University of São Paulo
"Luiz de Queiroz" College of Agriculture

Development and characterization of beef burger added with micronized salt and long-chain n-3 fatty acids

Juan Dario Rios Mera

Thesis presented to obtain the degree of Doctor in Science. Area: Food Science and Technology

Piracicaba
2021

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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RESUMO

Desenvolvimento e caracterização de hambúrguer bovino adicionado de sal micronizado e ácidos graxos n-3 de cadeia longa

O hambúrguer bovino é um produto cárneo muito apreciado pelos consumidores devido às suas características sensoriais. Além disso, oferece grande ingestão de proteínas, calorias, vitaminas e minerais. No entanto, o hambúrguer bovino, e geralmente os produtos cárneos, podem conter quantidade considerável de sódio derivado do sal adicionado e perfil lipídico pouco saudável. Esses componentes podem aumentar o risco de doenças da síndrome metabólica, como as doenças cardiovasculares. Nesse contexto, o desenvolvimento de produtos cárneos mais saudáveis é uma necessidade que atende às demandas dos consumidores. Nesta pesquisa, uma estratégia sequencial foi desenvolvida para obter um hambúrguer bovino com caracteristicas mais saudáveis. Na primeira etapa, sal micronizado foi parcialmente misturado com o toucinho suíno para proteger sua estrutura e utilizado para reduzir o teor de sal/sódio no produto, mostrando a possibilidade de reduzir 33% de sal no hambúrguer. Desse modo, a textura, o rendimento, as características físico-químicas e sensoriais determinadas pelos consumidores e a aceitação global foram ligeiramente ou nada afetados. A segunda etapa envolveu o desenvolvimento de um ingrediente fonte de ácidos graxos poliinsaturados n-3 de cadeia longa (eicosapentaenóico, EPA, e docosahexaenóico, DHA) para fortificar o hambúrguer. Micropartículas (MP) de proteína isolada de soja e inulina contendo óleo de peixe foram produzidas por coacervação complexa, obtendo-se um produto otimizado quanto à eficiência de encapsulação e rendimento. Além disso, as MP foram resistentes à condições específicas de pH e tratamento térmico, retendo mais de 81% do óleo de peixe encapsulado quando a transglutaminase foi usada para dar maior resistência às MP. Na terceira etapa, foi estudada a estratégia de fortificação do hambúrguer bovino com óleo de peixe fonte de EPA e DHA, em combinação com a estratégia de redução de sal/sódio da primeira etapa. Como resultado, algumas diferenças foram encontradas entre hambúrgueres com diferentes teores de sal (1,5% vs. 1,0%) quando adicionado óleo de peixe encapsulado ou não encapsulado, principalmente no perfil de ácidos graxos e compostos voláteis. No entanto, as MP liofilizadas falharam em vários parâmetros de qualidade do hambúrguer bovino, sendo os mais notáveis o aumento nos compostos voláteis de oxidação, perfil sensorial negativo e aceitação global com pontuação intermediária. Presume-se que o grande volume ocupado pelas MP liofilizadas na massa do hambúrguer bovino permitiu maior acesso a agentes oxidantes durante o processamento e tratamento térmico do produto. Porém, foi possível obter um hambúrguer bovino reduzido em sal e fortificado com óleo de peixe não encapsulado com boas características de qualidade e com alta aceitação global, mas que, após o cozimento, mantém apenas EPA. Portanto, foi possível obter um hambúrguer bovino com caracteristicas mais saudáveis e sensorialmente aceitáveis, no qual se destaca o resultado mais promissor: a fácil adaptação na indústria.

Palavras-chave: Produtos cárneos, Redução de sódio, Microencapsulação, Óleo de peixe, PUFA, EPA/DHA

ABSTRACT

Development and characterization of beef burger added with micronized salt and long-chain n-3 fatty acids

The beef burger is a popular meat product highly appreciated by consumers due to its sensory characteristics. In addition, it offers large intake of proteins, calories, vitamins and minerals. However, the beef burger, and generally meat products, may contain considerable sodium amount from the added salt and an unhealthy lipid profile. These components could increase the risk of metabolic syndrome diseases, such as cardiovascular disease. In this context, the development of healthier meat products is a necessity that responds to the demands of consumers. In this research, a sequential strategy was developed to obtain a beef burger with healthier characteristics. In the first stage, micronized salt was partially mixed with pork fat to protect its structure and used to reduce the salt/sodium content in the product, showing the possibility of reducing 33% of salt in the burger. Thus, texture, yield, physicochemical and sensory characteristics determined by consumers and the overall liking were slightly or not affected. The second step involved the development of an ingredient source of long-chain n-3 polyunsaturated fatty acids (eicosapentaenoic, EPA, and docosahexaenoic, DHA) to fortify the beef burger. Microparticles (MP) of soy protein isolate and inulin entrapping fish oil was produced by complex coacervation, obtaining an optimized product in terms of encapsulation efficiency and yield. In addition, MP were resistent to specific conditions of pH and heat treatment, retaining more than 81% of the encapsulated fish oil when transglutaminase was used to give greater resistence to the MP. In the third stage, the strategy of beef burger fortification with fish oil source of EPA and DHA, in combination with the salt/sodium reduction strategy of the first stage was studied. As a result, some differences were found between beef burgers with different salt content (1.5% vs. 1.0%) when unencapsulated or encapsulated fish oil was added, mainly in fatty acids profile and volatile compounds. However, the freeze-dried MP failed in several quality parameters of beef burger, the most notable being the increase in volatile oxidation compounds, negative sensory profile and overall liking with intermediate score. It is presumed that the large volume occupied by the freeze-dried MP in the beef burger dough allowed greater access of oxidizing agents during the processing and heat treatment of the product. However, it was possible to obtain a beef burger reduced in salt and fortified with unencapsulated fish oil with good quality characteristics and high overall liking, but that after cooking maintains only EPA. Therefore, a beef burger with healthier and sensorially acceptable characteristics can be obtained, in which the most promising result stands out: the easy adaptation in the industry.

Keywords: Meat products, Sodium reduction, Microencapsulation, Fish oil, PUFA, EPA/DHA

1. INTRODUCTION: OVERVIEW AND OBJECTIVES

Burger or hamburger is, perhaps, the most consumed meat product in the western diet, being part of the fast-food sector and also of the gourmet market, which have beef as the main ingredient. However, in times when the environmental situation is a concern, and with the inceased demand for a healthy diet, the previously considered "impossible foods" have arisen based on the use of only vegetable ingredients and even using cultured meat in order to reduce meat consumption. Under this scenario, will the consumption of meat and meat products be radically decreased or eliminated from people's diet in the future? Although the answer has two dimensions, the history shows that the consumption of any food has not been totally eliminated from the diet. An example is the consumption of butter that has margarine as a similar product (first marketed in 1911 by Procter & Gamble), developed with the aim of having a "healthier" and cheaper option. Until now, both products are regularly consumed by people. In addition to this example, the fact of radically reducing the consumption of meat would generate an imbalance in the economy and in the income of meat producers, as well as affect the human nutrition given the protein, caloric, vitamin and mineral contribution of meat. Therefore, the niche market for meat and meat products still continues and will continue in force, but due to the constant critics for these types of products, the meat industry needs to rethink its products to meet consumer demand, since certain aspects that scientific reports and the mainstream point out are of real concern about meat products: their high sodium and fat content, unhealthy lipid profile and use of synthetic additives. In this context, since the beef burger is widely consumed, the risk of developing metabolic syndrome diseases from the consumption of components that are harmful to health is increased.

The present PhD thesis was developed in Brazil, thus, it seeks to solve a problem in the context of the Brazilian meat products industry. The final objective of scientific publications related to healthier meat products should be the industrial application, but the main barrier to achieve this goal is the regulation that establishes the rules for the manufacture and use of ingredients and additives. Taking this aspect into account, the starting point of the research was to carry out a screening of scientific publications (with at least one author from a Brazilian institution) and their relationship with Brazilian regulations, in the sense of verifying and discussing the viability of industrial application of the most studied aspects in the reformulation of meat products:

reduction of salt/sodium, improvement of the lipid profile and use of natural antioxidants.

Important results were highlighted from publications in scientific journals indexed in Scopus, from 2011 to July 2019. Considering the real possibility of industrial application in Brazil, it is suggested, for each context, the use of the following ingredients:

1. Reduction of salt or sodium: Use of salt with a modified structure (size and shape).
2. Improvement of the lipid profile: Use of vegetable oils encapsulated with non-meat proteins or embedded in oleogels, using structuring agents of lipid and vegetable nature.
3. Natural antioxidants: Use of dyes such as carotene, turmeric, annatto, paprika and red betroot. Use of spices such as rosemary and oregano.

Inspired in the review reported by Inguglia, Zhang, Tiwari, Kerry, and Burgess (2017) and in the ConAgra Foods® patent (Jensen, Smith, Fear, Schimoeller, & Johnson, 2011), the use of salt of reduced crystal size was chosen as a sodium reduction strategy in beef burger. The principle of the strategy is based on the intensification of the salty taste using less salt, but a smaller crystal size means greater dissolution of the salt in the aqueous matrix of the product, with negative implications in the salty taste. Therefore, part of the micronized salt used in the formulation was mixed with pork fat to protect its solubilization in the product and to allow its dissolution in the mouth, thus optimizing the quantity of salt and the salty taste of the product. Several quality parameters were evaluated to define the potential of this strategy, which are shown in chapters 3 and 4, with emphasis on sensory quality determined by consumers and with the use of emerging techniques of sensory analysis with static and dynamic perspectives. The success of this strategy was reflected in the reduction of up to 33% of salt in the product, and to further reinforce its potential for industrial application in meat products and the call for future studies, a review of this strategy is presented in chapter 5.

For the lipid profile improvement, the Brazilian regulation allows the use of vegetable oils in several meat products, including burgers, as mentioned before. However, the effect that the bioactive compound present in the oils have on health should be considered, that is, the ability to have favorable and direct physiological effects on the organism. In this sense, long-chain n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexahenoic acid (DHA), have multiple functions, mainly preventing metabolic syndrome diseases (Simopoulos, 2016), but their main benefit is also their weakness, because due to the high unsaturation, they are more susceptible to lipid oxidation. In this context, other healthy lipid sources can be used, such as vegetable oils, which do not contain EPA and DHA, but whose main bioactive compound is α-linolenic acid (ALA), which is converted into EPA and DHA in the organism. However, as reviewed by Walker, Jebb, and Calder (2013), the conversion of ALA to EPA and DHA is poor. To improve this aspect, the production of fatty acids with higher conversion efficiency than ALA has been proposed, such as stearidonic (SDA) and gamma-linolenic (GLA) acids (Rincón-Cervera et al., 2020; Walker, Jebb, & Calder, 2013). Due to the commercial unavailability of SDA and GLA and the lack of food ingredients rich in these fatty acids, in the present thesis it was decided to use fish oil as source of EPA and DHA to be incorporated into beef burger. In this regard, the main issue based on oxidative stability and Brazilian regulation was the form of incorporation of fish oil: encapsulated or in oleogels.

The potential to incorporate healthy oils into meat products by microencapsulation and oleogels was recently reviewed by Heck et al. (2021) and López-Pedrouso, Lorenzo, Gullón, Campagnol, and Franco (2021), respectively. On one hand, microencapsulation could guarantee, at least partially, the oxidative stability of lipids (Heck et al., 2021). This effect is reinforced by the dehydration process that is commonly applied in the production of microparticles, increasing the shelf life of the active encapsulated. As encapsulating agents, the use of non-meat proteins, which can be incorporated up to 4% in burgers according to Normative Instruction N° 20 of the Ministry of Agriculture (Brazil, 2000) was suggested in chapter 2. On the other hand, despite the fact that oleogels may have a direct industrial application suggested in chapter 2, López-Pedrouso et al. (2021) highlighted that there are challenges to be optimized for their incorporation into meat products, such as textural properties, use of new organogelators, new methodologies for the production of organogels and use of antioxidants. Therefore, in the current scenario, the incorporation of healthy oils by microencapsulation seems to be more promising than oleogels to improve the lipid profile of meat products.

In this regard, the next step of the thesis was to develop microparticles containing fish oil as a functional ingredient to fortify the beef burger with EPA and DHA, shown in chapter 6. For this purpose, soy protein isolate was used as encapsulant, which is a commonly ingredient added into meat products; also, the fiber inulin was used as another encapsulating agent, which has the potential to replace fat in meat products. The microencapsulation technique used was complex coacervation, based on the electrostatic interaction between polymers, generally between proteins and polysaccharides, and which guarantees oxidative stability due to the use of temperatures no higher than 40 °C. Taking into account this last aspect, it was hypothesized that complex coacervation guarantees the oxidative stability of lipids, therefore the level of the antioxidant or the use of natural antioxidants was not investigated in this thesis, but this approach could be the subject of future studies.

The use of inulin through the complex coacervation technique was first reported in this study, generating a patent request due to the lower amount of inulin used compared to other fiber sources reported in the literature, which may have a favorable cost implication for the microencapsulated fish oil production (Appendix B). It is important to highlight that the ingredients added in formulation of the beef burger evaluated in this thesis were within the Brazilian regulation for burger manufacturing (Brazil, 2000, 2019), but fish oil and fibers, such as inulin are not specified in manufacturing of meat products in the Brazilian context. However, given the need to incorporate EPA and DHA and fibers in the diet, the main pathway to produce meat products with these bioactive compounds is through the addition of ingredients, trying to reinforce the scientific reports that are the basis for decisions regarding the regulation of ingredients and additives in food.

Finally, the last experimental phase of the thesis was the fortification of beef burger reduced or not in salt with fish oil in the unencapsulated or microencapsulated form, evaluating physicochemical, technological, nutritional and sensory characteristics (chapter 7). In this aspect, the effect of salt reduction and the fish oil incorporation on the fatty acid profile and lipid oxidation determined by volatile compounds were of special interest, which have implications on the sensory profile and overall liking of the product. Therefore, the expected result was to obtain a product reduced in salt and fortified with long chain n-3 fatty acids.

The objectives of the thesis were:

General objective: To produce and characterize beef burger added with micronized salt and fortified with long-chain n-3 fatty acids.

Specific objectives:

To obtain a salt-reduced beef burger using micronized salt, without compromising the physicochemical characteristics, yield properties, texture profile, static and dynamic sensory characteristics, and overall liking.

To optimize the production of microparticles of soy protein isolate and inulin entrapping fish oil, produced by complex coacervation, and to determine the fish oil retention under similar conditions of processing and heat treatment of the beef burger.

To successfully incorporate fish oil into beef burger reduced or not in salt, evaluating the physicochemical characteristics, yield properties, texture profile, fatty acids profile, volatile compounds, sensory characterization, and overall liking.

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2. ADVANCES AND GAPS IN STUDIES ON HEALTHY MEAT PRODUCTS AND THEIR RELATIONSHIP WITH REGULATIONS: THE BRAZILIAN SCENARIO

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Abstract

Background: Strategies for the reformulation of meat products respond to consumers' demand for healthier food alternatives. The reduction of salt or sodium and animal fat, and the substitution of synthetic antioxidants by natural ones have been a subject of study for Brazilian researchers for many years, but the industrial application of new technological alternatives presents the challenge of adapting to regulations related to the processing of meat products. *Scope and approach:* Reports by Brazilian researchers from 2011 to 2019 were collected from the Scopus database and their abstracts were submitted to a textual analysis to visualize the trend of studies over these years. The relationship between the approaches/ingredients proposed in the studies and the Brazilian regulation was discussed. *Key findings and conclusions:* Numerous alternatives for natural antioxidants and salt and animal fat substitutes have been proposed, but Brazilian regulation is restrictive with respect to the use of natural antioxidants and the most studied salt substitute in meat products. However, studies using alternatives more compatible with Brazilian regulations have been proposed. For industrial application purposes, some flavorings and spices may have antioxidant activity and act as natural antioxidants, delaying the lipid oxidation of meat products; the sodium content can be reduced using several morphologies or sizes of salt particles; and the incorporation of oils rich in polyunsaturated fatty acids, embedded in oleogels and microparticles using encapsulating proteins, seem to be a viable alternative for reducing animal fat in meat products.

Keywords: Salt reduction, Sodium reduction, Fat reduction, Natural antioxidants, Meat products.

2.1. Introduction

In recent years, it has become increasingly difficult not to associate the consumption of meat products with peoples' health. The presence of excessive amounts of sodium, saturated fats, cholesterol and synthetic additives in meat products (Paglarini et al., 2018) contributes to an increased risk of metabolic diseases, such as obesity, type-2 diabetes and coronary heart disease (Beriain et al., 2018). Moreover, the consumption of meat products has been associated with the occurrence of cancer (Bouvard et al., 2015), generating great controversy in public opinion worldwide.

In this context, the role of academia is to study and disseminate new viable alternatives that may reduce or eliminate harmful components in meat products, and thus, support the industry based on consumers' demands. Consumers today are more concerned about the relationship between food and health, that has led to the search for healthy foods that also have attractive characteristics; in addition, convenience, which corresponds to foods that require little or no preparation before consumption, is considered a very important factor in the food purchase decision by consumers (Saldaña et al., 2020). Meat products are examples of sensory-attractive and convenient foods, considering the little time used for any dish preparation. In this sense, the main challenge has been to convert traditional meat products into healthy foods, aiming to meet consumer expectations.

Many technological alternatives proposed by researchers have been successfully applied, resulting in healthy meat products. In this context, Brazil stands out for being a resource-rich country with high potential for the development of new ingredients for the manufacture of meat products. Moreover, the consumption of meat products in Brazil is considerable. According to the *Instituto Brasileiro de Geografia e Estatística* - IBGE (2020) (Brazilian Institute of Geography and Statistics), the average per capita consumption of processed meats is approximately 6.5 kg, which would be representing a daily consumption of 17.8 g. The most highly consumed meat product is *Linguiça* sausage, representing 33.2% of total processed meat consumption by Brazilians (Table 2.1). According to Table 2.1, meat products represent an important food in the diet of Brazilian people, justifying their reformulation to obtain healthy meat products. However, the proposal of the new reformulation alternatives should consider their practical application in the industry, which could be limited by regulations.

Table 2.1. *Per capita* consumption of meat products in Brazil during 2017–2018.

Product	*Per capita* consumption (kg/year)
Bacon	0.156
Breaded chicken	0.089
Burger	0.163
Cooked ham	0.463
Dried beef	0.373
Minced beef meat	1.306
Mortadella	0.581
Salami	0.112
Salted meat	0.072
Sausage	
Common sausage[1]	0.811
Linguiça sausage[2]	2.155
Paio[3]	0.009
Sun-dried meat	0.199
Total	6.489

[1] Meat product obtained from a meat emulsion of one or more species of livestock animals, added with ingredients, embedded in a natural or artificial wrap or by extrusion process, and subjected to an appropriate thermal process (Technical Regulations of Identity and Quality of Sausages – MAPA).
[2] Meat product obtained from meat from livestock animals, with or without adipose tissues, ingredients, embedded in a natural or artificial wrap, and subjected to the appropriate technological process (Technical Regulations of Identity and Quality of *Linguiça* Sausage – MAPA).
[3] Traditional sausage in Brazil and Portugal.

In this commentary paper, we address the Brazilian regulations regarding the manufacture of meat products and the additives allowed in these types of products. In this sense, we analyzed the relationship between these regulations and scientific studies that present at least one researcher affiliated with a Brazilian institution. The sources of information regarding the Brazilian regulations were the Technical Regulations of Identity and Quality of Meat Products of the *Ministério da Agricultura, Pecuária e Abastecimento* – MAPA (Ministry of Agriculture, Livestock and Food Supply), obtained through the Legislation Consultation System (http://sistemasweb.agricultura.gov.br/sislegis), and the guidelines of the *Ministério da Saúde* (Ministry of Health), on the use of authorized additives in meat products.

The reported studies were collected from Scopus database, from 2011 to July 15th, 2019, using the keyword "meat products". Our research considered studies on natural antioxidants, salt or sodium reduction, and animal fat reduction or lipid profile improvement. Another aspect that was taken into account was the effect of reformulation on the physical, chemical, technological and sensory parameters of the meat products. Through a textual analysis of the abstracts of the collected articles, the main approaches and ingredients studied by Brazilian academia were analyzed, and from this information, the possibility of industrial applications was discussed and alternatives for new studies more compatible with Brazilian regulation were suggested.

2.2. Textual analysis

The textual analysis was performed on a body of 144 texts (article abstracts). The 26,065 co-occurrences (number of times the words were repeated) are displayed in Fig. 2.1, reflecting a general trend of studies released in this period, making it clear that the most frequently occurring terms are: *product*, *fat*, *meat*, *content*, *antioxidant*, *sodium*, *lipid*. Moreover, it is possible to observe the occurrence of the words *burger* and *sausage*, highlighting that these foods are the most studied by Brazilian researchers in the context of healthy meat products. The occurrence of the meat product “sausage” is in line with its *per capita* consumption in Brazil (Table 2.1).

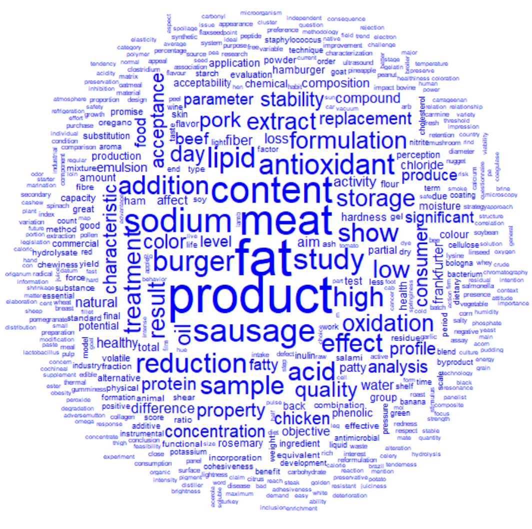

Figure 2.1. Cloud of more salient words present in the abstracts.

Even though the word cloud showed a global trend of the research carried out in Brazil, it did not show specific trends. To find these specific trends, text segments were considered using the software IRaMuTeQ (Pélissier, 2016). Texts segments are groups of words separated by a punctuation marks (in this study, a total of 740 segments were found). Then, the co-occurrences of lemmatized words were calculated based on active forms (911 nouns and adjectives). The number of segments after the reduction (average frequency of segments equal to or greater than 35.22) was 676, being subsequently classified using the Reinert method (Pélissier, 2016). Following the recommendation of the software creator, 483 active forms were found with a frequency equal to or greater than 3, classified in 5 classes shown in Fig. 2.2.

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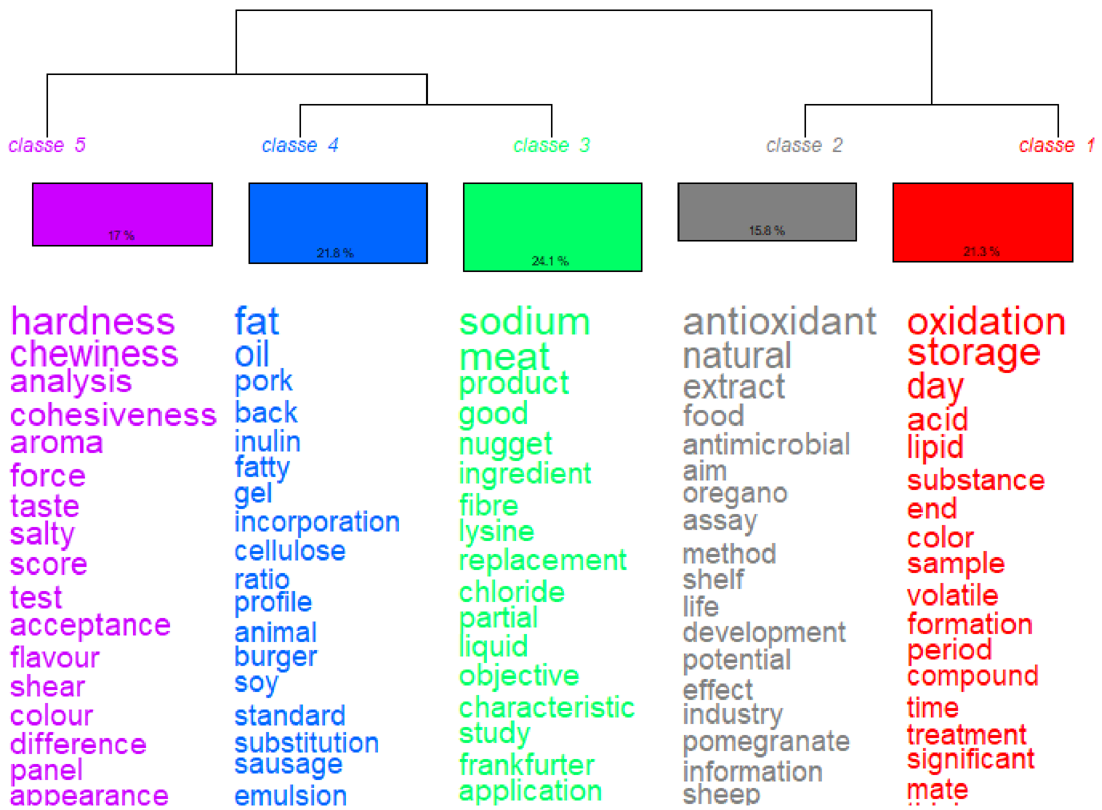

Figure 2.2. Dendrogram of words based on segment texts.

The size of the words is proportional to their contribution to the respective classes, estimated by the frequency that was tested through the chi-square test. The class with the highest participation is “sodium” (Class 3), followed by “fat” (Class 4) and “oxidation during storage” (Class 1), while the groups with the lowest participation are related to physical and sensory properties (Class 5) and antioxidants (Class 2). It is interesting to note that study of the addition of natural antioxidants is accompanied by the study of oxidation during storage, indicating that the effectiveness of antioxidants in meat products is assessed by the lipid oxidation over time. The study of sodium and fat reduction is accompanied by study of the physical and sensory properties of the product. These ingredients are known to play a fundamental role in the texture and sensory properties of meat products.

The co-occurrence matrix of words was subjected to a Correspondence Analysis (CA) to identify the associations between words, considering the hierarchical clustering previously performed (Reinert, 1983) to define the main clusters of words (Fig. 2.3).

Fig. 2.3 shows the first two dimensions of the CA of the active forms (substantives and adjectives), accounting for 60.78% of explained variance contained in the original data matrix. The words are based on study approaches and show the ingredients used to obtain healthy meat products.

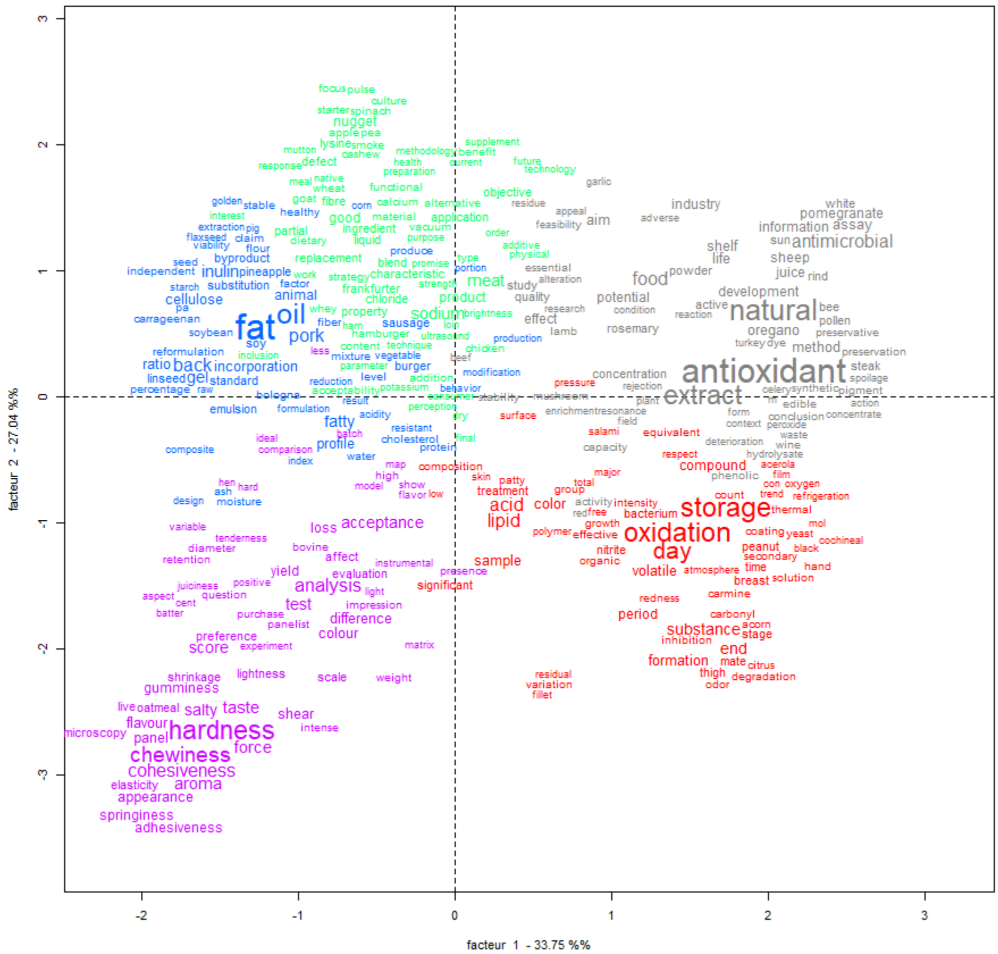

Figure 2.3. Correspondence analysis of the words. Classes are represented by different colors.

2.2.1. Interpretation of word classes by the study approach

To facilitate interpretation, the words extracted from the CA are presented in bold.

Class 1. The **lipid** oxidation during storage is highly influenced by the fatty **acid** profile and its quantification is based on the determination of **substances** or **organic compounds**, mainly **secondary** products of oxidation, such as **volatile** compounds. During storage, protein oxidation also occurs, which is usually measured by **carbonyl** determination. Since lipid and protein oxidation are affected by the presence of **oxygen**, most studies are performed under controlled **atmospheric** conditions, with the use of **oxygen**-barrier **polymeric coatings**; in addition, meat products subjected to processing, such as grinding and **thermal** treatment are especially susceptible to oxidative reactions. Studies focused on the evaluation of the lipid oxidation of meat products are usually accompanied by the determination of **color** and **odor** in the product due to the possible development of rancid odor, off-flavors and myoglobin oxidation.

Class 2. Natural antioxidants are usually obtained from plant sources, including their agro-industrial **residues**, applied in **powder** or as **extracts**, most of which also have **antimicrobial** activity. These biological activities are related to the presence of bioactive substances, such as **phenolic** compounds, which, depending on the **concentration,** have the potential to replace **synthetic** antioxidants in the **industry**. To evaluate the efficiency of these natural compounds as antioxidants, multiple **assays** are carried out to characterize the different mechanisms of the antioxidant action. The study of natural antioxidants in meat products is often associated with the measurement of lipid oxidation during storage, which is usually determined by the **peroxide** index (for primary oxidation compounds) and TBARS value (for secondary oxidation compounds).

Class 3. The words in this class show the association between **health** and salt consumption. Thus, there is interest in salt **replacement**, often as **partial** replacement**,** with alternatives such as other **chloride salts** or **blends** of salt substitutes to compensate the ionic **strength** of the sodium chloride (NaCl) reduction. Some emerging technologies have been used along with sodium reduction to improve product quality, such as **ultrasound.** However, it is important to consider the **consumer perception** and the **acceptability** of low-sodium products.

Class 4. As in Class 3, animal fat reduction is studied for **health** reasons. The reduction of animal fat is widely studied through its replacement by various plant sources, at several **levels** of **substitution.** Substitute ingredients include **oils**, **emulsions**, **gels**, **byproducts**, and **fibers**. According to the data, it is important to determine the **fatty** acid **profile**, **cholesterol** content and the **ratio** between fatty acids of interest, such as n–6 and n–3 polyunsaturated fatty acids.

Class 5. For the study of physical properties, **instrumental analysis** predominates, through the determination of Texture Profile Analysis and **Shear Force**, which provide information such as the degree of **hard**ness or **tenderness** of meat products. It is also important to determine **yield** parameters, such as **weight** loss, degree of **shrinkage** or **diameter** reduction. Sensory analysis is performed with **panelists,** either with trained assessors or consumers, who determine the sensory properties and **acceptance** of the product with the use of **scales**. Some studies determine the **purchase** intention of the product or ask consumers to define the **ideal** product, which is important information for future reformulation strategies. It is interesting to note the presence of the sensory attributes **salty** and **juiciness**, which suggests the extreme relevance of these parameters to the consumer. Finally, the word **map** involves the use of sensory maps that facilitate the interpretation of the sensory characterization of meat products.

2.2.2. Ingredients used for the development of healthy meat products

Sources of natural antioxidants were found in Classes 1 and 2. One of the most frequently used ingredients is rosemary. Its antioxidant properties are present in quantities that do not exceed 0.5% in the product, either in the form of extracts (Bertol et al., 2012) or as essential oils (Vital et al., 2016). However, Fruet, Nörnberg, Calkins, & de Mello (2019) observed that acerola or citrus extracts (lime, lemon, and orange) can outperform rosemary in the oxidative stability of lipids.

It is possible to increase the antioxidant capacity by applying technologies that maximize the extraction of antioxidant compounds, such as ultrasound (Heck et al., 2019, 2018) and pressurized liquid extraction (Horita et al., 2016). Moreover, the form of presentation of the antioxidant source (extract, powder, essential oil) is crucial to improving the oxidative stability of meat products (Borella et al., 2019; Horita et al., 2016).

Other antioxidant sources studied include oregano (Fernandes, Trindade, Lorenzo, & Melo, 2018; Fernandes, Trindade, Lorenzo, Munekata, & Melo, 2016), pomegranate (Firuzi et al., 2019; Shahamirian et al., 2019), agro-industrial residues from peanut skin (Munekata et al., 2015, 2017; Serrano-León et al., 2018) and acerola (Zegarra, Santos, Silva, & Melo, 2018), mate (Beal et al., 2011), garlic (Horita et al., 2016), bee pollen (Almeida et al., 2017), dealcoholized wine (Arcanjo et al., 2019; Arcanjo, Ventanas, González-Mohíno, Madruga, & Estévez, 2018), mushrooms (Stefanello et al., 2015), protein hydrolysates or peptide fractions of soy protein (Oliveira et al., 2014), chia (Coelho, Aquino, Latorres, & Salas-Mellado, 2019), and fish (Quadros et al., 2019).

In Class 3, the most studied salt substitute is potassium chloride (KCl). Technologically, KCl plays a role quite similar to NaCl, but it negatively affects the taste of meat products, even when partially replacing the NaCl content. However, several authors used the following flavor enhancers to minimize the negative sensory effects of KCl: lysine, taurine, disodium inosinate, disodium guanylate, monosodium glutamate, liquid smoke, and a combination of herbs and spices (Alves et al., 2017; Campagnol, dos Santos, Morgano, Terra, & Pollonio, 2011; Campagnol, dos Santos, Terra, & Pollonio, 2012; Carvalho et al., 2013).

Another strategy to reduce sodium is to use potassium and calcium chloride blends. However, the reported studies show unpromising results for sensory aspects (Horita, Messias, Morgano, Hayakawa, & Pollonio, 2014; Santos et al., 2015). Also, it was observed that calcium chloride decreased the oxidative stability of meat products, as reported by Santos, Campagnol, Fagundes, Wagner, & Pollonio (2017) in fermented sausages, and by Vidal et al. (2019) in jerked beef.

In fat reduction, the most highlighted ingredient is inulin, but in combination with other components. Guedes-Oliveira et al. (2019) observed that a mixture of 5.8% fat, 0.8% carboxymethylcellulose, and 3.3% inulin improved the cooking yield and diameter reduction without affecting the texture profile and color parameters of lamb patties. Inulin was also used to produce fat-substitute gels. These gels were prepared with the following components: soybean oil, soy protein isolate or chia flour, carrageenan, inulin, pectin, sodium caseinate, sodium tripolyphosphate, and soy lecithin (Paglarini et al., 2018; Paglarini, Martini, & Pollonio, 2019; Paglarini et al., 2019). Paglarini et al. (2018) observed that emulsion gels with high inulin content and unsaturated fatty acids slightly affect the technological properties of meat products. In another study, Paglarini et al. (2019) demonstrated that the combination of emulsion gels with pork fat improves the fatty acid profile without affecting the texture and rheological properties of Bologna sausages. However, when incorporated as a total animal fat substitute, the acceptance of frankfurters is compromised (Paglarini, Martini, & Pollonio, 2019).

