall assessment	0.067	0.415	0.250	0.720	-

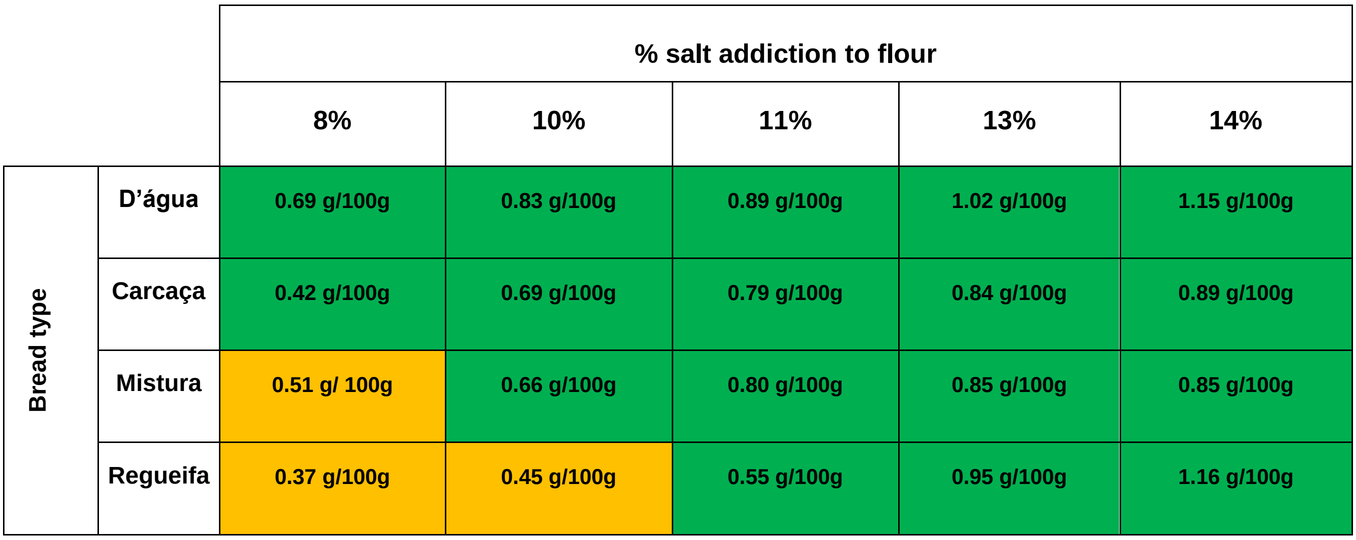
[1] Latent variables with significant weight in the model and correlation with Y-variable; highly influential latent variables (variable importance for the projection>1) are represented in bold and the remaining are moderately influential latent variables (0.8<variable importance for the projection<1). (+), positive correlation with Y-variable; (-), negative correlation with Y-variable. Latent variables were only considered for good models

BI, Browning index; Large size, Cell area > 10.0 (mm^2); Medium size, 3.0 < Cell area ≤ 10.0 (mm^2); ns, not significant; Q2, cumulative predictive variation from internal cross-validation; R2X, cumulative explained variation of X explained in terms of sum of squares; R2Y, cumulative explained variation of Y explained in terms of sum of squares; RMSE, Root mean square error; Small size, 0.2 < Cell area ≤ 3.0 (mm2); Very small size, Cell area ≤ 0.2 (mm2).

16. The Guide: a proposal

Based on the values estimated in the sodium analysis by flame photometry and in the results obtained from External Preference Mapping, a guide is proposed in order to help bakery professionals.

The guide, in the table form, use data from external preference mapping that indicate the lowest salt concentration with better percentage of satisfied assessors and the percentage of salt that bakery professionals could use when are making bread. In practical terms, bakery professionals could see in first line de % of salt (8%, 10%, 11% and 14%) that could use in bread recipe to obtain a final product with estimated salt concentration and with estimated potential risk to affect consumer satisfaction according to our laboratorial results. Additionally, the guide presents, through color signaling, the limits of lowest salt addition without potential impact on consumer preference. Thus, green color means that it is possible to lower the salt level without potential negative impact on consumer satisfaction, while yellow color represents the level of salt from which there is a potential risk to affect consumer satisfaction.



		% salt addiction to flour				
		8%	10%	11%	13%	14%
Bread type	D’água	0.69 g/100g	0.83 g/100g	0.89 g/100g	1.02 g/100g	1.15 g/100g
	Carcaça	0.42 g/100g	0.69 g/100g	0.79 g/100g	0.84 g/100g	0.89 g/100g
	Mistura	0.51 g/ 100g	0.66 g/100g	0.80 g/100g	0.85 g/100g	0.85 g/100g
	Regueifa	0.37 g/100g	0.45 g/100g	0.55 g/100g	0.95 g/100g	1.16 g/100g

Data expressed as median

🟩 Without potential risk to affect consumer satisfaction 🟨 With potential risk to affect consumer satisfaction

Figure 6. Guide proposal

Chapter IV

Publications

Poster

Impact of salt reduction on bread physicochemical properties and crumb color and structure (see annex 7)

C. Monteiro, Z. E. Martins, O. Pinho, C. Gonçalves

2nd prize in XVII Congress of Food and Nutrition & I International Congress of Food and Nutrition (see annex 7)

Impact of salt reduction on bread physicochemical properties and crumb colour and structure.

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Introduction: Bread is a staple component of the Portuguese diet but is also a major source of dietary salt. Many countries have chosen bread as one of the priority foods for reducing salt content in their national salt reduction initiatives, including Portugal. However, salt has specific properties that are essential for bread processing and quality in the final product.

Objectives: This study aimed to evaluate the impact of salt reduction on bread physicochemical parameters, as well as crumb colour and structure.

Methods: Four types of bread formulations were tested: “D’água”, “Carcaça”, “Mistura”, and “Regueifa”, produced with different salt concentrations (0.0%, 0.8%, 1.0%, 1.1%, 1.3%, and 1.4% of salt per wheat flour). Bread physicochemical characteristics evaluated included weight, volume, moisture and salt content. To study bread crumb characteristics (colour and structure), a single 200x200 pixel field of view (FOV) was cropped from each bread slice. The FOV was then: (i) converted to CIElab system; (ii) converted to a 256 level grey scale and segmented and cell morphological parameters were analysed. Statistical comparison was performed using as control bread with 1.4% of salt (legal value allowed).

Results: In general, salt reduction had significant impact on every bread type for moisture (“D’água”, $p<0.001$; “Carcaça”, $p<0.001$; “Mistura”, $p=0.010$; and “Regueifa”, $p<0.001$) and salt content (“D’água”, $p<0.001$; “Carcaça”, $p<0.001$; “Mistura”, $p<0.001$; and “Regueifa”, $p<0.001$). Regarding bread specific volume, no

significant differences ($p>0.050$) were found for “Regueifa” bread (“D’água”, $p=0.033$; “Carcaça”, $p<0.001$; and “Mistura”, $p<0.001$). Salt reduction had limited influence on crumb morphology, except for cell area distribution (“D’água”, $p=0.003$; “Carcaça”, $p=0.047$; and “Regueifa”, $p=0.006$), but more impact on colour redness/greenness (“D’água”, $p=0.014$; “Carcaça”, $p<0.001$; “Regueifa”, $p=0.047$; and “Mistura”, $p=0.018$).

Conclusions: The results suggest that it is possible to reduce, to some extent, the salt concentration in all bread types without major impact on bread characteristics.

Oral communication

Sensory preference as a valuable tool to establish salt reduction in bread

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Sensory preference as a valuable tool to establish salt reduction in bread.

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Introduction: Bread is a staple food and one of the major contributors to dietary salt intake. Salt has impact on bread sensory properties as it acts as flavour modifier, and influences crust development and crumb structure. While reduction of salt content in bread is paramount, it may compromise bread organoleptic characteristics and sensory acceptance by consumers.

Objectives: The major goal of this study was to evaluate the impact of salt reduction on bread sensory evaluation, and select the reduction levels with best consumers' acceptance.

Methods: Four types of bread formulations were tested: "D'água", "Carcaça", "Mistura", and "Regueifa", produced with different salt concentrations (0.0%, 0.8%, 1.0%, 1.1%, 1.3% and 1.4% of salt per wheat flour). A sensory panel composed by 8 members was trained for descriptive analysis according to the guidelines in the ISO 8586 (2012). Sensory acceptability tests were carried out, with 80 non-trained members. Statistical models for sensory preference evaluation were developed using External Preference Mapping. Statistical comparison was performed using as control bread with 1.4% of salt (legal value allowed).

Results: Overall, salt reduction had limited impact on sensory evaluation. "Overall assessment" presented significant differences for "D'água" (p=0.005), "Carcaça" (p<0.001), and "Mistura" (p<0.001) breads. Results obtained from the consumer test

only showed significant differences for salt reduction with “Taste liking” (“D’água”, $p<0.001$; “Carcaça”, $p<0.001$; “Mistura”, $p<0.001$; and “Regueifa”, $p<0.001$) and “Overall linking” (“D’água”, $p=0.002$; “Carcaça”, $p=0.001$; “Mistura”, $p<0.010$; and “Regueifa”, $p=0.005$) attributes. External preference mapping indicated consumer preferences and enabled selection of the lowest salt concentration with best acceptance, namely 0.8% for “D’Água” bread; 0.8% for “Carcaça” bread; 1.0% for “Mistura” bread; and 1.1% for “Regueifa” bread.

Conclusions: The results of the present study indicate that it is possible reduce salt concentration in all bread types compared to the amount regulated (1.4%) without compromising consumers’ acceptance.

Article

Impact on sensory preference and physiochemical parameters of salt reduction on bread

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Impact on sensory preference and physicochemical parameters of salt reduction on bread

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[submitted for publication]

Abstract

Bread is one of the major contributors to dietary salt intake, and is often a priority food in national salt reduction initiatives. Salt reduction in bread may influence its organoleptic characteristics that will affect sensory acceptance by consumers. Four Portuguese types of bread formulations were tested: “D’água”, “Carcaça”, “Mistura”, and “Regueifa”, produced with different salt concentrations (0.0%, 0.8%, 1.0%, 1.1%, 1.3%, and 1.4% of salt per wheat flour). The impact of salt reduction was evaluated on bread physicochemical parameters weight, volume, moisture and crumb colour and structure. Sensory evaluation was also performed, and relationship between sensory attributes and colour and crumb structure were further evaluated.

For bread weight, no significant results were found, except for “Carcaça” bread (p = 0.002), but without a linear pattern. Specific volume seemed to increase with increasing added salt, although no linear pattern ($p > 0.050$) was observed, except for “Carcaça” bread ($R^2 = 0.953$, $p = 0.001$). Moisture content seemed to decrease as the level of added salt increased ($p < 0.050$). No linear relationship ($R^2 < 0.700$, $p > 0.050$) with salt addition levels was observed for any of the crumb morphology or colour parameters studied.

In general, salt reduction had significant impact on every bread type for moisture (“D’água”, “Carcaça”, and “Regueifa”, $p < 0.001$; and “Mistura”, $p = 0.010$) and salt content (“D’água”, “Carcaça”, “Mistura”, and “Regueifa”, $p < 0.001$). Regarding bread specific volume, no significant differences ($p > 0.050$) were found for “Regueifa” bread (“D’água”, $p = 0.033$; “Carcaça” and “Mistura”, $p < 0.001$). Salt reduction had limited influence on crumb morphology, except for cell area distribution (small size cells: “D’água”, $p = 0.003$. Very small size cells: “Carcaça”, $p = 0.047$), but more impact on colour parameters (L*: “D’Água”, $p = 0.006$; “Regueifa”, $p = 0.027$. a*: “Carcaça”, p <

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0.001; “Mistura”, $p = 0.047$; b*: “Carcaça”, $p < 0.001$. BI: “D’Água”, $p = 0.002$; “Regueifa”; $p < 0.001$; “Carcaça”, $p = 0.029$).

Salt reduction also had limited impact on sensory evaluation. *Overall assessment* presented significant differences for all bread types (“D’Água”, $p = 0.005$; “Carcaça”, “Mistura”, and Regueifa”, $p < 0.001$). Results obtained from the consumer test only showed significant differences for salt reduction with *taste liking* (“D’água, “Carcaça”, “Mistura”, and “Regueifa”, $p < 0.001$), *texture liking* (“Carcaça”, $p < 0.001$), and *overall linking* (“D’água”, $p = 0.002$; “Carcaça”, $p = 0.001$; “Mistura”, $p < 0.010$; and “Regueifa”, $p = 0.005$) attributes. External preference mapping indicated consumer preferences and enabled selection of the lowest salt concentration with best acceptance, namely 0.8% for “D’Água” bread; 0.8% for “Carcaça” bread; 1.0% for “Mistura” bread; and 1.1% for “Regueifa” bread.

The results suggest that it is possible to reduce the salt concentration in all bread types analysed without major impact on bread characteristics and without compromising consumers’ acceptance.

Key words – bread; salt; sensory analysis; image analysis; external preference mapping.

1. Introduction

High sodium intake has been widely pointed as the most important factor for high blood pressure, that's closely associated to increased risk of cardiovascular diseases and stroke (Chockalingam, Campbell, & Fodor, 2006; F. J. He et al., 2009; Poggio et al., 2015). Based on these facts, the World Health Organization (WHO), recommended a reduction of sodium intake to <2000 mg per day, approximately 5 g of salt per day (WHO, 2012). However, estimates of salt intake of the global level far exceed the recommendations (Brown, Tzoulaki, Candeias, & Elliott, 2009; Powles et al., 2013). In Portugal, mean salt intake in adult population is approximately twice as high as recommendations (Polónia J et al., 2006; Polonia, Martins, Pinto, & Nazare, 2014).

Bread is a staple food of the diet (Z. E. Martins, Pinho, & Ferreira, 2017; Quilez & Salas-Salvado, 2012), and due to its high consumption, is one the major contributors to dietary sodium intake (Belz, Ryan, & Arendt, 2012).

Following the Framework for National Salt Initiatives created by European Union, several countries have developed operational salt reduction programs, where bread is one of the priority foods to intervene (European Union, 2009). In Portugal a national legislation concerning salt content in bread set a maximum of 1.4 g of salt per 100 g of bread (Assembleia da República, 2009). But, considering its importance on diet, any further reduction of salt content is expected to have a significant impact on heath.

However, salt has specific properties essentials for bread processing and quality in the final product. Salt modulates yeast fermentation reducing rate of gas production, promotes a stronger inter-protein hydrophobic interactions, strengthening the gluten network and enhancing dough stability and improving texture of the final product (Farahnaky & Hill, 2007; Hutton, 2002; Man, 2007; S. A. Matz, 1992; Sluimer, 2005; Tuhumury, Small, & Day, 2016). Additionally, salt also acts a preservative by decreasing water activity and promoting shelf life (Farahnaky & Hill, 2007; Hutton, 2002; Man, 2007; S. A. Matz, 1992; Sluimer, 2005; Tuhumury et al., 2016).

Besides that, salt plays a significant role on bread sensory properties, acting a flavour modifier and influencing crust development and crumb structure (Belz et al., 2012; Zita E. Martins, Pinho, Ferreira, Jekle, & Becker, 2017; Mondal & Datta, 2008; Silow, Axel, Zannini, & Arendt, 2016).

For these reasons, reduction of salt in the bread formulation is paramount, but remains a major challenge for the baking industry make this without impact on the technological functions and baking performance and understand the influence of salt reduction on consumers' acceptance.

In this context, this study aimed to evaluate the impact of salt reduction on bread physicochemical parameters, as well as crumb colour and structure, and evaluate the impact of salt reduction on bread sensory evaluation using a trained panel.

2. Materials and Methods

2.1. Sampling

Samples of four types of Portuguese traditional breads, namely "D'água", "Carcaça", "Mistura", and "Regueifa" were produced in an experimental laboratory of Moagem Ceres (Porto, Portugal), under controlled conditions (humidity/ temperature). Considering that in the original bread recipes used by bakeries only the salt addition in manufacture was known but not the amount present in the final product, a preliminary study was carried out to determine it. Those values were then used to estimate the required quantity to obtain a bread with 1.4 g of salt/100 g of bread (considered as control bread; allowed salt concentration established by Portuguese legislation (Assembleia da República, 2009)), as well as breads with salt reduction (0.0, 0.8, 1.0, 1.1, 1.3, 1.4 g of salt/100 g of bread). Ingredients used in the production of bread samples (flour, water, fresh yeast, commercial powder improver and salt) were from the same brand and commercial supplier. Fresh yeast was obtained from a pure culture of yeast species *Saccharomyces Cerevisiae*, from FALA AZUL (Lasafree Ibérica S.A., Valladolid, Spain), with a fermentative power of the 135 cm^3 CO_2/2 h. The powder improver used was Cerpan (CERES, Portugal), contained acidity regulator (E170i, E341), emulsifier (E472), wheat flour, antioxidant (E300) and enzymes.

2.2. Bread Making

All samples from each type of bread were produced under the same conditions of humidity and temperature, using industrial machines: mixer, fermentation chambers and ovens (Sopaco, Rio Tinto, Portugal).

Each bread type was produced according to original recipes, as shown in Table 1, and specific technological details: i) for “D’água” bread, the ingredients were mixed and kneaded in a spiral mixer for 25 min a “2” speed and next dough was leavened at room temperature during 30 to 90 min; dough was mechanically shaped into portions of the approximate 65 g in the bun divider rounder, manually shaped in balls and placed in a refrigeration chamber for 60 min; breads were baked for 30 min at 200 ºC; ii) for “Carcaça” bread, ingredients were mixed and kneaded for 20 min in a spiral mixer; dough was mechanically shaped in portions of a 65 g in a bun divider rounder and posteriorly shaped manually into a ball and leaving to ferment at 30 ºC for 60 min; breads were baked for 10 min at 200 ºC; iii) for “Mistura” bread, dough was mixed for 25 min in a spiral mixer and then leavened at room temperature for 90 min; the dough was mechanically shaped in portions of approximately 70 g and then manually shaped in balls and rested at 30 ºC for 30 min; the dough balls were baked in an oven at 220 ºC for 30 min; iv) for

"Regueifa" bread, the ingredients were mixed and kneading for 13 min in a spiral mixer, 3 min at slower speed and 10 min at faster speed; dough was pressed in a dough sheeter and shaped manually in portions of 500 g and leavened for 60 min at 30 ºC; baking was performed for 10 min at 200 ºC. Every bread was cooled at room temperature during 90 min before further analysis. Additionally, samples were frozen before sodium analysis.

2.3. Bread Analysis

2.3.1. Bread weight, specific volume, and moisture

Samples of each type of bread and salt content were individually evaluated for weight in Ceres laboratory in a digital scale METO (Esselte Meto International GmbH, Hirschhorn, Germany). Ten breads of each type were evaluated (n=10), except with "Regueifa" bread, for which four samples were analysed (n=4).