Another type of gel is the amorphous cellulose gel. Campagnol, dos Santos, Wagner, Terra, & Pollonio (2012) reported that replacing 50% of pork fat with amorphous cellulose gel in fermented sausages was important for reducing the levels of fat, cholesterol and volatile compounds derived from lipid oxidation. Also, amorphous cellulose gel reduced the cooking loss and improved the emulsion stability of Bologna-type sausages, but increased hardness, gumminess, and chewiness when added at high concentrations (Faria et al., 2015). Almeida, Wagner, Mascarin, Zepka, & Campagnol (2014) suggested that it is possible to replace the total pork fat content with amorphous cellulose gel without affecting the sensory characteristics and acceptance of emulsified cooked sausages, with a significant improvement in the fatty acid profile of the product.

Linseed oil is also a well-studied ingredient for replacing fat in meat products. The strategies of its incorporation include pre-emulsion with sodium caseinate (Câmara & Pollonio, 2015), hydrogelled emulsion using kappa carrageenan and polysorbate 80 (Heck et al., 2019), microparticles of sodium alginate and calcium chloride (Heck et al., 2017), and sterol-based oleogels (oryzanol and sitosterol) (Martins et al., 2019). Considering the high levels of animal fat substitution, these studies showed positive results for emulsion stability, technological properties, fatty acid profile, texture profile and sensory profile of meat products.

The incorporation of golden flaxseed (oil, flour or seed) showed benefits in the lipid profile (Novello & Pollonio, 2013), without affecting the sensory quality of meat products (Hautrive, Piccolo, Rodrigues, Campagnol, & Kubota, 2019). Morais et al. (2013) suggest that pork fat can be completely replaced by soybean oil without harming the acceptance of Mortadella. Finally, Selani, Shirado, Margiotta, Rasera, et al. (2016) and Selani, Shirado, Margiotta, Saldaña, et al. (2016) used freeze-dried pineapple byproduct and canola oil as fat replacers in beef burgers, and reported improvements in the nutritional quality due to a decrease in the n-6/n-3 ratio and cholesterol levels, but low-fat burgers showed high hardness, chewiness and cohesiveness values.

2.3. Are the proposed new ingredients authorized for industrial manufacture of meat products?

2.3.1. Natural antioxidants

According to the RDC N° 272–2019 (Brasil, 2019), which regulates authorized additives in meat products, the antioxidant section does not specify the use of natural antioxidants, therefore none of the ingredients proposed by Brazilian academia in the studies evaluated in this commentary paper are in accordance with the regulation. However, in the dye section of the regulation, some components of natural sources are authorized, such as carotene extract (limit 0.002 g/100 g product), turmeric (limit 0.002 g/100 g), annatto (limit 0.002 g/100 g), paprika extract (limit 0.001 g/100 g), and red beetroot (*quantum satis*). According to international literature, studies evaluating these components in meat products have already been carried out (Aguirrezábal, Mateo, Domínguez, & Zumalacárregui, 2000; Figueirêdo, Trad, Mariutti, & Bragagnolo, 2014; Østerlie & Lerfall, 2005; Rey, Hopia, Kivikari, & Kahkonen, 2005; Sharma, Pazhaniandi, Tanwar, Das, & Goswami, 2011), showing their antioxidant potential and indicating that, in addition to their role as dyes, they have potential to act as natural antioxidants.

The flavoring section of RDC N° 272–2019 specifies the use of flavorings authorized by RDC N° 2–2007 (Brasil, 2007). This regulation mentions the possibility of updating the list of botanical species for potential use as flavoring additives. These botanical species include fruits and vegetables, either in whole or in part, as well as plants or their parts, such as herbs, spices and seasonings commonly added to food without any evidence of adverse effects (Brasil, 2007). However, the addition of natural flavorings may be limited by the presence of some active substances. For example, if the food contains fungi, the concentration of agaric acid should not exceed 100 mg/Kg food. Moreover, the limits of solvents commonly applied for the extraction of natural compounds used as flavorings are specified, such as acetone (2 mg/Kg), cyclohexane (1 mg/Kg), hexane (1 mg/Kg), and methanol (10 mg/Kg), among others. In short, this scenario opens the possibility of conducting studies focused on the flavoring potential of botanical species, especially those with antioxidant effects in meat products.

Ingredients with the designation of spices can also impart antioxidant action. The Normative Instruction N° 17 of MAPA (Brasil, 2018) defines spices as "*products consisting of parts (roots, rhizomes, bulbs, rinds, leaves, flowers, fruits, seeds, stalks) of one or more plant species traditionally used to impart flavor or aroma to the seasoned meat product*". As observed in Class 2 of CA, rosemary and oregano are spices widely studied as natural antioxidants in meat products, which confirms their multifunctionality due to the antioxidant action and their already being approved for use as flavorings and spices. In addition to their role in delaying oxidation, the new natural antioxidants proposed should also be studied for their effect on the sensory profile of meat products, in order to add value to those ingredients as spices and antioxidants. The lack of any relationship between Classes 1 and 2 with Class 5 (sensory profile) opens up the possibility of future studies.

2.3.2. NaCl substitutes

Despite the great potential of KCl as a NaCl substitute, this chloride is not specified for use in the processing of meat products according to the Technical

Regulations of Identity and Quality of Meat Products and the RDC N° 272–2019 (Brasil, 2019). Other salts, such as potassium lactate, potassium phosphate and calcium ascorbate have been approved by Brazilian regulations for use in other functions (as acidity regulators, stabilizers and antioxidants, respectively), but studies have already shown that they can be useful to reduce sodium in meat products (Ciriano, Berasategi, Navarro-Blasco, Astiasarán, & Ansorena, 2012; Choi et al., 2014; Falludosa, Serra, Gou, & Arnau, 2009; Ruusunen, Niemistö, & Puolanne, 2002; Ruusunen et al., 2005; Seman, Olson, & Mandigo, 1980). Flavor enhancers such as the widely used monosodium glutamate have also been studied, as they act to intensify the perceived saltiness (Chun et al., 2014; Santos, Campagnol, Morgano, & Pollonio, 2014). Yeast extract, a natural ingredient, also has potential as a flavor enhancer in sodium-reduced meat products. There are also flavor masking agents that are used in meat products together with KCl to minimize its bitter and metallic taste (Desmond, 2006; Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017). Since KCl is not approved for use in meat products, the use of these agents is limited.

Considering the current trend of developing more natural or clean label foods (free of artificial additives/ingredients), both academia and industry have the option of reducing salt without the use of synthetic substitutes. Inguglia et al. (2017) reviewed that, in addition to the use of salt substitutes, there are several strategies for reducing salt or sodium in meat products, including salt reduction over time, the application of new technologies, such as ultrasound or high pressure processing to counteract defects of low-salt meat products, and decreasing the size of salt particles and changing the salt morphology in order to increase the perception of saltiness. Thus, future studies on the application of these strategies in meat products are needed.

2.3.3. Animal fat substitutes

According to the textual analysis, the most commonly used ingredients as animal fat substitutes are fibers and oils. The Technical Regulations of Identity and Quality of Meat Products do not mention the use of fibers and oils. However, the Normative Instruction N° 17 (Brasil, 2018) authorizes the use of vegetable oils and fats, but it is important to mention that this regulation excludes sausages and cooked meat products. In this sense, the addition of vegetable oils sources of polyunsaturated fatty acids is an industrially viable alternative, which will depend on how the oil is added to the product.

Regarding linseed oil, the studies cited in section 2.2.2 evaluated the addition of certain components associated with the oil, but carrageenan and sodium alginate are the only additives authorized in meat products, at relatively small quantities (0.3 and 1.2 g/100 g, respectively) (Brasil, 2019). It is important to mention that Heck et al. (2019) used sodium alginate + calcium chloride microparticles, but the latter component is limited by the calcium content, which must not exceed 0.9% in several meat products.

The use of non-meat proteins could be an alternative for the incorporation of oils (as emulsions), since their maximum percentage of addition in meat products (2.0–4.0%) is higher than that of thickeners, such as carrageenan and sodium alginate. Câmara & Pollonio (2015) observed that the addition of linseed oil pre-emulsified with sodium caseinate in Bologna sausages was sensorially and technologically satisfactory. Other protein sources were studied by Youssef & Barbut (2011), who used canola oil pre-emulsified with soy protein, sodium caseinate, or whey protein isolate, with positive effects on yield and texture parameters of low-fat comminuted meat products.

Another promising alternative was reported by Martins et al. (2019), using sterol-based oleogels to incorporate linseed oil in hamburgers. In fact, this ingredient may undergo immediate implementation by the industry as it is within the scope of the Normative Instruction N° 17 (Brasil, 2018), which allows the addition of vegetable oils and fats in seasoned meat products. However, the previously cited studies on the addition of pre-emulsions and oleogels did not contemplate the oxidative stability of lipids, being an important issue to be addressed in order to evaluate the potential industrial application of these ingredients. Pérez-Palacios, Ruiz-Carrascal, Solomando, & Antequera (2019) suggest that the best alternative to ensure oxidative stability of polyunsaturated fatty acids in meat products would be microencapsulation. Considering this aspect and the possibility of using non-meat proteins, the addition of oils using proteins as encapsulating agents is an important strategy.

2.4. Conclusions and future perspectives

Textual analysis is a powerful tool that was found to be capable of analyzing trends in studies on healthy meat products by Brazilian researchers, based on the use of natural antioxidants, the reduction of salt or sodium and the reduction of animal fat or lipid profile improvement. Five groups of words associated with the study approach and the proposed ingredients were found.

Brazilian regulation has the option of including a wide range of ingredients and additives for use in meat products, but so far it has been shown to be quite restrictive, especially regarding natural antioxidants and salt substitutes. On the other hand, academia has the option of utilizing a new approach to antioxidants as flavoring ingredients or spices, in order to propose new studies based on the effect of natural antioxidants on the sensory profile of meat products, thus providing subsidies for industrial applications. Unfortunately, potassium chloride, which is the most studied NaCl substitute, cannot be applied in the manufacture of meat products; we therefore suggest that salt reduction studies should follow the trend of clean label products, using, for example, several morphologies and sizes of NaCl crystals. The replacement of animal fat by oils embedded in oleogels and microparticles has the potential to be applied in the industry, but the incorporation of microparticles in meat products needs further study aimed at the use of non-meat proteins as oil encapsulating agents.

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3. REDUCING THE SODIUM CONTENT WITHOUT MODIFYING THE QUALITY OF BEEF BURGERS BY ADDING MICRONIZED SALT

Chapter published in Food Research International.

Rios-Mera, J. D., Saldaña, E., Cruzado-Bravo, M. L. M., Patinho, I., Selani, M. M., Valentin, D., & Contreras-Castillo, C. J. (2019). Reducing the sodium content without modifying the quality of beef burgers by adding micronized salt. Food Research International, 121, 288–295. https://doi.org/10.1016/j.foodres.2019.03.044

Abstract

This study determined the effect of the incorporation of micronized salt on physicochemical, yield and consumer's sensory characteristics of beef burger. The micronized salt was obtained by sieving the commercial salt in a 60-mesh stainless steel sieve. The commercial (regular salt) and micronized salt presented differences in the mean size, size distribution and bulk density. Half of the amount of the micronized salt was mixed with pork back fat, and the other half was added to the meat batter in the beef burger manufacture. A Pivot profile method was used with consumers to describe the sensory properties of the burger samples (ranging from 0.5% to 1.5% NaCl). The Pivot profile data revealed that treatments with 0.75% and 0.5% micronized salt were mainly characterized as dry, besides showing the highest cooking loss and diameter reduction. However, beef burgers with 1.0% micronized salt and 1.5% regular salt had similar perceived salty taste. In terms of salt reduction, the results indicated that it would be possible to reduce salt from 1.5% to 1.0% when using micronized salt, without affecting the pH, color parameters, yield properties and some sensory characteristics of the burger, such as salty, tasty, juicy, fatty, and spicy. Therefore, this strategy promises great potential for industrial application in products that contain lipids in its composition, such as meat products.

Keywords: Meat products; NaCl reduction; consumer sensory characterization; Pivot profile.

3.1. Introduction

Beef burger is one of the most consumed meat products due to its sensory characteristics and fast preparation, but it contains high sodium amount in its composition. In Brazil, the average sodium amount of commercial beef burgers is 701 mg / 100 g of product (ANVISA, 2012), which is greater than the content reported in Australia (480 mg / 110 g) (Webster, Dunford, & Neal, 2010) and in the United States (290–400 mg / 100 g) (Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017). Excessive sodium intake is associated with the occurrence of several cardiovascular diseases (Aburto et al., 2013). Thus, the World Health Organization recommends that sodium intake should be less than 2 g (< 5 g salt) per day to avoid health risk (WHO, 2012).

To reduce sodium content in foods, sodium chloride (NaCl) substitutes are usually used, among which potassium chloride (KCl) is the most commonly studied. However, the partial replacement of NaCl by KCl generally has negative consequences on taste, conferring bitterness to meat products (Inguglia et al., 2017; Almeida, Montes-Villanueva, Pinto, Saldaña, & Contreras-Castillo, 2016). Additionally, in countries such as Brazil, the use of KCl is not specified in the legislation for the manufacture of meat products (ANVISA, 1998).

In this context, an alternative to reduce the sodium content of food without using other substitutes would be to reduce the size of the sodium chloride crystals. A reduction of the salt particle size leads to an improvement of the saltiness perception in foods due to a faster dissolution of the sodium in the saliva. Studies highlight this sodium reduction strategy for potato chips (salt <425 µm, Rama et al., 2013), cheese crackers (salt 1.5–15 µm, Moncada et al., 2015) and pâté (salt <250 µm, Shepherd, Wharf, & Farleigh, 1989). However, Galvão, Moura, Barretto, and Pollonio (2014) reported that the use of micronized salt (salt <840 µm) did not increase the saltiness perception of turkey ham, and that NaCl reduction above 30% resulted in products characterized as little salty, little seasoned, dry and brittle.

Thus, understanding the interactions of salt with food components is crucial for the development of strategies aimed at improving saltiness perception during the chewing of a meat product. In this sense, Jensen, Smith, Fear, Schimoeller, and Johnson (2011) patented the use of micronized salt encapsulated by a non-aqueous coating agent, such as oil or fat. The principle of the patent is based on the preservation of the structure and concentration of the salt encapsulated by the oil or fat, preserving its dissolution in an aqueous matrix. In this way, during mastication, the oil or fat layer is broken, and the salt is released for the dissolution in the mouth. Moreover, the salt encapsulation may also generate an inhomogeneous distribution of the crystals in the food matrix, an approach assessed by some studies that suggest that inhomogeneous distribution can enhance saltiness perception compared to salt homogeneously distributed in foods (Emorine, Septier, Thomas-Danguin, & Salles, 2013; Mosca, Bult, & Stieger, 2013; Noort, Bult, Stieger, & Hamer, 2010; Noort, Bult, & Stieger, 2012; Shepherd et al., 1989). As an example, the saltiness perception of breads manufactured with 1% salt encapsulated with fat, which presented an inhomogeneous salt distribution, was equal to or greater than breads made with 2% salt (Noort et al., 2012). Therefore, the interaction of micronized salt with fat may be a feasible strategy for salt reduction of meat products.

From the sensory point of view, an excessive salt reduction can affect the sensory characteristics of meat products (Cluff, Kobane, Bothma, Hugo, & Hugo, 2017; Delgado-Pando et al., 2018; Lorido, Estévez, Ventanas, & Ventanas, 2015), which, in turn, can drive the consumer's rejection (Almeida et al., 2016; Saldaña et al., 2019a). In the last years, several sensory techniques have been developed to understand consumer's perception. The development of recent methods of sensory characterization of foods has shown that consumers are, in fact, able to provide accurate and reliable assessment of the sensory properties of a product (Valentin, Chollet, Lelièvre, & Abdi, 2012; Varela & Ares, 2012). These methods do not require any training, have low financial impact, optimize time and resources in companies, and provide information highly correlated with traditional methods (Varela & Ares, 2012). Among the fast sensory methods, the Pivot profile (PP) proposed by Thuillier, Valentin, Marchal, and Dacremont (2015), describes the differences between samples by comparing them with a reference, called Pivot. The Pivot product is chosen within the range of products to be evaluated and serves as a standard to describe the other products (Lelièvre-Desmas, Valentin, & Chollet, 2017; Thuillier et al., 2015). Recent works have highlighted the effectiveness of PP to describe consumer-based sensory characteristics of products, such as ice cream (Balthazar et al., 2017; Fonseca et al., 2016), Greek yogurt (Esmerino et al., 2017) and honey (Deneulin, Reverdy, Rébénaque, Danthe, & Mulhauser, 2018). Following these preliminary studies, the present work proposes to study the sensory properties of beef burgers based on consumers' perception considering a standard burger as reference (Pivot).

According to the panorama presented, our first hypothesis is that it is possible to reduce sodium in beef burger using micronized salt, maintaining its sensory characteristics. In particular, the salt size and its mixture with pork back fat could increase the salty taste to a level comparable to that of burgers with higher salt content. Our second hypothesis is that the mixture of half of the micronized salt in the meat batter is sufficient to maintain the technological (solubilization and extraction of meat proteins, water holding capacity, texture, among others) and sensory properties of the beef burger. Thus, the objective of this study was to determine the effect of the incorporation of micronized salt on the consumer-based sensory characteristics, as well as on the physicochemical and yield properties of beef burgers.

3.2. Materials and methods

3.2.1. Raw materials and ingredients

Lean meat, pork back fat and white pepper powder were obtained from the local market (Piracicaba, SP, Brazil). Salt, monosodium glutamate, onion and garlic powder and sodium erythorbate were supplied by Ibrac (Rio Claro, SP, Brazil). The micronized salt (MS) was obtained from the commercial salt (here called regular salt (RS)), which was manually sieved using a 60-mesh stainless steel sieve.

3.2.2. Beef burger manufacture

Six beef burger treatments were manufactured according to the type and concentration of salt: 1.5% RS, 1.0% RS, 1.5% MS, 1.0% MS, 0.75% MS and 0.5% MS. Beef burgers were processed by mixing the following ingredients: lean meat (70%), pork back fat (20%), water (7.5%), regular or micronized salt, monosodium glutamate (0.28%), white pepper powder (0.15%), onion powder (0.28%), garlic powder (0.28%) and sodium erythorbate (0.01%). The MS was added in two parts: half of the amount was manually mixed with pork back fat, and the other half was added directly to the meat batter. Subsequently, all components were mixed for 5 minutes, then the beef burgers were molded, packed and kept at -18 °C for future analysis.

3.2.3. Salt characterization

3.2.3.1. Mean size and size distribution

The mean size and size distribution of RS and MS were obtained by light scattering, using a LV 950-V2 equipment (Horiba, Kyoto, Japan). The mean size was expressed as the mean volumetric diameter ($D_{4.3}$) in a dry dispersion, and the size distribution was calculated by the span index (Eq. 3.1), which indicates the polydispersity or width of the particle diameter distribution.

$$\text{Span} = \frac{D_{0.9} - D_{0.1}}{D_{0.5}} \times 100 \qquad (3.1)$$

Where $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$ are the diameters relative to 10, 50 and 90% of the accumulated size distribution (Alvim, Stein, Koury, Dantas, & Cruz, 2016). The measurements were made in five replications.

3.2.3.2. Bulk density

Bulk density of RS and MS was determined according to Lavoie, Cartilier, and Thibert (2002). For each type of salt, a 100 mL graduated cylinder was weighed, then 40 mL of salt particles were added, and the cylinder was reweighed. The graduated cylinder was closed with Parafilm, carefully inverted, and returned to the starting position for once. The salt particles were leveled without being compacted. The bulk density was calculated by dividing the mass of the salt by the total volume read. The measurements were made in five replications.

3.2.4. Beef burger characterization

The physicochemical parameters were measured in raw samples, while the yield properties and consumer sensory characterization were evaluated in cooked samples. Two beef burgers per treatment were used for sodium content determination, and three beef burgers per treatment were used for pH, color parameters and yield properties. Samples were cooked in an electric hot plate at 150 °C, until the internal temperature of 75 °C was achieved in the burger. Then, samples were cooled to room temperature (25 °C) for further determination of the physicochemical characteristics and yield properties, and at 45 °C for consumer sensory characterization.

3.2.4.1. Sodium content

The sodium content of beef burgers was determined as described by Almeida et al. (2016) and AOAC (2005). Five grams of each sample were converted to ash in a muffle furnace at 550 °C for 6 h. Subsequently, the ashes were cooled to room temperature, solubilized in 2.5 mL nitric acid and transferred to a 50 mL volumetric flask. A blank sample was used as a control. The readings were taken on a flame photometer (Micronal model B462, São Paulo, Brazil).

3.2.4.2. pH determination

The pH of beef burgers was measured using a potentiometer coupled to a glass puncture electrode, previously calibrated in pH 4.0, 7.0 and 10.0 buffer solutions.

3.2.4.3. Color parameters

The lightness (L*), green-red (a*) and blue-yellow (b*) parameters were determined using a colorimeter (Konica Minolta, Chroma Meter, CR-400, Mahwah, NJ, USA). A measuring area of 8 mm, observation angle of 10° and illuminant D65 were used.

3.2.4.4. Yield properties

The cooking loss corresponded to the burger's weight before and after cooking, which was expressed as percentage and calculated according to the equation 3.2 (Selani et al., 2016):

$$\%\text{Cooking loss} = \frac{\text{Raw burger (g)} - \text{Cooked burger (g)}}{\text{Raw burger (g)}} \text{ x } 100 \qquad (3.2)$$

The diameter reduction of the samples was measured before and after cooking and was calculated as follows (Sánchez-Zapata et al., 2010):

$$\%\text{ Diameter reduction} = \frac{\text{Raw burger (cm)} - \text{Cooked burger (cm)}}{\text{Raw burger (cm)}} \text{ x } 100 \qquad (3.3)$$

3.2.4.5. Microbiological analysis

Before performing the sensory analysis, the microbiological quality of the beef burgers was analyzed. Total coliforms, thermotolerant coliforms and coagulase-positive *Staphylococcus* counts were determined following the instructions of the Compendium of Methods for the Microbiological Examination of Foods (Downes & Itō, 2001). The presence/absence of *Salmonella* spp. in 25 g was determined using the 1-2 test® kit (BioControl Systems, inc.). The analyses were carried out in the Laboratory of Hygiene and Dairy of the *Escola Superior de Agricultura “Luiz de Queiroz”* (ESALQ) / *Universidade de São Paulo* (USP).

3.2.4.6. Consumer sensory characterization

The consumer-based sensory characterization of the beef burgers was conducted in the Sensory Analysis Laboratory of the ESALQ/USP, in individual booths under artificial white light, in a single session of approximately 10 – 20 min. Samples (~10 g) were served at 45 °C on disposable plastic plates coded with three random numbers, and presented monadically following a Williams Latin Square design

(Wakeling & MacFie, 1995). Water and biscuits were offered to consumers to clean their palate between samples.

3.2.4.6.1. Consumers

Ninety-eight regular burger consumers (64% women and 36% men, aged between 18 to 59 years) were recruited from the staff and visitors of the ESALQ/USP. Prior to the sensory analysis, participants read and signed an informed consent, approved by the Ethics Committee of Human Research of the ESALQ/USP (protocol No. 2.823.957). Data were collected with the *Compusense Cloud* software (Compusense Inc., Guelph, Canada) using tablets (Samsung Galaxy Tab E T560).

3.2.4.6.2. Pivot profile

The Pivot profile (PP) was conducted according to Thuillier et al. (2015). This method consists of a free description of a target sample compared to a reference sample called Pivot. PP is versatile regarding the choice of the Pivot, being a good option to choose a central product, if the type of product allows it (Lelièvre-Desmas et al., 2017). The Pivot chosen in this study was the beef burger with 1.0% regular salt (RS1.0), which represents the "central product" of the treatments studied (ranging from 0.5% to 1.5% salt). Participants received pairs of samples composed of a target sample and the Pivot. Then, consumers were asked to write sensory attributes that are less and more intense in the target sample compared to the Pivot, avoiding the use of hedonic terms.

3.2.5. Data analysis

3.2.5.1. Salt characterization

Salt characterization data (mean size, span index and bulk density) were analyzed using Student t-test at a 5% significance level.

3.2.5.2. Physicochemical and yield properties

The results of sodium content, pH, color parameters and yield properties were analyzed by the Analysis of Variance (ANOVA) considering a randomized complete block design. Treatments and blocks (two independent burger processing) were considered as sources of variation. Differences between the mean values were analyzed by Tukey's test at a 5% significance level.

3.2.5.3. Consumer sensory characterization

For the PP data, three independent researchers with previous experience in sensory analysis of meat products reduced the list of terms by lemmatization and categorization. To avoid irrelevant information only attributes with a frequency of mention greater than 5% were maintained (Balthazar et al., 2017). This type of analysis is common in qualitative data (Piqueras-Fiszman, 2015). Subsequently, the number of times each attribute was cited as "less than the pivot" (negative frequency) was subtracted from the number of times it was cited as "more than the pivot" (positive frequency) for each product. After this step, a contingency table was generated by translating the data to have only positive values. This was done by adding the absolute value of the minimum value obtained in the subtraction to all values. In this study, the lowest negative value was –23, corresponding to the *salty* and *tasty* attributes for the MS0.5 treatment. Thus, the minimum value was zero (0) for these attributes and the other attributes had positive values. These positive values reflect the intensity of the attribute in the sample when compared to the Pivot (Thuillier et al., 2015). A global χ^2 test was performed on the contingence table to evaluate the significant difference between rows (beef burgers) and columns (sensory attributes). Then, when the difference was significant at a 5% significance level, a χ^2 test per cell was performed to identify if each cell was significantly different from the theoretical value (Saldaña et al., 2019b; Symoneaux, Galmarini, & Mehinagic, 2012).

The contingency table was then submitted to a Correspondence Analysis (CA) to obtain the sensory map of the beef burgers and sensory attributes. The Pivot sample was included in the sensory map to improve the interpretation of the results (Esmerino et al., 2017). Confidence ellipses were projected around each treatment at 95% of confidence, in order to asses the stability of the sample configurations (Cadoret & Husson, 2013; Dehlholm, Brockhoff, & Bredie, 2012; Saldaña et al., 2019a).

3.2.5.4. Software

All data analyses were performed in the R software, version 3.5.1. For CA the ExPosition package (Beaton, Fatt, & Abdi, 2014) was used and a global χ^2 test and χ^2 test per cell were performed using XLSTAT 2015 (Addinsoft, New York, EEUU).

3.3. Results

3.3.1. Salt characterization

As expected, salts with different sizes (RS and MS) presented significant differences in their physical characteristics (Table 3.1). The use of the 60-mesh stainless steel sieve (250 microns) allowed to reduce the mean size of the RS by almost three times ($P < 0.05$). In this sense, the size distribution was also lower for the MS ($P < 0.05$), which had a more uniform size, shown by the span index. Bulk density of RS was lower than that of MS ($P < 0.05$).

Table 3.1. Mean size, polydispersity index (span) and bulk density of regular salt (RS) and micronized salt (MS).

Salt	Mean size ($D_{4.3}$; µm)	Span	Bulk density (g/mL)
RS	477.57 ± 21.00^{a}	1.97 ± 0.04^{a}	0.97 ± 0.01^{b}
MS	168.86 ± 1.66^{b}	1.45 ± 0.03^{b}	1.08 ± 0.01^{a}

Mean values with different letters in the same column differ from each other ($P < 0.05$) according to the Student t-test.

3.3.2. Physicochemical characterization and yield properties

Differences were observed ($P < 0.05$) in the sodium content of the treatments. RS1.5 and MS1.5 presented the highest mean sodium values (0.74 and 0.75 g / 100 g, respectively), which were significantly higher than the contents of the other treatments. There was no difference ($P > 0.05$) between the mean sodium values of RS and MS at the same concentration (treatments with 1.5% and 1.0% of salt).

The pH values and color parameters (L*, a* and b*) were not affected by the size and amount of salt added to the burger (Table 3.2). Moreover, the salt concentration affected the yield properties of the product. Cooking loss was greater for MS0.75 and MS0.5 ($P < 0.05$), with values above 39%. Similarly, the diameter reduction was significantly greater in these treatments (> 25%) (see Table 3.2).

Table 3.2. Effect of the type (RS and MS) and level of salt on sodium content, pH, color parameters and yield properties of beef burger

Beef burger	Sodium (g/100g)	pH	Color parameters			Yield properties (%)	
			L*	a*	b*	Cooking loss	Diameter reduction
RS1.5	0.74 ± 0.03[a]	5.95 ± 0.11[a]	51.36 ± 1.62[a]	24.56 ± 1.29[a]	16.47 ± 0.38[a]	33.18 ± 1.32[c]	21.97 ± 2.12[c]
MS1.5	0.75 ± 0.04[a]	5.95 ± 0.02[a]	51.40 ± 1.73[a]	24.58 ± 0.83[a]	16.57 ± 0.29[a]	33.11 ± 2.68[c]	22.35 ± 1.33[c]
RS1.0	0.50 ± 0.04[b]	5.92 ± 0.04[a]	51.52 ± 1.91[a]	24.51 ± 0.51[a]	16.35 ± 0.65[a]	35.60 ± 2.70[bc]	23.28 ± 2.18[bc]
MS1.0	0.45 ± 0.00[bc]	5.90 ± 0.01[a]	52.49 ± 2.74[a]	24.21 ± 0.93[a]	16.30 ± 0.28[a]	34.88 ± 2.37[c]	23.58 ± 1.83[bc]
MS0.75	0.41 ± 0.03[c]	5.96 ± 0.01[a]	51.91 ± 0.95[a]	24.16 ± 0.89[a]	16.20 ± 0.45[a]	39.46 ± 2.17[ab]	25.96 ± 1.97[ab]
MS0.5	0.34 ± 0.02[d]	5.97 ± 0.02[a]	50.35 ± 1.09[a]	24.54 ± 0.61[a]	16.44 ± 0.58[a]	41.16 ± 1.83[a]	27.61 ± 2.07[a]

Mean values with different letters between the same column differ from each other ($P < 0.05$) according to the Tukey's test.
[1]Beef burgers: RS1.5 and RS1.0 (1.5% and 1.0% of regular salt, respectively); MS1.5, MS1.0, MS0.75, and MS0.5 (1.5%, 1.0%, 0.75%, and 0.5% of micronized salt, respectively). RS = Regular salt; MS = Micronized salt.

3.3.3. Microbiological results

All samples were within the acceptable microbiological limits for human consumption (APHA, 1992). They showed average counts of $1.3x10^2$ NMP for thermotolerant coliforms, <0.3 NMP for thermotolerant coliforms, <0.1x10 CFU/g for positive coagulase *Staphylococcus* and absence of *Salmonella* spp. in 25 g.

3.3.4. Consumer sensory characterization

A corpus of 92 sensory terms was generated during the PP test. After the lemmatization and categorization processes, 25 terms were obtained (Table 3.3).

Table 3.3. Categorization of the terms generated in the Pivot profile of beef burgers.

Terms	*Related terms*
Aromatic	*Better smell, strong smell, soft smell, odor, odoriferous, striking aroma, smell, better odor, weak smell, strong aroma, intense aroma, good odor, weak aroma, highlighted odor, strong odor, aroma*
Beef	*Beef taste, beef smell, roast beef aroma, meat aroma, meat smell, meat taste, beef flavor*
Bitter	-
Characteristic	*Characteristic odor, characteristic taste, aroma of burger, burger smell*
Compact	*Dense, mouth cover, dense texture, consistent, viscous, homogeneity, uniformity, uniform, heterogeneous, inconsistent, compacted texture, brittle*
Crunchy	*Roast crust*
Dry	*Dried*
Fatty	*Pieces of fat, aroma of fat, smell of fat, oily, fatty smell, oil, fatty appearance, greasy, fatty taste, fat, greasy odor, presence of fat*
Fibrous	*Nervy, nervous, fiber, connective tissue*
Granulated	*Sandy, granulated texture, sanded*
Grilled	*Barbecue, barbecue taste, grilled aroma, fried, smell of frying, roast, cooked, toasted, grilled taste*
Hard	*Massive, dull, dully, rigid, firm, texture, thick texture, resistant, bad texture, rubbery, hard texture, firm texture*
Juicy	*Moist, wet, moistened*

Lumpy	*Gummy, lump*
Off-flavour	*Viscera aroma, chicken heart flavor, aroma of soy, aroma of old meat, rancid, earthy, sour smell, stinky, strange taste, artificial, soy protein taste, strong taste, taste of viscera, astringent, acid, bittersweet, sour*
Pleasant	*Appetizing, best, good, attractive, beautiful, homelike*
Residual	*Residual taste, residual flavor*
Salty	*Salted*
Seasoned	*Accentuated taste, condiment taste, weak in condiments, umami, seasoning aroma, glutamate aroma, seasoning, seasoning taste, seasoning odor, seasoning smell, glutamate*
Smoked	*Smoked aroma*
Spicy	*Chili, pungent, hot, peppery*
Sweet	*Sweetish*
Tasty	*Intense taste, accentuated taste, delicious, nice taste, taste*
Tender	*Soft, mild, flaccid, limp, soft texture, tenderness*
Unpleasant	*Bad, simple, unpleasant taste, horrible, ugly, unpleasant flavor*

Pivot profile results are shown in Table 3.4 and Fig. 3.1. According to the global $\chi 2$ test (P < 0.05), the attributes were significantly different between the samples. The $\chi 2$ test per cell performed on the contingency table (Table 3.4) showed that RS1.5 was characterized as *juicy* and *salty* and presented a low frequency of the attribute *dry*. MS1.5 had high and low frequency of *salty* and *dry*, respectively. MS1.0 was characterized as *salty* and *tasty*, but presented the lowest frequency of the attribute *aromatic*. Sample MS0.75 had high frequencies of the terms *aromatic* and *dry*, which in turn, was less *fatty*, less *salty* and less *tender*. MS0.5 was described as *dry* and *hard* and had negative frequencies for *fatty*, *juicy*, *salty*, *tasty*, and *tender*.

The CA sensory map (beef burgers and sensory attributes) obtained via CA is shown in Fig. 3.1. The first two dimensions accounted for 89.89% of the total variance. The Pivot sample had its theoretical position in the center of the sensory map, and close to it were the samples with the highest salt content (1.5%). The confidence ellipses around the samples were small, which indicates good data stability (Cadoret & Husson, 2013; Saldaña et al., 2019b). The sensory attributes were represented by triangles and their size was proportional to their contribution in the CA (Symoneaux et al., 2012). The first dimension (80% of the variance) separated the samples in two groups. The first group consisted of the treatments MS1.0, MS1.5 and RS1.5. The attributes strongly associated with these treatments were *salty* and *tasty*, followed by *juicy* and *fatty*. The *spicy* and *tender* attributes were also close to these treatments, but the contribution of these terms to the sensory map was small as illustrated by the small size of their triangles. The confidence ellipses around the burgers with higher salt content (1.5%) overlapped, indicating that these burgers were perceived as similar. There was also an overlap of the confidence interval of RS1.5 and MS1.0, indicating similarity between these treatments, unlike MS1.5 and MS1.0, which may be considered different. The second group was composed by the MS0.75 and MS0.5, which have overlapping confidence ellipses. CA shows that these treatments were considered *aromatic* and *dry*.