Bread specific volume (SV) was measured using a seed displaced method and the following formula

$$\mathrm{SV}\ (\mathrm{cm}^3\ \mathrm{g}^{-1}) = \frac{\mathrm{S}\ (\mathrm{g}) \times 1.35\ (\mathrm{cm}^3\ \mathrm{g}^{-1})}{\mathrm{P}\ (\mathrm{g})} \qquad (1)$$

where P is the bread weight, S is the weight of displaced seeds, and 1.35 is the specific volume of *Phalaris canariensis* seeds (Z. Martins et al., 2015). Measurements were made with 10 samples for each type of bread (n=10).

Determination of bread moisture was performed according to the AACC International Method - Moisture-Air-Oven Methods (International, 1999). A representative part of each bread sample was conveniently comminuted with a Moulinex shredder, obtaining an aliquot as homogeneous as possible. A 6 g portion of the sample was placed in an aluminum dish for moisture balance (KERN DLB 160-3A, Ziegelei, Germany). The sample was tested at a temperature of 130 °C, and the percentage moisture being calculated automatically. Sample readings were made in triplicate.

2.3.2. Sodium analysis

Bread samples were analysed by flame photometry, carried out according to the method validated by Vieira, Soares, Ferreira, and Pinho (2012). Briefly, 2 g of sample (grounded and homogenized) were directly weighed in a 50 ml tube and 4 ml of nitric acid (HNO_3) (Fluka, France) were added. The mixture was shaken every 10 min for 60 min. The volume was completed up to 45 ml with deionized water and a preparation was homogenized using a Ultra Turrax blender (Ultra Turrax blender T25, Sotel, Germany). Calibration curves were established daily from standard sodium solutions with concentrations of 0.2, 0.5, 1.0, 1.5 and 5.0 µg/ml (Fluka, France).

2.3.3. Crumb structure and colour image analysis

For crumb structure and colour image analysis, three breads of every formulation were cut in slices of 1.6 cm thickness and analysed. Each slice was analysed in pre-standardized conditions: positioned on the flatbed scanner and a black cardboard was placed over the slice in order enhance contrast (Russ, 2011). Images were captured in the RGB (24 bit) standard format with a resolution of 300 dpi and saved in JPG format. Each image was processed and analyzed using Matlab R2015a (MathWorks) as described by Zita E. Martins et al. (2017). Briefly, a single 300 x 300 pixel (51 x 51mm) field of view (FOV) was cropped, converted to a 256 level grey scale and segmented. Cell morphological parameters were analyzed and recorded values for crumb structure analyses included: number of cells, mean cell area (mm^2), and cell density ($cells/mm^2$). Additionally, cells were divided into different classes as a function of their area: very small size (cell area $\leq 0.2\ mm^2$); small size ($0.2\ mm^2 \leq$ cell area $\leq 3.0\ mm^2$); medium size ($3.0\ mm^2 \leq$ cell area $\leq 10.0 mm^2$); large size (cell area $> 10.0\ mm^2$).

To study the crumb colour, for the second approach, each single 300 x 300 pixel FOV obtained from bread image analysis was converted from RGB to CIElab system – lightness (L*), redness (a*) and yellowness (b*) using code written in Matlab R2015a (MathWorks). Furthermore, crumb L*, a* and b* values were combined in the browning index (BI) parameter (Buera, Retriella, & Lozano, 1985) according to equations 2 and 3

$$BI=\frac{100(X-0.31)}{0.172} \qquad (2)$$

$$X=\frac{a^{*}+1.75\ L^{*}}{5.645\ L^{*}+a^{*}-3.012\ b^{*}} \qquad (3)$$

2.3.4. Bread sensory analysis

Sensory profile was evaluated in order to understand the influence of salt reduction on sensory characteristics of each type bread. A sensory panel composed by 8 members was trained for descriptive analysis according to the guidelines in the ISO 8586 (2012).

Prior to start of the study, assessors partook in the development of a descriptive vocabulary, compiling a list of attributes associated with the breads. Two sessions were performed to facilitate the acquisition of an accurate concept and refined terms in association with detection and recognition of smells, tastes, textures, among others. Redundant descriptive terms were removed. Sensory attributes were classified based on four characteristics, as shown in Table 2: appearance (visual perception), odour (olfactory perception), texture (tactile and oral texture) and flavour (oral and retronasal). Seventeen attributes were defined for a descriptive sensory analysis of the bread: *crust colour intensity*, *crumb colour intensity*, *number of large cells*, *number of small cells*, *cell circularity*, *cell homogeneity*, *odour intensity*, *crunchy crust*, *cohesiveness*, *adhesiveness*, *crumb elasticity*, *shape recovery*, *salty*, *sweet*, *bread aroma*, *aftertaste* and *overall assessment* (Table 2). Throughout two sessions, a score card was developed to evaluate attributes intensities using a 1 – 7 unstructured scale (1 representing the lowest intensity and 7 the highest intensity) and ballot anchors were established for each selected attribute. The bread sample used as control, 1.1 g/ salt per 100 g of bread, was select because it represented the midpoint of salt concentrations range used in this study.

In evaluation sessions, four breads, each one with their six salt concentrations (0.0, 0.8, 1.0, 1.3, 1.1, 1.4 % bread) were assessed. All samples were presented in similar conditions: at room temperature and similar size with approximately 7 g (a slice with 1.5 cm of thickness) including the crust and crumb in a random three-digit coded covered glass dish. These sessions were carried out individually under white light at room temperature. Assessors were provided with mineral water and instructed to cleanse their palate between tastings. All bread samples were analysed in triplicates, over six sessions.

2.3.5. Consumer test

To evaluate consumer acceptability for bread with different salt levels, the sensorial acceptance hedonic test was applied to students, professors and employees of the University of Porto Campus. Eighty consumers participated in the trial; each type of bread and its respective salt concentrations was evaluated by 20 consumers (n=20 for each bread type). In this way, six samples of bread in three-coded in plastic dishes were randomly presented to each consumer. Acceptability tests were conducted using a hedonic scale of 7 points, where 1 corresponds to “dislike extremely” and 7 to "like extremely”, to assess the five following attributes: *appearance liking*, *aroma liking*, *taste liking*, *texture liking* and *overall liking*.

2.3.6. Preference Mapping

The consumer test data were submitted to cluster analysis and then external preference mapping was done to find a regression between consumer acceptability variables (dependent) and the main dimensions obtained by Principal Component Analysis (PCA) carried out on sensory data by trained panel (explanatory variables). Thus, mapping the consumer data in the assessor's space and obtaining the sensory properties that influence consumer acceptability. Four preference models were used: vector, circular, elliptical, and quadratic (Z. E. Martins et al., 2017).

2.3.7c. Statistical Analysis

All dependent variables from bread parameters analysed were tested for residuals distribution with Shapiro-Wilk test. Different samples were studied using a one-way analysis of variance (ANOVA), if normal distribution was confirmed. Welch correction was applied when homogeneity of variances was not verified. Whenever statistical significance was found, Tukey's test was applied for mean comparison on equal variance assumption, and Dunnett's T3 post hoc when not equal variance assumption.

If normal distribution was not found, different samples were studied using a Kruskal Wallis test. Whenever statistical significance was found, Mann-Whitney post hoc test was applied for median comparison.

Overall acceptability from consumers was also used to select the lowest salt concentration with best sensory performance for each bread type studied. Sensory data collected was treated using External Preference Mapping technique (Greenhoff & MacFie, 1994; Schlich, 1995; XLSAT, 2014).

PLS regression was also used to study the relationships between sensory attributes (Y-matrix) and physicochemical parameters, colour and crumb structure (X-matrix) in terms of prediction of Y-variables from X-variables. Random validation was also applied to identify relevant X-variables. Scores and loading plots were analyzed, as well as, calibration and validation coefficients. Auto scaling was used for data pretreatment i.e., data was converted to fluctuations around zero, subtracting the mean of a variable to the original values and divided by the square root of the standard deviation.

All statistical analyses were conducted with the XLSTAT for Windows version 2016.02 (Addinsoft, Paris, France) at 10% (External preference mapping) and 5% (ANOVA, Kruskal–Wallis, and PLS regression) significance level.

3. Results and Discussion

3.1. Bread physicochemical characteristics

The parameters determined to evaluate physicochemical characteristics of each bread type with different salt levels are presented in Table 3.

Considering bread physical characteristics, the impact of salt addition at different levels had differed between bread types. Moreover, no linear pattern ($R^2 < 0.700$, $p > 0.050$) was observed, except for specific volume ($R^2 = 0.953$, $p = 0.001$) in "Carcaça" bread, where specific volume values increased as the level of added salt increases. Comparing to the control (1.4%), significant differences were found for specific volume in "D'Água" ($p = 0.033$), "Carcaça" ($p < 0.001$), and "Mistura" ($p < 0.001$) breads; and moisture in "D'Água", "Carcaça", and "Regueifa" ($p < 0.001$) breads.

Regarding specific volume, comparing to control bread, specific volume was higher than 0.0% in "Carcaça" bread and 0.8% in both "D'Água" and "Carcaça" breads, whereas it was lower than 1.1% and 1.3% in "Mistura" bread. The results are in accordance with McCann and Day (2013), where volume increased with increasing salt concentration, for breads prepared with 0%, 1% and 2% sodium chloride (w/w, flour base). Moreover, studies carried out by Czuchajowska, Pomeranz, and Jeffers (1989), Miller and Hoseney (2008), and H. He, Roach, and Hoseney (1992) are also in agreement with these findings. However, other research studies point out to a possible absence of effect on volume with a decrease in the salt concentration (Beck, Jekle, & Becker, 2012; Lynch, Dal Bello, Sheehan, Cashman, & Arendt, 2009). Therefore, it is difficult to establish the impact of salt on bread volume. At some extent, technological variability, such as dough mixing time, formulation, proofing and baking time, might explain the difference in results reported.

Moisture content seemed to decrease as the level of added salt increased. Control bread had lower moisture than 0.0%, 0.8%, and 1.0% for "D'Água" and "Carcaça" breads; 1.1% for "Carcaça" and "Regueifa" breads; and 1.3% for "D'Água" bread. Results obtained are not in agreement with those reported by Lynch et al. (2009), where breads with salt reduction, from 1.2% to 0.6%, 0.3% and 0% addition did not present significant differences in moisture content.

Regarding to bread weight, significant results were found only for "Carcaça" bread ($p = 0.002$), however without linear pattern. In addition, when comparing these results to the control (1.4%), no differences were observed.

Concerning chemical characteristics, i.e. salt concentration, in general, salt addition at different levels had significant impact on salt concentration for every bread type ($p < 0.001$). As it would be expected, salt concentration increased with increasing salt addition levels, following a linear trend for every bread type ("D'água", $R^2 = 0.727$, $p < 0.001$; "Carcaça", $R^2=0.770$, $p < 0.001$; "Mistura", $R^2=0.808$, $p < 0.001$; "Regueifa", $R^2=0.996$, $p < 0.001$).

3.2. Crumb structure and colour image analysis

Crumb structure and colour are essential bread quality parameters along with taste and crumb texture (Paraskevopoulou, Chrysanthou, & Koutidou, 2012; Skendi, Biliaderis, Papageorgiou, & Izydorczyk, 2010).

Bread colour is influenced by factors such as formulation or baking conditions, while brown colour (measured using the browning index, BI) results from Maillard reactions and crust caramelization (Habibi Najafi, Pourfarzad, Zahedi, Ahmadian-Kouchaksaraie, & Haddad Khodaparast, 2016; Mohd Jusoh, Chin, Yusof, & Abdul Rahman, 2009; Ramírez-Jiménez, Guerra-Hernández, & García-Villanova, 2000; Razavizadegan Jahromi, Karimi, Tabatabaee Yazdi, & Mortazavi, 2014).

Overall, salt addition at different concentrations had limited impact on crumb morphology, but more influence on colour parameters (Table 4). Furthermore, no linear relationship ($R^2 < 0.700$, $p > 0.050$) with salt addition levels was observed for any of the crumb morphology or colour parameters studied.

Regarding cell morphology, salt has been described as having a fundamental role on the formation of an even crumb (S. A. Matz, 1992). However, significant differences comparing to the control breads were only found for cell distribution as a function of their area, namely very small size cells in “Carcaça” bread ($p = 0.047$) and small size cells in “D’Água” bread ($p = 0.003$). Control bread had higher percentage of very small size cells than 1.0%, in “Carcaça” bread, and small size cells than 0.0% and 0.8% in “D’Água” bread. Results obtained are in agreement with Yovchev et al. (2017) that found no significant differences on total number of cells in breads with salt reduction. However, the absence of significant differences on the percentage of large cells is not in agreement with what is described by Lynch et al. (2009), where bread without salt resulted in a smaller number of larger cells when compared to bread containing salt.

Concerning colour, salt influences Maillard reactions that occur throughout baking (Silow et al., 2016). Although salt impact is more described for bread crust, it would also be expected, at some extent, for bread crumb. Salt reduction resulted in significant differences for every parameters when comparing to respective controls, i.e. L* for

“D’Água” ($p = 0.006$) and “Regueifa” ($p = 0.027$); a* for “Carcaça” ($p < 0.001$) and “Mistura” breads ($p = 0.047$); b* for “Carcaça” ($p < 0.001$); BI for “D’Água” ($p = 0.002$), “Regueifa” ($p < 0.001$), and “Carcaça” ($p = 0.029$) breads. Control bread was lighter than 0.8% for “D’Água” bread, 1.0% and 1.1% for “Regueifa”. While control bread was greener than 0.0%, 0.8%, and 1.3% in “Carcaça” bread, it was redder in “Regueifa” bread. Salt reduction affected L*, a*, and b* differently and, together with the inherent influence of factors such as formulation or baking conditions on bread colour, the comparison with literature was not possible.

3.2. Bread sensory analysis

3.2.1. Descriptive Sensory Analysis

Several studies (Antúnez, Giménez, & Ares, 2016; La Croix et al., 2014; Lynch et al., 2009; Pflaum, Konitzer, Hofmann, & Koehler, 2013; Rødbotten et al., 2015) have shown that salt reduction has a negative impact on bread characteristics, which can potentially affect its sensory characteristics and, consequently on consumer's preferences.

Values for sensory analysis scores for each bread type with different salt addition levels are presented in Table 5. Overall, salt addition at different concentrations had limited impact on sensory evaluation of the different bread types. This was even more perceptible when comparisons were made with respective controls (1.4%). Considering appearance attributes, significant differences were only found for *large number of cells* in “Mistura” bread ($p = 0.040$) and *cell homogeneity* in “D’Água” bread ($p = 0.028$). For “Mistura” bread, the control had more large cells the 1.1%, whereas for “D’Água” bread cells distribution was less homogenous in control than for 0.0% and 0.8%. As for odour attribute, no significant differences were observed. With texture attributes, significant differences were detected for *crunchy crust* in “Carcaça” ($p = 0.020$) and “Mistura” ($p =$

0.010) breads, and *cohesiveness* in “D’Água” ($p = 0.005$). While the crust of control bread was crunchier than 0.8% and 1.0% in “Carcaça” bread, it was less crunchy than 0.0% and 1.3% in “Mistura” bread. Although with the same median values, the mean of ranking of *cohesiveness* was significantly higher for control than for 0.8% in “D’Água” bread, therefore control was more cohesive than 0.8%. Aroma attributes was the category where salt reduction had more impact, with significant differences found for *salty* in all bread types ($p < 0.001$), *sweet* in “Mistura” bread ($p = 0.005$) and *bread aroma* in “D’Água” ($p = 0.002$), in “Mistura” ($p < 0.001$), and in “Regueifa” ($p < 0.001$) breads. Though control bread was perceived as saltier than: 0.0% for all breads, 0.8% for all breads, except “Carcaça”, and 1.0% for “D’Água” bread; it was less sweet than 0.0% in “Mistura” bread. Moreover, control breads had more bread aroma than 0.0%, except for “Carcaça” bread. Finally, for *overall assessment*, significant differences were found for all bread types ($p = 0.005$, “D’Água” bread; $p < 0.001$, “Carcaça”, “Mistura”, and Regueifa” breads). Breads without salt addition (0.0%) were less preferable than control in “D’Água”, “Mistura”, and Regueifa” breads, while this was observed with 1.1% for “Carcaça” bread. Globally, the effect of salt reduction was not consistent across sensory characteristics evaluated by the trained panel evaluation, which makes comparison with literature difficult and meaningless.

When sensory profile was compared with image analysis, the sensory panel was not able to identify differences between control and breads with salt reduction for some parameters, including cell distribution as a function of their area and crumb colour. Thus, data gathered from image analysis provided relevant information that would not be possible to obtain from sensory data.

3.2.2. Consumer test

Consumer hedonic perception of salt reduced products is relevant. While the ability to identify differences among samples by trained assessors outperform consumers, they may be too conservative (Ishii, Kawaguchi, O'Mahony, & Rousseau, 2007; Oliveira et al., 2015). Thresholds estimated with trained assessors are based on differences that may not be relevant for consumers' liking preferences.

The results obtained from the consumer test are shown in Table 6. Although significant differences were found between the different concentrations of salt for some liking attributes, they were not as evident when comparing to the control (1.4%). Apart from "Regueifa" bread, breads with 0.0% of salt were the only ones with significant lower scores, comparing to respective control. The lack of salt addition had a negative effect on the *overall liking*, for D'água" (p = 0.002), "Carcaça" (p = 0.001), and "Mistura" ($p <$ 0.001) breads. This negative effect was also observable for other liking attributes, such as *appearance liking* (p = 0.013) and *texture liking* ($p <$ 0.001) for "Carcaça" bread, and *taste liking* for both "Carcaça" ($p <$ 0.001) and "Mistura" ($p <$ 0.001). Overall, from consumer's point of view, only the lack of salt addition was relevant, which was more noticeable for "Carcaça" bread and less evident for "Regueifa" bread. This results are in agreement with what is described in literature that, even if perceived, small changes in the sensory characteristics of products do not significantly affect their hedonic perception (Oliveira et al., 2015).

3.2.3. External Preference Mapping

External preference mapping includes three sequential steps: creates the sensory map, groups the consumers and creates the preference map using PREFMAP method. PCA was applied on sensory data of attributes evaluated by the trained panel for create the sensory map. Considering *overall liking* attribute, consumers were grouped into homogeneous groups according to their preference, using Agglomerative Hierarchical Clustering (AHC). PREFMAP method was employed using the sensory attribute coordinates in the two-dimensional facto space, resulting from PCA, and average *overall liking* scores for each 3 clusters, obtained from AHC. As result, four different regression models were tested to predict each consumer group *overall liking*: vector model, circular model, elliptical model and quadratic surface method (Resano, Sanjuán, Cilla, Roncalés, & Albisu, 2010).