Table 3.4. Contingency table of the translated frequencies of the attributes of beef burger

Attribute	Beef burger				
	RS1.5	MS1.5	MS1.0	MS0.75	MS0.5
Aromatic	10	19	**3(–)**	**24(+)**	18
Beef	18	19	21	26	18
Bitter	24	23	22	26	24
Characteristic	22	21	24	22	23
Compact	26	27	26	26	22
Crunchy	23	24	24	26	30
Dry	**22(–)**	**21(–)**	27	**40(+)**	**50(+)**
Fatty	35	35	30	**10(–)**	**15(–)**
Fibrous	23	21	25	25	27
Granulated	23	22	22	23	26
Grilled	24	22	21	26	26
Hard	36	26	24	31	**43(+)**
Juicy	**40(+)**	32	23	17	**8(–)**
Lumpy	23	24	21	23	25
Off-flavour	27	23	28	29	20
Pleasant	21	22	20	22	23
Residual	26	23	25	25	22

Salty	**32(+)**	**36(+)**	**31(+)**	**3(–)**	**0(–)**
Seasoned	21	31	27	24	20
Smoked	23	23	22	25	25
Spicy	25	24	31	17	20
Sweet	23	29	27	31	29
Tasty	25	19	**34(+)**	6	**0(–)**
Tender	37	39	39	**22(–)**	**31(–)**
Unpleasant	25	23	25	24	24

Values in bold presented translate frequencies higher (+) or lower (–) than the expected theoretical value according to the chi-square per cell test at $P < 0.05$.

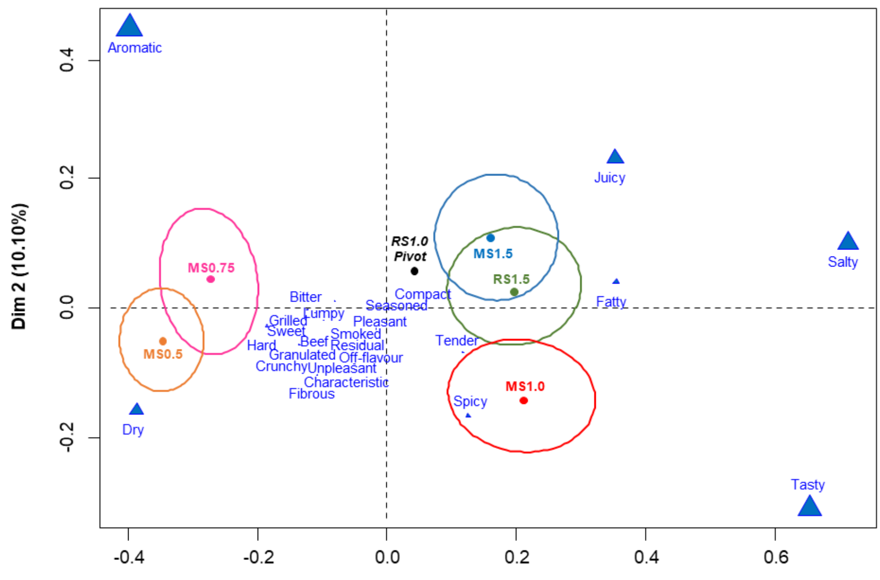

Figure 3.1. Correspondence analysis of Pivot profile of beef burger added with regular salt and micronized salt.

3.4. Discussion

Salt is one of the main constituents of meat products and is responsible for providing unique sensory characteristics, texture and shelf life to them (Desmond, 2006; Inguglia et al., 2017). Salt reduction from 1.5% to 0.5% was performed in beef burger using micronized salt in order to obtain a healthier product, while maintaining the quality of the burger.

Only two previous studies used micronized salt as an approach to reduce sodium content of meat products, but negative aspects of the use of this type of salt were found. The first study dates back to 1989, in which Shepherd et al. (1989)

reported that small salt particles incorporated in the surface of pâté provided higher saltiness than the same amount of salt (large crystals) incorporated into the product, but samples with salt on the surface were less liked by the respondents. This strategy is clearly based on the inhomogeneous distribution of micronized salt in foods to increase saltiness perception, but its application in meat products suggests that there may be problems of liking. In the other study, Galvão et al. (2014) reported that the use of micronized salt did not influence the saltiness perception of turkey ham. Probably, the micronized salt used in that study was dissociated in chlorine and sodium much faster in the meat batter than larger particles (regular salt), which may be involved in the final saltiness perception. Chemically, it is assumed that saltiness perception is attributed to the Na^+ ion, but the Cl^- ion has the role of modulating this perception (Miller & Barthoshuk, 1991; Ruusunen & Puolanne, 2005). As reported by Murphy, Cardello, and Brand (1981), anions associated with sodium with molecular weights higher than that of sodium chloride may cause a decrease in salty taste, suggesting the importance of the sodium-chlorine interaction for saltiness perception. Due to this, the saltiness perception is probably increased when salt particles are solubilized in the saliva instead of being dissociated in the meat batter during processing. This would involve controlling salt solubilization for sodium release in the mouth. This approach was considered by Jensen et al. (2011), who patented the use of micronized salt encapsulated by any lipid coating to avoid salt solubilization in an aqueous matrix.

Considering the studies of Shepherd et al. (1989), Galvão et al. (2014) and Jensen et al. (2011), we can deduce that to maintain a balance between the saltiness perception and the product liking, the micronized salt must be added to the whole product, but its solubilization in the aqueous matrix should be avoided. For this reason, in this study half of the micronized salt particles were mixed with the pork back fat, and the other fraction was added to the meat batter. In addition, due to the particle size, the micronized salt had a more homogeneous particle size distribution and greater bulk density than the regular salt (Table 3.1), i.e., it is assumed that MS particles will be relatively more scattered in the mouth than the RS particles when the beef burger is consumed, thus enhancing the salty taste.

From our results, salt reduction did not affect pH, color parameters and the microbiological results. However, negative aspects were found for cooking loss and diameter reduction of the products when using less than 1.0% salt (Table 3.2). Salt has the property of solubilizing the myofibrillar proteins of the meat, which are capable of binding water and fat, retaining them in the structure of the product (Desmond, 2006). In addition, according to Besbes, Attia, Deroanne, Makni, and Blecker (2008), the diameter reduction occurs due to the denaturation of meat proteins with the loss of water and fat. MS was not in total contact with the protein fraction because half of the MS was mixed with pork back fat. Thus, it is likely that for samples MS0.75 and MS0.5 the solubilization of myofibrillar proteins was not intense, which led to the release of water and fat during cooking, affecting the yield and the size of the product.

Regarding the sensory aspect, the differences between treatments were multidimensionally marked by 8 sensory attributes (*aromatic*, *tasty*, *salty*, *juicy*, *fatty*, *dry*, *spicy*, and *tender*), which contribute to the first dimension of the CA (Fig. 3.1). In general, beef burgers with salt equal to or greater than 1.0% (including the theoretical position of the Pivot sample) were related to attributes that could be positive for consumers' liking. These sensory attributes that discriminate the samples were related to the functional properties of salt in meat products. Salt solubilizes myofibrillar proteins, increasing their hydration and water retention capacity (Desmond, 2006). Salt concentrations lower than 1.0% increase the cooking loss of the product, which, in part, corresponds to water loss. This may have occurred because salt in concentrations of 1.5%–2.5% increases water retention in meat products (Inguglia et al., 2017; Ruusunen & Puolanne, 2005). However, micronized salt at 1.0% was enough to maintain this functional property in the product in a similar way to treatments with 1.5% salt (Fig. 3.1). These three treatments (RS 1.5, MS1.5 and MS1.0) were characterized as *juicy*. Among other properties, salt improves the taste and texture of meat products (Inguglia et al., 2017). In the PP results, consumers characterized the treatments RS1.5, MS1.5 and MS1.0 as more *tasty* and slightly more *tender* than the treatments MS0.75 and MS0.5. It is well known that salt enhances the taste of meat products, and may interact with other components of the formulation, such as certain seasonings. In our study, the white pepper powder, which imparts a spicy flavor, was highlighted by the presence of salt, since according to the consumers, the spicy flavor was more associated to samples with a higher salt content. The increase in tenderness of beef burgers with salt concentrations greater than 1.0% can be attributed to the presence of fat and water, since RS1.5, MS1.5 and MS1.0 were perceived as *fatty* and *juicy* (Fig. 3.1). The water and fat retention in treatments with more than 1.0% salt may be related to their higher yields (Table 3.2). Salt could favor the formation of fat/water emulsion

(Phan et al., 2008), and the retention or loss of these components during cooking may vary, depending on the salt content of the formulation. Another characteristic that stood out was the attribute *aromatic*, which was associated to treatments with lower salt content in the sensory map. However, MS1.5 had an aromatic score similar to that of MS0.5 (Table 3.4). Thus, it is not possible to establish a relationship between this attribute and the size and/or content of salt.

Regarding the *salty* attribute, the use of 1.0% micronized salt partially mixed with fat helped to maintain the salty taste as the *salty* score of MS1.0 was similar to that of the treatment 1.5% regular salt. It is also important to note that MS1.0 and the Pivot sample (RS1.0) had the same salt concentration, but consumers agreed that the salty taste was more intense in MS1.0 than in the Pivot, which can be observed by the higher positive frequency of MS1.0 (Table 3.4). According to Phan et al. (2008), the release of sodium in the saliva is influenced by the fat content, which acts as a barrier that limits the saltiness perception. Lorido et al. (2015) found similar results for Iberian and Serrano dry-cured hams. These authors observed that the dynamic saltiness perception was affected by the content of intramuscular fat, since the solubilization of salt in the saliva decreased when the fat content was higher. Our results suggest that micronized salt diffuses more quickly in the mouth, despite being mixed with fat. This may be related to the partial fat melting during sample cooking. The melting point of the pork fat is about 40 °C (Bozinovic & Méndez, 1997), but the fatty acids present in that fat have a wide melting temperature range (–11.58 to 71.2 °C) (USDA, 2018; Knothe & Dunn, 2009). Technologically, a melting temperature of 40 °C is interesting because it avoids the contact of the micronized salt with the moisture of the meat dough during the processing and commercialization of the product. During cooking, the fatty protection may have been broken due to partial fat melting, promoting salt release. Since cooking of this type of product is performed right before consumption, it is likely that some of that salt released by the lipid matrix is dissolved in the mouth and a small part remains intact after cooking, being released in the chewing process. However, the intensity of saltiness perception was not directly measured in this work, and a study of the dynamic sensory properties could confirm the hypothesis that micronized salt has advantages in reducing NaCl in meat products, such as beef burger. Nevertheless, PP results showed that the salty taste is maintained when micronized salt is used at 1.0% as compared to 1.5% regular salt. This can be attributed to the smaller particle size of the salt, to its mixture with pork back fat, which may have diminished the aqueous salt solubilization and generated an inhomogeneous distribution of the MS in the beef burger. However, these hypotheses need to be tested in a future study, especially to know what happens to the salt structure and the salt-fat interaction in the beef burger before and after cooking.

In terms of sodium reduction, the results suggest that the use of 1.0% micronized salt has the same impact as the use of 1.5% regular salt or micronized salt, but the theoretical position of the Pivot sample on the sensory map (in addition to the technological aspect) entails to the following question: Should micronized salt be used to reduce the sodium content in beef burger? The answer is yes, as long as it is of interest to intensify the salty taste in the product, since according to the consumers of the PP test, there is an indication that the sample with 1.0% micronized salt is saltier than the Pivot sample, being very similar to the sample with 1.5% regular salt.

3.5. Conclusion

The use of micronized salt partially mixed with pork back fat has the potential to be used as a sodium reduction strategy in beef burger. The addition of 1.0% micronized salt in two parts of the product (pork back fat and meat batter) resulted in burgers with yield properties (cooking loss and diameter reduction), pH, color and consumer-based sensory profile comparable to those with the highest salt levels (1.5%). The salty taste of samples with 1.0% micronized salt and 1.5% regular salt was similar, suggesting an advantage in the use of micronized salt over regular salt to intensify this sensory attribute. Thus, by replacing regular salt with micronized salt, it would be possible to reduce 33% of salt content of the product (from 1.5% to 1.0%). We suggest that this simple strategy has great potential for industrial application in products containing lipid fraction in its composition, in order to reduce sodium in foods.

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4. IMPACT OF THE CONTENT AND SIZE OF NaCl ON DYNAMIC SENSORY PROFILE AND INSTRUMENTAL TEXTURE OF BEEF BURGERS

Chapter published in Meat Science.

Rios-Mera, J. D., Saldaña, E., Cruzado-Bravo, M. L. M., Martins, M. M., Patinho, I., Selani, M. M., Valentin, D., & Contreras-Castillo, C. J. (2020). Impact of the content and size of NaCl on dynamic sensory profile and instrumental texture of beef burgers. Meat Science, 161, 107992.

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Abstract

The objectives of this study were to determine the effect of reducing the content and size of NaCl on the instrumental texture and dynamic sensory profile, and to determine the temporal drivers of liking (TDL). The reduction of the NaCl content decreased the hardness and chewiness parameters, and affected the dynamic sensory profile of the product. The NaCl reduction (<1.0% NaCl) was related to a higher incidence of the attributes *off-flavor* and *dry*. In general, the overall liking was driven by the *juicy* and *tasty* attributes, the latter being associated with the presence of the sensory attributes *salty* and *seasoned* and the texture parameters hardness and chewiness. According to the results, among the treatments with NaCl reduction, the beef burger added with 1.0% micronized salt stood out, since it did not affect considerably the texture parameters, the dynamic sensory profile during chewing and the consumers' liking.

Keywords: Meat products; Sodium reduction; Micronized salt; Texture Profile Analysis; TCATA; Consumers' liking.

4.1. Introduction

4.1.1. Sodium reduction in meat products

Currently government agencies, industry, academia, and consumers are increasingly concerned about the high sodium content of foods (Guardià, Guerrero, Gelabert, Gou, & Arnau, 2006). Meat products, specifically beef burgers, represent a high source of sodium due to their widespread consumption. Therefore, efforts should be made to reduce the sodium content without modifying the quality of this product. Sodium chloride (NaCl) is the main source of sodium of meat products, directly influencing the water holding capacity, sensory properties, texture, and shelf life of the product (Desmond, 2006; Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017; Ruusunen & Puolanne, 2005). For this reason, the reduction and/or replacement of NaCl should be carefully studied. In this regard, different approaches have been explored to reduce the sodium content of burgers, such as the incorporation of flavor enhancers (Mattar et al., 2018), or the substitution of NaCl by KCl (Lilic et al., 2015), among others. However, the sensory characteristics of the sodium-reduced meat products were always negatively affected.

A new approach to reduce sodium content in foods is to use NaCl crystals with low-circularity morphologies, such as flat/pyramidal shape (Quilaqueo, Duizer, & Aguilera, 2015) or to reduce the size of their particles (Rios-Mera et al., 2019), the latter being, perhaps the best alternative due to its low-cost and easy processing. On this subject, Rios-Mera et al. (2019) suggested that it is possible to decrease the NaCl content of beef burgers by 33% without affecting their sensory properties using size-reduced NaCl, also called micronized salt (MS). This approach is based on the mixing of half of the MS with pork back fat and the other half is added in the meat batter, in order to decrease the solubilization of MS in the aqueous matrix and to maintain its action on myofibrillar proteins, respectively (Rios-Mera et al., 2019).

However, further research should be done to obtain an in-depth understanding of the implications of this approach on texture of beef burger, since this parameter is strongly influenced by the physical structure of the product (Almeida et al., 2016; Stanley, Bower, & Sullivan, 2017; Yotsuyanagi et al., 2016). Considering the instrumental texture, several studies indicate that a decrease of NaCl without the use of NaCl substitutes, can increase (Cluff, Kobane, Bothma, Hugo, & Hugo, 2017; Delgado-Pando et al., 2018; Lobo, Ventanas, Morcuende, & Estévez, 2016) or decrease the instrumental hardness (Fellendorf, Sullivan, & Kerry, 2015; Sofos, 1983, Tobin, Sullivan, Hamill, & Kerry, 2012), depending on the meat product under study. In our previous study, we observed that NaCl influenced the sensory texture of the product. Beef burgers with NaCl <1.0% were negatively related to the sensory attribute *tender*, which could be explained by the high cooking losses (Rios-Mera et al., 2019).

4.1.2. Sensory perception as a temporal process

One of the most critical aspects of reducing sodium in foods is trying to maintain the saltiness perception. In our previous study, we emphasized that the use of MS resulted in a product with a higher frequency of salty taste when compared to treatments with regular salt (RS) at the same concentration. However, the salty taste was determined from a static sensory approach, which does not respond to the temporal phenomenon of saltiness perception during chewing (Rios-Mera et al., 2019).

Sensory perception is a temporal process (mastication task, flavor release, etc.) (Piggott, 2000; Lawless & Heymann, 2010). Hence, to consider this aspect, several methodologies have been developed (Castura, 2018). Among the temporal methods, time-intensity provides additional information on sensory perception of meat products compared to quantitative descriptive analysis, but the sensory attributes are evaluated individually (Lorido, Estévez, & Ventanas, 2014). More recently, the Temporal Dominance of Sensations (TDS) technique was applied to explore the simultaneous interactions among attributes during product consumption (Lorido, Estévez, & Ventanas, 2018; Schlich, 2017). However, according to Ares et al. (2015), TDS allows assessors to focus their attention only on the dominant attributes, allowing the loss of information of other attributes that are perceived simultaneously. To overcome this concern, Castura, Antúnez, Giménez, and Ares (2016) proposed the extension of Check-All-That-Apply (CATA) questions as a temporal approach, called Temporal-Check-All-That-Apply (TCATA).

TCATA allows the evaluation of multiple sensory attributes at a time and along the consumption time, using a list of pre-established terms, thus allowing a more complete and detailed temporal sensory description (Ares et al., 2015; Castura et al., 2016). TCATA has the potential to be used with consumers to describe foods, such as probiotic chocolate-flavored milk (Oliveira et al., 2015), low-alcohol beer (Ramsey et al., 2018), orange juice, strawberry yogurt, and vanilla milk desserts (Alcaire et al., 2017) and fermented dairy products (Esmerino et al., 2017). However, this consumer-based approach is still little explored for complex products, such as meat products.

The TCATA results are interpreted through curves that represent the proportion of attribute citation along time. These curves can be analyzed to visually explain the significant differences between samples through pairwise comparisons (Castura et al., 2016). In a similar context, Galmarini, Visalli, and Schlich (2017) proposed the quantification of the duration of dominant attributes for TDS data, allowing to infer about the differences between products for a given attribute. Merlo et al. (2018) and Saldaña, Soletti, et al. (2019) expanded the application of Galmarini et al. (2017) to measure the emotions affected by the packaging color of hamburgers using Temporal Dominance of Emotions and to understand the dynamic sensory perception of bacon smoked with different Brazilian woods using TDS, respectively. Following the same approach, in this work we applied the duration of attribute citation instead of the dominance, as reported by Galmarini et al. (2017), in the attempt to provide additional insights to the citation proportions of the sensory attributes.

The temporal sensory properties of foods can be complemented with the changes in the food structure, through the measurement of the instrumental texture, such as the texture profile analysis (Tang, Larsen, Ferguson, & James, 2017; Ningtyas, Bhandari, Bansal, & Prakash, 2019; Panouillé, Saint-Eve, Déléris, Bleis, & Souchon, 2014; Rizo, Jimenez-Pérez, et al., 2019; Rizo, Peña, Alarcon-Rojo, Fiszman, & Tarrega, 2019). These aspects, along with the overall liking measurement, will help to determine the real effect of the sensory and texture properties on the consumers' liking (Bemfeito, Rodrigues, Silva, & Abreu, 2016; Nguyen, Wahlgren, Almli, & Varela, 2017; Meyners, 2016).

4.1.3. Objectives and hypothesis

The objectives of this study were to investigate changes in the instrumental texture and temporal sensory profile, and to determine the TDL of beef burger, due to the reduction of the content and size of NaCl. The associations between TDL with instrumental texture and sensory attributes were also explored. Moreover, based on the objectives, the hypotheses proposed for this study are:

1. NaCl reduction will greatly modify the sensory and instrumental properties, being the samples with more NaCl the most liked by consumers.
2. At the same NaCl concentration, differences between the size of the NaCl crystals will cause changes in the sensory and instrumental properties. The NaCl size reduction will cause an increase in the salty taste and, therefore, may intensify positive sensory attributes, with MS burgers being the most liked by consumers.
3. The sensory attributes will drive the overall liking, and they will be the TDL, which could serve as a reference for future reformulations of beef burger with NaCl reduction.

4.2. Materials and methods

4.2.1. Raw materials and ingredients

Lean beef, pork back fat, water and white pepper powder were purchased in Piracicaba, SP, Brazil. Monosodium glutamate, NaCl, onion and garlic powder and sodium erythorbate were supplied by Ibrac (Rio Claro, SP, Brazil). The MS was obtained by sieving the commercial salt (regular salt, RS) using a 60-mesh stainless steel sieve. The mean sizes of MS and RS are 168.86 µm and 477.57 µm, respectively (Rios-Mera et al., 2019).

4.2.2. Beef burger manufacture

Six beef burger treatments were manufactured in two batches or independent processes (n = 20 beef burgers / treatment / batch) performed at different days, according to Rios-Mera et al. (2019): 1.5% regular salt (RS1.5), 1.5% micronized salt (MS1.5), 1.0% regular salt (RS1.0), 1.0% micronized salt (MS1.0), 0.75% micronized salt (MS0.75), and 0.5% micronized salt (MS0.5). The ingredients and their concentrations in the beef burger manufacture were: lean beef (70%), pork back fat (20%), water (7.5%), regular or micronized salt, monosodium glutamate (0.28%), white pepper powder (0.15%), onion powder (0.28%), garlic powder (0.28%) and sodium erythorbate (0.01%). However, due to NaCl reduction, the components beef, pork fat and water slightly increased 0.7%, 0.2% and 0.08%, respectively, in treatment MS0.5 compared to treatments with 1.5% NaCl.

Beef burgers were manufactured as follows: the lean beef and pork back fat were ground separately using a 0.8 cm plate. Then, the beef was manually mixed with salt for 2 min. For MS treatments, half of the MS was mixed with pork back fat (Rios-Mera et al., 2019) and also homogenized for 2 min. Subsequently, the other ingredients were added and all the components were manually mixed for 3 min. Finally, 100 g portions were manually shaped using a burger-maker (10 cm diameter and 1 cm thickness). Beef burgers were stored at –18 °C for 2 hours before being vacuum packed to prevent sample deformation. Then, the burgers were placed in vacuum packages (–73 cm Hg) and stored at –18° C for future analysis.

The samples were cooked in an electric hot plate at 150 °C, until the internal temperature of 75 °C was achieved in the burger. For texture profile analysis, the samples were cooled at 25 °C, and for TCATA and overall liking, the samples were served to consumers at 45 °C.

4.2.3. Texture profile analysis

The texture profile analysis (TPA) was conducted in the Texture Analyzer TA-XT (Stable Micro Systems, Godalming, United Kingdom) following the parameters established by Selani et al. (2016). Five beef burgers / treatment / batch were used for TPA measurement. Cylindrical samples (2.5 cm diameter, 1 cm height) were extracted from the burgers to be compressed twice using a probe of 3.5 cm diameter (P/35, Stable Micro Systems, Godalming, United Kingdom) coupled to the texturometer. The samples were compressed up to 75% of the original sample height at a constant speed of 20 cm/min (pre-test speed and post-test speed: 40 cm/min). The following parameters were determined: 1) hardness (Newton, N): defined as the force necessary to reach a deformation (maximum force during the first cycle of compression); 2) springiness: the distance that the food recovers during the time between the end of the first compression and the beginning of the second compression; 3) cohesiveness: ratio between the area under the second curve to that of the first curve; and 5) chewiness (N): the product of hardness x cohesiveness x springiness (Bourne, 1978; Saldaña et al., 2015).

4.2.4. Sensory analysis

4.2.4.1. Consumers

Ninety-eight regular consumers (71% women and 28% men, with ages ranging from 18 to 65 years) of beef burger were recruited at the *Escola Superior de Agricultura ''Luiz de Queiroz'' (ESALQ) / Universidade de São Paulo (USP)*, based on self-reported consumption of beef burger (35% of consumers consume burger 1–3 times a week, 33% each 15 days, 19% once a month, 9% rarely, and 4% daily or others), interest and availability to participate in the study. Before conducting the sensory analysis, consumers read and signed an informed consent approved by the Ethics Committee of Human Research at ESALQ/USP (protocol No. 2.823.957).

4.2.4.2. Procedure

TCATA and overall liking tests were conducted under artificial white light in individual booths in a single session of about 15 min. Samples (~10 g) were presented monadically to the participants on plates coded with three random numbers following a Williams Latin Square design (Wakeling & MaFie, 1995). First, the participants performed the TCATA test and then rated their overall liking. Water and biscuits were offered to consumers for palate cleansing between samples. The data were collected through the *Compusense Cloud* software (Compusense Inc., Guelph, Canada) using tablets (Samsung Galaxy Tab E T560) with android system.

4.2.4.3. Temporal Check-All-That-Apply (TCATA)

To familiarize consumers with the TCATA method, a preliminary session, consisting of a general explanation and a practical activity of the TCATA method was carried out, using the same tasting protocol (software collection and tablets) of the formal session (Jaeger et al., 2017).

In the formal session, consumers were asked to put the sample in the mouth and simultaneously click the start button to start chewing. Then, they selected and re-selected (if necessary) the sensory attributes during the tasting time. The attributes were automatically deselected after 3 s and if consumers considered that the deselected attribute persisted, had to select it again (Ares et al., 2016). Fifteen attributes (*aromatic*, *beef*, *characteristic*, *compact*, *dry*, *fatty*, *grilled*, *hard*, *juicy*, *off-flavor*, *salty*, *seasoned*, *spicy*, *taste*, and *tender*) were used in the TCATA task, obtained from the consumers description of the same beef burgers (Rios-Mera et al., 2019).

4.2.4.4. Overall liking

After the TCATA task, consumers rate their overall liking using a 9-point category hedonic scale, ranging from “dislike extremely” to “like extremely” (Peryam & Pilgrim, 1957).

4.2.5. Scanning Electron Microscopy (SEM)

In our previous study (Rios-Mera et al., 2019) we suggested that it is possible to maintain the salty taste of beef burger using 1.0% micronized NaCl compared to 1.5% regular NaCl, which could be attributed to the protection of the structure of micronized NaCl by fat, allowing the dissolution of the NaCl in the mouth instead of the meat dough. For this reason, the structure of the NaCl crystals in both raw and cooked burgers was observed via SEM. The samples were prepared as described in item 4.2.2, but to eliminate interferences from other ingredients, samples were produced with beef, fat, water and NaCl, to visualize only the salt crystals on raw and cooked burgers. The treatments RS1.5 and MS1.0 were evaluated, with the intention of demonstrating that it is possible to reduce NaCl in beef burger using micronized NaCl.

Once the burgers were prepared, samples (at 25 °C) of 6.0 x 3.0 x 3.0 mm were extracted and fixed overnight in Karnovsky solution (Karnovsky, 1965), prepared with small modifications (2.5% glutaraldhyde [v/v] and 2.5% formaldehyde in 0.05 M cacodylate buffer, pH 7.0; and 1 mM calcium chloride). Subsequently, the samples were washed 3 times (5 min each) in 0.05 M cacodylate buffer. Then, the samples were placed in 30% glycerol (v/v) for 4 h, and then the samples were cryofractured in liquid nitrogen. After this, the samples were dehydrated at increasing concentrations of ethanol (30, 50, 70, 90, three times 100%, 20 min each concentration) and dried to the critical point of CO_2. Then, the samples were sputtered with a 30 nm gold layer, and observed in a scanning electronic microscope (LEO 435 VP, Leo Electron Microscopy Ltd., Cambridge, England) at an acceleration voltage of 20 kV. The samples were photographed at 1500 × magnification.

4.2.6. Data analysis

Data analysis was performed to test the hypotheses proposed for this study, detailed in Fig. 4.1. The first two hypotheses concern to the effect of the NaCl content and size on the texture properties and dynamic sensory profile, and as a consequence, the third hypothesis seeks to define the TDL and their associations with instrumental texture parameters and sensory attributes.

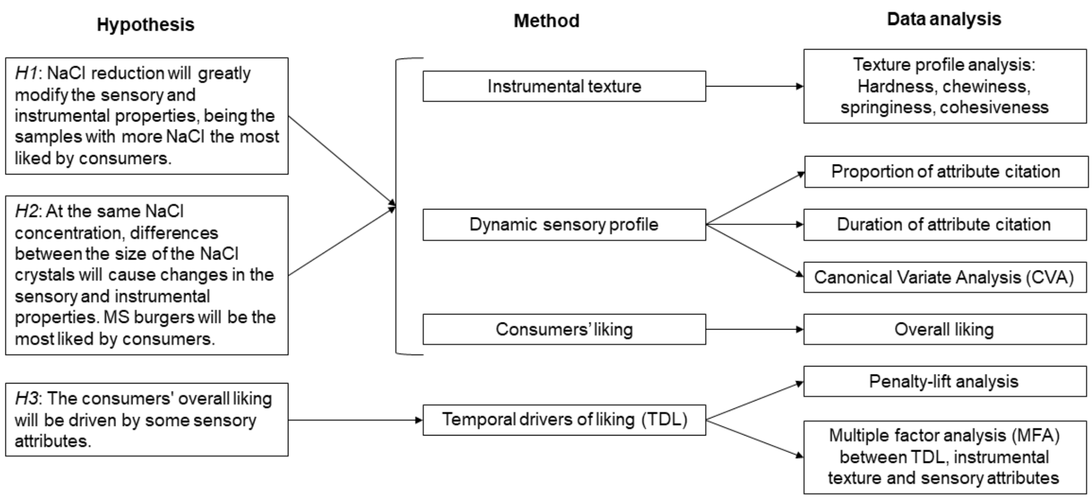

Figure 4.1. Work schema including the hypotheses, topics and analysis to test them.

4.2.6.1. Texture profile analysis (TPA)

The TPA results were submitted to a mixed analysis of variance (ANOVA), considering treatments (fixed) and batch (random) as sources of variation. Pairwise comparisons were performed according to the Tukey's test at 5% of significance level.

4.2.6.2. Temporal Check-All-That-Apply (TCATA)

Before data processing, tasting time was standardized between 0 (first check) and 1 (stop) to remove individual differences in duration task. Then, the citation proportion was calculated for each attribute and sample at a given timepoint. Thus, citation proportion was displayed per sample over standardized time.

The duration of the standardized citation was calculated for each attribute and sample and then, an ANOVA was performed considering sample (fixed) and consumer (random) as sources of variation. The citation duration was also represented at the multivariate level, through the Canonical Variate Analysis (CVA) and multivariate ANOVA (Consumers + Beef burgers + Interaction, with consumers as random effect) at 5% significance level (Galmarini et al., 2017; Merlo et al., 2018). Also, confidence ellipses were drawn around the product using the Barycentric approach.

4.2.6.3. Overall liking

The consumers' overall liking of the beef burgers was analyzed through the mixed ANOVA considering the treatments and presentation order as fixed factors, and consumers as random factor, followed by the Tukey's test to compare mean liking at 5% of significance level.

4.2.6.4. Penalty-lift analysis

To evaluate the effect of the sensory attributes on consumers' liking, data of citation proportions, coded as CATA data and the mean overall liking values were subjected to a penalty-lift analysis in order to obtain positive and negative TDL (Meyners, 2016).

4.2.6.5. Multiple factor analysis (MFA)

The correlation between instrumental and sensory data was performed by Multiple Factor Analysis (MFA), considering the effect of treatments, following the procedure described by Saldaña et al. (2015) and Saldaña, Saldarriaga, et al. (2019). In addition, the mean overall liking was included in the MFA map as supplementary group (Abdi, Williams, & Valentin, 2013).

4.2.6.6. Software

Sensory data were analyzed using TimeSense© software (INRA, Dijon, France). Instrumental data, penalty-lift analysis, and MFA were performed in the R environment (R Development Core Team, 2017).

4.3. Results

4.3.1. Texture profile analysis (TPA)

The TPA results are shown in Table 4.1. Significant effects on the texture were observed. Treatments with 1.5% NaCl were harder than burgers with 0.5% NaCl, and treatments with 1.0%–1.5% NaCl were chewier than 0.75%–0.5% NaCl burgers. On the other side, springiness and cohesiveness were not affected by the treatments. At the same NaCl concentration, i.e. samples RS1.5 vs. MS1.5 and RS1.0 vs. MS1.0, slight changes were observed. MS1.5 was found to be more cohesive and less hard than RS1.5 ($P < 0.05$), while MS1.0 had a higher springiness value than RS1.0 ($P < 0.05$).

Table 4.1. Mean values (± SE) of the effect of the type (RS and MS) and content of NaCl on instrumental texture of cooked beef burgers.

Beef burger[1]	Texture properties			
	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)
RS1.5	96.11 ± 3.91^{a}	0.80 ± 0.01^{ab}	0.61 ± 0.02^{bc}	46.55 ± 2.51^{a}
MS1.5	86.71 ± 0.15^{b}	0.84 ± 0.03^{a}	0.66 ± 0.04^{a}	47.96 ± 4.26^{a}
RS1.0	85.68 ± 1.09^{bc}	0.78 ± 0.02^{b}	0.64 ± 0.01^{ab}	42.32 ± 2.22^{a}
MS1.0	78.68 ± 0.17^{cd}	0.85 ± 0.00^{a}	0.63 ± 0.01^{ab}	42.28 ± 0.90^{a}
MS0.75	76.80 ± 0.44^{d}	0.80 ± 0.00^{ab}	0.58 ± 0.02^{c}	35.72 ± 0.73^{b}
MS0.5	55.16 ± 1.33^{e}	0.80 ± 0.02^{ab}	0.65 ± 0.03^{ab}	28.92 ± 1.02^{c}

Mean values with different letters in the same column differ from each other ($P < 0.05$) according to the Tukey's test.
[1]Beef burgers: RS1.5 and RS1.0 (1.5% and 1.0% regular salt, respectively); MS1.5, MS1.0, MS0.75, and MS0.5 (1.5%, 1.0%, 0.75%, and 0.5% micronized salt, respectively). N = Newtons.

4.3.2. Citation proportions

The citation proportions of the sensory attributes are shown in Fig. 4.2. Consumers focused their attention on the texture of the product at the beginning of the evaluation, where the attribute *tender* had a higher citation proportion compared to the other attributes for samples with ≤1.0% NaCl. Then, the citation proportions were increasing as the NaCl concentration in the samples increased, indicating associations between NaCl and the intensification of the sensory attributes evaluated in this study. It is possible to note for example that, the citation proportion of *seasoned* and *tasty* increased as the NaCl concentration increased in the product (≥1.0%). Individually, MS1.0 was the juiciest sample, because it exceeds 50% of the citation proportion from half of the standardized time; while MS0.5 was the driest sample, reaching a 50% citation proportion before the end of the evaluation. The samples MS1.5 and MS1.0 had higher citation proportions of *salty* during the standardized time than their RS counterparts. In addition, all samples received low citation proportions of the attributes *off-flavor* and *hard*.