For each bread type, the resulting preference map (see Figure 1) shows the best fitting model for each cluster and consumers preference. For “D’Água” bread (Figure 1a), the vector model was the best fit for cluster 1 (C1) and cluster (C3) but only significant (*p* = 0.097) for C1, while elliptical model was the best (*p* = 0.094) for cluster 2 (C2). In C1 and C3, the vector indicated the direction in the map where *overall liking* increased. In C1, the preference order was A1.4 > A0.8 > A1.3 > A1.0 > A1.1 > A0.0, while in C3 was A1.4 > A1.1 > A1.3 > A1.0 > A0.8 > A0.0. The elliptical model for C2 showed a saddle point, where the thicker lines indicated the direction in which *overall liking* increased, and the thinner ones to the direction in which it decreased. Here, the preference order was A1.1 > A0.8 > A1.0 > A1.3 > A1.4 > A0.0. The best fitting models for “Carcaça” bread (Figure 1b) were the elliptical for C1 (*p* = 0.089), circular for C2 (*p* = 0.069), and vector for C3 (*p* = 0.078). In C1, the preference order was C1.4 > C1.0 > C1.3 > C1.1 > C0.8 > C0.0. The circular model for C2 showed a maximum in terms of preference, known as the ideal point, with circular lines of isopreference drawn around it. Here, the preference order was C1.4 > C0.8 > C1.1 > C1.3 > C1.0 > C0.0. For C3, preference order was C0.8 > C0.0 > C1.4 > C1.3 > C1.0 > C1.1. Considering “Mistura” bread (Figure 1a), the vector model was the best fit for C1 (*p* = 0.020) and C2 (*p* = 0.071), while circular was the best for C3 (*p* = 0.025). The preference order for this bread was M1.4 > M1.1 > M1.3 > M1.0

> M0.8 > M0.0 for C1 and C2, and M0.8 > M1.0 > M1.4 > M1.1 > M1.3 > M0.0 for C3. As for "Regueifa" bread, the vector model was the best fit for all clusters, but they were not significant ($p > 0.100$). The preference order for the different clusters was R1.4 > R1.3 > R0.8 > R1.1 > R1.0 > R0.0 for C1; R0.8 > R1.4 > R1.1 > R1.0 > R1.3 > R0.0 for C2; and R1.4 > R1.3 > R1.0 > R1.1 > R0.8 > R0.0 for C3. Finally, with information gathered from this analysis it was possible to establish the lowest salt concentration with better percentage of satisfied assessors (Figure 1) namely: 0.8% for "D'Água" bread (67% of satisfied assessors) 0.8% for "Carcaça" bread (100% satisfied assessors); 1.0% for "Mistura" bread (100% satisfied assessors); and 1.1% for "Regueifa" bread (100% of satisfied assessors).

3.3. Correlation of sensory characteristics with physicochemical, crumb structure and colour parameters

PLS regression model quality was performed to establish a simultaneous correlation between sensory attributes and analytical parameters: physicochemical parameters (weight, specific volume, moisture, and salt concentration), crumb structure (cell density; number of cells; mean area; circularity; very small size, small size, medium size, and large size cells) and colour (L*, a*, b*, and BI). This model is based on sensory data prediction (Y-variables) from analytical parameters data (X-variables). For a successful regression model, the values obtained for R^2Y and R^2X must be equal or superior to 0.7 and the prediction ability is achieved by Q^2 values, which must be equal or superior to 0.5.

Table 7 summarizes individual sensory attributes prediction models from analytical parameters. Of the 17 sensory attributes analyzed, 9 were found to be correlated with analytical parameters. Overall, models with good predictive quality were obtained for *crust colour intensity*, *crumb colour intensity*, *cohesiveness*, *adhesiveness*, *crumb elasticity*, *shape recovery*, *salty*, *bread aroma*, and *aftertaste*. These results indicate that, at some extent, assessors were able to evaluate the parameters evenly, regardless of the type of bread analysed. As for the attributes with lower quality values, they can be explained by a dispersion in the results, which may indicate that these parameters were considered in different ways for each type of bread. Consequently, the mathematical base cannot produce a model with good predictive quality.

The importance of X-variables (analytical parameters) in the projection and their correlation with Y-variables (sensory attributes) was also determined, and latent variables were identified (Table 7). Moreover, analytical parameters common in regression models with good performance for each group of sensory attributes were identified. Regarding appearance attributes, crust and crumb colour were more intense for redder and browner breads, and less intense for darker breads. As for appearance attributes related to crumb structure, models were poorly fitted (R^2Y and $R^2X < 0.50$), and with poor ($0.00 < Q^2 < 0.50$) or lacking ($Q^2 < 0.00$) predictive ability. Odour and texture characteristics were not evaluated analytically, and therefore, it would be less likely to find correlations or successful predictive model for these parameters. Although models for *odour intensity* and *crunchy crust* models were poorly fitted ($R^2X < 0.50$) and with poor ($0.00 < Q^2 < 0.50$) predictive ability, it was possible to find good predictive models for other texture attributes, namely *cohesiveness*, *adhesiveness*, *crumb elasticity* and *shape recovery*. Lighter and heavier breads were the ones with higher *cohesiveness*, *adhesiveness*, and *crumb elasticity*, but also with lower *shape recovery*. Moreover, breads with higher percentage of large size cells were less cohesive, adhesive, and with lower *crumb elasticity*. Regarding aroma sensory attributes, breads with higher specific volume were saltier and with higher bread aroma and aftertaste. Furthermore, it was interesting to observe that saltier breads were the ones with higher salt concentration, as it would be expected. As regards to sensory attribute *overall assessment*, no association models with good fitting and predictive ability were found. This sensory attribute, unlike others, is more susceptible to a subjective evaluation and therefore, is a more difficult to standardize. Globally, these results could be expected at some extent; nevertheless, it is important to highlight that they show associations and not cause-effect relationships.

5. Conclusion

Salt reduction in the different bread formulations had limit impact on physicochemical and sensory characteristics. Results obtained from the consumer test only showed significant differences for salt reduction with *taste liking* and o*verall linking* attributes. Mathematical modelling was shown as a relevant tool to study bread acceptability and understand relationships between sensory and analytical data. External preference mapping was appropriate to study consumer preferences and to select the lowest salt concentration with best acceptance, namely 0.8% for “D’Água” bread; 0.8% for “Carcaça” bread; 1.0% for “Mistura” bread; and 1.1% for “Regueifa” bread. The results suggest that it is possible to reduce, to some extent, the salt concentration in all bread types without major impact on bread characteristics and without compromising consumers’ acceptance.

Additionally, PLS regression provided information on the relationship between sensory and analytical data (physicochemical, crumb structure and colour). Successful models were obtained for *crust colour intensity*, *crumb colour intensity*, *cohesiveness*, *adhesiveness*, *crumb elasticity*, *shape recovery*, *salty*, *bread aroma*, and *aftertaste*. However, these relationships should be interpreted as associations and not as direct cause and effect, once observed correlations do not necessarily imply causality.

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Figure captions

Fig. 1. External preference mapping for a) “D’Água”, b) “Carcaça”, c) “Mistura”, and d) “Regueifa” breads. 3 clusters are ilustrated: 1 and 3 (vector), and 2 (elliptical (○); where the circle indicates a point of low variability in preference, located immediately before a decrease or increase in preference area) and the 5 regions of the global average value of acceptance.

0.0, Breads with 0.0% salt; 0.8, Breads with 0.8% salt; 1.0, Breads with 1.0% salt; 1.1, Breads with 1.1% salt; 1.3, Breads with 1.3% salt; 1.4, Breads with 1.4% salt; A, “D’Água” bread; C, “Carcaça”; C1, Cluster 1; C2, Cluster 2 ; C3, Cluster 3; M, “Mistura”; R, “Regueifa”.

Table Captions

Table 1. Original recipes of the different bread types in study and salt reduction levels

Type of bread	Flour type	Water (%)*	Yeast (%)*	Powder improver (%)*	Salt reduction levels (%)*
"D'água" bread	Mixture 70:25:5 of type 65 wheat flour, type 80 wheat flour and 70 rye flour	80%	3%	1%	1.4%; 1.3%; 1.1%; 1.0%; 0.8%; 0.0%
"Carcaça" bread	Type 65 wheat flour	60%	5%	1%	1.4%; 1.3%; 1.1%; 1.0%; 0.8%; 0.0%
"Mistura" bread	Mixture 79:20:1 of type 65 wheat flour, type 70 rye flour and barley flour	75%	3%	1%	1.4%; 1.3%; 1.1%; 1.0%; 0.8%; 0.0%
"Regueifa" bread	Mixture of wheat flour, vegetable oil palm powder and milk protein	50%	3%	1%	1.4%; 1.3%; 1.1%; 1.0%; 0.8%; 0.0%

* Percentages applied to the total flour used

Table 2. Descriptive sensory attributes developed by trained sensory panel

Sensory attributes	**Definitions**
Appearance	
Crust colour intensity	Degree of colour darkness in the crust – light to dark
Crumb colour intensity	Degree of colour darkness in the crumb – light to dark
Number of Large Cells	Amount of large cells – low to high
Number of Small Cells	Amount of small cells – low to high
Cell circularity	Level of perfection of the circular shape of the crumb cells/ number of circular crumb cells
Cell homogeneity	Homogeneity of the size of the crumb cells
Odour	
Odour intensity	Degree of intensity of odour of the sample – low to high
Texture	
Crunchy crust	Degree of perceived noise when chewing the crust sample
Cohesiveness	Level of mass formation in the mouth before breaking
Adhesiveness	Degree in which the material adheres to the palate
Crumb elasticity	Ability to return to initial shape after being pressed
Shape recovery	Resistance to the crumb pressure on the finger
Aroma	
Salty	Perception of taste sensation for sodium chloride
Sweet	Perception of taste sensation for sugars
Bread aroma	Degree of perception of the intensity of the characteristic bread flavour
Aftertaste	Flavour remaining after tasting

Table 3. Physicochemical parameters values for each bread type, with different salt addition levels (% bread).

Bread type/ Salt (%)	**Weight (g)**	**Specific volume (cm^3/g)**	**Moisture (%)**	**Salt concentration (g/100 g**
		"D'Água"		
0.0	52.23 ± 3.91	7.36 ± 1.01^{ab}	35.71 ± 0.21^{a}	0.000^{a} (0.000 – 0.000)
0.8	52.41 ± 3.45	6.64 ±0.40^{a}	34.98 ± 0.24^{a}	0.685^{ab} (0.656 – 0.692)
1.0	53.89 ± 6.65	6.88 ± 0.45^{ab}	40.51 ± 5.23^{a}	0.834^{ab} (0.813 – 0.864)
1.1	52.19 ± 6.31	7.36 ± 1.01^{ab}	33.00 ± 1.03^{b}	0.891^{abc} (0.875 – 0.899)
1.3	51.48 ± 4.91	7.00 ± 0.50^{ab}	34.26 ± 0.36^{c}	1.024^{bc} (1.003 – 1.050)
1.4	52.07 ± 7.40	7.67 ± 0.88^{b}	31.88 ± 0.65^{b}	1.146^{c} (1.094 – 1.159)
p	ns	0.033**	< 0.001**	<0.001***
		"Carcaça"		
0.0	48.46^{a} (44.19 – 52.11)	3.54 ± 0.88^{a}	32.47 ± 0.19^{a}	0.000^{a} (0.000 – 0.012)
0.8	47.43^{ab} (44.82 – 49.96)	6.27 ± 0.26^{b}	33.46 ± 0.29^{b}	0.420^{ab} (0.223 – 0.530)
1.0	43.05^{b} (37.89 – 47.81)	6.65 ± 0.59^{bc}	33.01 ± 0.17^{ab}	0.693^{ab} (0.281 – 0.843)
1.1	43.24^{b} (38.55 – 47.88)	7.15 ± 0.29^{c}	32.71 ± 0.22^{a}	0.788^{b} (0.559 – 1.000)
1.3	44.94^{ab} (35.35 – 48.17)	7.15 ± 0.86^{c}	30.66 ±0.26^{c}	0.843^{b} (0.799 – 0.938)
1.4	46.48^{ab} (39.14 – 48.13)	7.24 ± 0.56^{c}	31.22 ± 0.37^{c}	0.893^{b} (0.625 – 1.075)
p	0.002***	< 0.001**	< 0.001*	<0.001***
		"Mistura"		
0.0	52.41 ± 5.44	4.68 ± 0.23^{a}	29.89^{a} (27.16 – 29.91)	0.000^{a} (0.000 – 0.000)
0.8	52.05 ± 0.35	5.07 ± 0.23^{b}	32.37^{b} (32.14 – 32.22)	0.508^{ab} (0.376 – 0.575)
1.0	53.00 ± 4.88	5.27 ± 0.40^{bc}	31.61^{ab}(31.61 – 32.22)	0.659^{ab} (0.299 – 0.659)
1.1	50.94 ± 7.99	5.56 ± 0.40^{cd}	31.32^{ab} (30.88 – 31.74)	0.804^{b} (0.557 – 0.864)
1.3	51.80 ± 4.38	6.14 ± 0.34^{d}	31.09^{ab} (30.13 – 31.15)	0.850^{b} (0.585 – 1.051)
1.4	52.70 ± 4.76	4.91 ± 0.60^{ab}	30.16^{ab} (30.12 – 30.74)	0.851^{b} (0.442 – 0.955)
p	ns	< 0.001**	0.010***	<0.001***
		"Regueifa"		
0.0	404.50 (383.00 – 440.00)	4.69 ± 0.34	35.08 ± 0.57^{ab}	0.000^{a} (0.000 – 0.000)
0.8	433.00 (395.00 – 481.00)	4.91 ± 0.40	33.59 ± 0.29^{c}	0.367^{ab} (0.149 – 0.509
1.0	420.00 (415.00 – 470.00)	4.84 ± 0.12	34.54 ± 0.16^{ad}	0.449^{abc} (0.418 – 0.459)
1.1	470.00 (410.00 – 485.00)	4.28 ± 0.23	35.68 ± 0.13^{b}	0.528^{abc} (0.469 – 0.654)
1.3	412.50 (385.00 – 450.00)	4.62 ± 0.31	33.70 ± 0.36^{cd}	0.945^{bc} (0.764 – 1.312)
1.4	432.50 (365.00 – 460.00)	4.84 ± 0.14	33.60 ± 0.23^{c}	1.160^{c} (0.914 – 1.535)
p	ns	ns	< 0.001*	<0.001***

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=10).

ns, not significant.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* p Values from one-way ANOVA analysis. Means were compared by Tukey's, since homogeneity of variances was confirmed by Levene's test ($p > 0.05$).

** p Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene's test ($p < 0.05$).

*** p Values from Kruskal-Wallis analysis. Medians were compared by Dunn's test.

Table 4. Values for crumb structure and colour parameters for each bread type, with different salt addition levels (% bread).