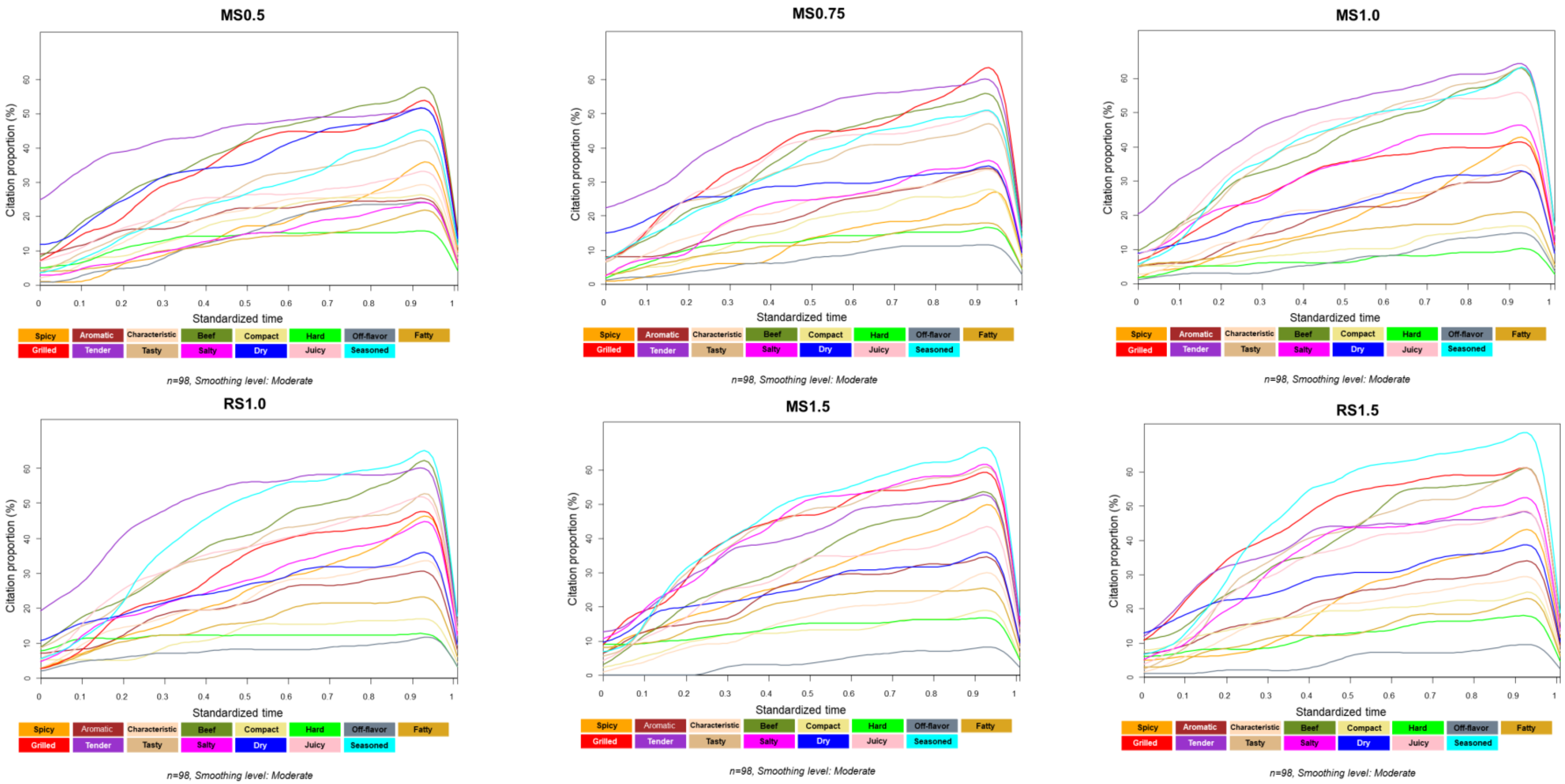

Figure 4.2. Citation proportions of sensory attributes for beef burger with regular salt (RS) and micronized salt (MS). Beef burgers: RS1.5 and RS1.0 (1.5% and 1.0% regular salt, respectively); MS1.5, MS1.0, MS0.75, and MS0.5 (1.5%, 1.0%, 0.75%, and 0.5% micronized salt, respectively).

4.3.3. Duration of the standardized citation of the attributes

To complement the citation proportion information, the duration of the standardized citation of the attributes was calculated to express univariate and multivariate statistical differences between treatments (Galmarini et al., 2017; Merlo et al., 2018). According to Table 4.2, significant univariate differences were found between samples in 7 sensory attributes. MS1.5 presented high citation duration for *salty* compared to treatments RS1.0, MS0.75 and MS0.5 ($P < 0.05$); MS1.5 had higher citation duration of the attribute *spicy* than MS0.75 and MS0.5 ($P < 0.05$). In addition, MS1.5 and MS1.0 were tastier than MS0.5 ($P < 0.05$). MS0.5 was the lest *juicy* treatment and was statistically different from the MS1.0. The duration of the attribute *seasoned* was greater in treatments with ≥1.0% NaCl, but statistical differences were observed between RS1.5 and MS0.75, and between RS1.5 and MS0.5. *Grilled* received the lowest citation duration in samples with 1.0% NaCl, being different from RS1.5 ($P < 0.05$). The citation duration of *off-flavor* was low for all samples; however, it was significantly higher for the MS0.5 compared to 1.5% NaCl samples. At the same NaCl concentration, no differences were observed ($P > 0.05$) between treatments (RS vs MS).

Table 4.2. Mean values (± SE) of the citation duration of the sensory attributes during the tasting of beef burgers.[1]

Attributes	MS0.5	MS0.75	MS1.0	RS1.0	MS1.5	RS1.5
Salty	**0.14 ± 0.03(a)**	**0.23 ± 0.03(ab)**	**0.32 ± 0.03(bcd)**	**0.27 ± 0.03(bc)**	**0.44 ± 0.03(d)**	**0.36 ± 0.03(cd)**
Seasoned	**0.26 ± 0.03(a)**	**0.34 ± 0.03(ab)**	**0.42 ± 0.03(bc)**	**0.43 ± 0.03(bc)**	**0.46 ± 0.03(bc)**	**0.50 ± 0.03(c)**
Spicy	**0.16 ± 0.03(ab)**	**0.13 ± 0.03(a)**	**0.20 ± 0.03(abc)**	**0.24 ± 0.03(bc)**	**0.29 ± 0.03(c)**	**0.22 ± 0.03(abc)**
Grilled	**0.36 ± 0.03(ab)**	**0.40 ± 0.03(ab)**	**0.30 ± 0.03(a)**	**0.30 ± 0.03(a)**	**0.43 ± 0.03(ab)**	**0.47 ± 0.03(b)**
Off-flavor	**0.14 ± 0.02(b)**	**0.07 ± 0.02(ab)**	**0.08 ± 0.02(ab)**	**0.08 ± 0.02(ab)**	**0.04 ± 0.02(a)**	**0.05 ± 0.02(a)**
Tasty	**0.26 ± 0.04(a)**	**0.33 ± 0.04(ab)**	**0.42 ± 0.04(b)**	**0.35 ± 0.04(ab)**	**0.43 ± 0.04(b)**	**0.39 ± 0.04(ab)**
Juicy	**0.24 ± 0.04(a)**	**0.36 ± 0.04(ab)**	**0.42 ± 0.04(b)**	**0.35 ± 0.04(ab)**	**0.29 ± 0.04(ab)**	**0.34 ± 0.04(ab)**
Compact	0.18 ± 0.03	0.17 ± 0.03	0.10 ± 0.03	0.12 ± 0.03	0.12 ± 0.03	0.18 ± 0.03
Tender	0.44 ± 0.04	0.47 ± 0.04	0.50 ± 0.04	0.49 ± 0.04	0.39 ± 0.04	0.39 ± 0.04
Dry	0.36 ± 0.04	0.28 ± 0.04	0.23 ± 0.04	0.25 ± 0.04	0.26 ± 0.04	0.29 ± 0.04
Fatty	0.13 ± 0.03	0.12 ± 0.03	0.14 ± 0.03	0.16 ± 0.03	0.19 ± 0.03	0.14 ± 0.03
Beef	0.39 ± 0.03	0.37 ± 0.03	0.41 ± 0.03	0.39 ± 0.03	0.33 ± 0.03	0.41 ± 0.03
Characteristic	0.20 ± 0.03	0.22 ± 0.03	0.21 ± 0.03	0.22 ± 0.03	0.16 ± 0.03	0.19 ± 0.03
Hard	0.13 ± 0.03	0.12 ± 0.03	0.07 ± 0.03	0.12 ± 0.03	0.14 ± 0.03	0.12 ± 0.03
Aromatic	0.20 ± 0.03	0.21 ± 0.03	0.20 ± 0.03	0.21 ± 0.03	0.24 ± 0.03	0.22 ± 0.03

Mean values with different letters in the same raw for the attributes in bold differ from each other ($P < 0.05$) according to the Tukey's test. [1]Beef burgers: RS1.5 and RS1.0 (1.5% and 1.0% regular salt, respectively); MS1.5, MS1.0, MS0.75, and MS0.5 (1.5%, 1.0%, 0.75%, and 0.5% micronized salt, respectively).

In order to obtain a synthetic representation of the treatments based on the citation duration of the sensory attributes, a sensory map was constructed via CVA (Fig. 4.3). The two canonical components explain 81.78% of the variability of the data, and confidence ellipses were added around the samples to explain differences or similarities between them. The vectors represent the sensory attributes and their lengths are proportional to the contribution to the sensory map. Overall, the treatments were distributed on the sensory map according to the NaCl content. The sensory attributes followed the trend observed in the citation proportions and durations. On the left side of the map are the treatments with the lowest NaCl contents (MS0.75 and MS0.5) with their ellipses overlapped, and close to them are the attributes *dry*, *off-flavor*, *characteristic*, *beef*, *tender* and *compact*. RS1.0 is located in the middle of the map (which its ellipse overlapping those of MS0.75, MS1.0 and RS1.5), characterized by, *beef*, *characteristic, tender*, *tasty*, and *seasoned.* In addition, MS1.0 was the sample with the highest juiciness duration. Finally, the treatments with the highest NaCl content (MS1.5 and RS1.5) were characterized as *salty*, *seasoned*, *tasty*, *spicy*, *fatty*, *hard* and *aromatic*.

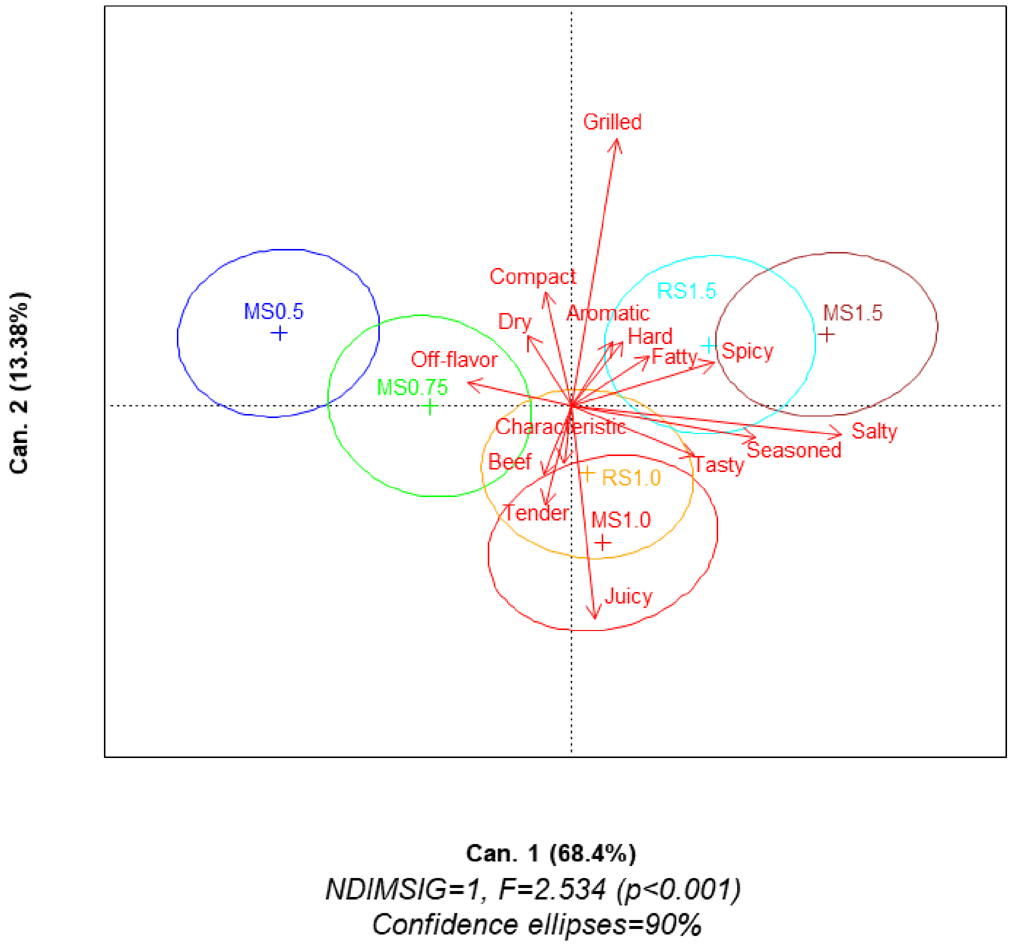

Figure 4.3. CVA of citation durations of the attributes and their association with the beef burgers.

4.3.4. The temporal drivers of liking

The overall liking ranged from 5.88 to 6.84, which was lower for MS0.5. This sample was significantly different (P < 0.05) from the other samples, except for MS0.75, demonstrating that in this study consumers preferred beef burger with NaCl levels (RS or MS) greater than 1.0% in the product. However, at the same NaCl concentration, both NaCl types were liked similarly by consumers.

The penalty-lift analysis revealed that the positive TDL were the attributes *juicy* and *tasty*, while *off-flavor* had a strong negative relationship with liking, and to a lesser extent, with other negative drivers, such as *dry*, *compact*, *beef*, and *fatty* (Fig. 4.4).

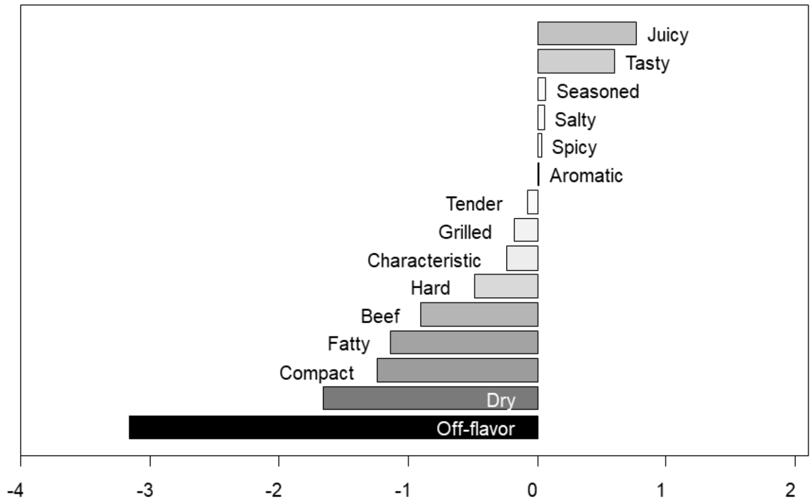

Figure 4.4. Penalty-lift analysis on the mean overall liking and citation proportions of sensory attributes for beef burgers with regular (RS) and micronized salt (MS).

4.3.5. Correlation between instrumental texture, attribute duration and liking

Fig. 4.5 shows the relationships between the instrumental texture and the citation durations of the sensory attributes; in addition, the overall liking was included as supplementary group in the map. In this section, the positive and/or negative associations involving the TDL was obtained, based on the size of the vectors. In terms of duration, the sensory attributes were associated with overall liking in a similar way to the TDL. As a result, it was observed that in the first dimension of the MFA the overall liking was positively correlated with *tasty*, *seasoned* and *salty*, and negatively with the attributes *dry* and *off-flavor*. Among the positive TDLs, *juicy* was negatively associated with *dry*, *off-flavor* and cohesiveness, while *tasty* was positively correlated with *salty*, *seasoned*, hardness, and chewiness. *Tasty* was also negatively correlated with the attributes *dry* and *off-flavor*, being those attributes associated with each other. The differences between the treatments were also expressed via MFA. Fig. 4.6 shows that the differences between treatments were noticeable by the concentration of NaCl in the first dimension, but RS1.5, RS1.0 and MS1.0 are similar due to the proximity between them, even observing variability in the instrumental and sensory responses for the RS1.5 and MS1.0 treatments.

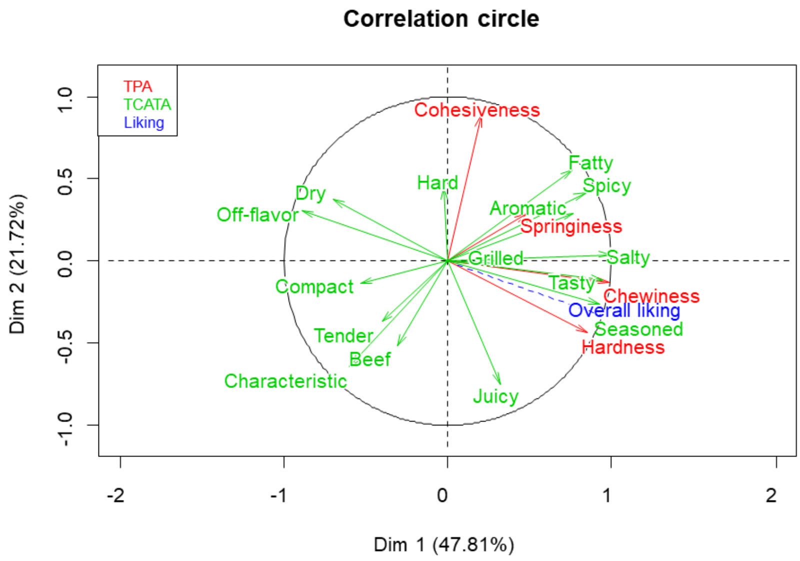

Figure 4.5. Multiple factor analysis (MFA) of the citation duration of the sensory attributes, instrumental texture and overall liking.

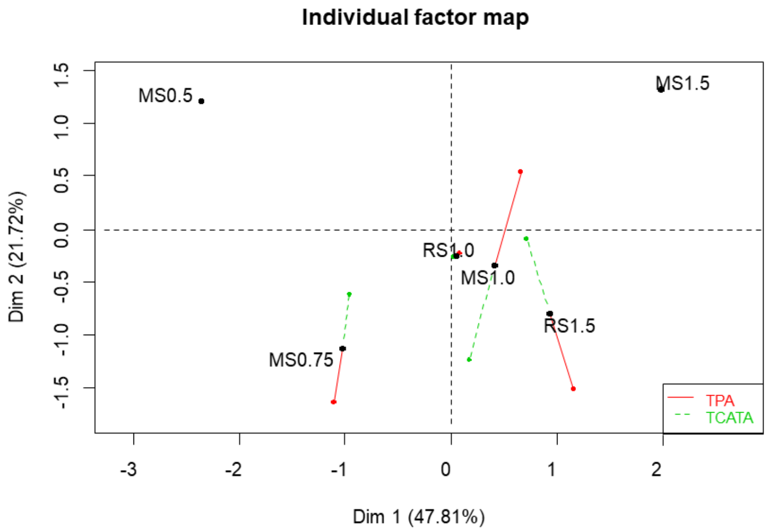

Figure 4.6. Multiple factor analysis (MFA) of the beef burgers considering instrumental texture and TCATA perspectives.

4.4. Discussion

As a healthy approach, we propose to reduce NaCl in beef burger by using micronized salt, but the reduction of the content or size of NaCl could compromise the consumers' liking. Since the consumers' liking is driven by the sensory properties of meat products (Almeida et al., 2016), it is necessary to know the dynamic sensory attributes drivers of liking of beef burger, but seen from a temporal perspective in this study. Besides, the texture and flavor are improved by the addition of NaCl (Desmond, 2006; Inguglia et al., 2017; Ruusunen & Puolanne, 2005), which suggests the determination of associations between texture parameters and sensory attributes with the TDL, in the sense of not compromise these associations when NaCl is reduced in beef burger.

Our first hypothesis is based on the reduction of the NaCl content. Due to the function of the NaCl in the texture of meat products, its reduction caused changes in the TPA parameters. Basically, NaCl has the capacity of solubilizing the myofibrillar proteins of the meat, increasing their capacity of binding water and fat (Desmond, 2006), which affect attributes such as hardness and juiciness of the product. It also promotes the formation of a protein gel during heating (Terrell, 1983), which results in a product with a firmer structure. In this sense, our results showed that NaCl increased the values of hardness and chewiness, similar to that reported by Tobin et al. (2012), who found that hardness and chewiness were significantly correlated to beef patties with 1.0%–1.5% NaCl.

The dynamic sensory profile was considered using the citation proportion and duration of attributes per sample. In general, burgers with NaCl ≥1.0% were related to positive sensory attributes (*juicy*, *tasty*, *seasoned*, *salty*, *spicy*), whereas samples <1.0% NaCl were characterized by the attributes *dry* and *off-flavor*. In the citation proportions, the attribute *tender* attracted the attention of the consumers at the beginning of the tasting. Also, *tender* was inversely related to burgers with >1.0% NaCl, both in the citation proportions and the sensory map, which agrees with the instrumental texture of the product. However, in terms of univariate differences in the citation duration of the attribute *tender*, there was no significant difference between treatments. From a static sensory approach, our previous results showed that *tender* was inversely related to burgers with <1.0% NaCl. In the Pivot Profile test, the presence of *juicy* and *fatty* attributes in burgers with ≥1.0% NaCl was related with tenderness perception (Rios-Mera et al., 2019). However, in Pivot profile, tenderness is not considered as a temporal sensory attribute, an aspect addressed in the citation proportions of TCATA, where samples with ≤1.0% NaCl were highlighted as *tender* at the beginning of the tasting. Finally, the samples with NaCl ≥1.0% were the most liked by consumers, confirming our first hypothesis. This fact implies that a reduction of less than 1.0% NaCl in beef burger would be detrimental to the consumers' liking.

The second hypothesis includes the same aspects of the first, but seen from the point of view of the two NaCl crystal sizes in the same concentration (1.0% and 1.5% NaCl). The sodium reduction strategy proposed in this study and in our previous study indicates that MS should be used, but its incorporation in the beef burger manufacturing should avoid aqueous solubilization, but allowing its action on the myofibrillar proteins of the meat (Rios-Mera et al., 2019). For this reason, MS was added in two stages: 1) half of the MS was mixed with pork back fat; 2) half was added in the meat batter. The protective action of fat on NaCl is confirmed by SEM micrographs, as the NaCl crystals in the RS1.5 decreased in size due to cooking, which could be attributed to their dissolution in the meat dough, while in the 1.0% micronized salt sample, the NaCl crystals maintained practically the same size in raw and cooked burger (Fig. 4.7).

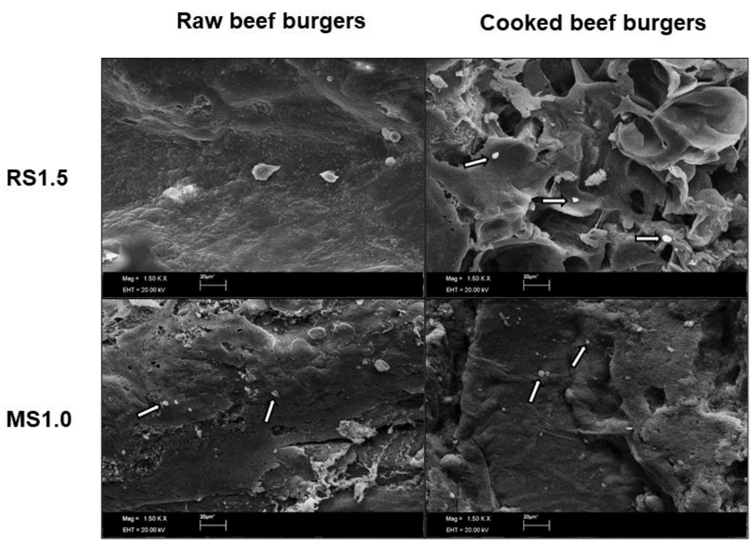

Figure 4.7. Scanning electron micrographs of raw and cooked beef burgers added with 1.5% regular NaCl and 1.0% micronized NaCl. Some arrows were placed in the figure for better visualization of NaCl crystals.

Through our strategy of adding micronized NaCl in the beef burger manufacture, it is expected that, unlike the addition of RS, MS will have less contact with the myofibrillar proteins, consequently there will be less formation of protein gel (Terrell, 1983) and the texture parameters will be influenced. The results showed that there were differences in hardness but statistical differences were observed only between MS1.5 and RS1.5 (Table 4.1).

One of the most important attributes when reducing NaCl in meat products is the *salty* taste (Inguglia et al., 2017). The citation proportions suggest that MS burgers were saltier than the RS burgers during tasting. The citation durations of *salty* in MS burgers at 1.0% and 1.5%, were slightly higher than of RS burgers. However, statistical differences were found only between MS1.5 and RS1.0. The literature shows that the saltiness perception is improved when the NaCl size is decreased, especially for dry products (Moncada et al., 2015; Rama et al., 2013). In addition, a patent developed by ConAgra Foods® shows that the saltiness perception in foods is improved when the contact of NaCl with aqueous food matrices is avoided, which suggested protect NaCl with some hydrophobic component to allow the dissociation of NaCl in the mouth, rather than the food matrix (Jensen, Smith, Fear, Schilmoeller, & Johnson, 2011). We applied these fundaments in the beef burger manufacturing and according to the consumer responses, we suggest that the salty taste was improved by the decrease of almost three times the size of the NaCl particles, either by static (Rios-Mera et al., 2019) or dynamic sensory approaches. Other studies in high-moisture foods indicated that MS may improve the saltiness perception if MS is placed on the surface of paté, but this strategy generated problems of consumers' liking (Shepherd, Wharf, & Farleigh, 1989). On the other hand, Galvão et al. (2014) reported that MS did not improve the saltiness perception in turkey ham. In our study, there were no statistical differences between RS burgers and MS burgers at the same concentration for the overall liking. In this sense, hypothesis 2 is not confirmed, except in the citation proportions for salty taste.

The third hypothesis assumes that there are sensory attributes related to overall liking, called temporal drivers of liking. The Penalty-lift analysis was performed to found the TDL (Meyners, 2016), while the MFA was carried out to explain the associations between TDL with sensory attributes and texture parameters. The positive drivers of liking were only *juicy* and *tasty*, which suggests that, in addition to maintaining the saltiness perception when NaCl is reduced in beef burger (and perhaps in other meat products), the components related to texture, as juicy, and flavor, as tasty, should be maintained. On one hand, *juicy* was negatively correlated with cohesiveness, *off-flavor* and *dry*. The relationship between *juicy* and cohesiveness is difficult to explain because the results do not indicate a tendency for the content or type of NaCl in the instrumental cohesiveness of beef burger; however, *off-flavor* and *dry* were positively correlated according to the consumer responses, *dry* being associated with beef burgers <1.0% NaCl, which obtained the highest cooking losses (Rios-Mera et al., 2019). On the other hand, a chewy and hard texture, and the presence of *salty* and *seasoned* attributes were influenced by the presence of NaCl; these instrumental and sensory parameters together helped to increase the presence of the *tasty* attribute in the product, and consequently *tasty* increased the overall liking.

In summary, to obtain *juicy* and *tasty* beef burgers, the *salty* and *seasoned* attributes should be maintained, as well as the *off-flavor* and *dry* attributes should be avoided when NaCl is reduced in beef burger. An alternative to avoid the presence of *dry* would be to compensate the cooking losses with the addition of water or ingredients that retain water, as an approach of NaCl reduction in meat products. Moreover, the product must have a desired texture in terms of hardness and chewiness, specifically with the values observed in burgers with ≥1.0% NaCl (Table 4.1). Therefore, the third hypothesis is confirmed because the temporal drivers of liking were determined and they were associated with the texture parameters and sensory attributes. Finally, which treatment presented suitable characteristics when NaCl is reduced in beef burger? According to the results, MS1.0 is the most appropriate formulation, especially because it received the highest values of *tasty* and *juicy* in the citation durations.

4.5. Conclusion

The NaCl reduction from 1.5% to 0.5% affected the instrumental texture parameters and the sensory attributes from a temporal perspective; this fact generated the identification of temporal drivers of consumers' liking. Tasty and juicy attributes were the consumers' drivers of liking. Tasty received positive associations with salty and seasoned and with the texture parameters hardness, and chewiness. Considering these associations, our results suggest the possibility of reduce 33% of NaCl in beef burger (from 1.5% to 1.0% NaCl) by using micronized salt, without compromising the consumers' liking.

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5. MODIFICATION OF NaCl STRUCTURE AS A SODIUM REDUCTION STRATEGY IN MEAT PRODUCTS: AN OVERVIEW

Chapter published to Meat Science.

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

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Abstract

Sodium chloride (NaCl) is an indispensable ingredient in meat products, but the consumption of high doses of sodium contained in their formulations may bring about negative health implications. The replacement of NaCl by other salts in meat products has been a technological challenge. Accordingly, this review highlights the importance of NaCl over other sodium and non-sodium salts in the saltiness perception and proposes the use of reduced-size and shapes of NaCl to maximize saltiness perception, while using less NaCl dosages in meat products. However, the effect of matrix components (water, proteins and fats) on the final salty taste is of special consideration. To counteract the effect of the matrix components, two main routes of incorporation of different NaCl types in meat products are discussed: encapsulation and protection of NaCl by the hydrophobic component of the meat product. Given the limited number of publications using this potential strategy, more studies on the application of these technological strategies are required.

Keywords: Salt reduction; Micronized salt; Microparticulated salt; Nanosalts; Salt morphologies; Encapsulation.

5.1. Introduction

Sodium is an essential mineral that maintains the blood volume and pressure and its consumption (up to 5 g of NaCl per day) is required (Bigiani, 2020). However, the current scenario shows an excess of sodium consumption, mainly from table salt (NaCl) which may increase the risk of cardiovascular diseases (WHO, 2012).

Most meat products present a high NaCl content to confer suitable texture, to facilitate emulsion formation, to increase the production yield, to reduce microbial growth and to provide unique sensory characteristics (Desmond, 2006; Inguglia, Zhang, Tiwari, Kerry, & Burgess, 2017). Therefore, its direct reduction or substitution by other salts is a challenging technological task. Moreover, the growing demand for clean label foods limits the use of synthetic ingredients, and the cost of production derived from the use of some of these ingredients makes the substitution of NaCl a complex approach.

A practical example of sodium reduction strategy is the gradual reduction over time, in which consumers adapt to the levels of NaCl applied to food (Busch, Yong, & Goh, 2013; Inguglia et al., 2017); however, this strategy requires consensus and compromise between industry and the health authorities. The use of NaCl crystals of reduced size and distinct shapes represents another potentially immediate alternative to be adopted in the food industry. By using this approach, the saltiness intensity can be maximized and the use of less NaCl amounts is achieved. Previous reports show the feasibility of this approach in the manufacture of dry products (Moncada et al., 2015; Rama et al., 2013). Nonetheless, it is still debatable the use of reduced size NaCl in meat products containing intermediate or high amount of water, because water dissociates NaCl into ions during the processing and storage of the product, thus decreasing the saltiness intensity (Guilloux et al., 2013; Mueller, Koehler, & Scherf, 2016; Rios-Mera et al., 2020; Rios-Mera et al., 2019).

In this sense, the strategy addressed here includes the protection the NaCl crystals from water by preventing possible dissolution of NaCl. Some studies showed that the encapsulation or mixture of NaCl with hydrophobic components represents an interesting strategy to reduce the use of NaCl up to 33% in meat products (Gaudette, Pietrasik, & Johnston, 2019; Rios-Mera et al., 2020; Rios-Mera et al., 2019; Soteras, Cunzolo, Carduza, & Grigioni, 2019). However, it is important to consider any possible NaCl interaction with the components of meat products. For example, the mixture of NaCl and fat (hydrophobic component) could decrease the product's saltiness, since fats act as a barrier in the oral cavity, thus retarding the saltiness perception (Chabanet, Tarrega, Septier, Siret, & Salles, 2013). Additionally, any mixture of NaCl and fats may reduce the sodium-protein interaction, affecting the yield properties such as water retention (Gaudette et al., 2019).

This review shows and discusses reports that were exhaustively searched without considering the year of publication, indexed mainly in Scopus and Web of Science. First, the review highlights the effects of NaCl and other sodium and non-sodium salts on the saltiness perception mechanism. For this purpose, available studies on the physiology of saltiness perception carried out in rodents and humans were extensively reviewed, which show, in synthesis, the advantages of NaCl over other salts on the perceived salty taste. Additionally, the effect of the meat products matrix on the salty taste is discussed, considering the interactions between sodium and other components, more specifically moisture, proteins and fats. In this context, studies carried out on meat products that determined proximal analysis of the cooked or ready-to-eat product were cited, but the data extraction was limited to the control treatments of these studies. Finally, the possibility of including different salts, either encapsulated or protected by fat is comprehensively discussed. In this regard, studies on the application of different sizes of NaCl (in nano and micro scale) and shapes, not only in meat products, but also in foods that suggest the effect of the food matrix on the perception of salty taste were included. Those studies that carried out the encapsulation or protection of the NaCl with hydrophobic components were also considered in order to improve the salty taste, in addition, the available brands that market salt of various shapes/morphologies are cited, and their application in meat products is suggested. The technological approach covered herein unravels the possibility for further studies to corroborate the viability of this strategy to reduce sodium, while maintaining the saltiness in meat products.

5.2. NaCl and salty taste

It is known that salty taste is generated from sodium cation (Na^+), but the exact transduction mechanism of the salty taste perception by humans has not yet been fully described. However, studies in other mammals, mainly rodents, show that the saltiness perception is mediated by Na^+ detecting channels that are located in the cell receptors of taste buds in the oral cavity (Dötsch et al., 2009). Subsequently, the identification of saltiness includes the production of electrical impulses, transported by the neurons to the brain, which are then decoded (Liem, Miremadi, & Keast, 2011).

A sodium recognition channel is able to detect Na^+ at low concentrations. This channel is sensitive to amiloride, a diuretic drug that blocks the detection of sodium, widely used in salt perception studies. Thus, this route is known as the amiloride-sensitive pathway (Bigiani, 2020). However, amiloride has been recognized to be ineffective in blocking the salty taste in humans (McCaughey, 2019). In this context, the oral-antiseptic chlorhexidine can reduce the salty taste of NaCl in humans (Breslin & Tharp, 2001; Helms, Della-Fera, Mott, & Frank, 1995), but the mechanism of action of chlorhexidine is still not known (McCaughey, 2019).

The amiloride-insensitive pathway represents another route that detects sodium in high concentrations, and can also detect a wide variety of cations, such as potassium (K^+) (Bigiani, 2020; Liem et al., 2011). Although the transduction mechanism of this pathway has not yet been explained in detail, scientific reports over the years have suggested that it is dependent on the size of the anion that accompanies sodium. This fact was observed by Schiffman, McElroy, and Erickson (1980) and Murphy, Cardello, and Brand (1981), who investigated the saltiness perception in a wide variety of sodium salts and observed that saltiness decreases as anion size increases. Roebber, Roper, and Chaudhari (2019) highlight that both ions (Na^+ and Cl^-) act in parallel in separate transduction mechanisms (one specific for Na^+ and the other amiloride-insensitive for Cl^-) that together contribute to salty taste. Sodium salts used in the processing of meat products (Table 5.1) have a higher molecular weight than that of NaCl, that is, the salty taste contribution of these salts is lower than that of NaCl, which is a clear example of the effect of the anion on saltiness perception. However, it is important to consider that most of the sodium salts mentioned in Table 1 do not have the function of enhancing the salty taste of meat products.

Fig. 5.1 summarizes the pathways of sodium and non-sodium salts in the salty taste. It is important to note that NaCl uses both channels that detect Na^+, benefiting from both the presence of Na^+ and Cl^-. The most common salt used to substitute NaCl is KCl, but its saltiness perception is only favored by the non-selective route of Na^+ (amiloride-insensitive pathway). Among sodium salts, monosodium glutamate stands out as an ingredient that enhances flavor and provides salty taste, but because of its high molecular weight compared to NaCl (Table 5.1), the saltiness perception mechanism would be favored only by the selective pathway of Na^+ (amiloride/chlorhexidine-sensitive pathway). However, the combined use of these alternative salts with NaCl could enhance the saltiness perception, but given the multifunctional role of NaCl in meat products (in addition to cost), its partial or total replacement could be compromised. Thus, to maximize the salty taste and maintain the technological role of NaCl, the most viable alternative is hypothesized to be to reduce sodium using less amount of NaCl.

Table 5.1. Molecular weight of main sodium salts used in meat products.