Bread type/ Salt (%)	**Number of cells**	**Mean area (mm^2)**	**Cell density (cells/mm^2)**	**Circularity**	**Cell area (% of total cells)**				**Crumb colour**			
					Very small size	Small size	Medium size	Large size	L*	a*	b*	BI
“D’Água”												
0.0	190 (105 – 274)	17.21 (12.70 – 32.08)	11.71 (3.27 – 21.57)	0.69 ± 0.11	28.50 ± 7.65	40.00^{a} (36.70 – 40.51)	11.45 (8.39 – 18.35)	18.27 ± 5.35	61.20 ± 2.26^{ab}	-1.98^{a} (-2.64 – -1.65)	18.32 ± 0.70^{ab}	31.61 ± 1.59^{a}
0.8	120 (92 – 302)	29.58 (11.19 – 36.68)	4.52 (2.51 – 26.99)	0.67 ± 0.14	30.39 ± 8.82	38.07^{ab} (38.04 – 46.51)	12.59 (8.16 – 15.22)	17.19 ± 6.58	56.99 ± 5.59^{a}	-1.67^{ab} (-1.82 – -0.83)	19.03 ± 0.47^{b}	37.34 ± 3.14^{b}
1.0	243 (196 – 345)	14.37 (10.02 – 17.40)	16.93 (11.26 – 34.45)	0.75 ± 0.11	29.94 ± 4.35	46.90^{bc} (43.37 – 48.70)	12.20 (6.75 – 12.76)	12.58 ± 3.24	64.36 ± 2.84^{ab}	-1.42^{ab} (-2.00 – -0.92)	19.01 ± 0.99^{b}	31.98 ± 1.97^{a}
1.1	154 (86 – 288)	23.91 (12.29 – 39.71)	7.47 (2.17 – 23.43)	0.70 ± 0.09	27.66 ± 7.50	43.17^{abc} (34.88 – 47.22)	11.79 (8.14 – 18.61)	17.54 ± 7.91	58.69 ± 4.09^{ab}	-1.89^{ab} (-1.98 – -1.41)	18.00 ± 0.31^{a}	33.07 ± 3.04^{ab}
1.3	224 (121 – 441)	15.99 (7.57 – 28.05)	15.99 (4.31 – 58.29)	0.77 ± 0.04	33.01 ± 6.74	42.49^{abc} (40.00 – 51.10)	9.51 (6.35 – 13.94)	13.16 ± 4.39	66.00 ± 6.30^{b}	-1.39^{b} (-1.60 – -0.95)	18.74 ± 0.37^{b}	31.00 ± 3.73^{a}
1.4	120 (87 – 412)	28.61 (8.33 – 38.82)	4.22 (2.24 – 49.44)	0.69 ± 0.12	25.42 ± 7.26	47.18^{c} (40.71 – 52.87)	10.16 (5.83 – 13.27)	17.43 ± 6.02	64.90 ± 4.65^{b}	-1.58^{ab} (-1.74 – -0.95)	18.57 ± 0.49^{ab}	29.83 ± 1.49^{a}
p	ns	ns	ns	ns	ns	0.003***	ns	ns	0.006*	0.014***	0.034*	0.002*
“Carcaça”												
0.0	411 (149 – 605)	11.36 ± 6.57	56.07 ± 48.09^{a}	0.55 ± 0.05	37.12 ± 10.18^{a}	44.27 ± 3.46	8.64 ± 4.96	9.98 ± 6.29	71.84 (57.40 – 71.84)	-0.94 ± 0.28^{a}	20.74 ± 0.88^{a}	33.30 ± 2.92^{a}
0.8	231 (119 – 428)	17.14 ± 8.71	21.53± 16.66^{ab}	0.59 ± 0.08	32.80 ± 6.96^{ab}	45.92 ± 2.63	9.31 ± 3.27	11.97 ± 4.27	73.01 (67.95 – 73.01)	-1.77 ± 0.12^{bc}	19.08 ± 0.65^{b}	27.53 ± 1.23^{b}
1.0	169 (117 – 316)	19.52 ± 5.52	11.48 ± 9.45^{b}	0.54 ± 0.08	26.52 ± 5.74^{b}	45.36 ± 3.97	11.84 ± 3.22	16.30 ± 3.67	69.15 (61.88 – 69.15)	-1.96 ± 0.12^{bd}	18.06 ± 0.49^{c}	27.37 ± 1.55^{b}
1.1	154 (89 – 409)	23.92 ± 11.18	13.06 ± 17.62^{b}	0.57 ± 0.10	27.78 ± 9.06^{ab}	45.89 ± 4.12	10.93 ± 3.87	15.40 ± 4.90	69.31 (58.64 – 69.31)	-1.96 ± 0.11^{bd}	18.01 ± 0.31^{c}	27.33 ± 2.70^{b}
1.3	307 (129 – 523)	14.55 ± 8.45	35.00 ± 31.35^{ab}	0.63 ± 0.11	33.76 ± 9.06^{ab}	47.10 ± 3.11	9.11 ± 2.69	10.03 ± 4.98	73.89 (61.86 – 77.89)	-1.67 ± 0.17^{c}	19.08 ± 0.48^{b}	26.47 ± 0.75^{b}
1.4	354 (127 – 592)	13.94 ± 9.34	42.34 ± 38.15^{ab}	0.61 ± 0.08	37.19 ± 9.06^{a}	45.95 ± 3.74	6.91 ± 1.70	9.96 ± 5.64	75.63 (66.55 – 78.59)	-2.05 ± 0.15^{d}	18.04 ± 0.42^{c}	25.26 ± 2.38^{b}
p	ns	ns	0.004**	ns	0.047*	ns	ns	ns	ns	<0.001**	<0.001*	<0.001*
“Mistura”												
0.0	197 (133 – 380)	16.53 ± 6.53	11.38 (5.31 – 43.29)	0.65 ± 0.10	35.95 ± 7.79	43.78 ± 5.63	8.42 (5.98 – 11.28)	11.96 ± 4.21	53.28 (50.64 – 53.28)	1.50 ± 0.07^{a}	20.45 ± 0.56	47.89 ± 3.02
0.8	218 (109– 551)	16.88 ± 8.51	10.49 (3.62 – 26.84)	0.63 ± 0.07	34.27 ± 11.05	45.51 ± 5.50	8.92 (5.81 – 10.84)	9.69 ± 2.49	56.10 (46.62 – 58.41)	1.50 ± 0.25^{bc}	20.38 ± 0.63	47.81 ± 3.64
1.0	162 (126 – 505)	17.63 ± 8.90	7.97 (4.58 – 76.47)	0.67 ± 0.07	38.16 ± 10.70	41.04 ± 8.21	6.35 (5.40 – 15.87)	12.03 ± 5.80	49.95 (48.44 – 59.23)	1.59 ± 0.13^{bd}	20.47 ± 0.48	50.44 ± 4.58
1.1	154 (93 – 390)	21.52 ± 11.13	7.07 (2.54 – 45.71)	0.65 ± 0.06	33.04 ± 11.03	42.13 ± 4.94	9.10 (5.90 – 14.41)	15.28 ± 7.04	50.41 (44.74 – 56.35)	1.59 ± 0.20^{bd}	20.02 ± 0.69	50.02 ± 3.25
1.3	139 (93 – 383)	23.04 ± 9.29	4.77 (2.63 – 13.18)	0.71 ± 0.13	36.22 ± 5.55	38.48 ± 6.04	8.11 (4.96 – 13.28)	13.58 ± 2.82	47.18 (45.76 – 52.52)	1.11 ± 0.19^{c}	19.35 ± 0.42	50.80 ± 3.55
1.4	180 (121 – 219)	18.29 ± 7.28	9.67 (4.35 – 13.98)	0.75 ± 0.12	31.09 ± 7.42	46.54 ± 3.26	10.78 (5.42 – 13.70)	13.63 ± 2.73	52.98 (49.01 – 56.73)	1.58 ± 0.12^{d}	19.87 ± 0.57	46.93 ± 2.48
p	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.047*	ns	ns
“Regueifa”												
0.0	294 ± 112	10.14 (8.73 – 27.89)	28.40 ± 16.97	0.57 ± 0.13	34.94 ± 12.75	50.41 ± 6.45	6.50 ± 1.20^{a}	3.75 ± 0.89	90.55^{a} (81.05 – 92.19)	-2.85 ± 0.16^{ab}	19.41 ± 0.52	21.18^{ab} (19.44 – 24.35)
0.8	270 ± 106	14.26 (8.39 – 23.96)	24.23 ± 17.82	0.56 ± 0.14	32.52 ± 9.40	50.45 ± 3.07	9.66 ± 3.73^{ab}	7.37 ± 4.84	90.11^{ab} (84.36 – 92.30)	-2.69 ± 0.19^{a}	18.83 ± 0.36	20.79^{a} (19.35 – 21.41)
1.0	196 ± 63	16.18 (12.44 – 30.08)	12.31 ± 6.96	0.47 ± 0.09	27.33 ± 8.41	49.31 ± 3.70	11.16 ± 3.20^{ab}	12.21 ± 8.02	84.63^{b} (80.98 – 90.68)	-2.60 ± 0.11^{ab}	19.86 ± 0.22	23.52^{ab} (20.99 – 24.87)
1.1	247 ± 106	12.00 (9.36 – 38.46)	20.84 ± 13.93	0.62 ± 0.10	34.57 ± 4.18	50.82 ± 6.15	7.41 ± 1.45^{ab}	5.35 ± 1.50	91.54^{b} (79.44 – 92.79)	-2.62 ± 0.19^{b}	20.10 ± 0.88	22.51^{ab} (20.95 – 24.21)
1.3	218 ± 83	16.46 (10.25 – 26.19)	15.89 ± 11.07	0.53 ± 0.10	29.55 ± 7.31	49.79 ± 2.48	9.52 ± 3.29^{b}	9.15 ± 2.99	86.74^{ab} (56.54 – 90.97)	-2.69 ± 0.13^{b}	20.02 ± 1.01	23.61^{b} (21.38 – 33.82)
1.4	219 ± 121	18.98 (8.55 – 33.38)	17.57 ± 17.27	0.54 ± 0.08	29.91 ± 6.75	52.50 ± 5.92	8.82 ± 4.36^{ab}	8.77 ± 4.65	87.55^{a} (81.44 – 92.97)	-2.87 ± 0.18^{ab}	19.75 ± 0.49	22.37^{ab} (19.97 – 24.63)
p	ns	ns	ns	ns	ns	ns	0.006**	ns	0.027*	0.018*	ns	0.029***

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=36).

BI, Browning index; Large size, Cell area > 10.0 (mm^2); Medium size, 3.0 < Cell area ≤ 10.0 (mm^2); ns, not significant; Small size, 0.2 < Cell area ≤ 3.0 (mm^2); Very small size, Cell area ≤ 0.2 (mm^2).

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey’s, Fisher’s or Duncan’s test, since homogeneity of variances was confirmed by Levene’s test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene’s test ($p < 0.05$).

*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn’s test.

Table 5. Values for sensory analysis scores for each bread type, with different salt addition levels (% bread).

Bread type/ Salt (%)	**Appearance**						**Odour**	**Texture**					**Aroma**				**Overall assessment**
	Crust color intensity	**Crumb color intensity**	**Number of large cells**	**Number of small cells**	**Cell circularity**	**Cell homogeneity**	**Odour intensity**	**Crunchy crust**	**Cohesiveness**	**Adhesiveness**	**Crumb elasticity**	**Shape recovery**	**Salty**	**Sweet**	**Bread aroma**	**Aftertaste**	
								“D’Água”									
0.0	4.0^{ab} (2.0 – 5.0)	3.0 (2.0 – 3.0)	6.0 (1.0 – 7.0)	4.0 (2.0 – 7.0)	3.0 (2.0 – 5.0)	3.0^{a} (1.0 – 5.0)	4.0 (3.0 – 6.0)	2.0 (1.0 – 5.0)	4.0^{ab} (2.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 7.0)	6.0 (5.0 – 6.0)	2.0^{a} (1.0 – 4.0)	2.0 (1.0 – 3.0)	4.5^{a} (2.0 – 5.0)	4.0 (2.0 – 5.0)	4.0^{a} (1.0 – 6.0)
0.8	4.0^{a} (3.0 – 6.0)	3.0 (2.0 – 4.0)	5.0 (1.0 – 6.0)	4.0 (2.0 – 7.0)	3.0 (3.0 – 5.0)	3.0^{a} (2.0 – 6.0)	4.0 (3.0 – 6.0)	2.0 (2.0 – 5.0)	4.0^{a} (2.0 – 4.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 7.0)	3.0^{b} (2.0 – 4.0)	2.0 (1.0 – 2.0)	5.0^{ab} (4.0 – 5.0)	4.0 (2.0 – 6.0)	5.0^{b} (4.0 – 6.0)
1.0	4.0^{ab} (3.0 – 6.0)	3.0 (2.0 – 4.0)	5.0 (3.0 – 7.0)	4.0 (4.0 – 6.0)	3.0 (2.0 – 4.0)	2.0^{ab} (1.0 – 4.0)	4.0 (3.0 – 6.0)	2.0 (1.0 – 5.0)	4.0^{ab} (2.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 7.0)	3.0^{bc} (2.0 – 4.0)	2.0 (1.0 – 3.0)	5.0^{ab} (2.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (3.0 – 6.0)
1.1	4.0^{ab} (3.0 – 5.0)	3.0 (2.0 – 3.0)	6.0 (2.0 – 7.0)	4.0 (2.0 – 6.0)	3.0 (2.0 – 5.0)	2.0^{b} (1.0 – 5.0)	4.0 (2.0 – 5.0)	2.0 (2.0 – 4.0)	4.0^{b} (2.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 6.0)	6.0 (3.0 – 7.0)	4.0^{cd} (3.0 – 5.0)	2.0 (1.0 – 3.0)	5.0^{ab} (4.0 – 5.0)	4.0 (3.0 – 4.0)	5.0^{b} (4.0 – 6.0)
1.3	4.0^{b} (2.0 – 5.0)	3.0 (2.0 – 3.0)	5.5 (2.0 – 6.0)	4.0 (3.0 – 7.0)	3.0 (2.0 – 5.0)	2.5^{ab} (2.0 – 5.0)	4.0 (3.0 – 5.0)	2.0 (1.0 – 4.0)	4.0^{ab} (3.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 7.0)	4.0^{bcd} (1.0 – 5.0)	2.0 (1.0 – 5.0)	5.0^{b} (2.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (1.0 – 7.0)
1.4	4.0^{ab} (2.0 – 6.0)	3.0 (2.0 – 3.0)	6.0 (2.0 – 7.0)	4.0 (3.0 – 6.0)	3.0 (2.0 – 6.0)	2.0^{b} (1.0 – 5.0)	5.0 (2.0 – 6.0)	3.0 (1.0 – 5.0)	4.0^{b} (2.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 5.0)	6.0 (5.0 – 6.0)	3.0^{d} (3.0 – 6.0)	2.0 (1.0 – 3.0)	5.0^{b} (4.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 7.0)
p	0.005*	ns	ns	ns	ns	0.028**	ns	ns	0.005*	ns	ns	ns	<0.001*	ns	0.002*	ns	0.005*
								“Carcaça”									
0.0	3.0^{ab} (2.0 – 4.0)	2.0^{a} (2.0 – 4.0)	2.0 (1.0 – 4.0)	6.0 (4.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (3.0 – 7.0)	5.0 (3.0 – 7.0)	2.0^{ab} (1.0 – 5.0)	3.0 (2.0 – 4.0)	2.0 (2.0 – 4.0)	3.0 (1.0 – 4.0)	6.0 (4.0 – 6.0)	2.0^{a} (1.0 – 6.0)	2.0 (1.0 – 3.0)	4.0^{a} (1.0 – 6.0)	5.0 (3.0 – 6.0)	3.5^{a} (1.0 – 5.0)
0.8	3.0^{a} (2.0 – 5.0)	2.0^{ab} (2.0 – 3.0)	2.0 (1.0 – 3.0)	6.0 (5.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (1.0 – 6.0)	5.0 (3.0 – 6.0)	3.0^{a} (1.0 – 5.0)	3.0 (2.0 – 4.0)	2.0 (2.0 – 5.0)	3.0 (2.0 – 4.0)	6.0 (4.0 – 6.0)	4.0^{ab} (2.0 – 5.0)	2.0 (1.0 – 3.0)	5.0^{ab} (3.0 – 5.0)	5.0 (3.0 – 6.0)	5.0^{bc} (3.0 – 6.0)
1.0	3.0^{b} (1.0 – 4.0)	2.0^{b} (1.0 – 2.0)	2.0 (1.0 – 4.0)	6.0 (5.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (2.0 – 6.0)	5.0 (4.0 – 7.0)	3.0^{a} (1.0 – 5.0)	3.0 (2.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 5.0)	6.0 (4.0 – 6.0)	4.0^{ab} (2.0 – 5.0)	2.0 (1.0 – 2.0)	5.0^{ab} (4.0 – 6.0)	5.0 (4.0 – 7.0)	5.0^{bc} (3.0 – 5.0)
1.1	3.0^{ab} (2.0 – 4.0)	2.0^{b} (1.0 – 2.0)	2.0 (1.0 – 4.0)	6.0 (6.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (3.0 – 6.0)	5.0 (4.0 – 6.0)	2.0^{b} (1.0 – 5.0)	3.0 (2.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 5.0)	6.0 (4.0 – 6.0)	4.0^{b} (3.0 – 6.0)	2.0 (1.0 – 3.0)	5.0^{b} (4.0 – 5.0)	5.0 (4.0 – 5.0)	5.0^{c} (5.0 – 6.0)
1.3	3.0^{ab} (2.0 – 6.0)	2.0^{ab} (1.0 – 2.0)	2.0 (1.0 – 4.0)	6.0 (5.0 – 6.0)	4.0 (3.0 – 6.0)	4.0 (2.0 – 6.0)	5.0 (3.0 – 6.0)	2.0^{b} (1.0 – 4.0)	3.0 (2.0 – 4.0)	2.0 (2.0 – 5.0)	3.0 (2.0 – 4.0)	6.0 (4.0 – 6.0)	4.0^{b} (3.0 – 5.0)	2.0 (1.0 – 3.0)	5.0^{b} (4.0 – 6.0)	5.0 (4.0 – 6.0)	5.0^{bc} (4.0 – 6.0)
1.4	3.0^{ab} (2.0 – 4.0)	2.0^{ab} (2.0 – 2.0)	2.0 (1.0 – 5.0)	6.0 (5.0 – 5.0)	4.0 (3.0 – 5.0)	4.0 (3.0 – 6.0)	5.0 (3.0 – 6.0)	2.0^{b} (1.0 – 4.0)	3.0 (2.0 – 4.0)	2.0 (2.0 – 5.0)	3.0 (2.0 – 4.0)	6.0 (4.0 – 6.0)	4.0^{b} (1.0 – 6.0)	2.0 (1.0 – 5.0)	5.0^{ab} (3.0 – 6.0)	5.0 (4.0 – 6.0)	4.0^{ab} (2.0 – 6.0)
p	0.016*	0.008*	ns	ns	ns	ns	ns	0.020**	ns	ns	ns	ns	<0.001*	ns	0.002*	ns	<0.001*
								“Mistura”									
0.0	6.0 (3.0 – 7.0)	5.0 (3.0 – 6.0)	4.0^{ab} (2.0 – 5.0)	5.0^{ab} (4.0 – 6.0)	4.0 (1.0 – 5.0)	3.0 (2.0 – 6.0)	4.0 (3.0 – 6.0)	4.0^{a} (2.0 – 6.0)	4.0 (3.0 – 5.0)	2.0 (2.0 – 4.0)	3.0 (2.0 – 5.0)	6.0 (4.0 – 7.0)	2.0^{a} (1.0 – 3.0)	1.0^{a} (1.0 – 3.0)	3.5^{a} (2.0 – 4.0)	4.0 (2.0 – 5.0)	4.0^{a} (2.0 – 6.0)
0.8	6.0 (3.0 – 7.0)	5.0 (2.0 – 6.0)	4.0^{abc} (2.0 – 5.0)	5.0^{a} (4.0 – 6.0)	4.0 (2.0 – 6.0)	3.0 (2.0 – 5.0)	4.0 (3.0 – 5.0)	3.0^{ab} (2.0 – 5.0)	4.0 (3.0 – 6.0)	2.0 (2.0 – 5.0)	3.0 (2.0 – 6.0)	6.0 (5.0 – 7.0)	3.0^{b} (2.0 – 4.0)	2.0^{ab} (1.0 – 3.0)	4.0^{ab} (2.0 – 4.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 6.0)
1.0	6.0 (4.0 – 7.0)	5.0 (3.0 – 5.0)	4.0^{bc} (2.0 – 6.0)	5.0^{ab} (4.0 – 6.0)	4.0 (2.0 – 5.0)	3.0 (2.0 – 5.0)	4.0 (2.0 – 6.0)	3.0^{ab} (2.0 – 6.0)	4.0 (3.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 6.0)	4.0^{bc} (3.0 – 5.0)	2.0^{ab} (1.0 – 3.0)	4.0^{b} (2.0 – 6.0)	4.0 (3.0 – 5.0)	5.0^{b} (3.0 – 7.0)
1.1	6.0 (3.0 – 6.0)	5.0 (2.0 – 2.0)	4.0^{a} (2.0 – 4.0)	5.0^{ab} (3.0 – 6.0)	4.0 (2.0 – 5.0)	3.0 (2.0 – 4.0)	4.0 (3.0 – 6.0)	3.0^{b} (2.0 – 5.0)	4.0 (3.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 5.0)	6.0 (4.0 – 6.0)	4.0^{bc} (3.0 – 5.0)	2.0^{b} (1.0 – 3.0)	4.0^{b} (3.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 6.0)
1.3	6.0 (3.0 – 7.0)	5.0 (2.0 – 6.0)	4.0^{c} (3.0 – 6.0)	5.0^{b} (2.0 – 6.0)	4.0 (3.0 – 5.0)	3.0 (2.0 – 4.0)	4.0 (3.0 – 6.0)	4.0^{a} (2.0 – 5.0)	4.0 (2.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 6.0)	6.0 (3.0 – 6.0)	4.0^{bc} (3.0 – 5.0)	2.0^{b} (1.0 – 3.0)	4.0^{b} (4.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 6.0)
1.4	6.0 (4.0 – 7.0)	5.0 (2.0 – 5.0)	4.0^{bc} (2.0 – 5.0)	5.0^{ab} (4.0 – 6.0)	4.0 (2.0 – 6.0)	3.0 (2.0 – 5.0)	4.0 (3.0 – 6.0)	3.0^{b} (2.0 – 4.0)	4.0 (3.0 – 5.0)	2.0 (2.0 – 3.0)	3.0 (2.0 – 6.0)	6.0 (4.0 – 6.0)	4.0^{c} (3.0 – 6.0)	2.0^{b} (1.0 – 3.0)	4.0^{b} (4.0 – 5.0)	4.0 (3.0 – 5.0)	5.0^{b} (4.0 – 6.0)
p	ns	ns	0.040*	0.026*	ns	ns	ns	0.010**	ns	ns	ns	ns	<0.001*	0.005*	<0.001*	ns	<0.001*
								“Regueifa”									
0.0	4.0^{a} (2.0 – 6.0)	1.0 (1.0 – 1)	2.0^{ab} (1 – 2)	5.0 (4.0 – 7.0)	3.0^{a} (2.0 – 6.0)	4.0^{ab} (2.0 – 7.0)	4.5 (2.0 – 6.0)	4.0 (2.0 – 5.0)	6.0 (2.0 – 7.0)	5.0 (1.0 – 7.0)	5.0 (3.0 – 5.0)	3.0^{ab} (1.0 – 5.0)	1.0^{a} (1.0 – 2.0)	2.0 (1.0 – 5.0)	3.0^{a} (2.0 – 5.0)	3.0 (1.0 – 4.0)	3.0^{a} (2.0 – 6.0)
0.8	4.0^{a} (2.0 – 5.0)	1.0 (1.0 – 2)	1.5^{a} (1 – 2)	6.0 (5.0 – 7.0)	3.0^{ab} (1.0 – 6.0)	5.0^{a} (4.0 – 7.0)	4.0 (2.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (2.0 – 7.0)	5.0 (1.0 – 6.0)	5.0 (2.0 – 6.0)	3.0^{bc} (1.0 – 5.0)	3.0^{b} (2.0 – 4.0)	2.0 (1.0 – 6.0)	4.0^{ab} (2.0 – 6.0)	2.0 (2.0 – 5.0)	6.0^{b} (4.0 – 7.0)
1.0	4.0^{ab} (2.0 – 6.0)	1.0 (1.0 – 2)	2.0^{c} (2 – 3)	5.0 (4.0 – 7.0)	2.0^{ab} (2.0 – 5.0)	4.0^{b} (2.0 – 5.0)	4.0 (3.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (4.0 – 7.0)	5.0 (3.0 – 6.0)	5.0 (3.0 – 5.0)	3.0^{abc} (2.0 – 5.0)	3.0^{bc} (2.0 – 4.0)	3.0 (1.0 – 5.0)	4.0^{b} (3.0 – 5.0)	2.0 (2.0 – 4.0)	6.0^{b} (4.0 – 6.0)
1.1	4.0^{a} (1.0 – 5.0)	1.0 (1.0 – 2)	2.0^{abc} (1 – 2)	5.0 (4.0 – 7.0)	2.0^{b} (1.0 – 5.0)	4.0^{ab} (3.0 – 7.0)	4.0 (3.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (2.0 – 7.0)	5.0 (2.0 – 5.0)	5.0 (2.0 – 5.0)	3.0^{c} (2.0 – 6.0)	3.0^{bc} (2.0 – 5.0)	3.0 (2.0 – 5.0)	4.0^{b} (3.0 – 5.0)	2.0 (2.0 – 4.0)	6.0^{b} (5.0 – 7.0)
1.3	4.0^{b} (3.0 – 6.0)	1.0 (1.0 – 2)	2.0^{bc} (1 – 3)	5.0 (4.0 – 7.0)	2.0^{ab} (2.0 – 6.0)	4.0^{b} (2.0 – 6.0)	4.0 (2.0 – 6.0)	3.0 (2.0 – 5.0)	6.0 (3.0 – 6.0)	5.0 (3.0 – 6.0)	5.0 (3.0 – 7.0)	4.0^{a} (2.0 – 5.0)	3.0^{bc} (2.0 – 5.0)	4.0 (2.0 – 5.0)	4.0^{b} (4.0 – 5.0)	3.0 (2.0 – 4.0)	6.0^{b} (5.0 – 7.0)
1.4	4.0^{ab} (2.0 – 6.0)	1.0 (1.0 – 1)	2.0^{abc} (1 – 2)	5.0 (4.0 – 7.0)	2.5^{ab} (1.5 – 6.0)	4.0^{ab} (4.0 – 7.0)	4.0 (2.0 – 6.0)	3.0 (1.0 – 5.0)	6.0 (3.0 – 6.0)	5.0 (3.0 – 6.0)	5.0 (2.0 – 6.0)	3.0^{ab} (2.0 – 5.0)	4.0^{c} (2.0 – 6.0)	3.0 (2.0 – 5.0)	4.0^{b} (3.0 – 5.0)	2.5 (2.0 – 5.0)	6.0^{b} (2.0 – 6.0)
p	0.048*	ns	0.001*	ns	0.039*	0.003**	ns	ns	ns	ns	ns	0.028*	<0.001**	ns	<0.001**	ns	<0.001*

Data expressed as mean ± standard deviation or as median (minimum−maximum), (n=24).

ns, not significant.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene’s test ($p < 0.05$).

** *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn’s test.

Table 6. Overall liking attribute values from consumer acceptance testing for each bread type, with different salt addition levels (% bread) (n consumers=80).

Bread type/ Salt (%)	**Appearance liking**	**Aroma liking**	**Taste liking**	**Texture liking**	**Overall liking**
			“D’Água” (n=20)		
0.0	5.0 (2.0 – 7.0)	4.0 (1.0 – 6.0)	2.0^{a} (1.0 – 6.0)	4.0 (1.0 – 7.0)	3.0^{a} (1.0 – 6.0)
0.8	5.0 (2.0 – 7.0)	5.0 (2.0 – 7.0)	4.5^{b} (2.0 – 7.0)	5.0 (2.0 – 7.0)	5.0^{b} (2.0 – 7.0)
1.0	5.0 (2.0 – 7.0)	5.0 (2.0 – 6.0)	4.5^{b} (1.0 – 7.0)	4.5 (1.0 – 7.0)	5.0^{b} (2.0 – 7.0)
1.1	5.0 (2.0 – 7.0)	4.5 (3.0 – 6.0)	5.0^{b} (2.0 – 6.0)	5.0 (4.0 – 7.0)	5.0^{b} (3.0 – 6.0)
1.3	5.0 (2.0 – 6.0)	4.5 (2.0 – 6.0)	5.0^{b} (2.0 – 7.0)	5.5 (2.0 – 7.0)	5.0^{b} (2.0 – 6.0)
1.4	5.0 (2.0 – 7.0)	4.5 (1.0 – 6.0)	4.0^{ab} (1.0 – 7.0)	5.0 (1.0 – 7.0)	5.0^{b} (1.0 – 7.0)
p	ns	ns	<0.001*	ns	0.002*
			“Carcaça” (n=20)		
0.0	4.5^{a} (1.0 – 7.0)	4.0 (2.0 – 6.0)	3.0^{a} (1.0 – 6.0)	3.0^{a} (1.0 – 6.0)	3.0^{a} (1.0 – 7.0)
0.8	5.5^{ab} (2.0 – 7.0)	5.0 (3.0 – 7.0)	5.0^{b} (1.0 – 7.0)	5.0^{b} (3.0 – 7.0)	5.0^{b} (3.0 – 7.0)
1.0	5.5^{ab} (2.0 – 7.0)	5.0 (3.0 – 7.0)	5.0^{b} (2.0 – 7.0)	5.0^{b} (3.0 – 7.0)	5.0^{b} (3.0 – 7.0)
1.1	6.0^{b} (2.0 – 7.0)	5.0 (3.0 – 6.0)	5.5^{b} (3.0 – 7.0)	5.0^{b} (2.0 – 6.0)	5.0^{b} (2.0 – 6.0)
1.3	6.0^{b} (3.0 – 7.0)	5.0 (1.0 – 7.0)	5.0^{b} (1.0 – 7.0)	4.0^{b} (1.0 – 7.0)	5.0^{b} (1.0 – 7.0)
1.4	6.0^{b} (4.0 – 7.0)	5.5 (2.0 – 7.0)	5.0^{b} (1.0 – 7.0)	6.0^{b} (1.0 – 7.0)	5.5^{b} (3.0 – 7.0)
p	0.013*	ns	<0.001*	<0.001*	0.001*
			“Mistura” (n=20)		
0.0	5.0 (2.0 – 7.0)	5.0 (2.0 – 6.0)	3.0^{a} (2.0 – 5.0)	4.5 (2.0 – 7.0)	4.0^{a} (2.0 – 6.0)
0.8	6.0 (4.0 – 7.0)	6.0 (4.0 – 7.0)	6.0^{b} (5.0 – 7.0)	6.0 (3.0 – 7.0)	6.0^{b} (4.0 – 7.0)
1.0	6.0 (4.0 – 7.0)	6.0 (4.0 – 7.0)	5.5^{b} (3.0 – 7.0)	6.0 (3.0 – 7.0)	5.5^{b} (4.0 – 7.0)
1.1	6.0 (4.0 – 7.0)	6.0 (4.0 – 7.0)	6.0^{b} (3.0 – 7.0)	6.0 (3.0 – 7.0)	6.0^{b} (4.0 – 7.0)
1.3	6.0 (2.0 – 7.0)	6.0 (4.0 – 7.0)	6.0^{b} (4.0 – 7.0)	5.5 (3.0 – 7.0)	5.5^{b} (4.0 – 7.0)
1.4	6.0 (4.0 – 7.0)	5.0 (4.0 – 7.0)	6.0^{b} (4.0 – 7.0)	6.0 (3.0 – 7.0)	6.0^{b} (4.0 – 7.0)
p	ns	ns	<0.001*	ns	<0.001*
			“Regueifa” (n=20)		
0.0	6.0 (3.0 – 7.0)	5.0 (2.0 – 7.0)	3.0^{a} (1.0 – 6.0)	5.0 (2.0 – 7.0)	4.0^{a} (2.0 – 6.0)
0.8	5.0 (4.0 – 6.0)	5.0 (3.0 – 7.0)	6.0^{b} (3.0 – 7.0)	6.0 (4.0 – 7.0)	6.0^{b} (4.0 – 6.0)
1.0	5.5 (3.0 – 7.0)	5.0 (3.0 – 7.0)	5.5^{b} (4.0 – 7.0)	5.0 (3.0 – 7.0)	5.0^{ab} (3.0 – 7.0)
1.1	5.5 (3.0 – 7.0)	5.5 (3.0 – 7.0)	5.5^{b} (2.0 – 7.0)	5.5 (3.0 – 7.0)	5.0^{ab} (3.0 – 7.0)
1.3	5.5 (3.0 – 7.0)	5.0 (4.0 – 7.0)	5.0^{b} (2.0 – 7.0)	6.0 (3.0 – 7.0)	6.0^{b} (4.0 – 7.0)
1.4	6.0 (3.0 – 7.0)	5.0 (3.0 – 7.0)	4.5^{ab} (2.0 – 7.0)	5.0 (2.0 – 6.0)	5.0^{ab} (3.0 – 6.0)
p	ns	ns	<0.001*	ns	0.005*

Data expressed as median (minimum−maximum), (n=80).

ns, not significant.

Different letters for each extract in a row show statistically significant differences ($p < 0.05$) between means in normal distribution and median in non-normal distribution.

* *p* Values from one-way ANOVA analysis. Means were compared by Tukey’s, since homogeneity of variances was confirmed by Levene’s test ($p > 0.05$).

** *p* Values from one-way Welch ANOVA analysis. Means were compared by Tamhane's T2 test, since homogeneity of variances was not confirmed by Levene’s test ($p < 0.05$).

*** *p* Values from Kruskal-Wallis analysis. Medians were compared by Dunn’s test.

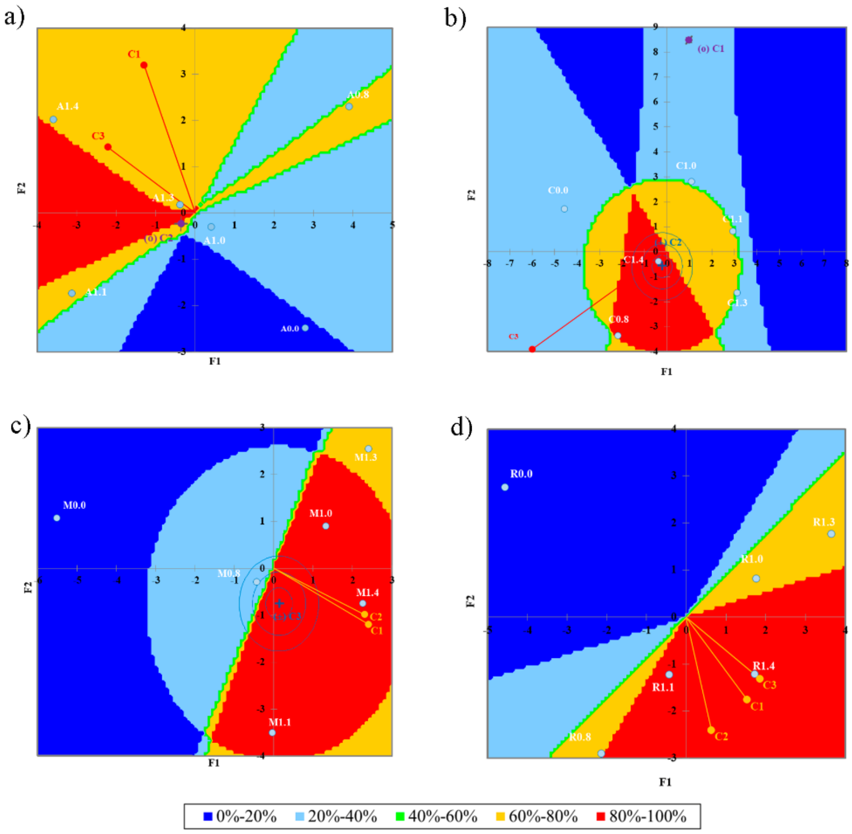
Table 7. Results of PLS regression between bread analytical parameters (X-variables) and sensory attributes (Y-variables) for all bread formulations.

Sensory attributes	**Q^2**	**R^{2}Y**	**R^{2}X**	**RSME**	**Latent variables**[1]
Appearance					
Crust colour intensity	0.836	0.929	0.808	0.225	**L*(-)**; **a*(+)**; **b*(+)**; **BI (+)**; Weight(+); **Specific volume(-)**
Crumb colour intensity	0.925	0.986	0.862	0.097	**Circularity (+)**; **L*(-)**; **a*(+)**; **BI (+)**
Number of large cells	0.415	0.579	0.406	0.597	-
Number of small cells	0.114	0.676	0.568	0.547	-
Cell circularity	0.465	0.593	0.332	0.558	-
Cell homogeneity	-0.451	0.841	0.601	0.314	-
Odour					
Odour intensity	0.296	0.888	0.531	0.353	-
Texture					
Crunchy crust	0.212	0.773	0.121	0.441	-
Cohesiveness	0.733	0.951	0.795	0.337	**Number of cells (-)**; **Cell density(-)**; **Small size(+)**; **Large size (-)**; Moisture (+); **L*(+); Weight (+)**; **Specific volume (-)**
Adhesiveness	0.879	0.949	0.817	0.222	**Large size(-)**; **L*(+)**; a*(-); **BI (-)**; **Weight(+)**;
Crumb elasticity	0.753	0.958	0.789	0.198	**Number of cells (-)**; **Cell density(-)**; **Large size (-)**; **L*(+)**; **a*(-)**; **BI (-)**; **Weight(+)**;
Shape recovery	0.805	0.945	0.767	0.249	Small size(-); **L*(-)**; b*(-); BI (+); **Weight (-)**; **Specific volume (+)**
Aroma					
Salty	0.618	0.958	0.854	0.242	**Moisture(-)**; **Specific volume (+); Salt (+)**
Sweet	0.156	0.837	0.880	0.239	-
Bread aroma	0.698	0.898	0.916	0.341	**Moisture(+)**; **Weight (-)**; **Specific volume (+)**
Aftertaste	0.628	0.940	0.762	0.290	Number of cells (+); Cell density(+); **L*(-)**; **Weight (-)**; **Specific volume (+)**
Overall assessment	0.067	0.415	0.250	0.720	-

[1] Latent variables with significant weight in the model and correlation with Y-variable; highly influential latent variables (variable importance for the projection>1) are represented in bold and the remaining are moderately influential latent variables (0.8<variable importance for the projection<1). (+), positive correlation with Y-variable;

(-), negative correlation with Y-variable. Latent variables were only considered for good models

BI, Browning index; Large size, Cell area > 10.0 (mm^2); Medium size, 3.0 < Cell area ≤ 10.0 (mm^2); ns, not significant; Q*2*, cumulative predictive variation from internal cross-validation; R^{2}X, cumulative explained variation of X explained in terms of sum of squares; R^{2}Y, cumulative explained variation of Y explained in terms of sum of squares; RMSE, Root mean square error; Small size, 0.2 < Cell area ≤ 3.0 (mm^2); Very small size, Cell area ≤ 0.2 (mm^2).


a)
C1
A1.4
C3
A0.8
A1.3
(o) C2
A1.0
A1.1
A0.0
F1
F2
b)
(o) C1
C1.0
C0.0
C1.1
(o) C2
C1.4
C1.3
C0.8
C3
F1
F2
c)
M1.3
M0.0
M1.0
M0.8
M1.4
(o) C2
C2
C1
M1.1
F1
F2
d)
R0.0
R1.3
R1.0
R1.4
C3
R1.1
C1
C2
R0.8
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🟩 40%-60%
🟨 60%-80%
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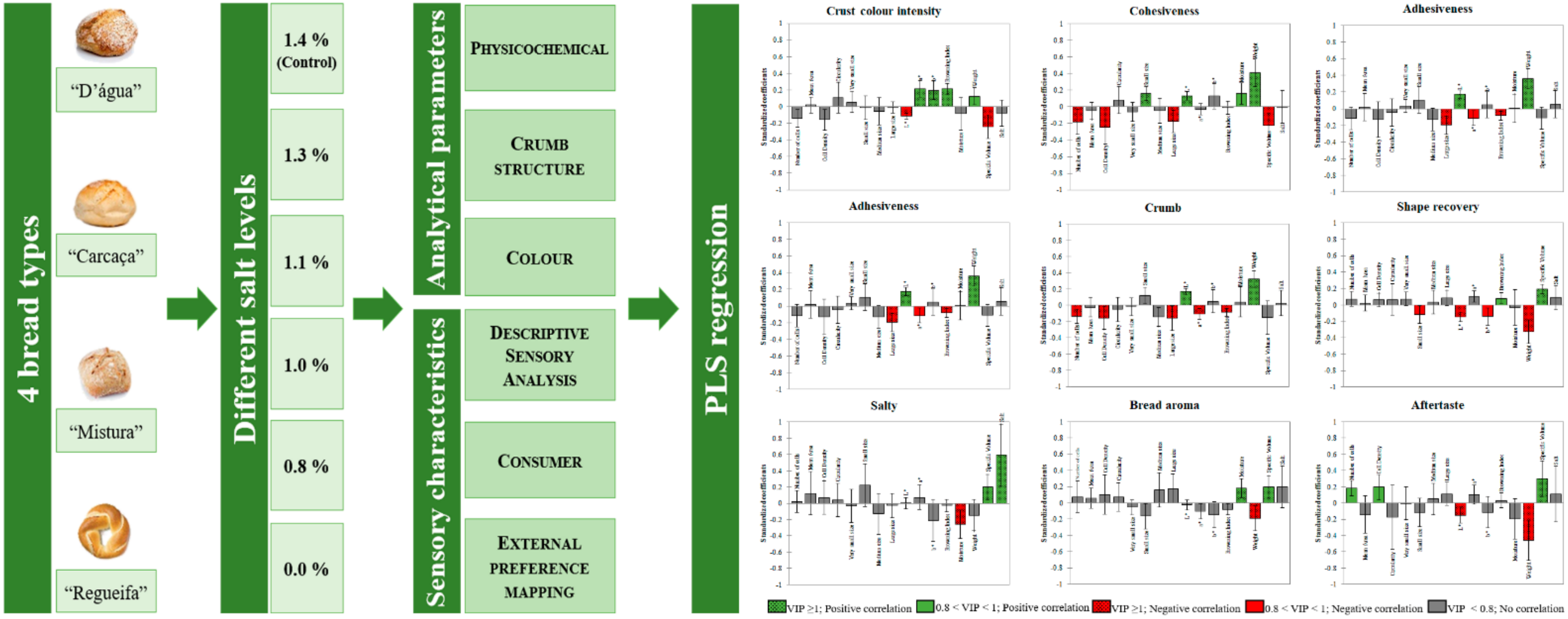

Graphical Abstract

[submitted for publication]

Chapter IV

Conclusions

The high consumption of salt is a recognized public health problem. A high sodium intake is widely pointed as the most important factor for high blood pressure, and is closely associated to increased risk of cardiovascular disease and stroke. Bread is a staple food of the diet and, therefore, one of the major contributors to dietary sodium intake. Considering its importance on diet, bread is a priority food to intervene and any reduction of salt content is expected to have a significant impact on health. It is known that salt plays an important role in bread processing and influences physicochemical and sensory characteristics of the final product. Therefore, this study aimed to understand the impact of different salt reductions on bread final quality and their consumer acceptance.