Sodium salt	Molecular weight (g/mol)	Technological function
Sodium acetate	82.03	Acidity regulator
Sodium ascorbate	198.11	Antioxidant
Sodium bicarbonate	84.01	Acidity regulator
Sodium citrate	294.10	Acidity regulator

Sodium carbonate	105.99	Acidity regulator
Sodium tripolyphosphate	367.86	Stabilizer
Sodium lactate	112.06	Acidity regulator
Sodium erythorbate	198.11	Antioxidant
Sodium nitrite	68.99	Preservative
Sodium nitrate	84.99	Preservative
Sodium chloride	58.44	Flavor enhancer, preservative
Monosodium glutamate	187.13	Flavor enhancer

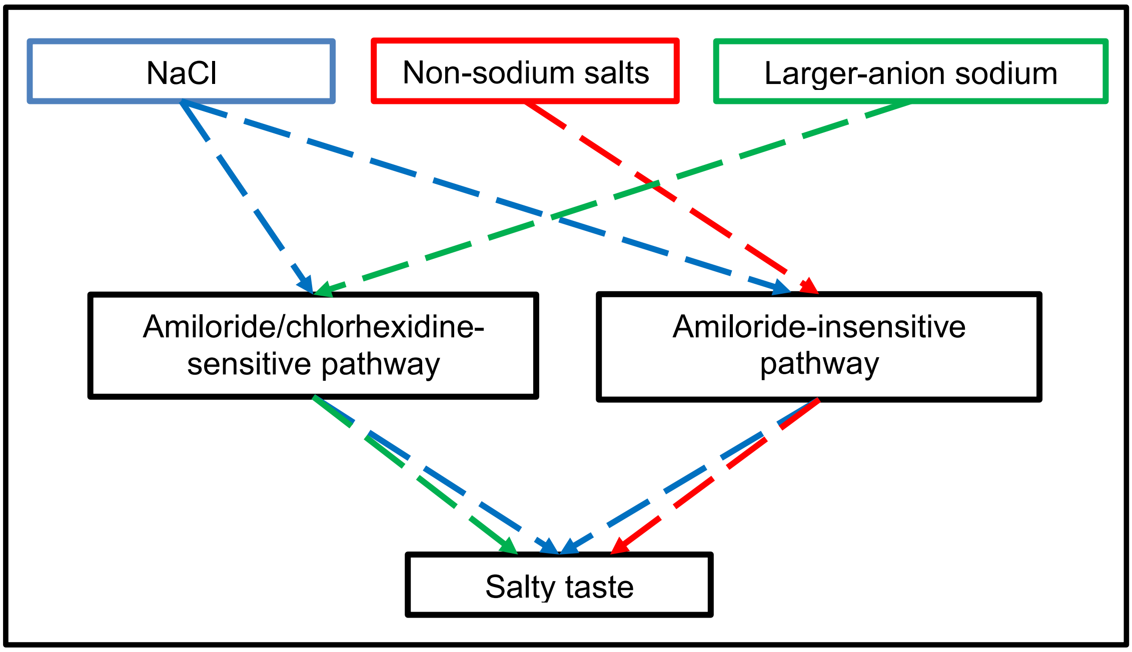

Figure 5.1. Pathways of sodium and non-sodium salts used to impart a salty taste.

5.3. Saltiness perception influenced by the meat product matrix

The food matrix is one of the factors involved in the sodium release and the consequent saltiness perception. Kuo and Lee (2014) proposed a three-stage model to measure the effect of the food matrix on the saltiness perception: (1) the release of sodium from the matrix into the oral cavity, (2) the delivery of sodium within the oral cavity, and (3) the detection of sodium by the receptor cells, thus generating the final salty taste. These three stages occur in solid foods and we invite the readers to review Kuo and Lee (2014) for more details. Water, proteins, and fats are the main components of meat products, and each component has a different role in the saltiness perception. In turn, these components have a direct implication on the texture, and it could be assumed that a product with a lower rupture strength or lower viscosity would release more sodium from the matrix and increase the saltiness perception, as generally happens with the sweet taste (Busch et al., 2013). However, Kuo and Lee (2014) concluded that a higher release of sodium from the matrix does not necessarily imply an increase in the saltiness perception, suggesting that the sensory perception of salty taste is much more complex.

The interaction of sodium with the components of the food matrix seems to be the key phenomenon in the saltiness perception of foods (Busch et al., 2013; Kuo & Lee, 2014). Ruusunen, Simolin, and Puolanne (2001) reported that an increase in the protein content of Bologna-type sausages implied a greater interaction of sodium with meat proteins, therefore less sodium was available to reach the taste buds reducing the saltiness perception. Some authors indicate that water improves the saltiness perception in foods. This behavior was corroborated by Phan et al. (2008) in cheeses. These authors used a dynamic sensory perspective and observed an increase in the release of sodium at the beginning of the chewing process (about 20 s), which may be a consequence of the release of the water that carries sodium from the food structure (Kuo & Lee, 2014). The same trend was observed by Chabanet et al. (2013) in chicken sausages, who found a significant effect of water on saltiness intensity in up to 50 s of chewing. Fats generally act as a barrier that prevents the migration of sodium to the taste buds, reducing the saltiness intensity (Chabanet et al., 2013; Hughes, Cofrades, & Troy, 1997; Phan et al., 2008). However, Kuo and Lee (2014) highlighted that there is divergence on the effect of fats on the saltiness perception of foods. Chabanet et al. (2013) observed a significant and negative effect of fat on saltiness intensity throughout chewing time. Conversely, Phan et al. (2008) observed that when half of the chewing time was reached (60 s) the effect of fat was positive for sodium release, as a consequence of greater matrix fragmentation (Kuo & Lee, 2014).

Table 5.2 shows the composition (moisture, proteins, lipids, and NaCl) of several meat products commonly marketed worldwide. It is important to note that the NaCl values correspond to the content added to the formulation and the other components correspond to the contents found in cooked or ready-to-eat products. Some products show high variability, as can be seen by the standard deviations of cooked beef burger (moisture) and salami (moisture, lipid and NaCl). Then, the NaCl content was plotted with the other components (Fig. 5.2). Fig. 5.2a shows that the lower the moisture content (up to 47%) the more NaCl is added to the product, and vice versa. This relationship suggests that an intermediate or high level of moisture is necessary to highlight the salty taste of meat products, which confirms the positive effect of water on the salty taste of foods (Chabanet et al., 2013; Phan et al., 2008). However, it is important to keep in mind that meat products added with high NaCl content reduces the water activity that prevents the growth of microorganisms, allowing these products to store at room temperature (Inguglia et al., 2017). Considering this information, it is possible to state that the high NaCl content is not only limited to the sensory characteristics of meat products but also to their shelf life and storage conditions.

Table 5.2. Moisture, protein, fat, and NaCl percentage in meat products.

Product	Moisture	Protein	Lipid	NaCl content in formulation	References
Bologna sausage	62.02 ± 4.41	15.14 ± 2.16	17.30 ± 5.29	1.65 ± 0.67	Câmara et al. (2020); da Silva et al. (2020); Fernández-López et al. (2020); Pires et al. (2017); Pires, Santos, Barros, and Trindade (2019).
Cooked beef burger	50.15 ± 13.24	22.38 ± 5.67	17.99 ± 4.95	1.20 ± 0.57	Antonini et al. (2020); do Prado et al. (2019); Selani et al. (2016); Moghtadaei, Soltanizadeh, and Goli (2018)
Cooked ham	75.55 ± 1.62	16.15 ± 2.32	3.30 ± 1.26	1.67 ± 0.71	Barretto et al. (2020; Pancrazio et al. (2016); Pateiro et al. (2019); Paula et al. (2019); Pietrasik and Gaudette (2014).
Cooked meatballs	58.75 ± 4.63	16.79 ± 4.96	18.39 ± 5.86	2.0 ± 0.00	Huang, Shiau, Liu, Chu, and Hwang (2005); Niu et al. (2020); Ran, Chen, Li, He, and Zeng (2020); Serdaroğlu (2006).

Dry fermented sausage	32.62 ± 2.37	27.16 ± 2.78	31.34 ± 0.96	2.71 ± 0.98	Coelho et al. (2019); Jiménez-Colmenero, Triki, Herrero, Rodríguez-Salas, and Ruiz-Capillas (2013); Mendoza, García, Casas, and Selgas (2001); Ruiz-Capillas, Triki, Herrero, Rodriguez-Salas, and Jiménez-Colmenero (2012).
Dry-cured ham	47.15 ± 3.16	33.17 ± 7.36	14.87 ± 3.63	5.98 ± 0.59	Benedini, Parolari, Toscani, and Virgili (2012); Fuentes, Ventanas, Morcuende, Estévez, and Ventanas (2010); Fuentes, Ventanas, Morcuende, and Ventanas (2013); Lucarini et al. 2013; Vestergaard and Parolari (1999).
Frankfurter sausage	63.75 ± 1.77	15.21 ± 2.64	15.84 ± 3.75	1.64 ± 0.27	Fernández-López et al. (2019); Firuzi et al. (2019; Kang, Wang, Li, Li, and Ma (2020); Ran et al. (2020); Sousa et al. (2017).

Mortadella	59.42 ± 5.09	15.43 ± 4.09	20.27 ± 8.51	2.38 ± 0.38	Cáceres, García, and Selgas (2008); Doménech-Asensi et al. (2013); Fontes et al. (2015); Horita, Morgano, Celeghini, and Pollonio (2011); Novelli et al. (1998); Pereira et al. (2014).
Salami	36.08 ± 12.29	30.88 ± 5.39	30.35 ± 14.59	3.35 ± 1.35	Bedia, Méndez, and Bañón (2011); Coelho et al. (2019); Zanardi, Ghidini, Conter, and Ianieri (2010)

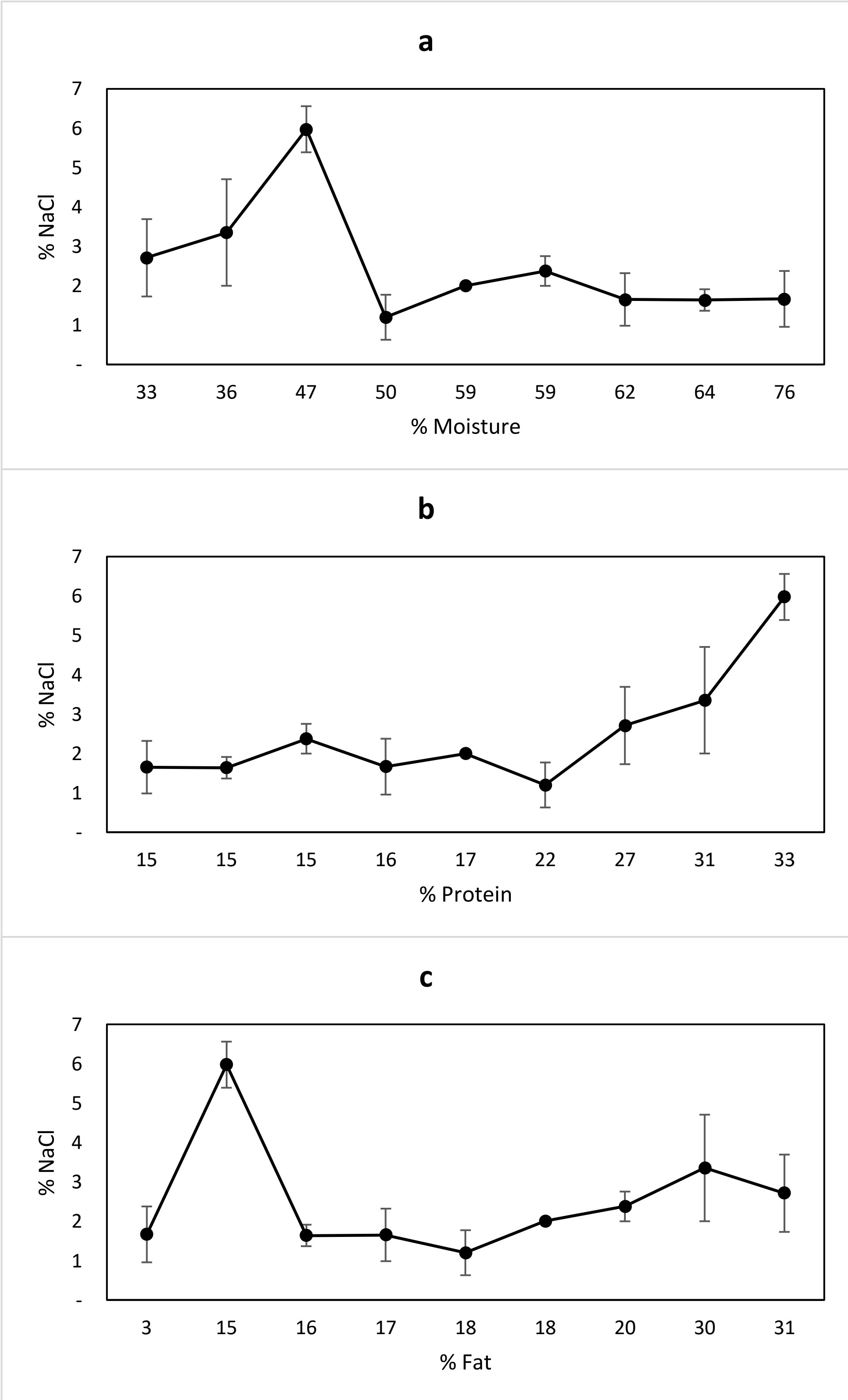

Figure 5.2. Relationship of NaCl with (a) moisture, (b) protein and (c) fat in meat products extracted from Table 5.2.

The relationship between NaCl and proteins is somewhat linear. At levels greater than 22% of protein, more salt is necessary in the meat product (Fig. 5.2b).

This necessity is related to the role of NaCl in the technological properties of meat products, mainly yield, but more interaction of sodium with proteins can reduce the saltiness perception, as already mentioned (Ruusunen et al., 2001). On the other hand, as observed in Fig. 5.2c, there is no linear relationship between fat and the amount of NaCl added in the meat product. At high levels of fat (up to 31%) a higher NaCl content would be expected in the product (due to fat barrier effect), but the relationship suggests that, in general, the fat content is not associated to the salty taste in meat products.

Therefore, strategies to reduce sodium in meat products should be based on the proportions of proteins and water added to the product. However, optimizing the meat product formulation would be challenging as their microbiological stability and sensory properties would be changed considerably. From the technological standpoint it implies that when the protein content is decreased, there should be a proportional increase in moisture and/or lipids and, therefore, there could be a greater susceptibility to microbiological and oxidative deterioration in the meat product, respectively. In this sense, another alternative would be the partial replacement of the meat by other protein sources, but the interaction of sodium with proteins would remain constant and would not have a positive effect on the salty taste. These characteristics make sodium reduction by lowering protein levels in meat products unfeasible.

Reducing the fat content of meat products would represent a suitable strategy to develop more healthy-conscious products and, from the technological standpoint, it can be obtained by replacing fat with other ingredients with similar characteristics. For example, the use of fibers and gels as alternatives for reducing saturated fats in meat products has been reported, with improvements in yield through the reduction of cooking losses (Campagnol, dos Santos, Wagner, Terra, & Pollonio, 2012; Guedes-Oliveira et al., 2019). Thus, the presence of components, such as water would not be affected by the addition of these fat substitutes, and in terms of intensification of the salty taste, it could bring positive results. However, would an increase in moisture content be favorable to intensify the salty taste when NaCl of different sizes and shapes are used? In the next sections this subject is discussed.

5.4. Salt sizes and shapes

The main reason for the use of smaller size NaCl crystals is the decrease in the amount of added NaCl without reducing the salty taste of the product. It is estimated that between 70 to 95% of NaCl remain in the food matrix without being dissolved by the saliva (Phan et al., 2008; Quilaqueo, Duizer, & Aguilera, 2015), indicating that most regular-sized NaCl crystals are swallowed without the consumer noticing the salty taste. The saltiness perception in the mouth increases when the size of the NaCl crystals decreases. These crystals are characterized by an increased surface area and low density, dissolving quickly in the mouth and allowing the efficient transference of sodium ions to the sodium receptors channels (Chindapan, Niamnuy, & Devahastin, 2018; Moncada et al., 2015; Rama et al., 2013). Based on time-intensity sensory technique, Rama et al. (2013) observed that snacks with NaCl crystal sizes less than 106 mm were characterized by presenting the fastest and highest maximum saltiness intensity, and maximum total saltiness. Salt reduction indexes using this approach are promising when applied to foods. For example, Moncada et al. (2015) demonstrated that NaCl can be reduced from 25 to 50% in cheese crackers when the size of the crystals is reduced to ~1.5 μm, without affecting the product’s color, aroma, crunchiness, and overall liking. Freire et al. (2014) reduced 51% of NaCl in shoestring potatoes using 26.1 μm NaCl crystals. For the same product, Rodrigues, Souza, Mendes, Nunes, and Pinheiro (2016) used a mixture of NaCl, KCl and monosodium glutamate with sizes of 117 μm and reduced the sodium content by up to 69%, without affecting the product’s sensory quality.

Reduced-size NaCl crystals can be obtained by various methods, including mechanical grinding, anti-solvent crystallization, laser ablation, vapor drying, spray drying, nanospray drying, electrospray techniques, cryochemical method, and precipitation in organic solvents (Chindapan et al., 2018; Li, Yang, & Liu, 2019; Moncada et al., 2015; Oliveira et al., 2019; Rama et al., 2013), or by simple size separation of the regular NaCl using sieves (Rios-Mera et al., 2019). However, Chindapan et al. (2018) and Quilaqueo and Aguilera (2016) mentioned that a wide distribution of particle sizes can be obtained in the grinding and in the anti-solvent crystallization processes. This effect is undesirable for the optimization of the reduction of the amount of added NaCl and the increase of the saltiness perception in foods. In this context, some companies have developed various salt shapes to obtain uniform particle sizes with specific characteristics.

Cargill Alberger® Flake Salt crystals is one of the brands available in the market. The product is characterized by its hollow pyramidal shape, large surface area and low bulk density, which guarantees better solubility, blend ability and adherence compared to the regular cubic NaCl (Inguglia et al., 2017). Cargill Fine Flake Salt is another type of NaCl with a cube agglomerate structure, and its application in meat products seems to be technologically advantageous. Compared to dendritic or regular salt, the Fine Flake Salt increased the yield, water and fat binding, increased protein functionality, reduced cooking losses, and did not cause detrimental effects on the sensory quality of Bologna-type meat products (Desmond, 2006). However, the final results depend on the type of meat product manufactured. For instance, Sofos (1983) did not observe differences between flaked and granulated NaCl on the sensory and instrumental characteristics of Frankfurters. In fact, a reduction of less than 2.0% NaCl was associated with a softer and less firm texture, and with lower scores for flavor and overall liking (Sofos, 1983). Tate & Lyle SODA-LO® Salt Microspheres is another type of commercially available NaCl, which has a free-flowing hollow characteristic and is encapsulated using dextrin (Lacey, Clark, Frewer, & Kuznesof, 2016; Tate & Lyle, 2017). According to the manufacturer, SODA-LO® is able to maintain the salty taste with up to 50% less sodium (Tate & Lyle, 2017). In meat products, Raybaudi-Massilia et al. (2019) reported that the partial reduction of regular NaCl by SODA-LO® (up to 50%) did not affect the sensory attributes evaluated by trained panelists and the microbiological characteristics of cooked ham, turkey breast and Deli-type sausages. However, it is known that when only SODA-LO® is used, that is, without being mixed with regular NaCl, the saltiness perception can be compromised, especially in products with high moisture.

Mueller et al. (2016) observed that a 25% NaCl reduction in pizza crust using SODA-LO® was significantly less salty than the 100% standard NaCl product. Those authors hypothesized that the NaCl microspheres may have dissolved in the water during the processing of the pizza dough, which could impair the dissolution of the NaCl crystals in the mouth and, consequently the saltiness of the product. The same hypothesis was mentioned by Guilloux et al. (2013) using extra fine NaCl crystals (<170 mm) in pizza dough. Moreover, it has been reported that the dissolution of NaCl crystals is faster in water than in artificial saliva, especially if the pyramidal crystals are considered (Quilaqueo & Aguilera, 2015).

During the manufacture of meat products, size-reduced NaCl crystals can be quickly dissolved during processing. Aheto et al. (2019) observed that fine flake NaCl crystals (0.55 mm) were dissolved quickly and had great penetration in dry-cured pork. A possible alternative to avoid the dissolution of NaCl in matrices with high water content would be the use of granular NaCl crystals of large sizes. Mueller et al. (2016) added coarse grained NaCl of 0.4–1.4 mm in pizza dough 30 s before the end of the mixing time and, as a result, the strategy was effective to obtain a saltier pizza dough with 25% reduction in NaCl. However, the timing of the addition of NaCl can be critical in the processing of meat products, since the incorporation of this salt almost at the end of the processing in order to maintain its structure could be technologically detrimental considering the role of NaCl in the solubilization of myofibrillar proteins, which influences important properties, such as texture and water and fat retention in the meat product (Desmond, 2006). The use of large crystal sizes (3 mm) in beef patties resulted in higher cooking loss and shrinkage, despite maintaining the salty taste with significant NaCl reduction (Gaudette et al., 2019). In addition, Noort et al. (2012) reported that NaCl crystals of 2000 µm improved the saltiness intensity in bread, but the sensory acceptance was negatively affected. These studies suggest that salt size should be carefully studied to find a balance between saltiness intensity, technological properties and sensory acceptance. Nonetheless, the use of large size NaCl impacts on incomplete solubilization of NaCl crystals in the mouth, implying that reduced-size NaCl crystals may be the most feasible alternative to reduce sodium in meat products.

In this context, Shepherd et al. (1989) reported that micronized salt crystals added to the pâté surface improved the saltiness perception compared to the addition of NaCl in the entire product, but this strategy showed negative effects on sensory acceptance. Galvão et al. (2014) observed that a 30% of reduction in NaCl by using micronized salt does not improve the saltiness perception in turkey ham, and the final product was characterized as less seasoned, dry, and brittle. In general, it is hypothesized that a large part of the micronized NaCl was solubilized in the meat product matrices mentioned. Therefore, considering that meat products contain an intermediate to high percentage of moisture (Table 5.2), it is recommended to use hydrophobic coating agents on the reduced-size NaCl crystals.

5.5. NaCl protection against moisture

The protection of NaCl against moisture involves the use of techniques, such as encapsulation to control sodium release, in which the encapsulating materials for NaCl should not dissolve in water, but at the same time soluble in saliva. Li et al. (2019) produced nanosalts using biocompatible polymers, such as polyethylene glycol. The interaction between NaCl crystals with the polymers was performed through ultrasound-controlled precipitation, resulting in supramolecular ion-dipole interaction. As a result, the nanosalts showed rapid dissolution in water and resistance to environmental moisture (up to 75%), which could be beneficial for the storage and transportation of the nanosalts (Li et al., 2019). However, because of the high solubility in water, the nanosalts proposed by Li et al. (2019) are possibly more compatible with dry products than those with high moisture contents. Other authors have reported the use of starch as a NaCl encapsulating agent. In this case, salivary amylase is able to dissolve starch during oral processing, thus releasing sodium in a controlled manner (Chiu et al., 2017). Cai and Lee (2020) and Chiu et al. (2017) observed that the esterification of starch with octenyl succinic anhydride (OSA) delayed sodium release because of the hydrophobic character of OSA. The encapsulation methods used by Cai and Lee (2019) and Chiu et al. (2017) were spray drying using maltodextrin and water-in-oil-in-water emulsions, respectively. The amount of OSA-treated starch to encapsulate NaCl depends on the desired degree of sodium release; for example, Chiu et al. (2017) suggested that 2% of OSA-treated starch is ideal for systems that require stability of NaCl crystals during processing. Christina and Lee (2016) also used starch to encapsulate NaCl via spray drying. Corn starch was enzymatically hydrolyzed to obtain a porous structure that would aid in the encapsulation of NaCl crystals. However, the authors observed that porous starch did not effectively encapsulate NaCl. In the same study, the freeze-dried gel formed by whey protein isolate and anhydrous milk fat was suitable for the NaCl encapsulation (Christina & Lee, 2016). In summary, various encapsulation techniques and encapsulating materials can be used to encapsulate NaCl and subsequently be incorporated into products with high moisture content, but obviously, the use of encapsulated NaCl would lead to an increase in the manufacturing cost of meat products, which could be unfeasible for commercial purposes.

Fat can modulate the saltiness perception in foods. In this sense, fat could be used as a NaCl coating agent, especially in high-fat meat products (Table 5.2). In fact, Tate and Lyle (2017) recommend that SODA-LO® dispersed in oils or fats helps to maintain the integrity of the structure of the microspheres. ConAgra Foods® patented the use of micronized NaCl encapsulated in oils and fats to improve the saltiness perception in foods. The approach is based on the hypothesis of maintaining the structure of NaCl with a hydrophobic agent (Jensen, Smith, Fear, Schimoeller, &

Johnson, 2011). The first report of this strategy in meat products was published by Rios-Mera et al. (2019) using beef burger as a study case. The addition of micronized NaCl with a size almost three times smaller than that of the regular salt (169 vs. 478 µm) was carried out in two steps: 1) 50% in the meat batter, for technological purposes; 2) 50% in the pork fat, to cover the NaCl particles, avoiding their contact with the aqueous matrix. The pH, instrumental color, cooking loss, diameter reduction and consumers sensory profile were not affected in burgers with NaCl reduction of up to 33%. Later, Rios-Mera et al. (2020) reported that the same level of NaCl reduction did not affect neither the dynamic sensory characteristics of the meat product nor the instrumental texture profile and the overall liking of beef burger. The same strategy was reported by Soteras et al. (2019). These authors manufactured lamb meat burger added with SODA-LO® mixed with fat and reduced 14.75% of the sodium content without modifying the salty taste, technological characteristics and acceptance of the product. Gaudette et al. (2019) also added NaCl coated with modified palm oil in beef patties. The addition of 3 mm NaCl crystals coated in palm oil in a ratio of 75:25 and 50:50 w/w, in comparison with the control NaCl (0.2–0.4 mm), maintained the saltiness perception and decreased the cooking loss of beef patties with NaCl reduction from 1% to 0.7%. However, at a higher ratio of 3 mm NaCl (from 25 to 100%), some consumers reported the presence of ‘salt chunks’ in the product, derived from the large-size NaCl added (Gaudette et al., 2019). The use of large-size NaCl in meat products could harm the viability of this strategy in future applications.

The application of encapsulated NaCl in food leads to a non-homogeneous distribution of the NaCl crystals or NaCl spots, which creates a gradient or sensory contrast that improves the saltiness intensity (Noort et al., 2012). However, little is known about how the structure of the encapsulated NaCl is prone to solubilization in high moisture meat products. Recently, Rios-Mera et al. (2020) observed that micronized NaCl crystals that were mixed with the fat maintain their size even after the burger is cooked, but to confirm this observation, more studies are necessary using coating agents and several sizes and shapes of NaCl.

5.6. Final considerations

NaCl is the primary source of salty taste available in our diet, being an indispensable ingredient in the manufacture of meat products. In this sense, its replacement by other salts can modify the traditional characteristics of meat products, as well as decrease the salty taste transduction mechanism that drives consumer’s preferences. NaCl impacts on the components of meat products, mainly proteins, but the effect of the product’s composition on the saltiness perception is a matter to be considered in sodium reduction strategies. Of the three major components present in meat products, proteins and water seem to exert negative and positive effects on the perceived saltiness in the product, respectively, while the relationship with fats is still not clearly understood.

In this review the use of small dimension of NaCl is proposed, but future studies should also consider using NaCl at different shapes and assess the saltiness perception. However, the use of size-reduced NaCl would imply a greater agglomeration of the NaCl crystals because of the effects of environmental moisture during storage, thus requiring encapsulation of the NaCl crystals (Li et al., 2019). In turn, given the moisture content of meat products, the saltiness perception may decrease as a consequence of the ionic dissociation effect of NaCl in water. This aspect leads to the need to encapsulate the NaCl crystals in matrices compatible with meat products, such as OSA-modified starch (Cai & Lee, 2019; Chiu et al., 2017). On the other hand, the protection of NaCl with added fat may be a simpler and cheaper alternative, but the effect of this protection must be tested in products with low and high fat contents in order to assess the protection of the NaCl crystals, to investigate the interference of fats on the saltiness perception, and to determine any possible interactions with other important technological parameters in meat products. Important details on the saltiness perception kinetics can be observed in studies of dynamic sensory properties, which also deserve to be considered in future studies (Saldaña et al., 2021). Given the limited number of publications so far, we encourage the meat (and food) scientific community to include NaCl sizes and shapes as a sodium reduction strategy in meat products or in products where water is a considerable component.

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6. ENCAPSULATION OPTIMIZATION AND pH- AND TEMPERATURE-STABILITY OF THE COMPLEX COACERVATION BETWEEN SOY PROTEIN ISOLATE AND INULIN ENTRAPPING FISH OIL

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Abstract

Fish oil presents health benefits but sensorially generates off-flavors and unpleasant odors originated from the oxidation of polyunsaturated fatty acids. To counteract this problem, microencapsulation by complex coacervation can be applied. Inulin is a prebiotic fiber, but its use in the complex coacervation process was unexplored. The objectives were to optimize the fish oil microencapsulation using soy protein isolate and inulin as wall materials, and to determine the effect of pH and temperature on the oil retention in the microparticles. A central composite rotatable design was used, in which the inulin:SPI ratio and the amount of oil added as a function of the amount of wall materials were the independent variables. A yield of 61% and encapsulation efficiency of 94% were obtained using small amounts of inulin (inulin:SPI = 0.4) and fish oil (20%). However, the optimized microparticles were not resistant when subjected to stress of pH (5.5–6.5) and temperature (50–100 °C). Conversely, the cross-linking with transglutaminase improved the resistance of the microparticles, helping to retain more than 81% of the microencapsulated oil. These cross-linked microparticles could be suitable for food matrices that have the pH range evaluated in this study and that receive thermal treatment.

Keywords: PUFAs, EPA / DHA, Fibers, Microencapsulation, Oil retention

6.1. Introduction

Cold-water fish oil is an important source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), polyunsaturated fatty acids (PUFAs) that have clinically proven health benefits (Eratte, Dowling, Barrow, & Adhikari, 2018; Simopoulos, 2016). PUFAs can decrease the risk of cardiovascular diseases, cancer, and inflammatory and autoimmune diseases, but currently the Western diet is not a venue for PUFAs (Simopoulos, 2016). The use of fish oil as an ingredient in foods is limited because PUFAs are very susceptible to hydroperoxidation and subsequent formation of volatile organic compounds related to off-flavors and unpleasant odors (Karlsdottir, Petty, &

Kristinsson, 2014). To counteract this problem, microencapsulation technology can be applied to mask the fishy taste and extend the shelf-life of PUFAs (Kolanowski, Świderski, & Berger, 1999).

Several methods have been proposed for the encapsulation of fish oil and other sources of omega 3, which have been reviewed by several authors (Chatterjee & Judeh, 2016; Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert, 2016; Kaushik, Dowling, Barrow, & Adhikari, 2015). The most widely used method is spray drying, due to its low cost, fast and continuous process (Comunian & Favaro-Trindade, 2016; Encina et al., 2016). Spray drying uses high temperatures but in very short times, which guarantees a low thermal degradation of the active encapsulated (Comunian & Favaro-Trindade, 2016). However, there are other encapsulation techniques that do not apply heat treatment, allowing to maximize the protection of heat-sensitive compounds. The ionic gelation is one of the encapsulation techniques that does not apply heat, based on the gelation of biopolymers such as alginate in the presence of calcium ions during an extrusion process (Comunian & Favaro-Trindade, 2016). Another technique is complex coacervation process, a liquid-liquid separation phenomenon that occurs between opposite charged biopolymers (generally produced from proteins and polysaccharides) through electrostatic interaction (Eghbal & Choudhary, 2018). Although both methods guarantee oxidative stability and healthy lipid profile in the microparticles production (de Conto et al., 2013; Vasile, Judis, & Mazzobre, 2018), the main difference is in the particle size obtained. While the size of the complex coacervates is around 0.1–500 μm, in the ionic gelation the particle size is between 1 to 10000 μm (Comunian & Favaro-Trindade, 2016), which represents an advantage for complex coacervation due to a narrower and more uniform particle size distribution. The complex coacervates are influenced by several factors such as pH, biopolymer concentration, biopolymer mixing ratio, molecular weight of biopolymers, ionic strength, temperature and homogenization (Eghbal & Choudhary, 2018; Eratte et al., 2018). Gelatin and gum Arabic are the most widely used biopolymers in the complex coacervation process (Eratte et al., 2018), however other sources are taking the interest of researchers, such as soy protein isolate (SPI). Molina Ortiz et al. (2009) suggest that SPI has functional properties for encapsulation, such as emulsification, solubility, film-forming and water binding capacity, in addition to presenting high nutritional value (Molina Ortiz et al., 2009). For the encapsulation of lipids, the emulsifying property of the biopolymer is important, a requirement that SPI complies, being even more effective than other protein source, such as the whey protein isolate (Kim & Morr, 1996). In addition, the use of SPI was optimized in relation to the pH and wall materials (de Conto, Grosso, & Gonçalves, 2013; Huang, Sun, Xiao, & Yang, 2012; Jun-xia, Hai-yan, & Jian, 2011; Mendanha et al., 2009; Nori et al., 2011).

On the other hand, polysaccharides with health benefits have been used for the encapsulation of bioactive compounds. In this aspect, inulin is a dietary fiber that displays prebiotic effects, in addition to being versatile for a wide range of pharmaceutical applications (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015a). There are reports on the use of inulin as an encapsulant agent of bioactive compounds with the spray drying method. These studies suggest that the effectiveness of inulin as an encapsulant is increased when it is associated with other biopolymers to form the protective wall (Botrel, Borges, Fernandes, & do Carmo, 2014; Paim, Costa, Walter, & Tonon, 2016; Zabot, Silva, Azevedo, & Meireles, 2016). Robert, Torres, García, Vergara, and Sáenz (2015) reported that the encapsulation of phenolic compounds from cactus pear pulp is enhanced when the blend of SPI and inulin is used as an encapsulant. However, to test the ability of inulin as an encapsulation material, it is necessary to extend its application to other encapsulation techniques.

In this study we propose inulin as a coacervating agent due to the presence of anomeric carbons (ketoses) in the fructose residues present in its structure (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2010), which could form interactions with cationic biopolymers. Mensink et al. (2015b) indicated that inulin increases its reducing activity applying hydrolysis, a condition that could favor the availability of ketoses to form interactions with cationic groups. In this aspect, it is important to note that to form complex coacervates is necessary the pH adjustment, being optimum conditions between pH 3.1 and 5.0 when are used aniomeric polysaccharides (Eghbal & Choudhary, 2018). Given these conditions, our hypothesis indicates that it is possible to form complex coacervates between SPI and inulin entrapping fish oil, but the optimal conditions will be subject to the stechiometry of the biopolymers.

Due to the industry's interest for the benefits of inulin, especially due to its prebiotic nature (Fernandes et al., 2016), its use could represent an advantage over other biopolymers used in complex coacervation. However, Timilsena, Wang, Adhikari, and Adhikari (2017) points out that the complex coacervation between proteins and polysaccharides is a reversible process that can be dissociated when the pH and temperature conditions are unfavorable. Thus, following a technological trend, it is necessary to define the stability of microparticles in terms of controlled release of the active encapsulated, especially for application of fish oil in food matrices, aiming to mask the fishy taste.

The instability of complex coacervates can be solved with additional treatments, such as protein cross-linking, based on the covalent bonds between glutamine and lysine through an isopeptide bond, which involves an acyl-transfer reaction where lysine is the acyl acceptor (McKerchar et al., 2019). Transglutaminase is an enzyme for food use and is able to catalyse the reaction between glutamine and lysine depending on the side-chain accessibility and structural conformation of the protein (McKerchar et al., 2019), providing resistance in the formation of complex coacervates (de Conto et al., 2013, Timilsena et al., 2016).

Therefore, this study aimed to optimize the encapsulation of fish oil by complex coacervation using SPI and inulin as wall materials, in terms of process yield and encapsulation efficiency, and to determine the effect of pH and temperature on the oil retention in the microparticles.

6.2. Materials and methods

6.2.1. Materials

Cod liver oil (each 5 mL of oil contains 557 mg EPA and 472 mg DHA, according to the supplier Holland & Barrett, Nuneaton, UK) was used as core material. Food grade soy protein isolate (92% protein content) (Ibrac, Rio Claro, SP, Brazil) and inulin (Orafti® GR, degree of polymerization ≥ 10, 92.9% inulin content) (SweetMix, Sorocaba, SP, Brazil) were used as wall materials. Microbial transglutaminase (Breatec, Vlijmen, Netherlands) was used as cross-linking agent.