The effect of salt reduction on dough properties and fermentation was studied on “Carcaça” dough using a rheofermentometer and provided relevant information about gas expansion and retention capabilities. These parameters can be also indicators that help to define the optimal conditions for fermentation, specifically time conditions. Results obtained indicated that doughs with 1.0% of salt showed good growth performance; nevertheless, based on gas retention capacity, dough with salt 1.1% of salt content would be stronger.

Overall, salt reduction in the different bread formulations had limit impact on physicochemical and sensory characteristics.

Concerning bread physicochemical characteristics, weight, specific volume, moisture, and salt concentration were evaluated. No significant results were found for bread weight, except for “Carcaça” bread. Salt reduction had significant impact on every bead type for moisture and salt content. Moreover, a linear pattern for specific volume parameter on “Carcaça” bread was found, i.e. specific volume increased as the level of added salt increases. These results are in agreement with the ones obtained from the analysis with the rheofermentometer. The performance of dough development and gas retention capabilities improved, to a certain extent, with increasing of salt addition. Additionally, as it would be expected, salt concentration increased with increasing salt addition levels, following a linear trend for every bread type.

Crumb structure and color are essential bread quality parameters that were also studied. Salt addition at different concentrations had limited impact on crumb morphology, except for cell area distribution. Control bread had higher percentage of very small cells than bread with 1.0% in “Carcaça” bread and small size cells than 0.0% and 0.8% in “D’Água” bread. Once more, for “Carcaça” bread, these results are in agreement with the ones obtained in the study of dough development, where the increase of the salt concentrations promoted the reduction of the fermentation rate, delaying the cells growth. Moreover, with absence of salt, the gluten network is fragile and the dough ceases to be able to retain the gas, leading to the disruption of the large cells. Concerning color, salt reduction resulted in significant differences for every parameters when comparing to respective controls. Control bread was lighter than 0.8% for “D’Água” bread, 1.0% and 1.1% for “Regueifa” bread; was greener than 0.0%, 0.8% and 1.3% in “Carcaça” bread; and it was redder than 0.0%, 0.8% and 1.3% in “Regueifa” bread.

While the evaluation of the physicochemical parameters is determinant to evaluate bread baking performance, the sensory analysis is essential to understand the level of salt reduction that can be achieved without affecting of the consumers’ acceptance.

A descriptive sensory analysis was performed with a trained panel. Overall, salt addition at different concentrations had limited impact on sensory evaluation of the different bread types. However, significant differences were found for all bread types and all attributes, except for odor attributes. Aroma attributes was the category where salt reduction had more impact and, globally, “Regueifa” bread seems to be the type of bread most affected by salt reduction.

Consumer test was also applied, providing additional and relevant information, since they may be less conservative than a trained panel. Results obtained only showed significant differences for salt reduction with *taste liking* and *overall liking* for all bread types. Comparing these results with the physicochemical analysis, it is possible to observed that physical parameters did not seem to have a significant impact on consumer perception, since only the lack of salt addition was relevant, which was more noticeable for “Carcaça” bread and less evident for “Regueifa” bread.

Mathematical modelling was shown as a relevant tool to study bread acceptability and understand the relationships between sensory and analytical data.

Multivariate PLS regression showed that *taste liking* had a significant positive impact on *overall liking* for every bread type. Moreover, different sensory attributes were related positively with *overall liking* by consumers, but, *bread aroma*, *salty* and *crumb color intensity* were important in every bread type. Additionally, “Mistura” bread was the bread type where sensory attributes have more impact on overall liking for consumers.

External preference mapping was appropriated to study consumer preferences and to select the lowest salt concentration with best acceptance, namely 0.8% for “D’Água” bread; 0.8% for “Carcaça” bread; 1.0% for “Mistura” bread; and 1.1% for “Regueifa” bread. These results suggest that it is possible to reduce, to some extent, the salt concentration in all bread types without major impact on bread characteristics and without compromising consumers' acceptance.

Additionally, PLS regression provided information on the relationship between sensory and analytical data (physicochemical, crumb structure and color). With this analysis, it was intended to evaluate the existence of a pattern of results associated with salt reduction, regardless of the type of bread studied. Successful models were obtained for *crust color intensity*, *crumb color intensity*, *cohesiveness*, *adhesiveness*, *crumb elasticity*, *shape recovery*, *salty*, *bread aroma*, and *aftertaste*. However, these relationships should be interpreted as associations and not as direct cause and effect, once observed correlations do not necessarily imply causality.

The information obtained from this study was essential for the construction of a support guide directed at baking professionals that will facilitate the gradual reduction of salt concentration on bread. It is important to note that salty taste is intrinsic and depends on several variables such as individual opinion and habits. Thus, the extent to which salt concentration can be reduced might have to be adjusted in accordance. In the long run, it is expected that this study achieves its ultimate goal, i.e. to contribute, in a pedagogic way, to the gradual reduction of salt in bread in order to impact salt consumption of the Portuguese population that could, in turn, have a significant positive impact on health.

Chapter VI

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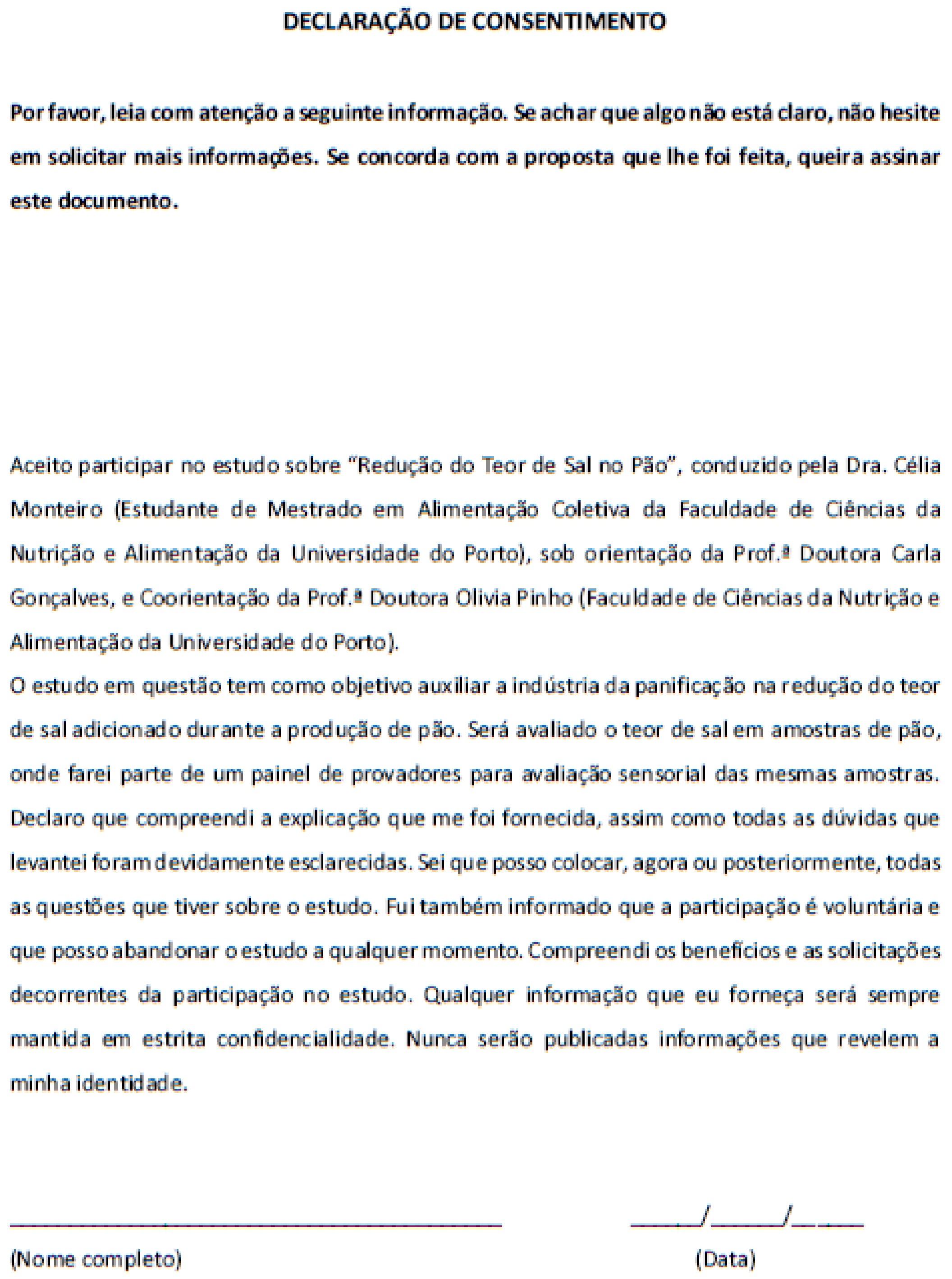
Chapter VI

Annex

Annex 1

Informed consent

Initial questionnaire for the tasters selection



DECLARAÇÃO DE CONSENTIMENTO

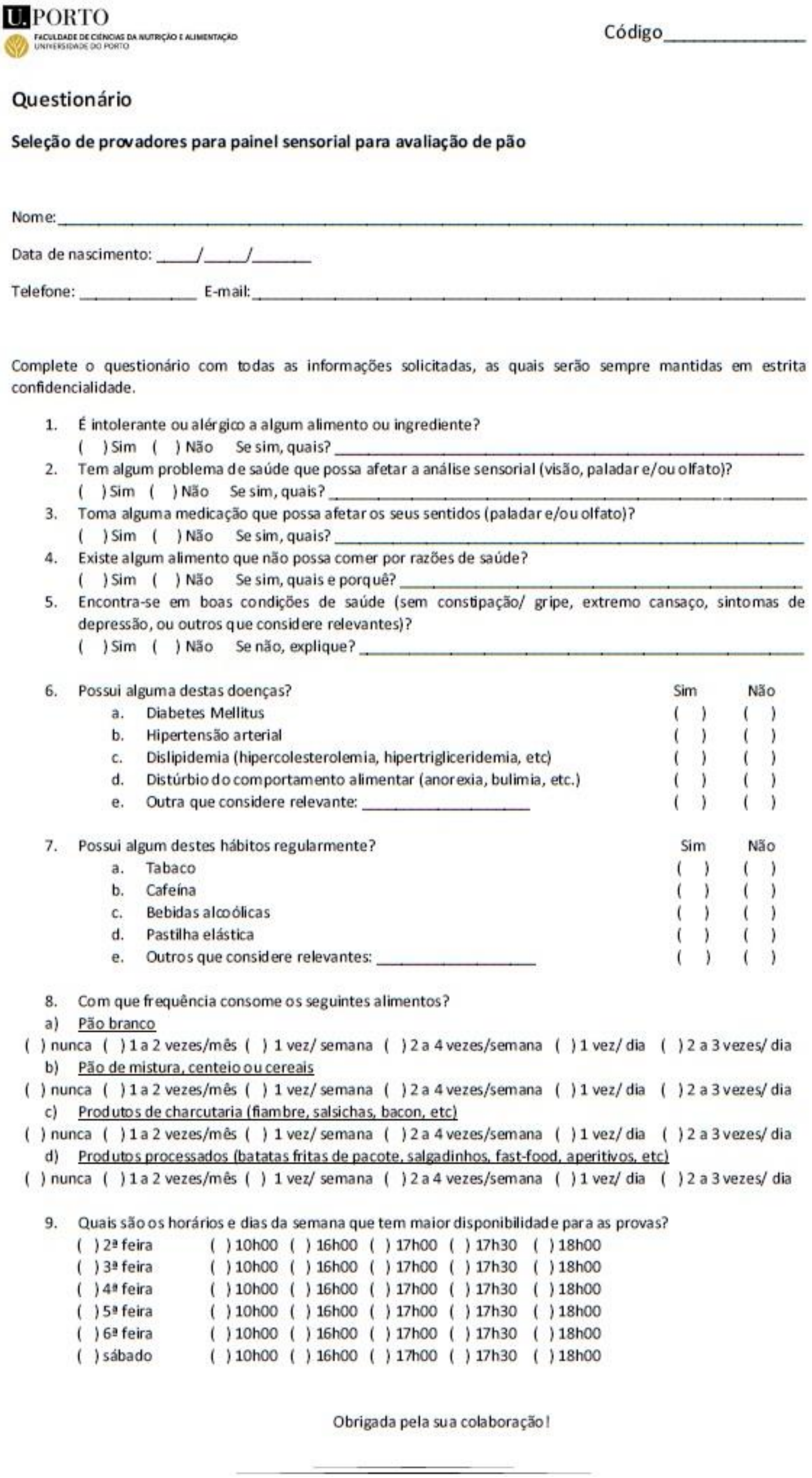
Por favor, leia com atenção a seguinte informação. Se achar que algo não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.

Aceito participar no estudo sobre "Redução do Teor de Sal no Pão", conduzido pela Dra. Célia Monteiro (Estudante de Mestrado em Alimentação Coletiva da Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto), sob orientação da Prof.ª Doutora Carla Gonçalves, e Coorientação da Prof.ª Doutora Olivia Pinho (Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto).

O estudo em questão tem como objetivo auxiliar a indústria da panificação na redução do teor de sal adicionado durante a produção de pão. Será avaliado o teor de sal em amostras de pão, onde farei parte de um painel de provadores para avaliação sensorial das mesmas amostras. Declaro que compreendi a explicação que me foi fornecida, assim como todas as dúvidas que levantei foram devidamente esclarecidas. Sei que posso colocar, agora ou posteriormente, todas as questões que tiver sobre o estudo. Fui também informado que a participação é voluntária e que posso abandonar o estudo a qualquer momento. Compreendi os benefícios e as solicitações decorrentes da participação no estudo. Qualquer informação que eu forneça será sempre mantida em estrita confidencialidade. Nunca serão publicadas informações que revelem a minha identidade.

_______________________________________ ______/______/______

(Nome completo) (Data)



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Código_______________

Questionário

Seleção de provadores para painel sensorial para avaliação de pão

Nome:____

Data de nascimento: _____/_____/_______

Telefone: ______________ E-mail:_________________________________

Complete o questionário com todas as informações solicitadas, as quais serão sempre mantidas em estrita confidencialidade.

1. É intolerante ou alérgico a algum alimento ou ingrediente?
   ( ) Sim ( ) Não Se sim, quais? ______________________________
2. Tem algum problema de saúde que possa afetar a análise sensorial (visão, paladar e/ou olfato)?
   ( ) Sim ( ) Não Se sim, quais? ______________________________
3. Toma alguma medicação que possa afetar os seus sentidos (paladar e/ou olfato)?
   ( ) Sim ( ) Não Se sim, quais? ______________________________
4. Existe algum alimento que não possa comer por razões de saúde?
   ( ) Sim ( ) Não Se sim, quais e porquê? ______________________________
5. Encontra-se em boas condições de saúde (sem constipação/ gripe, extremo cansaço, sintomas de depressão, ou outros que considere relevantes)?
   ( ) Sim ( ) Não Se não, explique? ______________________________

6. Possui alguma destas doenças?	Sim	Não
a. Diabetes Mellitus	( )	( )
b. Hipertensão arterial	( )	( )
c. Dislipidemia (hipercolesterolemia, hipertrigliceridemia, etc)	( )	( )
d. Distúrbio do comportamento alimentar (anorexia, bulimia, etc.)	( )	( )
e. Outra que considere relevante: ____________________	( )	( )

7. Possui algum destes hábitos regularmente?	Sim	Não
a. Tabaco	( )	( )
b. Cafeína	( )	( )
c. Bebidas alcoólicas	( )	( )
d. Pastilha elástica	( )	( )
e. Outros que considere relevantes: ____________________	( )	( )

8. Com que frequência consome os seguintes alimentos?

a) Pão branco
( ) nunca ( ) 1 a 2 vezes/mês ( ) 1 vez/ semana ( ) 2 a 4 vezes/semana ( ) 1 vez/ dia ( ) 2 a 3 vezes/ dia

b) Pão de mistura, centeio ou cereais
( ) nunca ( ) 1 a 2 vezes/mês ( ) 1 vez/ semana ( ) 2 a 4 vezes/semana ( ) 1 vez/ dia ( ) 2 a 3 vezes/ dia

c) Produtos de charcutaria (fiambre, salsichas, bacon, etc)
( ) nunca ( ) 1 a 2 vezes/mês ( ) 1 vez/ semana ( ) 2 a 4 vezes/semana ( ) 1 vez/ dia ( ) 2 a 3 vezes/ dia

d) Produtos processados (batatas fritas de pacote, salgadinhos, fast-food, aperitivos, etc)
( ) nunca ( ) 1 a 2 vezes/mês ( ) 1 vez/ semana ( ) 2 a 4 vezes/semana ( ) 1 vez/ dia ( ) 2 a 3 vezes/ dia

9. Quais são os horários e dias da semana que tem maior disponibilidade para as provas?
( ) 2ª feira ( ) 10h00 ( ) 16h00 ( ) 17h00 ( ) 17h30 ( ) 18h00
( ) 3ª feira ( ) 10h00 ( ) 16h00 ( ) 17h00 ( ) 17h30 ( ) 18h00
( ) 4ª feira ( ) 10h00 ( ) 16h00 ( ) 17h00 ( ) 17h30 ( ) 18h00
( ) 5ª feira ( ) 10h00 ( ) 16h00 ( ) 17h00 ( ) 17h30 ( ) 18h00
( ) 6ª feira ( ) 10h00 ( ) 16h00 ( ) 17h00 ( ) 17h30 ( ) 18h00
( ) sábado ( ) 10h00 ( ) 16h00 ( ) 17h00 ( ) 17h30 ( ) 18h00

Obrigada pela sua colaboração!