6.2.2. Methods

6.2.2.1. Microparticles production by complex coacervation

As a first step, the phase separation test induced by the electrostatic interaction between SPI and inulin was performed. For that purpose, both components were dissolved in deionized water at 2.5% (w/v). Previously, the SPI solution was adjusted to pH 8.0 using 0.1 M NaOH to achieve its maximum solubility and to form the complex coacervates (Molina Ortiz et al., 2009; Nori et al., 2011). The coacervation was performed in triplicate at pH values between 4.0 and 6.5 using 1 M HCl, and the pH was measured with a potentiometer (model pH 300, Oakton, Vernon Hills, USA).

The temperature was set at 40 °C and the system was maintained in a constant magnetic stirring (model RH basic 1, Ika, Wilmington, USA) at 400 rpm. Upon completion of pH adjustment, the solution was cooled at 10 °C overnight to promote decantation (Nori et al., 2011). The turbidity of the biopolymer poor-phase was evaluated via turbidimetric analysis using a spectrophotometer at 600 nm (model UVmini–1240, Shimadzu, Kyoto, Japan), according to Huang et al. (2012), in order to select the best treatment (less turbidity) for the microparticles production.

The parameters of complex coacervation reported by Nori et al. (2011) with some adaptations were used. Firstly, an emulsion was made using the SPI solution and the fish oil by means of an ultra turrax system (model T18 basic, Ika, Wilmington, USA) at 14000 rpm for 2 min. Then, the complex coacervates were formed adding the inulin solution and maintaining the conditions described above. The batch size for microparticles production was 100 mL of SPI solution plus 100 mL of inulin solution. Finally, the microparticles were sieved in a 63 µm sieve, washed with deionized water and stored at 10 °C. Additionally, after pH adjustment, cross-linked microparticles were produced using transglutaminase (10 U/g protein), following the methodology proposed by de Conto et al. (2013).

6.2.2.2. Process yield

The process yield (%PY) was calculated from the amount of microparticles collected after sieving. The wet microparticles were dehydrated at 100 °C for 24 h, and %PY was calculated by the amount of dry mass after dehydration relative to the amount of SPI, inulin and fish oil used for the microparticles production (Eq. 6.1):

$$\%PY = \frac{\text{Dehydrated microparticles (g)}}{\text{Initial mass of SPI, inulin and fish oil (g)}} \text{ x } 100 \tag{6.1}$$

6.2.2.3. Encapsulation efficiency

This analysis refers to the amount of oil encapsulated in relation to the initial amount of oil added in microparticles production. First, the oil from the microparticles was released by adding an alkaline solution to destabilize the interaction between the wall materials. Thus, 3% of sodium citrate solution (w/v) was added to 5 g of microparticles (Tello et al., 2015). Then, the oil quantification was performed according to the methodology of Bligh and Dyer (1959). To the microparticles were added 10 mL of chloroform, 20 mL of methanol and 8 mL of distilled water (the volume was corrected in relation to the moisture of the samples) with subsequent agitation for 30 min. Subsequently, 10 mL of chloroform and 10 mL of 1.5% sodium sulphate were added. This mixture was stirred for 2 min and the chloroform and methanol phases were allowed to separate naturally. The upper methanolic phase was discarded and the lower phase was filtered. From the filtrate, 5 mL was extracted and placed in a beaker and then taken to an oven at 100 °C for 2 h. The calculation of the total oil was made according to Eq. 6.2:

$$\%\text{Total oil} = \frac{\text{Oil weight (g)} \times 4 \times 100}{\text{Sample weight (g)}} \tag{6.2}$$

Then, the encapsulation efficiency (%EE) was determined by the Eq. 6.3:

$$\%\text{EE} = \frac{\text{Total oil in microparticles (g)}}{\text{Initial oil (g)}} \times 100 \tag{6.3}$$

6.2.2.4. Morphology of microparticles

The evaluation of the morphology of wet microparticles was performed by light microscopy (model L1000, Bioval, Brazil) at 10 X of magnification. The image was recorded using a microscope digital camera of 1.3 megapixels.

6.2.2.5. Microparticles stability

In this study, the microparticles stability means the oil retention (%OR) in the microparticles under stress conditions. Aqueous solutions of pH 5.5, 6.0 and 6.5 were prepared (10 mL each solution). Then, the microparticles were homogenized in these solutions (1:1 w/v) at 2400 rpm for 5 min using a vortex system and carried to a water bath at 50, 75 and 100 °C for 10 min, or without temperature treatment (room temperature or control treatment, 25 °C). Thereafter, the microparticles were filtered using a qualitative filter paper (porosity 15 µm) and rinsed with deionized water and absolute ethanol. Subsequently, the stability of microparticles was determined as a function of pH and temperature by the Bligh and Dyer (1959) method. Before, the oil was released from the microparticles by adding 3% of sodium citrate solution (w/v)

(Tello et al., 2015). The experiment was performed in duplicate and the %OR was calculated using Eq. 6.4:

$$\%OR = \frac{\text{Final oil in microparticles (g)}}{\text{Initial oil in microparticles (g)}} \times 100 \quad (6.4)$$

6.2.2.6. Experimental design and data analysis

A Central Composite Rotatable Design (CCRD) was applied according to Saldaña et al. (2018) to optimize the %PY and %EE of microparticles, considering the inulin:SPI ratio (0.5–1.5; being the SPI solution fixed at 2.5% [w/v]), and percentage of oil added in relation to the mass of the wall materials (20%–80%) as factors. A 2^2 factorial design including four assays under the axial conditions and three repetitions at the central point was used, totaling 11 assays. CCRD results were adjusted to a second-order polynomial model and the regression coefficients were obtained. The models were analyzed by one-way (ANOVA), and the lack of fit and the coefficient of determination (R^2) were considered for subsequent optimization of %PY and %EE. The non-significant factors in the ANOVA at $P < 0.05$ were excluded from the model, and the optimal conditions were determined using the response surface methodology. Data analysis was performed using the Statistica software (version 12.0, StatSoft, USA). The verification of the predictive model was carried out by producing the optimized microparticles in triplicate.

For turbidity data, a completely randomized design was used, considering the pH (pH 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5) as factor. For the assessment of the stability, a completely randomized design with a 3 x 4 factorial arrangement was used, considering three pH levels (pH 5.5, 6.0 and 6.5) and four temperature levels (Control, 50 °C, 75 °C and 100 °C) as factors and the %OR was the response. Data was analyzed by ANOVA and Tukey's test at a 5% of significance using the R software (R Core Team, 2017). Moreover, to explore the real effect of transglutaminase, %OR of treatments with and without cross-linking were compared via Student *t*-test at a 5% significance level, using the software XLSTAT (Addinsoft, New York, USA).

6.3. Results and discussion

6.3.1. Effect of pH on SPI-Inulin coacervation

To our knowledge, this is the first study that reports the use of inulin in microencapsulation by complex coacervation, therefore it is necessary to know the pH of maximum interaction between SPI and inulin. Fig. 6.1a shows that pH values above 4.5 reduced the interaction between the biopolymers, to the point of keeping turbid the whole system in the range of pH 5.0–6.5. The treatments with the lowest turbidity values were pH 4.0 and 4.5, but the minimum turbidity was obtained at pH 4.0 (mean turbidity 0.009) (Fig. 6.1b). This fact is also shown in the biopolymer-poor phase, where more suspended particles were observed at pH 4.5 compared to pH 4.0 (Fig. 1a). The formation of complex coacervates occurs when there are opposite charges between the biopolymers (Evans, Ratcliffe, & Williams, 2013), generally below the isoelectric point (pI) of the protein and above the pKa value of the polysaccharide, when the polysaccharide is anionic (de Kruif, Weinbreck, & Vries, 2004; Doublier, Garnier, Renard, & Sanchez, 2000; Evans et al., 2013). According to Huang et al. (2012) the pI of SPI is 4.8. However, since no reports exist about the pKa of inulin, we speculate that it is below pH 4.0. Eghbal and Choudhary (2018) mention that the formation of the protein-polysaccharide complex consists of three stages defined by pH. In the first and second pHs, soluble and insoluble complexes are formed, respectively, while in the third pH neutral complexes are formed with equivalent electrical charges between the biopolymers (Eghbal & Choudhary, 2018). It is possible that these three stages of SPI-inulin complex formation have occurred at pH 5.0, 4.5 and 4.0, respectively. Therefore, since the maximum interaction between SPI and inulin occurred at pH 4.0, the formation of complex coacervates for the fish oil encapsulation was performed at pH 4.0.

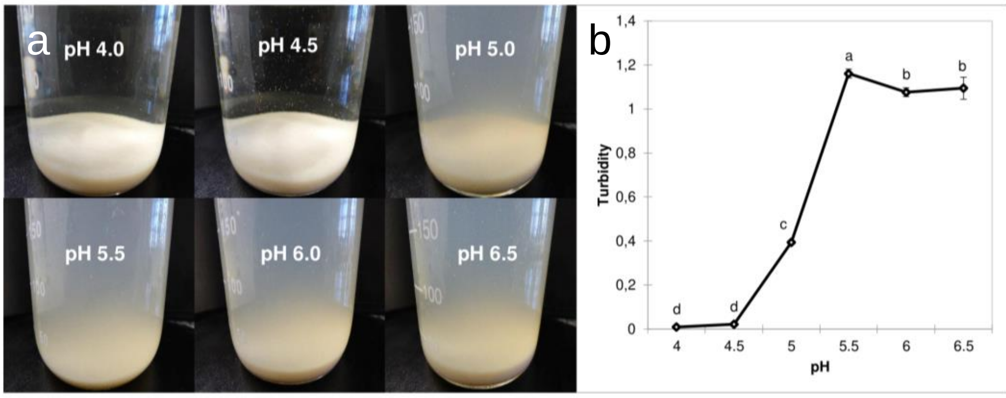

Figure 6.1. Complex coacervation between the soy protein isolate and inulin solutions at pH 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5. a) Phase separation; b) turbidity of the upper biopolymer-poor phase.

6.3.2. Optimization of microparticles production

The coded and decoded values and the experimental results of the CCRD are shown in Table 6.1.

Table 6.1. Central Composite Rotatable Design (CCRD) and experimental results (%PY and %EE) of the complex coacervation between SPI and inulin entrapping fish oil.

Assay	Coded values		Decoded values		Process yield (%PY)	Encapsulation efficiency (%EE)
	X1	X2	Inulin:SPI ratio	% Oil		
1	-1	-1	0.6	28.8	44.64	43.33
2	+1	-1	1.4	28.8	41.41	64.07
3	-1	+1	0.6	71.2	52.42	69.32
4	+1	+1	1.4	71.2	36.19	36.13
5	-1.41	0	0.5	50.0	55.60	63.34
6	+1.41	0	1.5	50.0	38.48	57.77
7	0	-1.41	1.0	20.0	40.31	65.47
8	0	+1.41	1.0	80.0	43.47	58.90
9	0	0	1.0	50.0	42.94	55.62
10	0	0	1.0	50.0	42.87	54.93
11	0	0	1.0	50.0	44.31	56.44

X1: Inulin:SPI ratio; X2: % Oil.

The results show that the maximum %PY (55.60%) was obtained when the minimum amount of inulin was used (experiment 5), and the minimum %PY (36.19%) was obtained using high amounts of inulin and fish oil (experiment 4). For %EE, the behavior was similar. The maximum %EE (69.32%) was obtained using a low concentration of inulin regardless of the oil concentration (experiment 3), whereas the minimum %EE was obtained using high amounts of inulin and oil (experiment 4).

ANOVAs for %PY and %EE are shown in Table 6.2. %EE response was discarded by lack of fit. %PY was mainly affected by the amount of inulin added (Table 6.2). Considering only the significant factors, %PY was optimized by the following model: %PY = 70.0812 – 41.3168X_1 + 13.2401X_1^2 + 0.01X_1X_2 (R^2=0.9234). This model was represented in Fig. 6.2, indicating that the lower the amount of inulin used, the higher the %PY.

Table 6.2. Analysis of variance of the quadratic model for process yield (%PY) and encapsulation efficiency (%EE) of the complex coacervation between SPI and inulin entrapping fish oil.

Factor	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
%PY					
X_1	234.3213	1	234.3213	355.4452	0.002802
X_1^2	13.3949	1	13.3949	20.3189	0.045857
X_2	6.1767	1	6.1767	9.3696	0.092205
X_2^2	6.7461	1	6.7461	10.2332	0.085390
X_1X_2	42.2500	1	42.2500	64.0896	0.015247
Lack of fit	10.3009	3	3.4336	5.2085	0.165283
Pure error	1.3185	2	0.6592		
Total SS	320.2661	10			
%EE					
X_1	52.8905	1	52.8905	92.558	0.010632
X_1^2	0.6690	1	0.6690	1.171	0.392357
X_2	15.8025	1	15.8025	27.654	0.034311
X_2^2	6.1757	1	6.1757	10.807	0.081392
X_1X_2	727.1112	1	727.1112	1272.434	0.000785
Lack of fit	141.4134	3	47.1378	82.490	0.012001
Pure error	1.1429	2	0.5714		
Total SS	946.5788	10			

X1: Inulin:SPI ratio; X2: % Oil.

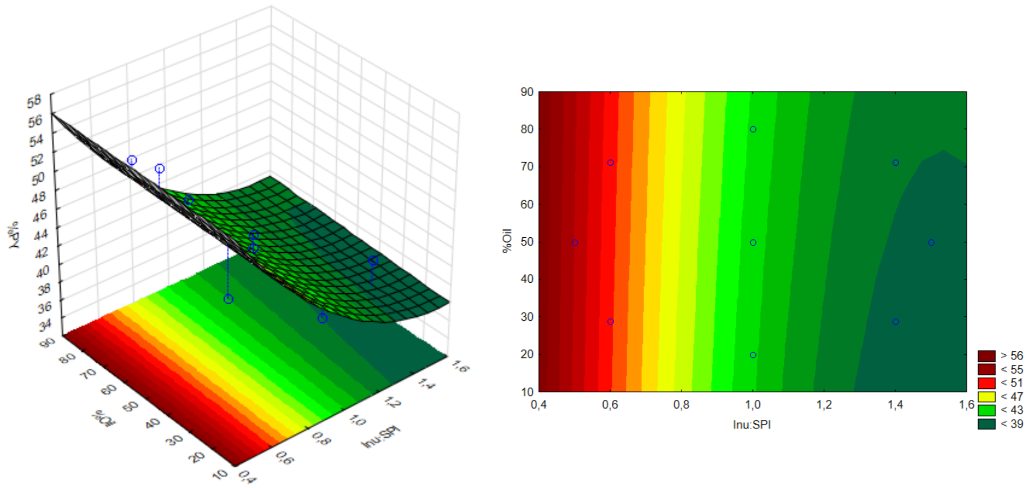

Figure 6.2. Response surface of the effect of inulin:soy protein isolate ratio and amount of fish oil added on the process yield (%PY) of microparticles.

According to the response surface plot for %PY, fish oil can be added at any of the range studied if the amount of inulin is low. Nonetheless, this could compromise the encapsulation efficiency. Several authors report that an excess of encapsulated material decreases the encapsulation efficiency (Jun-xia et al., 2011; Mendanha et al., 2009; Nori et al., 2011). In this sense, the optimized microparticles were produced at inulin:SPI ratio of 0.4 and 20% oil. Thus, the predicted %PY was 55.75%, but the real %PY was 60.74 ± 0.41%. In addition, at the optimized conditions a high %EE was obtained (93.57 ± 1.48%), which is contrary to that reported by some authors who used the spray drying method and observed that inulin decreases the encapsulation efficiency (Bakowska-Barczak & Kolodziejczyk, 2011; Fernandes, Borges, & Botrel, 2014; Fernandes et al., 2016).

It is interesting that using a small amount of inulin was enough to increase the %EE and %PY. Inulin consists of (2→1) linked β-D-fructosyl residues (n = 2–60) with an (1↔2) α-D-glucose end group. This chemical configuration makes inulin a non-reducing carbohydrate as it does not form any reactive aldehyde or ketone groups (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015b). The anomeric carbons of glucose and fructose are aldehyde and ketone, respectively, being ketoses (fructose) more reactive than aldoses (glucose) in solution (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2010). Therefore, it is necessary to release the ketoses from the structure of inulin to increase its reducing activity. Hydrolysis could trigger such activity by means of disrupting the β-D-fructosyl-(2→1)-β-D-fructosyl glycosidic bond, which is susceptible to acidic hydrolysis (Mensink et al., 2015b); then, the higher the hydrolysis degree the more reducing capacity. In addition, inulins with small sizes present a more remarkable reducing property because hydrolysis is faster (Mensink et al., 2015b). In this study, the complex coacervation between inulin and SPI was performed at pH 4.0, where inulin could be subject to hydrolysis forming shorter units to react with SPI and thus can form complex coacervates. However, it is likely that an excess of inulin units with a high degree of polymerization was the main reason for the decreased interaction with SPI, compromising the process yield and encapsulation efficiency. This also leads to the hypothesis that inulin with a high degree of polymerization could have been suspended in the biopolymer-poor phase of complex coacervation. Additionally, the role of SPI should not be disregarded because it retains lipids via emulsification process (Kim & Morr, 1996; Molina Ortiz et al., 2009).

The optimized wet microparticles presented from round to irregular shapes, with presence of oil inside and some agglomerations. The perimeter was well marked and they had approximately less than 100 μm (Fig. 6.3). Similar results were obtained by Mendanha et al. (2009) and Nori et al. (2011) in complex coacervates of SPI and pectin, in which microparticles were formed by different layers, which were attributed to the SPI.

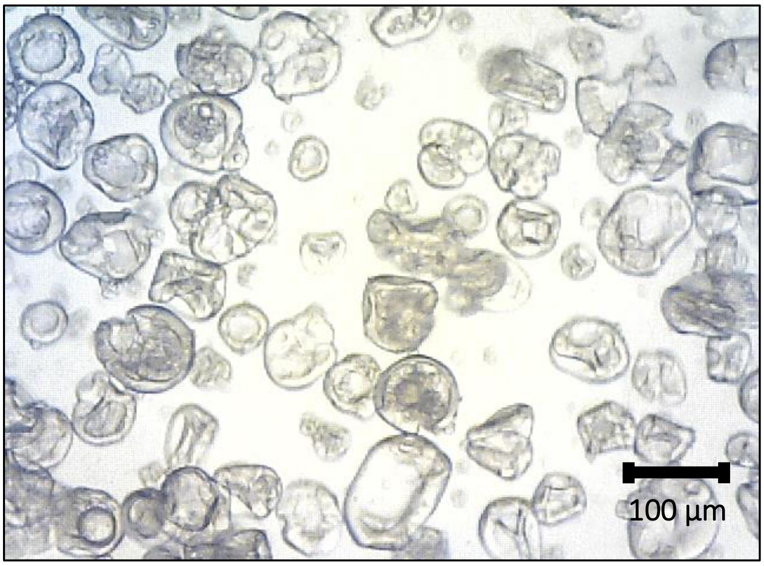

Figure 6.3. Micrographs at 10 X of magnification of wet microparticles of soy protein isolate and inulin entrapping fish oil, produced by complex coacervation.

6.3.3. The effect of pH and temperature on microparticles stability

The stability of microparticles was evaluated by the oil retention (%OR) methodology. The microparticles were produced with the parameters obtained from the optimization (inulin:SPI = 0.4; 20% fish oil). There were differences between the pH values at the control treatment, ranging from 51.34% to 80.54% for pHs 6.0 and 5.5, respectively (Fig. 6.4a). The temperature significantly decreased the %OR at pH 5.5 (~34.33%–36.62%), while at pH 6.0 the %OR was significantly increased at 100 °C (72.24 ± 4.45%) (Fig. 6.4a). Pathak, Priyadarshini, Rawat, and Bohidar (2017) relate that increasing the solubility of biopolymers induced by temperature decreases the efficiency of complex formation. Likewise, Jaramillo, Roberts, and Coupland (2011) observed that the SPI solubility increases from pH 5.0, and with thermal treatment (90 °C/30 min) the behavior was similar. On the other hand, the solubility of inulin increases linearly with temperature (Mensink et al., 2015b). Therefore, the decrease in oil retention may be related to the solubility of the SPI–inulin complex in the pHs and temperatures tested. However, at 100 °C, an increase in %OR can be observed at pHs 6.0 and 6.5. Jaramillo et al. (2011) reported that the SPI–pectin complex is highly soluble at pH 4–5, but that at pH 6–7 the solubility with thermal treatment is decreased. This phenomenon may also be related to the observed in this study.

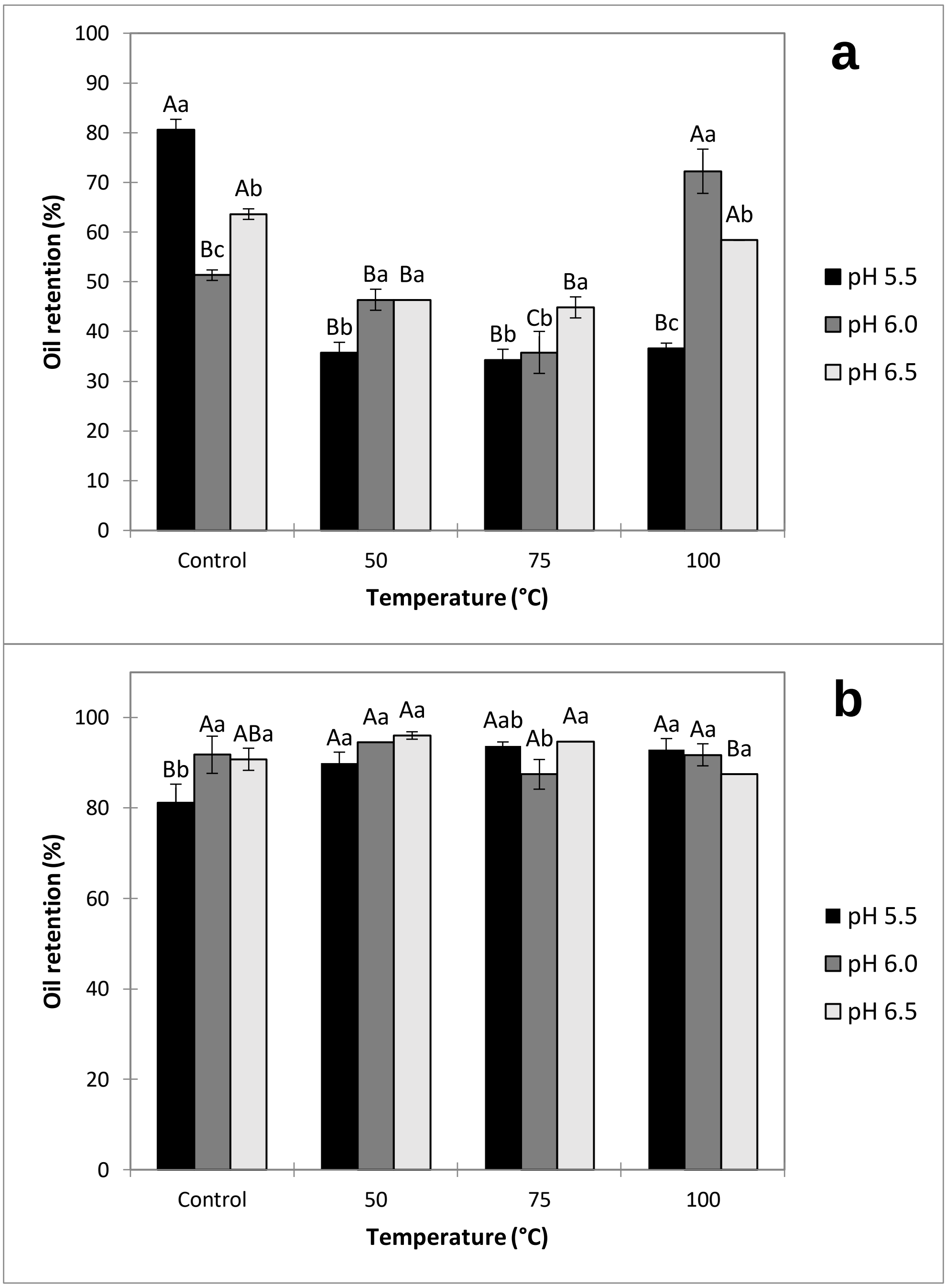

Figure 6.4. Oil retention of microparticles without (a) or with (b) cross-linking with transglutaminase, affected by pH and temperature. Different letters between the pH (a – c) and temperature (A – C) treatments indicate differences ($P < 0.05$) according to the Tukey's test.

Since the %OR was low in some specific stress conditions (Fig. 6.4a), additional treatments should be applied to give better resistance to the microparticles. For this reason, transglutaminase was used as a cross-linking agent in the manufacture of microparticles. The parameters reported by de Conto et al. (2013) were used to obtain maximum %EE rates: 10 U of transglutaminase per gram of protein. Under this condition, we observed that treatments with and without cross-linking presented significant differences ($P < 0.05$). The %OR values were 91.32 ± 4.23% and 49.15 ± 13.74% for cross-linked and non-cross-linked microparticles, respectively. The treatment with transglutaminase maintained the %OR above of 81%, with insignificant effects of pH and temperature (Fig. 6.4b). However, the significant difference observed in the pH 5.5 compared to the other pH values of the control treatment may be an indicator of slight breakdown of the inulin molecules induced by hydrolysis (Mensink et al., 2015b), which could destabilize the SPI–inulin complex. The cross-linked microparticles were stable through the heat treatment applied, which is in-line with the observations reported by Timilsena, Wang, Adhikari, and Adhikari (2016), who observed that transglutaminase improved the thermal stability of chia seed protein isolate–chia seed gum complex coacervates.

6.4. Conclusions

In this study it was possible to obtain microparticles by complex coacervation using inulin, a new coacervation agent proposed. The effect of this polysaccharide was determinant in the optimization of the process yield and the encapsulation efficiency of fish oil. A gram of inulin for 2.5 g of soy protein isolate combined with the small quantity of fish oil was sufficient to obtain suitable process yield and high encapsulation efficiency. On the other hand, the optimized microparticles were resistant to the fish oil release at a limited pH range (5.5–6.5) and temperatures (50–100 °C) when transglutaminase was added. The microparticles reported here can be used as a potentially functional ingredient (source of protein, fiber and EPA/DHA) in foods. However, future studies are necessary on the application in food matrices that present the stress conditions applied in this study, in terms not only of controlled release, but also of the fish oil oxidation. This approach will be tested by our group in a meat product model.

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7. ENRICHMENT OF NaCl-REDUCED BURGER WITH LONG-CHAIN POLYUNSATURATED FATTY ACIDS: EFFECTS ON PHYSICOCHEMICAL, TECHNOLOGICAL, NUTRITIONAL, AND SENSORY CHARACTERISTICS

Chapter submitted to Meat Science.

Rios-Mera, J. D., Saldaña, E., Patinho, I., Selani, M. M., & Contreras-Castillo, C. J. Enrichment of NaCl-reduced beef burger with long-chain polyunsaturated fatty acids: Effects on physicochemical, technological, nutritional, and sensory characteristics. Under review since November 17, 2020.

Abstract

This study aimed to determine the effect of NaCl reduction and addition of long-chain polyunsaturated fatty acids (PUFA) on the quality traits of burgers. Fish oil was either directly incorporated or added as encapsulated by freeze-dried microparticles (complex coacervates) composed of soy protein isolate and inulin. Despite the differences in some parameters associated with NaCl reduction (e.g., instrumental hardness), the quality of the burgers was mainly affected by the microparticles. Thus, a decrease in pH and increase in hardness and chewiness were observed, and a higher exposure of fish oil to oxidation was observed thus increasing volatile oxidation compounds and negatively impacting on the sensory profile and overall liking of the burgers. However, the encapsulation of the fish oil helped to retain EPA and DHA after cooking. The results of the NaCl-reduced burger with unencapsulated fish oil suggest the possibility of incorporating PUFAs, but only containing EPA after cooking.

Keywords: Meat products, salt reduction, micronized salt, EPA/DHA, microencapsulation, complex coacervation.

7.1. Introduction

The association between sodium consumption and cardiovascular diseases, in addition to the consumers' interest towards a healthier diet, has been the goal of research by food scientists, especially in meat products, which are widely consumed because of their unique sensory characteristics. However, meat products are considered to have a high sodium content, which is a limiting factor from the nutritional standpoint.

Recently, our research group has proposed the use of reduced-size NaCl as a viable sodium reduction strategy in meat products. This alternative is interesting considering the negative sensory effect of NaCl substitutes, such as KCl at high substitution levels, and the cost derived from the use of flavor maskers or enhancers to decrease the defects imparted by KCl (Rios-Mera et al., 2020; Rios-Mera, Saldaña, Cruzado-Bravo, et al., 2019). The only condition for the use of reduced-size NaCl or micronized salt is the protection of its structure using some hydrophobic component, such as the animal fat used in the processing of meat products. In this sense, we have reported a reduction of 33% of the NaCl content using micronized salt considering several quality characteristics of burgers (Rios-Mera et al., 2020; Rios-Mera, Saldaña, Cruzado-Bravo, et al., 2019).

In addition to NaCl reduction, meat products can be considered even healthier if bioactive compounds are added in the formulation. In this sense, Decker & Park (2010) have proposed that meat products can be considered functional foods by adding unsaturated fatty acids, dietary fibers, antioxidants, and bioactive peptides. Unsaturated fatty acids, especially long-chain polyunsaturated ones, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have direct and beneficial effects on health (Simopoulos, 2016); thus, the low consumption of these compounds is a concern of governmental institutions, mainly in western countries. For example, the *Southern Common Market* (Mercado Común del Sur, MERCOSUR) regulates the incorporation of EPA and DHA in foods: products between 40 and 80 mg EPA/DHA are considered "sources" and > 80 mg EPA/DHA are products with "high content" (MERCOSUR, 2012).

Marine sources, such as fish, are rich in polyunsaturated fatty acids, but fish consumption is very low in many countries, such as Brazil (Matos, Matos, & Moecke, 2019; Sartori & Amancio, 2012). Exposure of fatty acids present in fish to external factors, such as heat and oxygen during storage and processing, and elevated cooking temperatures, triggers the hydroperoxidation and the formation of volatile compounds related to unpleasant off-flavors (Karlsdottir, Petty, & Kristinsson, 2014). In this context, the use of encapsulated oils rich in PUFAs represents a suitable alternative to incorporate PUFAs in food matrices and, thus in the diet. According to Pérez-Palacios, Ruiz-Carrascal, Solomando, & Antequera (2019), one of the best approaches to incorporate PUFAs in meat products is the use of microencapsulation technology.

Bearing in mind the technological and sensory effect of the ingredients in meat products, as well as the effect of the encapsulation technique on the oxidative stability of lipids, we have recently reported the encapsulation optimization of fish oil using soy protein isolate (SPI) and inulin through the complex coacervation technique, resulting in high levels of encapsulation efficiency and yield (Rios-Mera, Saldaña, Ramírez, et al., 2019). The reasons for the use of these ingredients and the encapsulation technique were: 1) SPI enhances the yield of meat products (Petracci et al., 2013), and its use has been widely reported for the encapsulation of various bioactive compounds (de Conto et al., 2013; Huang, Sun, Xiao, & Yang, 2012; Jun-xia, Hai-yan, & Jian, 2011; Mendanha et al., 2009; Nori et al., 2011); 2) inulin acts as a fat substitute and improves the texture of meat products (Shoaib et al., 2016); 3) the use of temperatures that do not exceed 40 °C in the complex coacervation process can guarantee the oxidative stability of the encapsulated lipids and the microparticles have uniform size distribution (Comunian & Favaro-Trindade, 2016; Rios-Mera, Saldaña, Ramírez, et al., 2019). Therefore, it is expected that the microparticles containing fish oil improve the nutritional quality but do not affect negatively the technological characteristics of the burgers. However, the NaCl reduction could have an implication on the amount of fatty acids in the burger, since one of the factors that affects the stability of complex coacervates is the ionic strength (Eghbal & Choudhary, 2018). In this context, a higher salt concentration could destabilize the interaction between SPI and inulin, releasing fish oil from the interior of the microparticles. Additionally, NaCl added in meat products favors lipid oxidation, which directly impacts the sensory properties and shelf life of meat products (Mariutti & Bragagnolo, 2017).

There are few reports on the application of complex coacervates in foods, and to the knowledge of the authors, their application in meat products has been reported only by Bernardi et al. (2013) for encapsulation of propolis as an antioxidant agent in salami. Our hypothesis is that microencapsulation by complex coacervation protects PUFAs during the burger processing and cooking as microparticles have shown to be resistant to stress conditions of pH and temperature similar to those of the processing and heat treatment of meat products (Rios-Mera, Saldaña, Ramírez, et al., 2019). However, it is necessary to know whether a higher PUFAs retention will favor a higher incidence of volatile oxidation compounds, and consequently, have impact on the sensory profile and overall liking. Therefore, the objective of this work was to determine the effect of NaCl reduction and the incorporation of fish oil (uncapsulated or encapsulated) on several quality traits of the burgers.

7.2. Materials and methods

7.2.1. Raw materials and ingredients

Lean beef (*Quadriceps femoris*), pork back fat and white pepper powder were obtained from a supermarket in Piracicaba, SP, Brazil. NaCl (salt), monosodium glutamate, onion and garlic powder, sodium erythorbate and soy protein isolate (92% protein content) were supplied by Ibrac (Rio Claro, SP, Brazil). Micronized salt (MS) was obtained from the commercial regular salt (RS) by manually sieving in a 60-mesh (250 µm) stainless steel sieve. The mean sizes of MS and RS are 168.86 µm and 477.57 µm, respectively (Rios-Mera, Saldaña, Cruzado-Bravo, et al., 2019). Inulin (Orafti® GR, degree of polymerization ≥ 10; 92.9% inulin content) was supplied by SweetMix (Sorocaba, SP, Brazil). Fish oil was supplied by Holland & Barrett (Nuneaton, UK). Microbial transglutaminase (BakemyL® TG Conc, Breatec, Vlijmen, Netherlands) was used as a cross-linking agent for the microparticles production.

7.2.2. Microparticles production by complex coacervation

The production of the microparticles was carried out according to the conditions optimized by Rios-Mera, Saldaña, Ramírez, et al. (2019): inulin:SPI ratio = 0.4; 10% fish oil; pH 4.0; 10 U of transglutaminase per gram of protein. Subsequently, the microparticles were freeze-dried at –96 °C under vacuum conditions (Liotop L101 freeze drier), and stored at –18 °C for future use. The microparticles were produced in 3 batches in a single day, and freeze-dried for the next 3 days, but keeping the microparticles in dark conditions.

7.2.3. Burger manufacture

Six burger treatments (Table 7.1) were manufactured in two batches or independent processes (n = 20 burgers / treatment / batch), performed at different days, according to Rios-Mera et al. (2020). First, the beef and pork back fat were ground separately using a 0.8 cm plate. Then, the beef was manually mixed with salt for 2 min. For MS treatments, half of the MS was mixed with pork back fat (Rios-Mera, Saldaña, Cruzado-Bravo, et al., 2019) and also homogenized for 2 min. Subsequently, the other ingredients were added and all the components were manually mixed for 3 min. The amount of fish oil added was low, but sufficient to classify the food product as “source” of EPA/DHA (40–80 mg) (MERCOSUR, 2012). According to the supplier's specifications (Holland & Barrett), each 5 mL of fish oil contains 557 mg EPA and 472 mg DHA, thus we used 0.32 mL (0.25 g) of fish oil, which corresponds to 65.86 mg of the sum of EPA and DHA. The percentage of pork fat (20%) was reduced as the unencapsulated oil or microparticles were incorporated into the burger. The burgers were cooked in an electric hot plate at 150 °C, until the internal temperature of 75 °C was achieved. Thereafter, samples were cooled to 25 °C for subsequent analysis.

Table 7.1. Formulation (%) of burger treatments.

Component	Treatments					
	T1	T2	T3	T4	T5	T6
Beef	70	70	70	70	70	70
Pork back fat	20	20	19.75	19.75	17.5	17.5
Cold water	7.5	7.5	7.5	7.5	7.5	7.5
Regular salt	1.5	-	1.5	-	1.5	-
Micronized salt	-	1.0	-	1.0	-	1.0
Monosodic glutamate	0.28	0.28	0.28	0.28	0.28	0.28
White pepper powder	0.15	0.15	0.15	0.15	0.15	0.15
Garlic powder	0.28	0.28	0.28	0.28	0.28	0.28
Onion powder	0.28	0.28	0.28	0.28	0.28	0.28
Sodium erythorbate	0.01	0.01	0.01	0.01	0.01	0.01
Fish oil	-	-	0.25	0.25	-	-
Microparticles	-	-	-	-	2.5	2.5

7.2.4. Burger characterization

7.2.4.1. pH determination

The pH of raw and cooked burgers was measured using a potentiometer coupled to a glass puncture electrode, previously calibrated at pH 4.0, 7.0 and 10.0. The measurements were repeated five times.

7.2.4.2. Color parameters

A colorimeter (Konica Minolta, Chroma Meter, CR-400, Mahwah, NJ, USA) was used to assess the lightness (L*), green-red (a*), blue-yellow (b*), chroma (C*), and Hue (H*) on the surface of raw and cooked burgers. A measuring area of 8 mm, observation angle of 10° and illuminant D65 were used. The color analysis was performed five times.

7.2.4.3. Yield properties

Cooking loss, diameter reduction, moisture retention and fat retention were measured in five burgers using the following equations (Sánchez-Zapata et al., 2010; Selani et al., 2016):

$$\%\text{Cooking loss} = \frac{\text{Raw weight} - \text{Cooked weight}}{\text{Raw weight}} \times 100 \qquad (7.1)$$

$$\%\ \text{Diameter reduction} = \frac{\text{Raw diameter} - \text{Cooked diameter}}{\text{Raw diameter}} \times 100 \qquad (7.2)$$

$$\%\ \text{Moisture retention} = \frac{\text{Cooked weight x \%Moisture in cooked burger}}{\text{Raw weight x \%Moisture in raw burger}} \times 100 \qquad (7.3)$$

$$\%\ \text{Fat retention} = \frac{\text{Cooked weight x \%Fat in cooked burger}}{\text{Raw weight x \%Fat in raw burger}} \times 100 \qquad (7.4)$$

Moisture and fat (Soxhlet) of Eq. 7.3 and 7.4 were determined according to the methods issued by AOAC (2010).

7.2.4.4. Texture profile analysis

The texture profile analysis (TPA) was assessed using a Texture Analyzer TA-XT (Stable Micro Systems, Godalming, UK) according to Selani et al. (2016). Cylindrical samples (2.5 cm diameter, 1 cm height) were extracted from the burgers to be compressed using a probe of 3.5 cm diameter (P/35, Stable Micro Systems, Godalming, UK) coupled to the texturometer. The samples were compressed up to 75% of their original height at a constant speed of 20 cm/min (pre-test speed and post-test speed: 40 cm/min). The following parameters were determined: 1) hardness (Newton, N): defined as the force necessary to reach a deformation (maximum force during the first cycle of compression); 2) springiness: the distance that the food recovers during the time between the end of the first compression and the beginning of the second compression; 3) cohesiveness: ratio between the area under the second curve to that of the first curve; and 5) chewiness (N): the product of hardness x cohesiveness x springiness (Bourne, 1978; Saldaña et al., 2015). The measurements were conducted five times.

7.2.4.5. Profile of fatty acids

The lipid samples were extracted by the Bligh and Dyer (1959) method and were methylated according to Hartman and Lago (1973), with adaptations based on the AOCS (2003) Ce 1b-89 method. Comprehensive fatty acid analyses typically used for ruminant samples were not undertaken due to the high proportion of pork fat added to the burgers. Thus, the profile of fatty acids was determined in a gas chromatograph (Shimadzu, Series 2010 Plus) equipped with a Restek-Wax column (30 m x 0.32 mm i.d. x 0.25 µm film thickness) coupled to a flame ionization detector (FID). The temperature program started at 60 °C and reached 130 °C at a rate of 20 °C/min, remaining at that temperature for 7 min, then the program reached 240 °C at a rate of 30 °C/min, remaining at this condition for 18 min. The injector and detector temperatures were set at 250 °C. Hydrogen with a linear velocity of 21.0 cm/s was used as carrier gas. The injection volume was 1.0 µL in a split mode of 1/10. The identification of fatty acids was made by comparison of their retention times with those of a mix of fatty acid methyl ester standards (FAME C8-C22, Sigma-Aldrich), which was mixed with a methylated fish oil enriched with 250 mg DHA + 50 mg EPA to identify these fatty acids in the samples. The analysis was done within 15 days after the burgers processing, and the measurements were conducted using two burger samples / treatment / batch.

Indexes to measure the nutritional quality of the fatty acids were estimated: PUFA/SFA, n-6/n-3 and the atherogenic (AI) (Eq. 7.5) and thrombogenic indexes (TI) (Eq. 7.6) (Selani, Shirado, Margiotta, Rasera, et al., 2016; Ulbricht and Southgate, 1991):

$$\% \text{ AI} = \frac{\text{C12:0} + 4 \times \text{C14:0} + \text{C16:0}}{\text{MUFA} + \text{n-3 PUFA} + \text{n-6 PUFA}} \times 100 \tag{7.5}$$

$$\% \ TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{0.5 \times MUFA + 0.5 \times n\text{-}3\ PUFA + 3 \times n\text{-}6\ PUFA + \frac{n\text{-}3\ PUFA}{n\text{-}6\ PUFA}} \times 100 \qquad (7.6)$$

7.2.4.6. Volatile organic compounds

The profile of volatile organic compounds (VOCs) was determined according to Spada et al. (2017), with some adaptations. Raw and cooked burgers were minced and the sample (5 g) was placed in 20 mL SPME vials and then added with 1,2-dichlorobenzene in methanol (130.6 µL/mL) as an internal standard. Then, the vials were equilibrated at a temperature of 45 °C for 15 min. Thereafter, the fiber coated with carboxen/polydimethylsiloxane (CAR/PDMS) was exposed (1 cm) to the headspace above the samples for 30 min under magnetic agitation at 60 rpm.

The analysis was caried out in a GC-MS (Shimadzu QP2010 GC-MS) using a RTX5MS column (30 m, 0.25 mm i.d., 0.25 µm film thickness). VOCs were desorbed for 3 min at 200 °C in a splitless mode. The oven temperature was maintained at 40 °C for 8 min, then reached 200 °C at a rate of 4 °C/min, and finally at 280 °C at a rate of 10 °C/min, totaling 56 min. The mass spectrum was obtained by impact of electrons at 70 eV, in the range of 40 to 500 *m/z* in scan mode. Helium was used as carrier gas at a constant flow of 1 mL/min. Alkanes (C_7–C_{30}) were analyzed under the same conditions to obtain the linear retention indices (LRI) of the samples. The identification of VOCs was carried out by comparing their LRIs and/or mass spectrum with data of the computer library (Wiley 8 and FFNSC 1.3). The relative concentration of each compound was obtained by the ratio of the compound area to the standard area (1,2 dichlorobenzene). The analysis was done within 15 days after the burgers processing, and the measurements were conducted using two burger samples / treatment / batch.

7.2.4.7. Consumer sensory characterization

Before sensory analysis, the burger samples were microbiologically analyzed as described by Rios-Mera, Saldaña, Cruzado-Bravo, et al. (2019) to guarantee their microbiological quality (APHA, 1992).

7.2.4.7.1. Consumers

Seventy-two regular consumers of burgers (51% men and 49% women, with ages between 18 and 66) were recruited at the Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ) / University of São Paulo (USP), based on self-reported consumption of burgers (43% of consumers declared to consume burgers every 15 days, 25% 1–3 times a week, 19% once a month, 8% rarely, 3% 4–6 times a week, and 1% daily), interest and availability to participate in the study. Before conducting the sensory analysis, consumers read and signed an informed consent approved by the Ethics Committee of Human Research of ESALQ/USP (protocol No. 2.823.957).

7.2.4.7.2. Procedure

The sensory analysis was conducted under artificial white light in individual booths in a single session of about 15 min. Samples (~10 g) were presented monadically to the participants on plates coded with three random numbers following a Williams Latin Square design (Wakeling & MacFie, 1995). The samples were served at 45 °C, which were kept at that temperature in an electric stove for a maximum time of 15 min. The participants rated their overall liking using a 9-point category hedonic scale, ranging from “dislike extremely” to “like extremely” (Peryam & Pilgrim, 1957). Then, participants performed the Check-All-That-Apply (CATA) questions.

For the CATA questions, consumers were asked to check the sensory terms that they considered appropriate to describe the samples. The sensory terms were those reported by Rios-Mera et al. (2020). In addition, after discussing the attributes with the research team, the following terms related to the commonly sensory defects imparted by fish oil were added: *aftertaste*, *bad smell*, *bad taste*, and *rancid*. The term *acid* was also added because of the microparticle’s pH (4.0), totalling 20 sensory terms. The terms were presented to consumers monadically following a Williams Latin Square design (Wakeling & MacFie, 1995). Water and crackers were offered to consumers for palate cleansing between samples. Data were collected through the Compusense Cloud software (Compusense Inc., Guelph, Canada) using tablets (Samsung Galaxy Tab E T560) with android system.

7.2.5. Microparticles characterization

Color parameters, fat (Soxhlet), fatty acids profile and VOCs were determined in freeze-dried microparticles according to the procedures described above.

Additionally, the profile of fatty acids was determined in the unencapsulated fish oil. The bulk density of microparticles was determined according to Lavoie, Cartilier, and Thibert (2002). A graduated cylinder (capacity of 100 mL) was weighed and loaded with 40 mL of microparticles, and the cylinder was reweighed. The graduated cylinder was closed with Parafilm, carefully inverted, and returned to the starting position for once. The microparticles were leveled without being compacted. The bulk density was calculated by dividing the mass of the microparticles by the total volume.

7.2.6. Data analysis

Results of pH, color parameters, yield properties, TPA and profile of fatty acids were submitted to a mixed analysis of variance (ANOVA), considering treatments (fixed) and batch (random) as sources of variation. Pairwise comparisons were performed according to the Tukey's test at 5% of significance level.

For the CATA questions, the frequency that the sensory terms were mentioned was calculated by counting the number of consumers who used the terms to describe each sample (Meyners, Castura, & Carr, 2013). Thereafter, a Correspondence Analysis (CA) was performed using the frequency rate of the terms considering the Chi-square distances (Vidal, Tárrega, Antúnez, Ares, & Jaeger, 2015). A Penalty Analysis (PA) was performed using the consumers responses to determine the mean impact of the presence of sensory terms on the overall liking of burgers (Saldaña et al., 2018).

The mean values of fatty acids and VOCs were correlated using Multiple Factor Analysis (MFA) considering the effect of treatments, according to Saldaña et al. (2015) and Saldaña et al. (2019).

XLSTAT 2015 (Addinsoft, New York, EEUU) and R (R Development Core Team, 2017) were the software used for data analysis.

7.3. Results and discussion

7.3.1. Physicochemical characteristics, yield and texture properties

Color parameters, pH, yield and texture properties of the burgers are shown in Table 7.2. The pH and color parameters were not affected by the NaCl reduction, confirming our previous study (Rios-Mera, Saldaña, Cruzado-Bravo, et al., 2019). However, the incorporation of the microparticles affected the pH and the hue angle (H*) of raw burgers. Since the microparticles were produced at pH 4.0, a decrease in pH of the product was expected, but not to the extent of affecting the quality of the burger, since the normal pH range of meat is between 5.5 to 5.7 (Matarneh et al., 2017). The microparticles were characterized by the following color parameters: L* = 84.76; a* = –2.89; b* = 22.27; C* = 22.46; H* = 97.38. Thus, because of their high H* value, burgers with microencapsulated fish oil also had higher H* values (i.e., higher yellowness).

Table 7.2. Mean values of physicochemical characteristics, yield and texture properties of raw and/or cooked burger treatments.

Parameter	Treatments[1]						SEM	*P*-value
	T1	T2	T3	T4	T5	T6		
Physicochemical characteristics								
Raw burger								
pH	5.75^{a}	5.74^{a}	5.73^{ab}	5.74^{a}	5.58^{bc}	5.56^{c}	0.026	0.011
Lightness (L*)	44.23	45.87	45.76	44.70	42.70	42.65	0.644	0.413
Redness (a*)	18.56	19.34	18.54	17.71	16.52	16.95	0.420	0.129
Yellowness (b*)	11.52	12.15	11.83	11.76	11.34	12.28	0.234	0.596
Chroma (C*)	21.89	22.85	22.00	21.27	20.05	20.95	0.453	0.278
Hue (H*)	31.75^{b}	32.18^{b}	32.47^{b}	33.46^{ab}	34.49^{ab}	36.11^{a}	0.480	0.022
Cooked burger								
pH	5.93	6.04	5.93	6.04	5.85	5.89	0.024	0.096
Lightness (L*)	44.06	44.67	45.53	47.16	48.05	48.07	0.534	0.070
Redness (a*)	6.50	6.87	6.64	6.53	6.61	7.09	0.076	0.096
Yellowness (b*)	12.69	13.91	13.34	14.20	13.22	12.99	0.208	0.300
Chroma (C*)	14.28	15.53	14.92	15.64	14.80	14.82	0.193	0.306
Hue (H*)	62.59	63.53	63.30	65.15	63.23	61.14	0.427	0.230
Yield properties (%)								
Cooking loss	32.22	34.56	31.16	33.49	32.31	32.58	0.485	0.630
Diameter reduction	20.05^{b}	21.83^{ab}	21.21^{ab}	22.99^{a}	20.20^{b}	19.89^{b}	0.350	0.009

Moisture retention	56.40	55.60	56.60	55.42	56.53	56.47	0.579	0.998
Fat retention	92.08^{a}	86.03^{ab}	87.28^{ab}	81.85^{ab}	84.34^{ab}	75.21^{b}	1.791	0.047
Texture properties								
Hardness (N)	93.30^{c}	80.97^{de}	84.25^{cd}	72.83^{e}	127.36^{a}	105.03^{b}	5.457	0.001
Springiness	0.80	0.81	0.76	0.81	0.86	0.85	0.012	0.303
Cohesiveness	0.53	0.53	0.47	0.49	0.54	0.57	0.011	0.097
Chewiness (N)	39.47^{bc}	34.57^{c}	30.42^{c}	29.04^{c}	58.95^{a}	50.59^{ab}	3.329	0.001

[1]T1: 1.5% regular salt (RS1.5); T2: 1.0% micronized salt (MS1.0); T3: RS1.5 + uncapsulated fish oil; T4: MS1.0 + uncapsulated fish oil; T5: RS1.5 + encapsulated fish oil; T6: MS1.0 + encapsulated fish oil.
Different letters between mean values at the same row means statistical differences ($P < 0.05$).

On the other hand, no differences were observed in the physicochemical parameters of cooked burgers. Similar results were reported by Selani, Shirado, Margiotta, Saldaña, et al. (2016), who suggested that physicochemical alterations caused by cooking may mask the changes imparted by freeze-dried fruit byproduct and canola oil on the color of beef burger. In the present study, the pH of cooked burgers added with microparticles reached the less pH values compared to the other cooked treatments, but the changes were not significant ($P > 0.05$). However, it is necessary to know whether this slight change had implications in some quality parameters, such as the sensory profile.

Regarding the yield properties, there were no differences in cooking loss and moisture retention between treatments. The presence of SPI as a major component of the microparticles could have retained water in a similar way to meat proteins, since soy proteins retain water in meat products (Petracci et al., 2013) through the formation of hydrogen bounds with water (Herrero, Carmona, Cofrades, & Jiménez-Colmenero, 2008). In addition, the presence of inulin as a fiber may have a synergistic effect with proteins entrapping water. However, slight changes were observed for diameter reduction and fat retention as a consequence of the microparticles incorporation. The diameter reduction values of T5 and T6 were quite similar to that of the 1.5% NaCl treatment (T1), which is positive, but the lowest value of fat retention was observed for T6, being statistically different from T1. The combination of the increase in soy protein content and the NaCl reduction strategy applied in this study is a possible explanation for this result. Thus, it has been reported that elevating the soy protein content in emulsified beef batters increased cooking loss expressed as fluid loss and fat loss, as a consequence of gel formation before or during cooking of high-gelling soy protein (Youssef & Barbut, 2011). However, the reason for affecting the fat retention and not the moisture retention in the product may be related to the pH of the burgers. In the production of the microparticles, the pH of the SPI solution is adjusted to pH 8.0, which facilitates the emulsion with the fish oil due to an increase in the solubilization of the protein (Molina-Ortiz et al., 2009, Nori et al., 2011, Rios-Mera, Saldaña, Ramírez, et al., 2019). The solubilization of SPI is also slightly improved with heat treatment, which according to Jaramillo, Roberts and Coupland (2011) reaches maximum solubilization at pH 6–7. Thus, it can be deduced that the solubilization of SPI is improved at pH 6–8, but the pH values of the burgers with microparticles did not reach the pH 6. Therefore, a lower interaction between SPI and lipids (pork fat) is expected. In addition, NaCl solubilizes myofibrillar proteins with positive implications on water and fat retention (Desmond, 2006). As micronized salt was partially mixed with pork fat, its contact with myofibrillar proteins was reduced and therefore less myofibrillar solubilization can be obtained, thus these two factors (pH and salt reduction) can affect fat retention.

Hardness was affected by NaCl reduction, since samples manufactured with higher NaCl content were harder than NaCl-reduced burgers, corroborating the results obtained by Rios-Mera et al. (2020). Once again, the lower interaction of NaCl with meat proteins due to mixing of micronized salt with pork fat, or the NaCl reduction itself could reduce the gel formation of myofibrillar proteins (Terrel, 1983) impacting on the final texture of the burgers (Rios-Mera et al., 2020). Moreover, the microparticles significantly increased the hardness of the burgers, which is similar to the results reported by Herrero et al. (2008), who observed an increase in hardness when 3% and 6% of SPI were added to meat batter. The chewiness also increased by addition of microparticles, because chewiness is directly proportional to hardness.

7.3.2. Profile of fatty acids

The profile of fatty acids of raw and cooked burgers is shown in Table 7.3. In this table is also presented the mean fat content of the burger samples, in which the lowest value was observed in T6 after cooking, similar to the lowest value of % fat retention observed in this treatment (Table 7.2). In both raw and cooked treatments, monounsaturated fatty acids were the main compounds followed by saturated and polyunsaturated counterparts. Our data are in agreement with those reported for burgers (Aquilani et al., 2018; Selani, Shirado, Margiotta, Rasera, et al., 2016). It is not possible to observe a clear relationship between the NaCl reduction and the lipid profile of raw and cooked burgers. However, the profile of fatty acids appears to be driven by the mode of incorporation of the fish oil. In this aspect, the addition of microparticles increased the level of saturated fatty acids in raw burgers, mainly in T5, in which a significant increase in myristic, palmitic, stearic fatty acids and in ΣSFA was observed. Regarding the cooked treatments, T4 had a higher incidence of saturated fatty acids, being significantly higher for ΣSFA and palmitic acid. Monounsaturated fatty acids were higher in raw samples of T5 and T6, which tended to obtain significantly higher contents of palmitoleic, oleic and eicosanoic acids, while in cooked burgers only T2 had a significantly lower ΣMUFA than the other treatments. Despite the incorporation of fish oil, the treatments with microparticles showed significantly lower contents of ΣPUFA for raw samples, and for cooked samples, DHA was not detected in burgers with unencapsulated fish oil.

Table 7.3. Mean values (% of lipids extracted) of profile of fatty acids of raw and cooked burger treatments.

Fatty acids[2]	Treatments[1]						SEM	*P*-value
	T1	T2	T3	T4	T5	T6		
Raw burger								
Mean % Fat	17.68	18.06	18.15	18.10	17.18	18.17		
Myristic C14:0	1.592[d]	1.634[cd]	1.667[bc]	1.666[bc]	1.811[a]	1.695[b]	0.021	0.000
Palmitic C16:0	23.292[d]	23.645[b]	23.242[d]	23.438[c]	23.981[a]	23.487[c]	0.074	0.000
Palmitoleic C16:1	2.550[d]	2.568[cd]	2.619[bcd]	2.635[bc]	2.733[a]	2.686[ab]	0.019	0.001
Stearic C18:0	10.564[e]	10.947[c]	10.753[d]	10.701[d]	11.326[a]	11.018[b]	0.075	0.000
Oleic C18:1	41.576[b]	41.223[c]	41.105[c]	41.121[c]	41.560[b]	41.862[a]	0.084	0.000
Linoleic C18:2	18.632[a]	18.230[b]	18.347[b]	18.276[b]	16.633[d]	17.138[c]	0.219	0.000
Linolenic C18:3	1.043[a]	1.017[a]	1.031[a]	1.019[a]	0.897[b]	0.954[b]	0.016	0.001
Arachidic C20:0	0.145	0.148	0.149	0.146	0.142	0.151	0.001	0.421
Eicosanoic C20:1	0.606[b]	0.589[b]	0.664[ab]	0.660[ab]	0.644[ab]	0.700[a]	0.012	0.032
Eicosapentaenoic C20:5	ND	ND	0.215[a]	0.179[b]	0.169[b]	0.178[b]	0.027	0.000
Docosahexaenoic C22:6	ND	ND	0.209[a]	0.160[b]	0.103[d]	0.130[c]	0.024	0.000
ΣSFA	35.593[e]	36.373[b]	35.810[d]	35.951[c]	37.260[a]	36.352[b]	0.163	0.000
ΣMUFA	44.731[c]	44.379[d]	44.388[d]	44.416[d]	44.938[b]	45.249[a]	0.099	0.000
ΣPUFA	19.676[ab]	19.248[c]	19.802[a]	19.633[b]	17.802[e]	18.270[d]	0.230	0.000
PUFA/SFA	0.553[a]	0.529[c]	0.553[a]	0.546[b]	0.478[e]	0.503[d]	0.008	0.000
n-6/n-3	17.862[a]	17.925[a]	12.608[c]	13.463[bc]	14.233[b]	13.587[bc]	0.646	0.000

AI	0.460[e]	0.474[b]	0.466[d]	0.470[c]	0.498[a]	0.476[b]	0.004	0.000
TI	0.450[f]	0.468[c]	0.457[e]	0.460[d]	0.508[a]	0.484[b]	0.006	0.000
Cooked burger								
Mean % Fat	23.99	23.69	23.01	22.28	21.40	20.26		
Myristic C14:0	1.580[e]	1.597[e]	1.627[d]	1.722[b]	1.762[a]	1.699[c]	0.020	0.000
Palmitic C16:0	23.703[bc]	23.888[b]	23.870[b]	24.275[a]	23.592[c]	23.530[c]	0.075	0.000
Palmitoleic C16:1	2.584[e]	2.575[e]	2.627[d]	2.708[b]	2.751[a]	2.691[c]	0.020	0.000
Stearic C18:0	10.934[c]	11.272[a]	11.225[a]	11.291[a]	10.771[d]	11.038[b]	0.058	0.000
Oleic C18:1	42.226[a]	41.417[c]	42.037[ab]	42.076[ab]	41.847[b]	41.770[b]	0.080	0.002
Linoleic C18:2	17.322[ab]	17.554[a]	16.812[c]	16.197[d]	17.236[b]	17.170[b]	0.133	0.000
Linolenic C18:3	0.871[bc]	0.953[a]	0.827[cd]	0.800[d]	0.914[ab]	0.941[a]	0.017	0.001
Arachidic C20:0	0.150	0.154	0.155	0.149	0.146	0.151	0.001	0.214
Eicosanoic C20:1	0.629[ab]	0.591[b]	0.671[ab]	0.644[ab]	0.696[a]	0.701[a]	0.013	0.041
Eicosapentaenoic C20:5	ND	ND	0.148[b]	0.138[c]	0.179[a]	0.178[a]	0.023	0.000
Docosahexaenoic C22:6	ND	ND	ND	ND	0.105[b]	0.130[a]	0.017	0.000
ΣSFA	36.367[c]	36.910[b]	36.877[b]	37.438[a]	36.271[c]	36.418[c]	0.123	0.000
ΣMUFA	45.439[a]	44.583[b]	45.335[a]	45.428[a]	45.294[a]	45.163[a]	0.090	0.001
ΣPUFA	18.193[b]	18.507[a]	17.787[c]	17.134[d]	18.434[ab]	18.419[ab]	0.147	0.000
PUFA/SFA	0.500[a]	0.501[a]	0.482[b]	0.458[c]	0.508[a]	0.506[a]	0.005	0.000
n-6/n-3	19.883[a]	18.419[b]	17.237[c]	17.276[c]	14.383[d]	13.743[d]	0.650	0.000
AI	0.472[c]	0.480[b]	0.481[b]	0.498[a]	0.481[b]	0.477[bc]	0.002	0.000

TI	0.482^{c}	0.487^{c}	0.499^{b}	0.519^{a}	0.482^{c}	0.485^{c}	0.004	0.000

[1]T1: 1.5% regular salt (RS1.5); T2: 1.0% micronized salt (MS1.0); T3: RS1.5 + uncapsulated fish oil; T4: MS1.0 + uncapsulated fish oil; T5: RS1.5 + encapsulated fish oil; T6: MS1.0 + encapsulated fish oil.

[2]SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; AI: Atherogenic index; TI: thrombogenic index.

Different letters between mean values at the same row means statistical differences ($P < 0.05$).

The results reported herein are not in line with those reported by Aquilani et al. (2018), who did not observe significant differences in the sum of saturated, monounsaturated and polyunsaturated fatty acids of raw and cooked Cinta Senese burgers added with fish oil (unencapsulated and encapsulated). The variations in the contents of saturated and monounsaturated fatty acids between raw and cooked burgers do not seem to be related to the NaCl reduction nor with the incorporation of fish oil. Selani, Shirado, Margiotta, Rasera, et al. (2016) suggested that different physicochemical mechanisms related to the structural changes of fatty acids occur during cooking, such as oxidation, hydrolysis, polymerization of triacylglycerides, among others. Thus, these changes deserve to be studied in terms of NaCl reduction and incorporation of long-chain PUFAs in meat products.

The raw burgers manufactured with microparticles were negatively affected in the saturated and polyunsaturated fatty acids, which is directly related to the lipid profile of the microparticles. In fact, the microparticles contained 9.57% lipids, which represents a high encapsulation efficiency, as previously reported by Rios-Mera, Saldaña, Ramírez, et al. (2019). As shown in Table 7.4, the profile of fatty acids of microparticles consisted mainly of saturated fatty acids (50.97%), followed by the monounsaturated (43.76%) and polyunsaturated (5.27%) fractions. Linoleic acid was the main polyunsaturated fatty acid detected (68.97%), followed by DHA (18.17%), EPA (10.76%) and linolenic acid (2.11%). In contrast, the lipid profile of unencapsulated fish oil was mainly composed of polyunsaturated fatty acids (35.78%), followed by monounsaturated (32.23%) and saturated (31.99%) fractions. From the PUFAs fraction in unencapsulated oil, linoleic acid represents 5.10%, linolenic acid 2.81%, EPA 52.86%, and DHA 39.24%. The low PUFAs fraction in microparticles also decreased the ratio PUFA/SFA, and increased the n-6/n-3 ratio and AI and TI indexes. Therefore, the increase in SFA of raw burgers added with microparticles may be associated with the higher proportion of saturated fatty acids in the microparticles. As PUFAs were the minor components of the microparticles, raw burgers with unencapsulated oil showed higher values of long-chain PUFAs, such as DHA. These results were not expected, since the addition of the microparticles should have improved the lipid profile of the product.

Table 7.4. Profile of fatty acids (%) of unencapsulated and encapsulated fish oils.

Fatty acids[1]	Unencapsulated fish oil	Encapsulated fish oil
Myristic C14:0	9.398	12.541
Palmitic C16:0	19.055	31.732
Palmitoleic C16:1	12.326	14.670
Stearic C18:0	3.384	6.445
Oleic C18:1	12.027	19.782
Linoleic C18:2	1.826	3.637
Linolenic C18:3	1.004	0.111
Arachidic C20:0	0.156	0.248
Eicosanoic C20:1	7.876	9.308
Eicosapentaenoic C20:5	18.911	0.567
Docosahexaenoic C22:6	14.038	0.958
ΣSFA	31.992	50.966
ΣMUFA	32.229	43.760
ΣPUFA	35.779	5.274
PUFA/SFA	1.118	0.103
n-6/n-3	0.054	2.222
AI	0.833	1.670
TI	0.557	1.489

[1]SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; AI: Atherogenic index; TI: thrombogenic index.

A possible explanation for the higher proportion of saturated fatty acids in the microparticles may be linked to the complex coacervation process. Despite using temperatures that guarantee the oxidative stability of the oil, complex coacervation requires a long production time, exposing the compound of interest to oxidizing factors, such as light and oxygen. In fact, a recently published meta-analysis showed that the most popular methods for encapsulation of fish oil (coacervation and spray drying) protect less than the spray granulation method, but the latter is little explored (Łozińska, Głowacz-Różyńska, Artichowicz, Lu, & Jungnickel, 2020). Nevertheless, if the complex coacervation process interferes with the oxidative stability of the fish oil, the immediate response will be the increase in oxidation compounds, with negative impacts on the sensory profile and overall liking of the burgers.

The absence of DHA in cooked burgers with unencapsulated fish oil suggests that microencapsulation was effective in retaining this fatty acid during cooking, confirming the oil retention under stress of temperature tested in our previous study (Rios-Mera, Saldaña, Ramírez, et al., 2019). EPA also suffered alteration (decrease in %) in burgers with unencapsulated fish oil after cooking, but not to the degree of being eliminated from the fatty acid profile. The difference in the amount of double bonds in both fatty acids (EPA and DHA) can explain the differences observed, since the higher the content of double bonds, the greater the instability of the fatty acid (DHA) during cooking.

Regarding salt reduction, T6 obtained a higher DHA content than T5 ($P < 0.05$) in raw and cooked samples. Eghbal and Choudhary (2018) reviewed that the formation of complex coacervates is dependent on the ionic strength. Thus, NaCl has an adverse effect on the electrostatic interaction that occurs in the formation of complex coacervates, through the interaction between NaCl ions and macromolecules. It is also possible to observe that T6 had a higher affinity for long-chain fatty acids than short fatty acids compared to T5 (raw and cooked samples), which suggests that the release of fatty acids from microparticles due to ionic strength affected those with longer chains.

Raw and cooked burgers with unencapsulated fish oil and higher NaCl content obtained higher ($P < 0.05$) contents of EPA and DHA in comparison with their NaCl-reduced counterpart. This result is contrary to those reported by Souza and Bragagnolo (2014), who observed a decrease in EPA and DHA in shrimp because of the salting and drying process. With the pro-oxidant action of NaCl, a transition of fatty acids to the formation of oxidation compounds is expected (Mariutti & Bragagnolo, 2017), consequently the amount of fatty acid would be decreased. However, Kristensen and Purslow (2001) observed that NaCl prevents the release of iron (an oxidant in meat products, Mariutti & Bragagnolo, 2017) from the heme molecule in cooked minced pork. Following this observation, Kanner, Harel, and Jaffe (1991) suggested that in a muscle residue with low iron concentration, NaCl reduced the amount of iron ions in contact with fatty acids, thus decreasing the lipid peroxidation. Thus, when less iron is released from the heme complex, less losses of fatty acids content will occur because of a lower degree of oxidation. This phenomenon could have occurred in T3, especially in polyunsaturated fatty acids, in which significantly higher ΣPUFA values were observed compared to T4, but this statement needs to be reinforced with the level of oxidation in the product.

The indexes to measure the nutritional quality of fatty acids in the prevention of diseases, especially of cardiovascular origin are the PUFA/SFA (> 0.45) and n-6/n-3 (<4) ratios, and the atherogenic (AI) and thrombogenic indexes (TI) (<1) (Cifuni, Napolitano, Riviezzi, Braghieri, & Girolami, 2004; Pleadin et al., 2017; Selani, Shirado, Margiotta, Rasera, et al., 2016; Simopoulos, 2016). In raw samples, the burgers with microparticles, mainly T5, showed the lowest PUFA/SFA values, due to the decrease in PUFAs as previously discussed. This result also affected AI and TI in T5, with significantly higher values compared to the other treatments. The n-6/n-3 ratio was higher in burgers without the addition of fish oil because of the absence of EPA and DHA in these formulations. In cooked burgers, T4 had a similar behavior to raw T5, where a significant decrease in PUFA/SFA and a significant increase in AI and TI was observed. Similar to raw burgers, the samples without fish oil obtained higher contents of n-6/n-3 ($P < 0.05$), in which the treatments added with microparticles presented the lowest n-6/n-3 ratio ($P < 0.05$).

Beyond the differences between treatments, the PUFA/SFA and AI and TI indexes were at the recommended levels for the prevention of cardiovascular diseases, but the n-6/n-3 ratio was higher than 4 in all treatments. This result was expected because the aim of this study was not to greatly reduce the amount of pork fat in the product, in order to avoid as much as possible changes in the technological and sensory characteristics of the burger.

7.3.3. Volatile organic compounds and MFA

The VOCs of raw and cooked burgers and microparticles are shown in Table 7.5. Five families of compounds were found in beef burgers: alcohols, aldehydes, ketones, sulfides, and terpenes.

Table 7.5. Mean values of relative concentration of volatile organic compounds of microparticles and raw and cooked burgers[1].