Annex 2

Basic tastes test

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Código_______________

Teste de Identificação de Sabores Básicos

Nome:____

Data:____/____/_______

Por favor, classifique a sensação percebida de cada uma das amostras apresentadas.

	Doce	**Ácido**	**Salgado**
__ __ __	_______	_______	_______
__ __ __	_______	_______	_______
__ __ __	_______	_______	_______
__ __ __	_______	_______	_______
__ __ __	_______	_______	_______
__ __ __	_______	_______	_______
__ __ __	_______	_______	_______

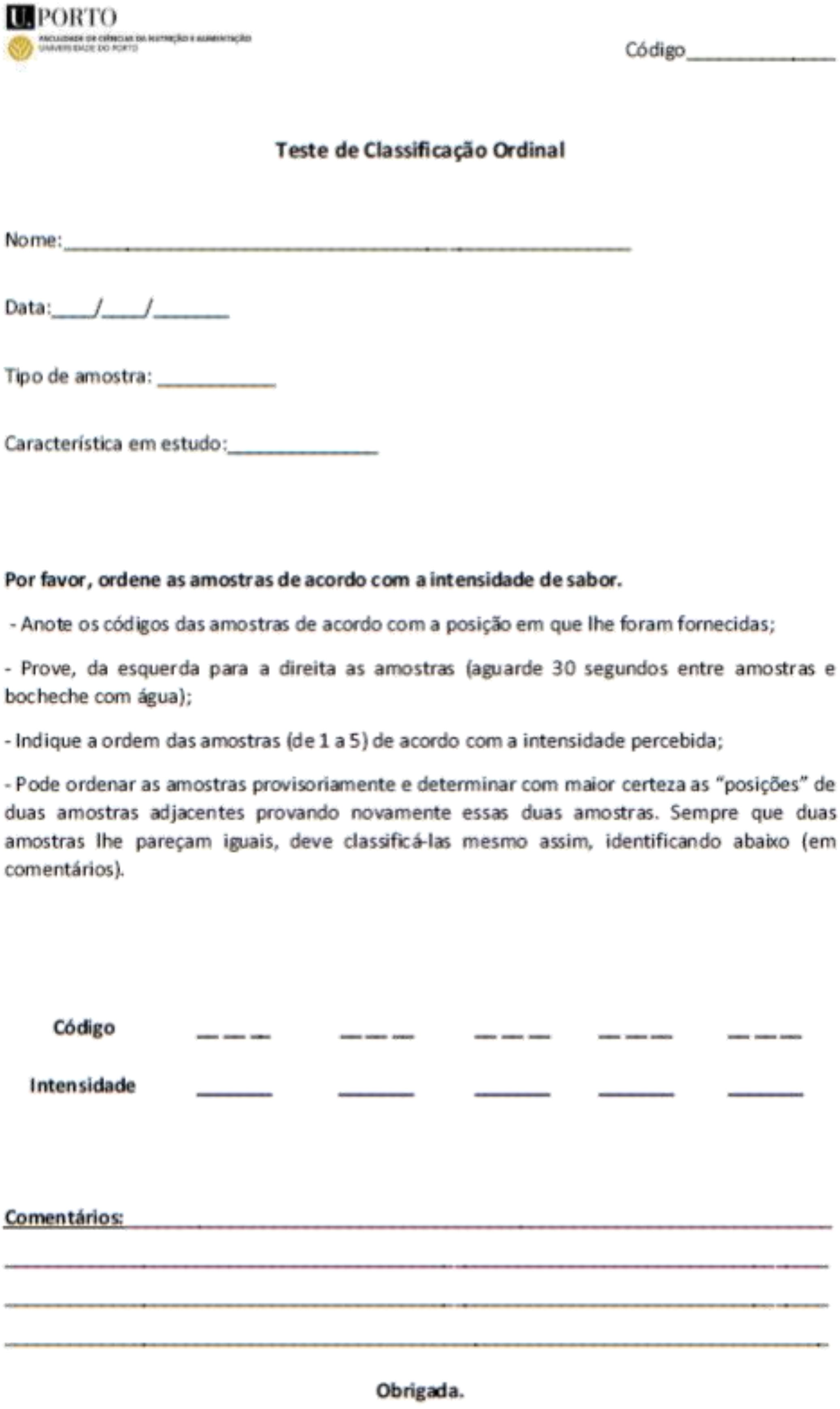
Obrigada.

Annex 3

Ranking test: aqueous solutions

Ranking test: “Carcaça” bread

Ranking test: “D’água”, “Mistura” and “Regueifa” breads



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Código______________

Teste de Classificação Ordinal

Nome:____

Data:____/____/_______

Tipo de amostra: ___________

Característica em estudo:______________

Por favor, ordene as amostras de acordo com a intensidade de sabor.

- Anote os códigos das amostras de acordo com a posição em que lhe foram fornecidas;

- Prove, da esquerda para a direita as amostras (aguarde 30 segundos entre amostras e bocheche com água);

- Indique a ordem das amostras (de 1 a 5) de acordo com a intensidade percebida;

- Pode ordenar as amostras provisoriamente e determinar com maior certeza as "posições" de duas amostras adjacentes provando novamente essas duas amostras. Sempre que duas amostras lhe pareçam iguais, deve classificá-las mesmo assim, identificando abaixo (em comentários).

Código	__ __ __	__ __ __	__ __ __	__ __ __	__ __ __
Intensidade	_______	_______	_______	_______	_______

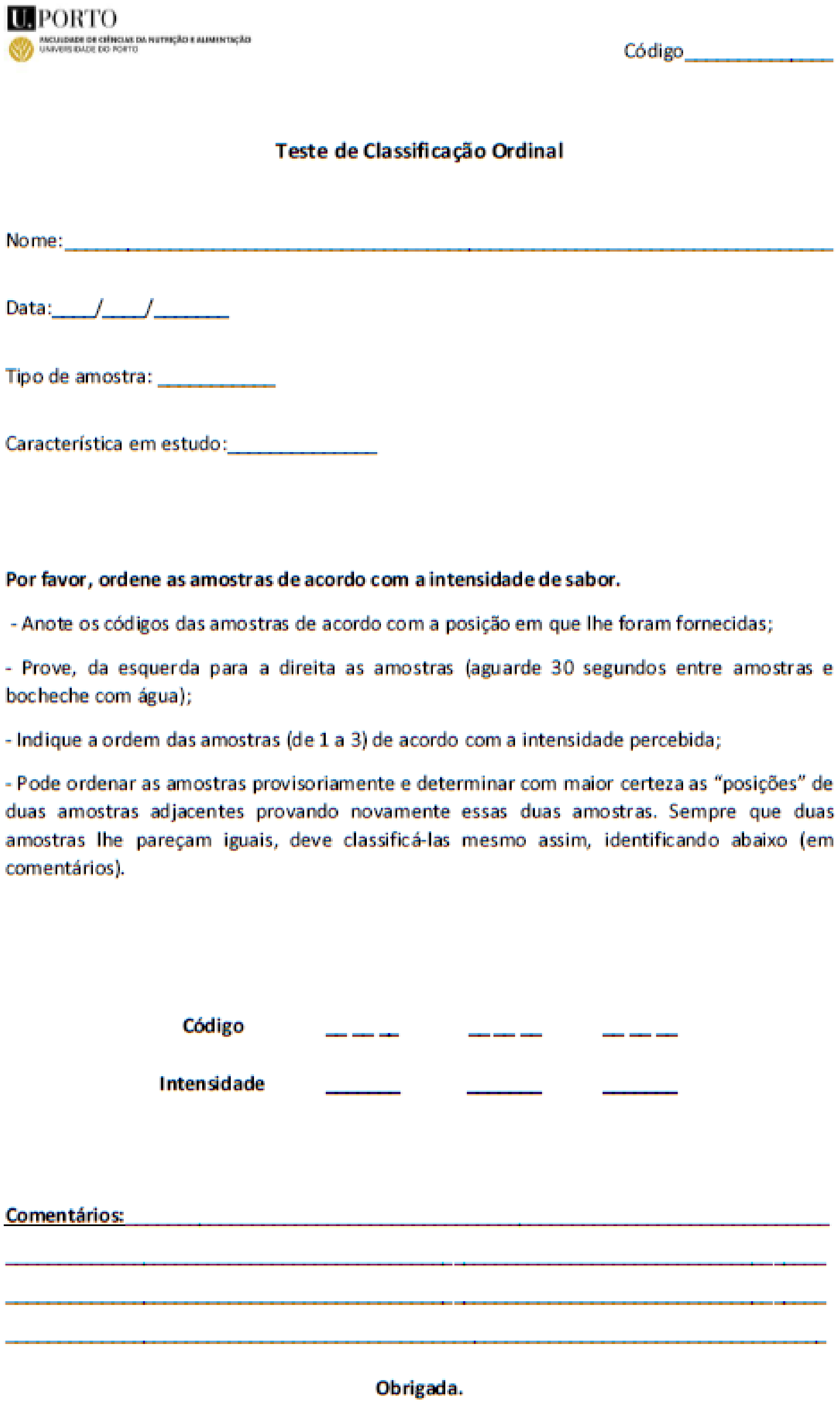
Comentários:____

____

____

____

Obrigada.



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Código______________

Teste de Classificação Ordinal

Nome:____

Data:____/____/_______

Tipo de amostra: ___________

Característica em estudo:______________

Por favor, ordene as amostras de acordo com a intensidade de sabor.

- Anote os códigos das amostras de acordo com a posição em que lhe foram fornecidas;
- Prove, da esquerda para a direita as amostras (aguarde 30 segundos entre amostras e bocheche com água);
- Indique a ordem das amostras (de 1 a 3) de acordo com a intensidade percebida;
- Pode ordenar as amostras provisoriamente e determinar com maior certeza as "posições" de duas amostras adjacentes provando novamente essas duas amostras. Sempre que duas amostras lhe pareçam iguais, deve classificá-las mesmo assim, identificando abaixo (em comentários).

Código	__ __ __	__ __ __	__ __ __
Intensidade	_______	_______	_______

Comentários:____

____

____

____

Obrigada.

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Código______________

Teste de Classificação Ordinal

Nome:____

Data:____/____/_______

Característica em estudo:______________

Por favor, ordene as amostras de cada tipo de pão de acordo com a intensidade de sabor.

- Anote os códigos das amostras de acordo com a posição em que lhe foram fornecidas;

- Prove, da esquerda para a direita as amostras (aguarde 30 segundos entre amostras e bocheche com água);

- Indique a ordem das amostras (de 1 a 3) de acordo com a intensidade percebida;

- Pode ordenar as amostras provisoriamente e determinar com maior certeza as "posições" de duas amostras adjacentes provando novamente essas duas amostras. Sempre que duas amostras lhe pareçam iguais, deve classificá-las mesmo assim, identificando abaixo (em comentários).

Pão 1	**Código**	__ __ __	__ __ __	__ __ __	__ __ __
	Intensidade	______	______	______	______

Pão 2	**Código**	__ __ __	__ __ __	__ __ __	__ __ __
	Intensidade	______	______	______	______

Pão 3	**Código**	__ __ __	__ __ __	__ __ __	__ __ __
	Intensidade	______	______	______	______

Comentários:____

____

____

Obrigada.

Annex 4

Triangular test

Triangular test: trained panel validation

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Código______________

Teste Triangular

Nome:____

Data:____/____/_______

Foram-lhe dadas três amostras, em que duas são iguais e uma é diferente.

Por favor prove as amostras pela ordem abaixo, e identifique a amostra diferente.

Este teste é de resposta obrigatória.

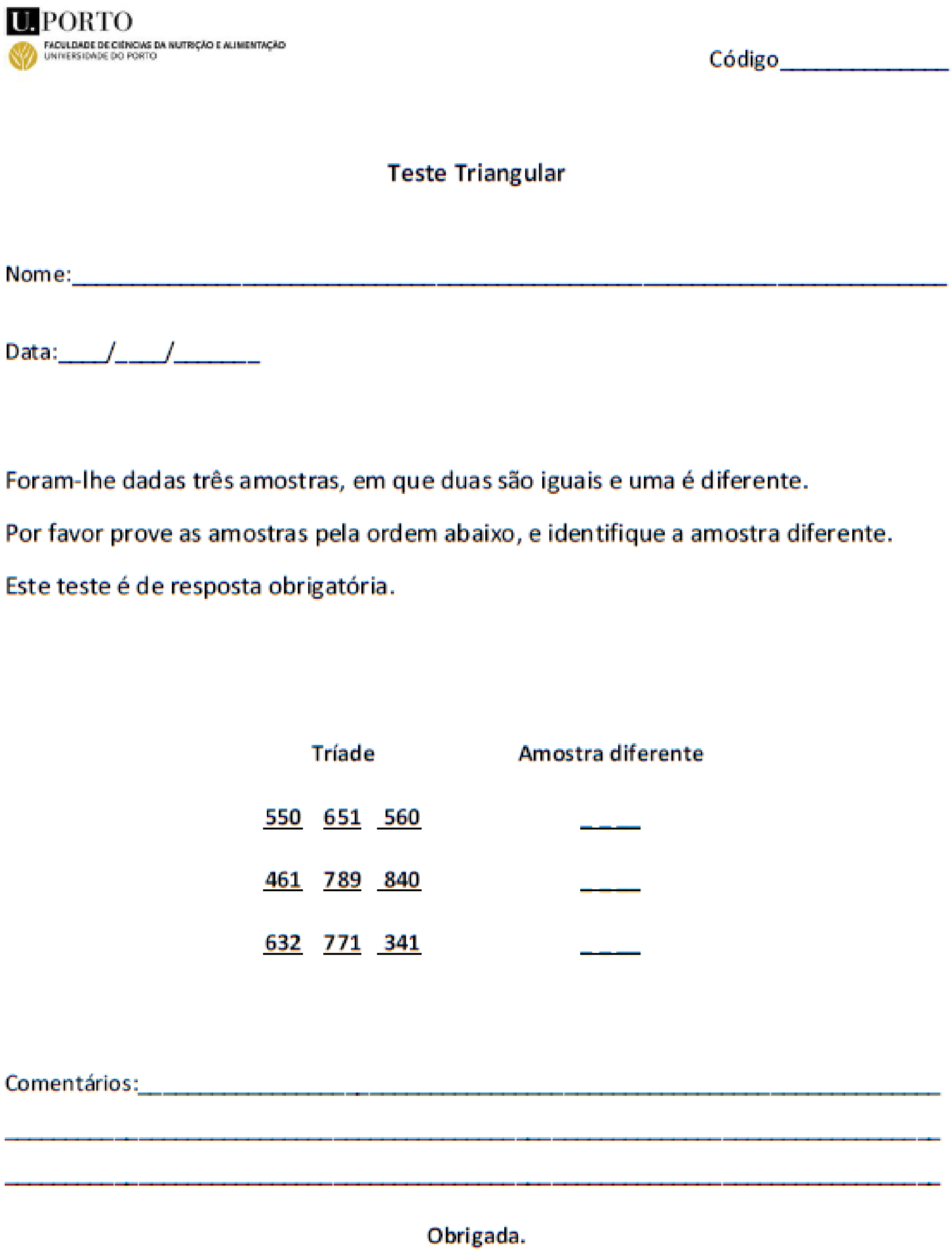
Tríade	Amostra diferente
280 631 470	___

Comentários:____

____

____

Obrigada.



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Código_______________

Teste Triangular

Nome:____

Data:____/____/_______

Foram-lhe dadas três amostras, em que duas são iguais e uma é diferente.

Por favor prove as amostras pela ordem abaixo, e identifique a amostra diferente.

Este teste é de resposta obrigatória.

Tríade	Amostra diferente
550 651 560	___
461 789 840	___
632 771 341	___

Comentários:____

____

____

Obrigada.

Annex 5

Descriptive sensory analysis: toast

Descriptive sensory analysis: answer sheet for attributes definition

Descriptive sensory analysis: final scale

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Código______________

Ficha de avaliação - Tostas

Nome:____

Data:____/____/_______

Código da amostra: __ __ __

Aspeto geral

Intensidade da cor da crosta	1	2	3	4	5	6	7
Intensidade da cor do interior	1	2	3	4	5	6	7
Homogeneidade da cor interior	1	2	3	4	5	6	7
Rugosidade da superfície	1	2	3	4	5	6	7

Características olfativas

Intensidade do odor	1	2	3	4	5	6	7
Odor a pão	1	2	3	4	5	6	7
Odor a cereais	1	2	3	4	5	6	7
Odor a tostado	1	2	3	4	5	6	7

Código______________

Características da textura

Crocante	1	2	3	4	5	6	7
Dureza	1	2	3	4	5	6	7
Elasticidade	1	2	3	4	5	6	7
Coesividade	1	2	3	4	5	6	7
Adesividade	1	2	3	4	5	6	7

Características do aroma

Salgado	1	2	3	4	5	6	7
Doce	1	2	3	4	5	6	7
Aroma a pão	1	2	3	4	5	6	7
Aroma a cereais	1	2	3	4	5	6	7
Aroma a tostado	1	2	3	4	5	6	7
Sabor residual	1	2	3	4	5	6	7

Apreciação global	1	2	3	4	5	6	7

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Código______________

Ficha de avaliação - Pão

Nome:____

Data:____/____/_______

Código da amostra: __ __ __

Aspeto geral

__________-____________	1 2 3 4 5 6 7
__________-____________	1 2 3 4 5 6 7
__________-____________	1 2 3 4 5 6 7
__________-____________	1 2 3 4 5 6 7
__________-____________	1 2 3 4 5 6 7
__________-____________	1 2 3 4 5 6 7

Características olfativas

__________-___________	1 2 3 4 5 6 7
__________-___________	1 2 3 4 5 6 7
__________-___________	1 2 3 4 5 6 7
__________-___________	1 2 3 4 5 6 7

Código______________

Características da textura

__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

Características do aroma

__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

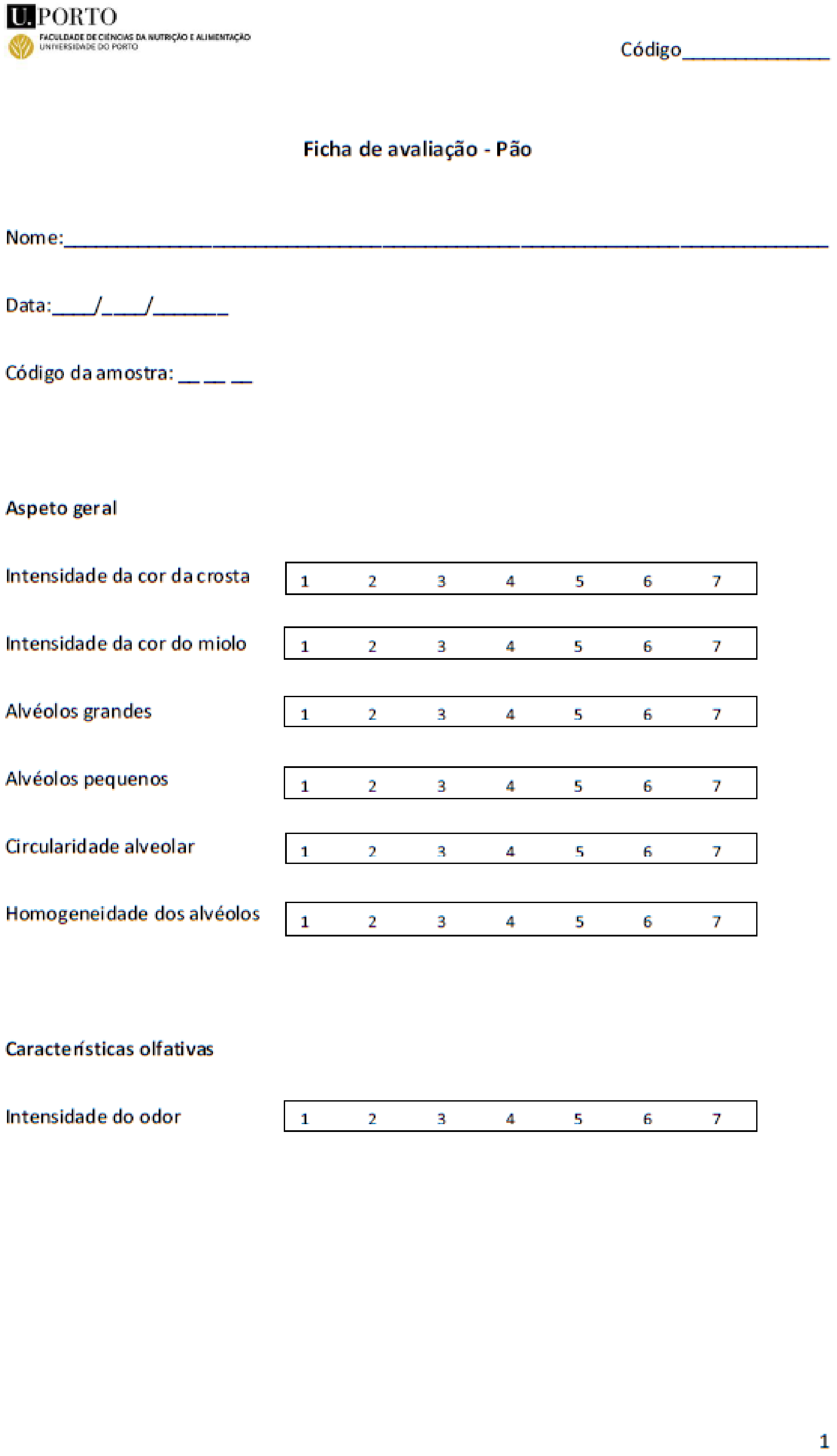
__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

__________-__________ 1 2 3 4 5 6 7

Apreciação global 1 2 3 4 5 6 7



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Código______________

Ficha de avaliação - Pão

Nome:____

Data:____/____/_______

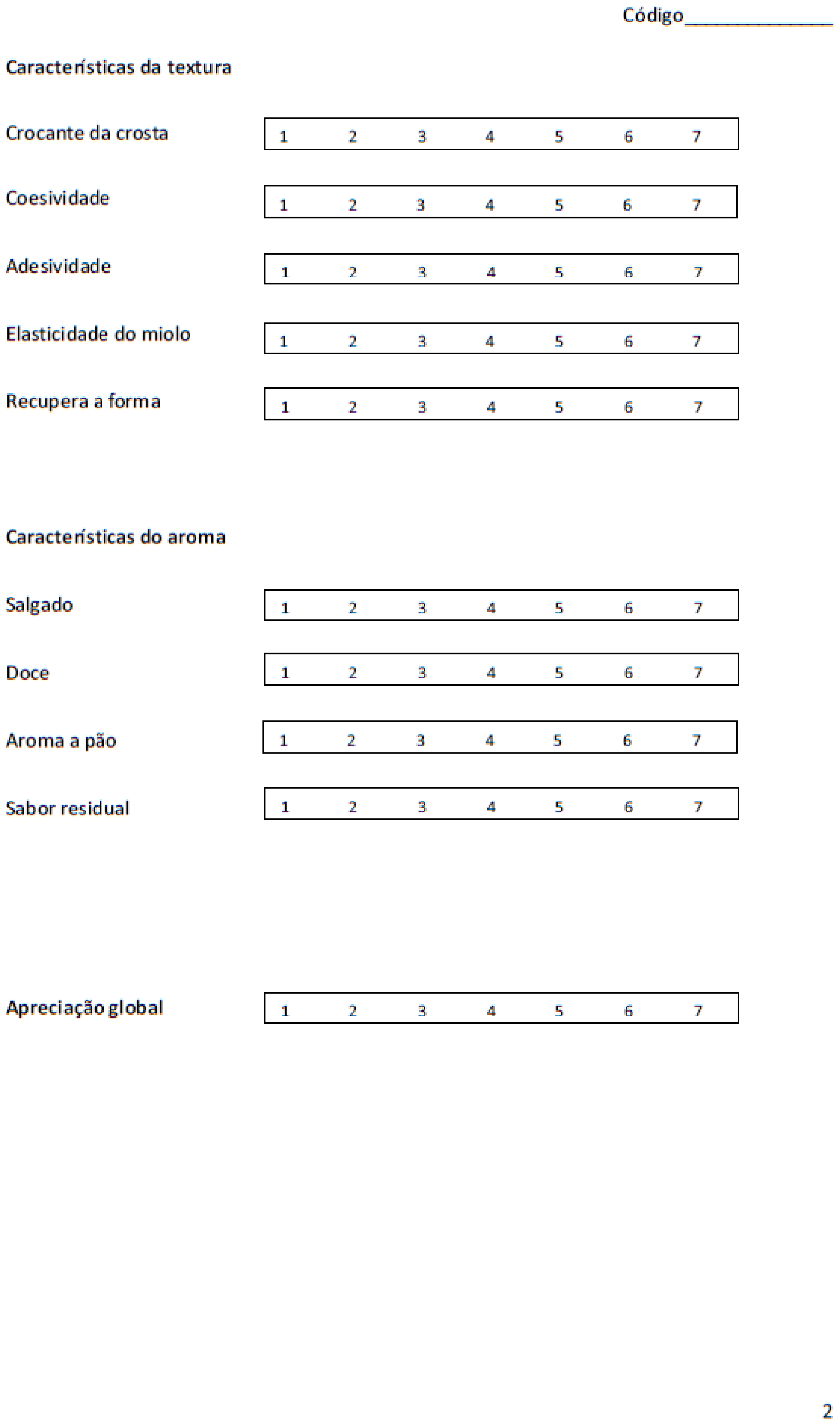
Código da amostra: __ __ __

Aspeto geral

Intensidade da cor da crosta	1	2	3	4	5	6	7
Intensidade da cor do miolo	1	2	3	4	5	6	7
Alvéolos grandes	1	2	3	4	5	6	7
Alvéolos pequenos	1	2	3	4	5	6	7
Circularidade alveolar	1	2	3	4	5	6	7
Homogeneidade dos alvéolos	1	2	3	4	5	6	7

Características olfativas

Intensidade do odor	1	2	3	4	5	6	7



Código______________

Características da textura

Crocante da crosta	1	2	3	4	5	6	7
Coesividade	1	2	3	4	5	6	7
Adesividade	1	2	3	4	5	6	7
Elasticidade do miolo	1	2	3	4	5	6	7
Recupera a forma	1	2	3	4	5	6	7

Características do aroma

Salgado	1	2	3	4	5	6	7
Doce	1	2	3	4	5	6	7
Aroma a pão	1	2	3	4	5	6	7
Sabor residual	1	2	3	4	5	6	7

Apreciação global	1	2	3	4	5	6	7

Annex 6

Consumer test

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Código______________

Análise sensorial de pão

Sexo: ___F ___M Idade:_____ Profissão:__________________________________

Prove cada amostra de pão que lhe é apresentada e avalie para cada parâmetro.

Amostra <u>154</u>		**Não gosto nada**						**Gosto muito**	
Apreciação da aparência	☹	1	2	3	4	5	6	7	☺
Apreciação do aroma		1	2	3	4	5	6	7	
Apreciação do sabor		1	2	3	4	5	6	7	
Apreciação da textura		1	2	3	4	5	6	7	
Apreciação global		1	2	3	4	5	6	7	

Amostra <u>141</u>		**Não gosto nada**						**Gosto muito**	
Apreciação da aparência	☹	1	2	3	4	5	6	7	☺
Apreciação do aroma		1	2	3	4	5	6	7	
Apreciação do sabor		1	2	3	4	5	6	7	
Apreciação da textura		1	2	3	4	5	6	7	
Apreciação global		1	2	3	4	5	6	7	

Amostra <u>192</u>		**Não gosto nada**						**Gosto muito**	
Apreciação da aparência	☹	1	2	3	4	5	6	7	☺
Apreciação do aroma		1	2	3	4	5	6	7	
Apreciação do sabor		1	2	3	4	5	6	7	
Apreciação da textura		1	2	3	4	5	6	7	
Apreciação global		1	2	3	4	5	6	7	

Código_______________

Amostra 167

		Não gosto nada						Gosto muito	
Apreciação da aparência	☹	1	2	3	4	5	6	7	☺
Apreciação do aroma		1	2	3	4	5	6	7	
Apreciação do sabor		1	2	3	4	5	6	7	
Apreciação da textura		1	2	3	4	5	6	7	
Apreciação global		1	2	3	4	5	6	7	

Amostra 125

		Não gosto nada						Gosto muito	
Apreciação da aparência	☹	1	2	3	4	5	6	7	☺
Apreciação do aroma		1	2	3	4	5	6	7	
Apreciação do sabor		1	2	3	4	5	6	7	
Apreciação da textura		1	2	3	4	5	6	7	
Apreciação global		1	2	3	4	5	6	7	

Amostra 108

		Não gosto nada						Gosto muito	
Apreciação da aparência	☹	1	2	3	4	5	6	7	☺
Apreciação do aroma		1	2	3	4	5	6	7	
Apreciação do sabor		1	2	3	4	5	6	7	
Apreciação da textura		1	2	3	4	5	6	7	
Apreciação global		1	2	3	4	5	6	7	

Annex 7

Poster - Impact of salt reduction on bread physicochemical properties and crumb color and structure

2nd prize in XVII Congress of Food and Nutrition & I International Congress of Food and Nutrition

IMPACT OF SALT REDUCTION ON BREAD PHYSICOCHEMICAL PROPERTIES AND CRUMB COLOR AND STRUCTURE

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INTRODUCTION

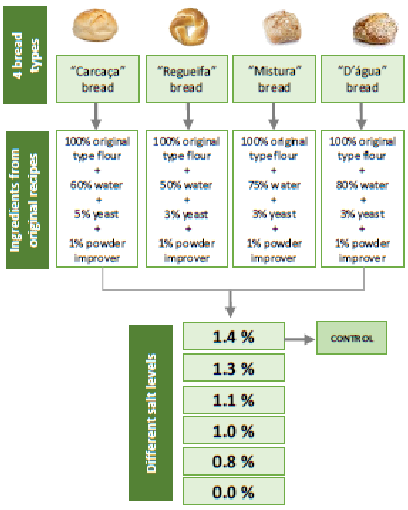
Bread is a staple component of the Portuguese diet but is also a major source of dietary salt [1,2]. Many countries have chosen bread as on the priority foods for reducing salt content in their national salt reduction initiatives, including Portugal [3]. However, salt has specific properties that are essential for bread processing and quality in the final product [4,5].

OBJECTIVES

This study aimed to evaluate the impact of salt reduction on bread physicochemical parameters, as well as crumb colour and structure.

MATERIAL AND METHODS

1. Bread production



4 bread types

"Carcaça" bread	"Regueifa" bread	"Mistura" bread	"D'água" bread
100% original type flour + 60% water + 5% yeast + 1% powder improver	100% original type flour + 50% water + 3% yeast + 1% powder improver	100% original type flour + 75% water + 3% yeast + 1% powder improver	100% original type flour + 80% water + 3% yeast + 1% powder improver

Ingredients from original recipes

Different salt levels

- 1.4 % → CONTROL
- 1.3 %
- 1.1 %
- 1.0 %
- 0.8 %
- 0.0 %

2. Bread analysis

Physicochemical characteristics

SODIUM ANALYSIS BY FLAME PHOTOMETRY [6]

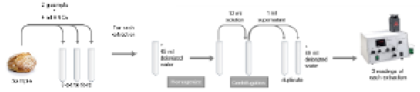


Fig. 1 Process of sample preparation for sodium analysis by photometry flame

BREAD WEIGHT

- Sampling for each salt level: 10 samples for "Carcaça", "Mistura" and "D'água" breads; 4 samples for "Regueifa" bread;
- Method: digital scale.

SPECIFIC BREAD VOLUME

- Sampling for each salt level: 10 samples for all bread types;
- Method: seed displaced method using the formula $SV(\text{cm}^3\text{g}^{-1}) = S(g) \times \frac{1{,}35(\text{cm}^3\ \text{g}^{-1})}{P(g)}$, where P is bread weight, S is weight of the displaced seeds, 1.35 is specific volume of the *Phalaris canariensis* seeds, and SV is specific volume of the bread [7].

BREAD MOISTURE

- Sampling for each salt level: 3 readings for all bread types;
- Method: moisture balance at 130°C (6 g portion of the homogeneous aliquot sample) [8].

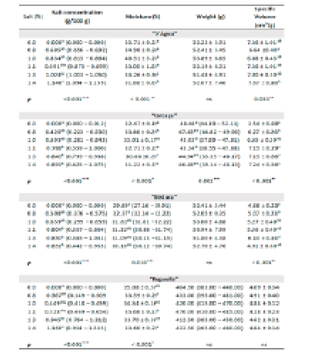
Bread crust color and crumb structure

- Sampling for each salt level: 3 samples (1 slice of 1.6 cm thickness for each sample);
- Method: A single 200x200 pixel field of view (FOV) was cropped from each slice;
  For bread color, the FOV was converted to CIElab system and L*, a*, and b* parameters were analyzed;
  For crumb, the FOV was converted to a 256 level gray scale and segmented and cell morphological parameters were analyzed, including number of cells, mean cell area (mm²), cell density (cells/ mm²), and cell area (% of total cells) [9].

RESULTS

Physicochemical characteristics

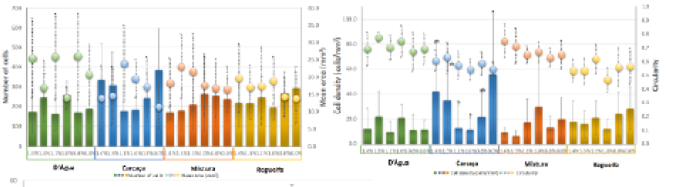
Table. 1 Physicochemical parameters values for each type, with different salt levels (% wheat flour)



[illegible]

- Salt reduction had significant impact on every bread type for salt content and moisture;
- The increase in concentration of salt measured *versus* salt added followed a linear trend for every bread type ("D'água", R^2=0.996; "Carcaça", R^2=0.808; "Mistura", R^2=0.770; "Regueifa", R^2=0.727);
- No significant differences were found for bread weight;
- Regarding bread specific volume, no significant differences were found for "Regueifa" bread.

Bread crust color and crumb structure



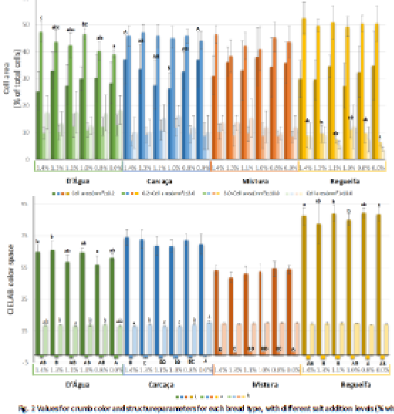


Fig. 2 Values for crumb color and structure parameters for each bread type, with different salt addition levels (% wheat flour); Different letters for each bread type in a set of parameters show statistically significant differences (p < 0.05).

- Salt reduction had limited influence on crumb morphology, except for cell area distribution:
  - number of cells, mean area and circularity → *p*>0.050, for every bread types;
  - cell density → *p*<0.050, for "Carcaça" bread between 0.0%, 1.3% and control;
  - Very small cell (cell area≤0.2 mm²) → *p*<0.050, for "Carcaça" bread between control and 1.0%;
  - Small cell (0.2<cell area≤3.0 mm²) → *p*<0.050, for "D'água" bread between 0.0%, 0.8% and control;
- Regarding color, more impact was found on redness/ greenness.
  - "D'água" bread → *p*<0.050, for L* between control and 0.8% and 1.3%;
  - "Carcaça" and "Mistura" breads → *p*<0.050, for a* and b* between control and 0.0%, 0.8%, and 1.3%;
  - "Regueifa" bread → *p*<0.050, for L* between control and 1.0% and 1.1%;

CONCLUSION

The results suggest that it is possible to reduce, to some extent, the salt concentration in all bread types without major impact on bread physicochemical characteristics.

REFERENCES

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CERTIFICATE

It was presented the poster entitled by **"Impact of salt reduction on bread physicochemical properties and crumb colour and structure."** during the XVII Congress of Food and Nutrition & I International Congress of Food and Nutrition, held by Associação Portuguesa de Nutrição, at Lisbon Congress Center, on May 10 and 11, 2018. This poster received the 2nd prize. The authors are: C. Monteiro[1], Z. E. Martins[2], O. Pinho1,2, C. Gonçalves[1,3]

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[3] Escola Superior de Tecnologia e Gestão do Instituto Politécnico de Viana do Castelo

Lisbon, 11th May 2018

Célia Craveiro (President)
Organizing Committee

XVII CONGRESS OF FOOD AND NUTRITION
I INTERNATIONAL CONGRESS OF FOOD AND NUTRITION

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M.FCNAUP **2018**

Impact on sensory preference and physicochemical parameters of salt reduction on bread

Impacto na avaliação sensorial e nos parâmetros físico-químicos da redução de sal no pão

Célia Regina Oliveira Monteiro

FACULDADE DE CIENCIAS DA NUTRIÇAO E ALIMENTAÇAO