Compound	RT[2]	LRI[3]	Microparticles	Raw burgers						Cooked burgers					
				T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6
Alcohols															
1-Pentanol	6.37	764	ND	0.103	0.119	0.079	0.120	0.251	0.182	0.123	0.074	0.093	0.267	0.086	0.247
1-Hexanol	11.93	874	ND	0.014	0.020	0.007	0.025	0.028	0.016	ND	ND	ND	ND	0.008	0.025
1-Octen-3-ol	17.54	982	ND	0.042	0.054	0.045	0.082	0.098	0.107	0.051	0.014	0.050	0.091	0.036	0.082
1-Octanol	21.65	1073	ND	0.009	0.018	0.013	0.033	0.038	0.057	0.006	0.003	0.012	0.023	0.009	0.032
Total				0.168	0.211	0.144	0.260	0.415	0.362	0.180	0.091	0.155	0.381	0.139	0.386
Aldehydes															
Hexanal	7.81	802	ND	1.252	0.591	1.018	1.831	2.771	2.614	4.193	1.428	2.619	7.386	2.601	7.298
(E)-2-hexenal	11.07	859	ND	0.154	0.153	0.123	0.104	0.191	0.113	0.255	0.231	0.163	0.292	0.145	0.246
Heptanal	13.53	903	ND	0.045	0.036	0.062	0.128	0.156	0.181	0.191	0.056	0.159	0.403	0.159	0.492
Benzaldehyde	16.44	960	ND	ND	ND	ND	ND	ND	ND	0.027	0.018	0.026	0.019	0.028	0.057
Octanal	18.59	1003	ND	0.094	0.092	0.060	0.089	0.143	0.124	0.263	0.172	0.129	0.257	0.109	0.319
Nonanal	23.00	1104	ND	0.085	0.074	0.074	0.132	0.183	0.184	0.159	0.077	0.131	0.222	0.096	0.304
Decanal	26.97	1206	ND	ND	ND	ND	ND	0.002	0.005	ND	ND	0.002	0.002	0.002	0.033
Total				1.630	0.946	1.337	2.284	3.446	3.221	5.088	1.982	3.229	8.581	3.140	8.749
Ketones															
2,3-Pentanedione	4.15	703	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.531	1.287

2,3-Octadione	17.77	987	ND	0.052	0.037	0.023	0.073	0.065	0.123	0.044	0.009	0.041	0.095	0.030	0.107
Total				0.052	0.037	0.023	0.073	0.065	0.123	0.044	0.009	0.041	0.095	0.561	1.394
Sulfides															
Methional	14.16	915	ND	0.094	0.071	0.077	0.074	0.128	0.077	0.112	0.104	0.059	0.147	0.074	0.106
Diallyl disulfide	21.89	1078	ND	0.163	0.179	0.209	0.181	0.470	0.191	0.279	0.222	0.233	0.327	0.272	0.300
Total				0.257	0.250	0.286	0.255	0.598	0.268	0.391	0.326	0.292	0.474	0.346	0.406
Terpenes															
Hydroperoxide, 1-mehtylhexyl	2.73	-	0.193	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
α-Thujene	14.83	928	ND	0.295	0.329	0.244	0.253	0.384	0.386	0.384	0.312	0.193	0.338	0.190	0.226
α-Pinene	15.11	934	ND	0.146	0.130	0.090	0.077	0.108	ND	0.286	0.207	0.088	0.195	0.078	0.097
Sabinene	17.21	976	ND	0.235	0.192	0.178	0.328	0.244	0.089	0.522	0.400	0.223	0.502	0.182	0.224
Myrcene	18.06	992	ND	0.078	0.078	0.049	0.053	0.104	0.066	0.187	0.168	0.093	0.141	0.095	0.133
δ-3-Carene	18.85	1009	ND	0.416	0.381	ND	ND	ND	ND	0.944	0.790	ND	ND	ND	ND
α-Terpinene	19.16	1016	ND	0.050	0.056	0.045	0.037	0.062	0.021	0.123	0.114	0.065	0.098	0.052	0.065
p-Cymene	19.52	1025	ND	0.185	0.187	0.065	0.057	0.098	0.039	0.431	0.359	0.097	0.154	0.080	0.115
Limonene	19.72	1029	ND	0.636	0.614	0.558	0.510	0.840	0.360	1.514	1.271	0.840	1.306	0.668	0.935
γ-Terpinene	21.06	1060	ND	0.078	0.082	0.086	0.087	0.131	0.074	0.156	0.141	0.113	0.175	0.089	0.124
Isoterpinolene	22.33	1088	ND	0.036	0.037	0.027	0.027	0.041	0.013	0.070	0.069	0.039	0.057	0.032	0.045
Linalool	22.83	1100	ND	0.015	0.013	0.018	0.022	0.039	0.021	0.019	0.017	0.023	0.023	0.018	0.032
Terpinen-4-ol	25.90	1178	ND	0.086	0.104	0.052	0.047	0.087	0.058	0.154	0.139	0.067	0.065	0.044	0.086

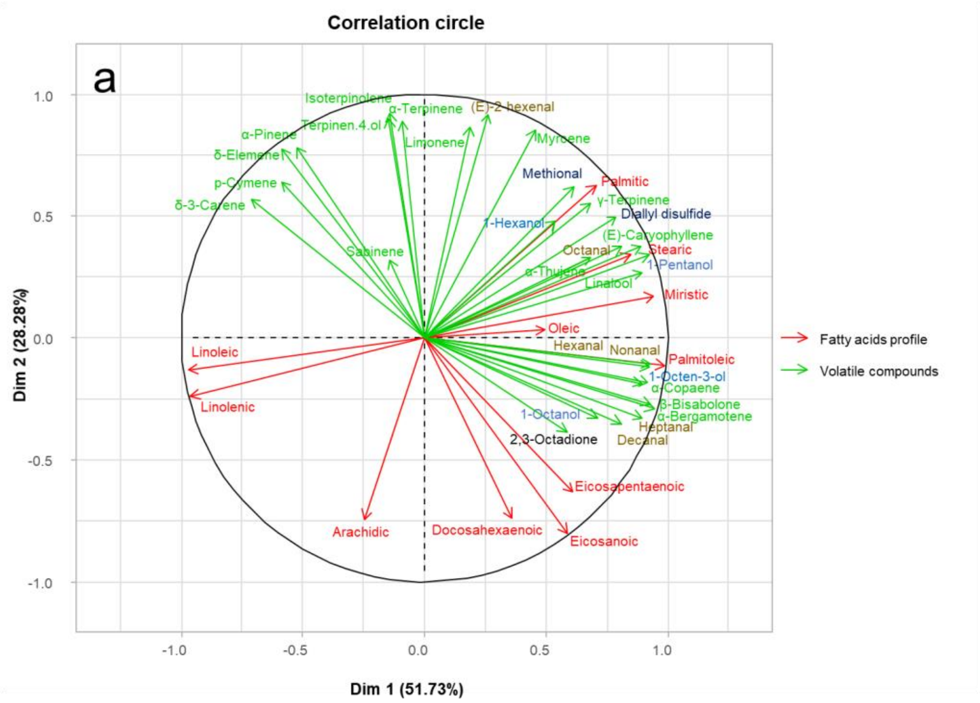
δ-Elemene	31.73	1340	ND	0.013	0.022	0.002	0.002	0.004	0.002	0.035	0.033	0.003	0.002	0.002	0.004
α-Copaene	33.05	1380	ND	0.002	0.002	0.031	0.032	0.057	0.037	0.005	0.003	0.040	0.043	0.030	0.054
α-Bergamotene	34.30	1418	ND	ND	ND	0.005	0.005	0.009	0.007	ND	ND	0.006	0.004	0.004	0.008
(E)-Caryophyllene	34.50	1425	0.014	0.144	0.184	0.184	0.180	0.322	0.206	0.320	0.266	0.222	0.261	0.164	0.319
β-Bisabolone	37.20	1511	ND	ND	ND	0.008	0.009	0.016	0.014	ND	ND	0.010	0.006	0.006	0.011
Total			0.207	2.415	2.411	1.642	1.726	2.546	1.393	5.150	4.289	2.122	3.370	1.734	2.478
Hydrocarbons															
2,2,5-Trimethylhexane	20.85	1055	0.027	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,3,5-Trimethylheptane	21.80	1076	0.032	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5-Trimethylheptane	22.35	1089	0.035	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2,5,5-Tetramethylhexane	23.83	1125	0.018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3,6-Dimethyloctane	25.45	1166	0.019	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Undecane	26.76	1200	0.006	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total			0.137												

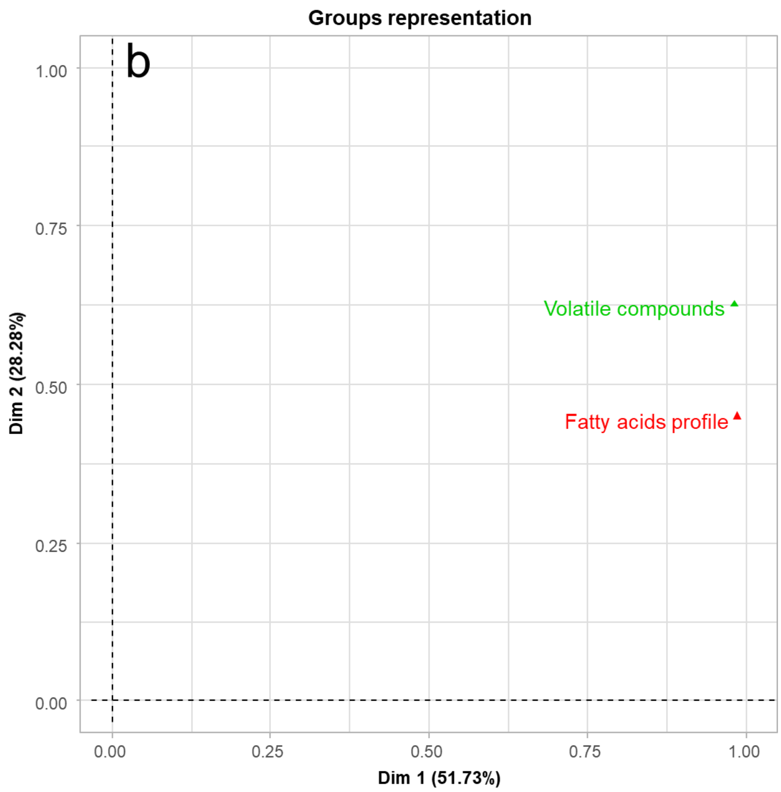
[1]T1: 1.5% regular salt (RS1.5); T2: 1.0% micronized salt (MS1.0); T3: RS1.5 + uncapsulated fish oil; T4: MS1.0 + uncapsulated fish oil; T5: RS1.5 + encapsulated fish oil; T6: MS1.0 + encapsulated fish oil.
[2]RT: Retention time.
[3]LRI: Linear retention index.

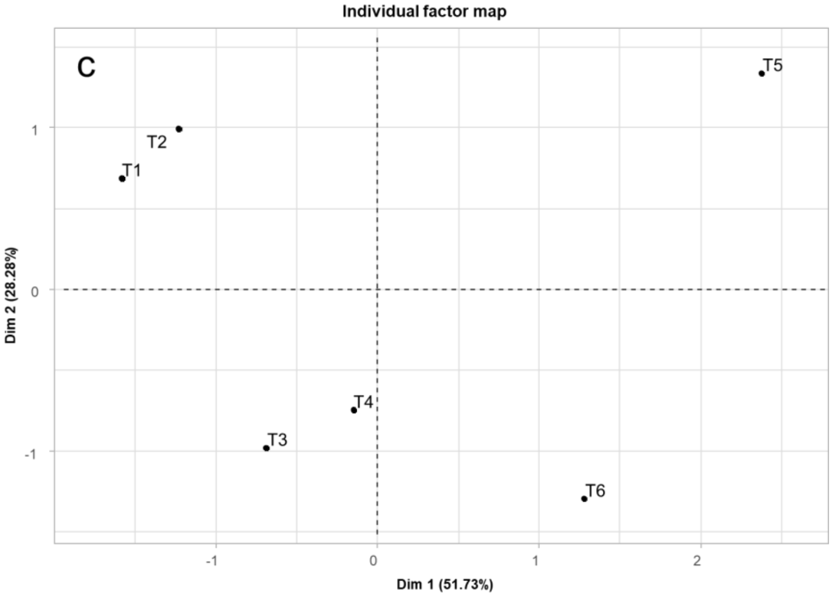
The latter group showed the highest number of compounds, whose presence can be attributed to seasonings, such as white pepper powder, since the terpene profile is very similar to that reported by Liu, Zeng, Wang, Wu, and Tan (2013), in various genotypes of white pepper. Other ingredients, such as onion and garlic, are known to contain sulfur compounds, such as diallyl disulfide, which was found in the burgers (Leyva, Ortega-Ramirez, & Ayala-Zavala, 2016; Liu, Su, & Guo, 2018). Methional is another sulfide found in the samples and it is derived from the degradation of amino acids (Söllner & Schieberle, 2009).

Alcohols, aldehydes, and ketones are secondary oxidation products formed from fatty acids that first react with molecular oxygen to form hydroperoxides or primary oxidation compounds (Ladikos & Lougovois, 1990). Among these compounds, aldehydes were found in abundance in the burgers, especially after cooking (Table 7.5).

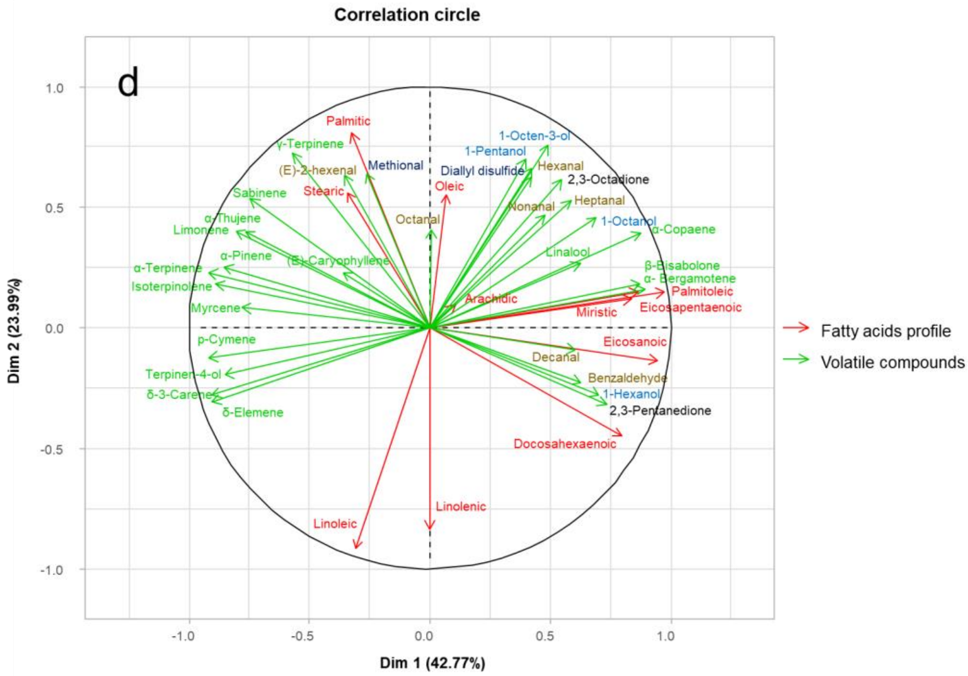
To explore the relationship between fatty acids and VOCs, an MFA was performed (Fig. 7.1). For raw and cooked burgers, the first two dimensions represent 80.01% and 66.76% of the data variability, respectively. Despite the literature suggests that aldehydes, such as hexanal come from linoleic acid (C18:2) (Shahidi, 2001; Vasta & Priolo, 2006), no relationship between this fatty acid and VOCs was observed for raw and cooked burgers (Fig. 7.1a, d). This result may be because the samples were not stored for a long period of days, or may be associated with the complexity of the meat product, which has a considerable contribution of VOCs derived from seasonings, mainly terpenes, in addition to the presence of long-chain PUFAs, which could have directed the relationship between fatty acids and VOCs. In this context, the groups representation (Fig. 7.1b, e) shows proximity in the two dimensions, that is, fatty acids and VOCs were closely correlated.


Correlation circle
a
Dim 1 (51.73%)
Dim 2 (28.28%)
Fatty acids profile
Volatile compounds
Isoterpinolene
α-Terpinene
(E)-2-hexenal
Terpinen.4.ol
Limonene
Myrcene
α-Pinene
δ-Elemene
p-Cymene
δ-3-Carene
Methional
Palmitic
γ-Terpinene
Diallyl disulfide
1-Hexanol
(E)-Caryophyllene
Octanal
Stearic
1-Pentanol
Sabinene
α-Thujene
Linalool
Miristic
Oleic
Hexanal
Nonanal
Linoleic
Palmitoleic
1-Octen-3-ol
α-Copaene
Linolenic
β-Bisabolone
α-Bergamotene
1-Octanol
Heptanal
2,3-Octadione
Decanal
Eicosapentaenoic
Arachidic
Docosahexaenoic
Eicosanoic

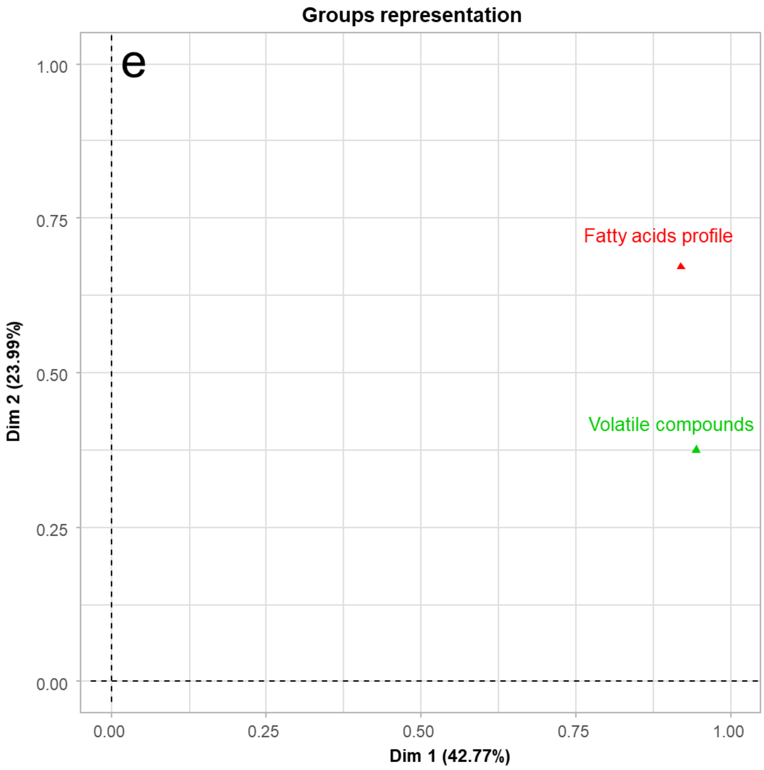

Groups representation
b
Dim 1 (51.73%)
Dim 2 (28.28%)
Volatile compounds
Fatty acids profile



Individual factor map
c
T1
T2
T3
T4
T5
T6
Dim 1 (51.73%)
Dim 2 (28.28%)



Correlation circle
d
Dim 1 (42.77%)
Dim 2 (23.99%)
Fatty acids profile
Volatile compounds
Palmitic
γ-Terpinene
(E)-2-hexenal
Methional
Stearic
Sabinene
α-Thujene
Limonene
α-Pinene
(E)-Caryophyllene
α-Terpinene
Isoterpinolene
Myrcene
p-Cymene
Terpinen-4-ol
δ-3-Carene
δ-Elemene
Octanal
Oleic
Diallyl disulfide
1-Pentanol
1-Octen-3-ol
Hexanal
2,3-Octadione
Nonanal
Heptanal
1-Octanol
α-Copaene
Linalool
β-Bisabolone
α- Bergamotene
Palmitoleic
Eicosapentaenoic
Miristic
Arachidic
Eicosanoic
Decanal
Benzaldehyde
1-Hexanol
2,3-Pentanedione
Docosahexaenoic
Linolenic
Linoleic



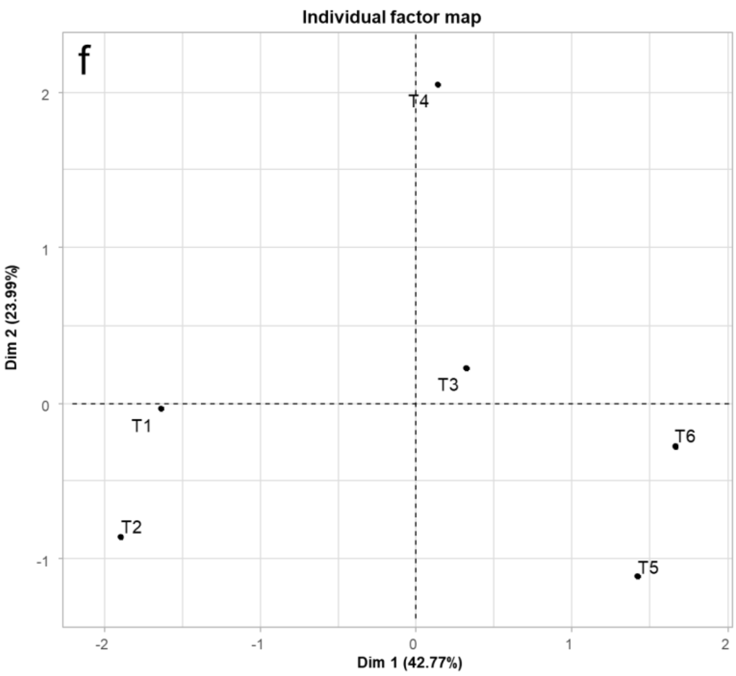


Figure 7.1. Multiple factor analysis (MFA) of the burgers considering profile of fatty acids and volatile organic compounds. a – c: raw burgers; d – f: cooked burgers. Compounds with the same color belong to the same compound family.

Figures 7.1c and 7.1f show that treatments without the addition of fish oil were related to the presence of terpenes. Burgers with uncapsulated fish oil (T3 and T4) show a relationship with linoleic, linolenic and arachidic fatty acids in raw samples, but after cooking, it is not possible to observe a clear relationship of these treatments with the fatty acids or VOCs. However, cooked T4 seems to be close to some oxidation compounds in the second dimension of MFA, such as 1-pentanol, 1-octen-3-ol and hexanal (Fig. 1d, f), which would explain the difference with cooked T3 in the fatty acids (Table 7.3).

In raw burgers added with microparticles, a variable lipid profile was obtained because of the presence of saturated fatty acids (palmitic, stearic and myristic) and PUFAs (EPA, DHA), but the presence of the last group allowed a higher incidence of volatile oxidation compounds, such as aldehydes (hexanal, heptanal, octanal, nonanal, and decanal), mainly in the NaCl-reduced burger (T6). Similarly, the microparticles also affected the profiles of VOCs and fatty acids of the cooked burger, but the proximity between T5 and T6 suggests that these treatments were similar. In these samples, the presence of eicosanoic and DHA fatty acids was accompanied by decanal, benzaldehyde, 1-hexanol and 2,3-pentanedione. These results suggest that the microencapsulation process was not effective in maintaining the oxidative stability of fish oil.

The VOCs in microparticles were composed by 2 terpenes (hydroperoxide 1-mehtylhexyl and (E)-caryophyllene) and 6 aliphatic hydrocarbons. The hydroperoxide 1-mehtylhexyl, belonging to the terpene family (Hanifah, Maharijaya, Putri, Laviña, & Sobir, 2018), may be linked to the fish diet. In this regard, hydrogen peroxide benefits fishes in their growth and survival (Jainab et al., 2019). The aliphatic hydrocarbons are secondary products of lipid oxidation (Shahidi, 2011). However, Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Ruiz-Carrascal, and Antequera (2015) reported that the correlation between the values of aliphatic hydrocarbons and Thiobarbituric Acid Reactive Substances (TBARS) was negative for the lipid oxidation of fish oil, which suggests that the presence of aliphatic hydrocarbons does not indicate the extend of lipid oxidation of fish oil. Nevertheless, the presence of these compounds in the microparticles indicates alteration of the fish oil in the complex coacervation process, which resulted in a decrease in polyunsaturated fatty acids (Table S1). However, to have an increase in volatile oxidation compounds such as aldehydes in burgers with microparticles, other factors related to the manufacture and heat treatment of the burgers may also be involved.

Márquez-Ruiz, Velasco, & Dobarganes (2000) and Velasco, Holgado, Dobarganes, & Márquez-Ruiz (2009) observed greater lipid oxidation in microencapsulated fish oil than in uncapsulated fish oil. These authors suggested that fish oil is more exposed to oxidation because of the porous and spongy structure of the freeze-dried microparticles and the higher surface-to-volume ratio, allowing greater access of oxygen to the fatty acids (Márquez-Ruiz et al., 2000; Velasco et al., 2009). In this context, the bulk density of the microparticles was very low (0.34 g/mL), indicating the occupation of a large volume in the burger, clearly illustrated at the bottom of Fig. 7.2. Therefore, it is suggested that the freeze-dried microparticles were greater exposed to lipid oxidation during processing and cooking of the burgers than unencapsulated fish oil, limiting the use of the freeze-dried microparticles as carriers of fish oil in meat products. However, the addition of natural antioxidants is an approach not explored here, which can counteract the oxidation process in meat products (Ribeiro et al., 2019), or they can be added in the fish oil encapsulation process, which suffered alteration in the encapsulation process that resulted in the presence of aliphatic hydrocarbons as VOCs.

Figure 7.2. Burger ingredients. The microparticles at 2.5% of the beef burger are shown at the bottom of the figure.

7.3.4. Sensory profile and overall liking

The sensory profile of the burgers is shown in Fig. 7.3a. The first two dimensions of the CA explained 93.33% of the data variability. The response of consumers reflects that they were able to differentiate the samples according to the NaCl reduction and the type of fish oil added to the products. Thus, burgers with regular salt (1.5%) (T1 and T3) were associated with the attributes *salty*, *fatty*, *spicy*, *juicy*, *tender* and *seasoned*. On the other hand, burgers with micronized salt (1.0%) were characterized by *grilled*, *beef*, *characteristic*, *aromatic* and *tasty*. Since burgers added with microparticles were associated with oxidation compounds, the presence of negative attributes in the product was expected, such as *bad smell*, *off-flavor*, *bad taste*, *aftertaste* and *rancid*. Additionally, these samples were associated with the *acid* attribute because of the lower pH value in cooked burgers (Table 7.2).

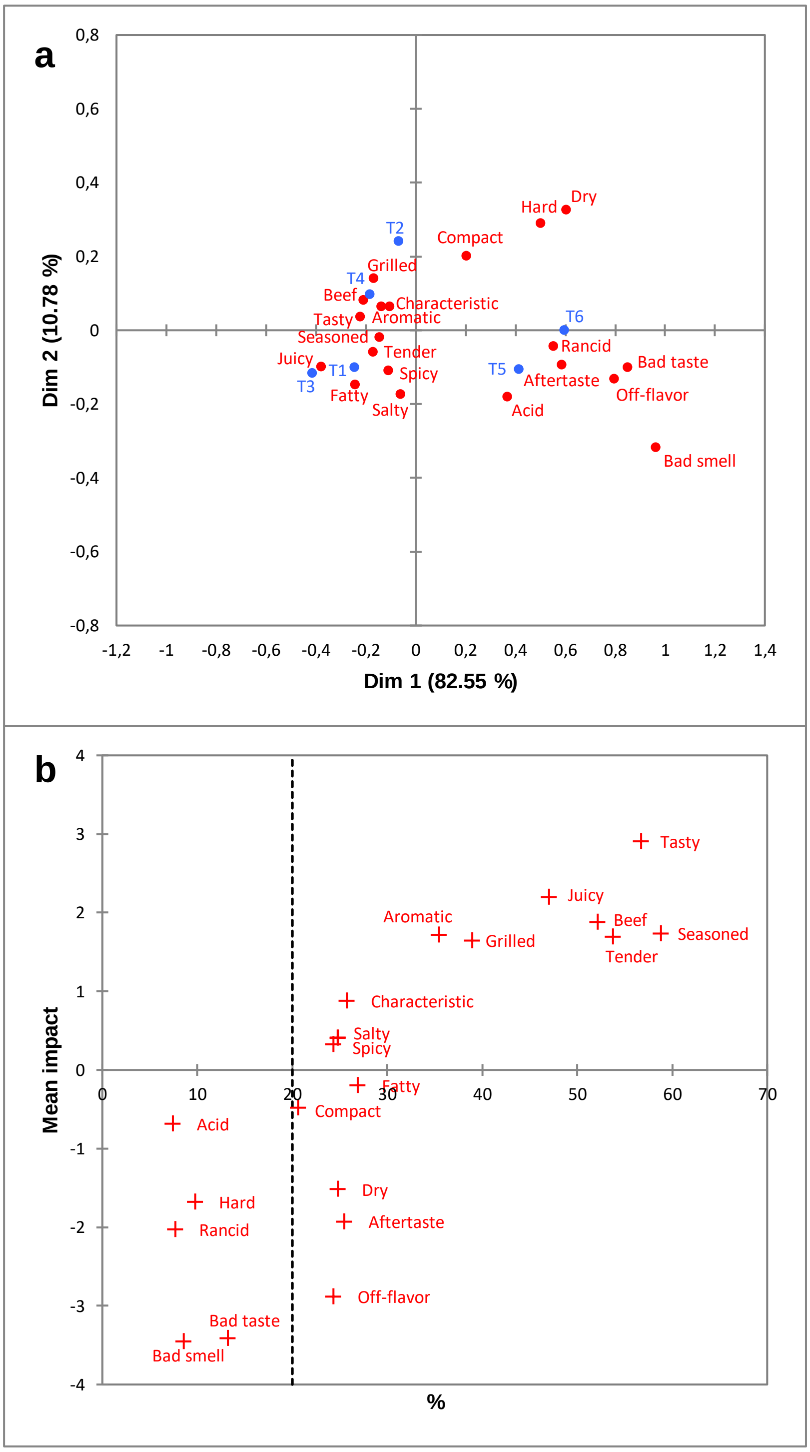


Figure 7.3. Correspondence analysis (a) of Check-All-That-Apply (CATA) questions of burger treatments and penalty analysis (b) of the mean impact of sensory attributes on overall liking.

The sensory profile of T1 to T4 is similar to the result obtained by Rios-Mera et al. (2020) and Rios-Mera, Saldaña, Cruzado-Bravo, et al. (2019), including the presence of temporal drivers of liking (*tasty* and *juicy*) (Rios-Mera et al., 2020) regardless of the NaCl reduction or the incorporation of uncapsulated fish oil. As a result, the mean overall liking for T1 to T4 ranged from 6.27 to 7.13, while for T5 and T6 the mean liking scores were 4.70 and 4.36, respectively, which corresponds to 'neither liked nor disliked'. Finally, the mean impact of the sensory attributes on overall liking revealed that the sensory attributes *tasty*, *juicy*, *beef*, *seasoned*, *tender*, *aromatic*, *grilled*, *characteristic*, *salty*, and *spicy* (almost all present in T1–T4), were important to the overall liking for more than 20% of consumers (Fig. 7.3b).

In synthesis, the results show that it is possible to reduce salt (33%) and to incorporate EPA into the burgers without harming the quality parameters evaluated in this study. However, for the product to meet the claim defined by MERCOSUR (2012), it is necessary to quantify the EPA (mg/100 g) present in the product reduced in salt and after cooking, and in case of not reaching the necessary amount to be considered a "source" food (40–80 mg EPA / DHA), more fish oil should be added. In the latter case, there will be a greater risk of lipid oxidation, so it will be necessary to define the level of antioxidants (natural or synthetic) and the shelf-life of the product, aspects to be considered in future studies.

7.4. Conclusion

The results show that it is possible to reduce 33% NaCl and add long-chain PUFAs in the burger, but to no compromise the quality of the burgers, the condition is to incorporate uncapsulated fish oil, which after cooking conserves only eicosapentaenoic acid (EPA). The freeze-dried microparticles, despite maintaining EPA and DHA after cooking, increased the hardness, chewiness and the oxidation process, which negatively impacted on the sensory attributes and overall liking of the burgers. The physical state of the microparticles (low density) could have exposed more fish oil to oxidation before and after cooking. Therefore, the use of freeze-dried microparticles as an approach for incorporating long-chain PUFAs in meat products such as burgers is not recommended.

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8. GENERAL CONCLUSION

Two approaches to obtain a healthy beef burger were investigated in the this PhD thesis: salt/sodium reduction and fortification with long-chain n-3 polyunsaturated fatty acids (PUFAs).

The first specific objective was based on the reduction of salt using micronized salt. Chapters 3 and 4 show that this objective was achieved, that is, it was possible to obtain beef burger with 33% less salt content without affecting most of the quality parameters evaluated. Although there were already previous studies on the use of micronized salt in meat products, the novelty of the strategy was to protect the structure of the micronized salt against the aqueous component of the burger, mixing half of the salt mass with the pork fat, optimizing the amount of salt added and the sensory attributes, such as the salty taste.

The second specific objective sought to obtain an optimized functional ingredient, source of EPA and DHA. Thus, microparticles of soy protein isolate and inulin entrapping fish oil were obtained through the complex coacervation technique, resulting in high encapsulation efficiency and suitable yield. Moreover, the microparticles were expected to resist to stress conditions similar to those of processing and heat treatment of the beef burger. In this regard, the enzyme transglutaminase helped to improve the fish oil retention in the microparticles. Therefore, the second specific objective was achieved.

For the third specific objective, it was expected to fortify the beef burger with fish oil source of EPA and DHA, unencapsulated or encapsulated using the microparticles developed in chapter 6, without significantly affect the quality parameters evaluated. The results of chapter 7 show that in the presence of fish oil, some differences between beef burgers with different salt content were observed, mainly in the fatty acids profile and volatile compounds. However, the most outstanding result was that the incorporation of freeze-dried microparticles failed in several quality parameters, probably due to the presence of aliphatic hydrocarbons as volatile organic compounds, in addition to the low density of microparticles, resulting in the occupation of a large volume in the burger, presumably increasing the lipid oxidation of the product and having negative implications on the sensory profile and overall liking. In this regard, the greatest oxidation of the beef burgers with microparticles may have been from the combined effect of the complex coacervation process, the physical state of the microparticles, the matrix components, processing and heat treatment. In this scenario, it is recommended to incorporate the antioxidant approach to solve this problem. The beef burger formulation included sodium erythorbate as an antioxidant. Also, some spices such as white pepper contributed to a large amount of terpenes to the product, but the level of these components does not seem to be sufficient to promote oxidative stability in the products added with microencapsulated fish oil. Nevertheless, the specific objective 3 was achieved, which was to obtain a product enriched with long-chain n-3 PUFAs, but in the sense of not affecting the quality parameters of beef burger, the use of unencapsulated fish oil stands out, which only maintains EPA after cooking.

Finally, it was possible to obtain a healthier beef burger reduced in salt and incorporated with long-chain n-3 PUFAs, which can be easily adapted in the industry. The next step in future studies is to evaluate the oxidative stability considering the storage time and to study the level of added antioxidants, especially those of natural origin and that are allowed by Brazilian regulation, aiming the adaptation of the product in the industry.

APPENDIX

Appendix A. Ettics Committee for Human Research.

USP - ESCOLA SUPERIOR DE AGRICULTURA "LUIZ DE QUEIROZ" DA UNIVERSIDADE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Desenvolvimento e caracterização de hambúrguer bovino adicionado de sal micronizado e micropartículas de proteína isolada de soja, inulina e óleo de peixe

Pesquisador: Carmen Josefina Contreras Castillo
Área Temática:
Versão: 3
CAAE: 90113518.1.0000.5395
Instituição Proponente: "Escola Superior de Agricultura ""Luiz de Queiroz"" - ESALQ da Universidade
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.823.957

Apresentação do Projeto:
O projeto será desenvolvido em três etapas para a obtenção de hambúrguer bovino com apelo à saudabilidade. Na primeira etapa, será utilizado sal micronizado para reduzir sal no hambúrguer. Na segunda etapa, serão utilizadas micropartículas de proteína isolada de soja, inulina e óleo de peixe produzidas por coacervação. Na terceira etapa será estudada a redução de gordura saturada e fortificação com as micropartículas, combinando com a redução de sódio da primeira etapa. No teste de Pivot profile, cada consumidor irá avaliar pedaços pequenos de 5 amostras de hambúrguer mais 5 amostras de uma amostra referência chamada de Pivot (5 g por amostra), num total de aproximadamente 50 g de hambúrguer por consumidor. Neste teste, será solicitado aos consumidores escrever atributos sensoriais menos intensos e mais intensos que o Pivot. Na DTS (Dominância temporal de Sensações), para avaliação das amostras, os provadores receberão treinamento em várias sessões sobre o uso do software em tabletes. O teste consta de caracterizar as amostras com atributos sensoriais de uma lista pré-estabelecida ao longo do tempo da mastigação. Cada provador avaliará seis tratamentos de hambúrguer com 3 repetições, resultando em 18 degustações e aproximadamente 90 g de hambúrguer degustado por provador. No teste de questões CATA será fornecido aos participantes aproximadamente a mesma quantidade de hambúrguer avaliado na DTS, e avaliados com uso de tabletes. Este teste é baseado na caracterização sensorial das amostras com uso de uma lista pré-estabelecida de atributos

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sensoriais. A lista de atributos será definida pela equipe do estudo. Ao final da descrição de cada amostra, será pedido aos consumidores indicar a aceitabilidade global como uso de escala não estruturada de 9 pontos, variando de desgostei extremamente (1) até gostei extremamente (9).

Objetivo da Pesquisa:

Objetivo principal:

Produzir e caracterizar a qualidade global de hambúrguer bovino com apelo à saudabilidade, com uso de sal micronizado, redução de gordura saturada e fortificado com micropartículas de proteína isolada de soja, inulina e óleo de peixe.

Objetivos secundários:

1) Determinar a qualidade física, química, de textura e rendimento e sensorial com uso de provadores treinados e consumidores, de hambúrguer adicionado de sal micronizado.

2) Otimizar a produção de micropartículas de proteína isolada de soja, inulina e óleo de peixe, produzidas por coacervação complexa, e determinar a estabilidade oxidativa e de rendimento em condições similares de processamento e tratamento térmico do hambúrguer.

3) Determinar a qualidade física, química, de textura e rendimento e sensorial com uso de provadores treinados e consumidores, de hambúrguer adicionado de sal micronizado, com redução de gordura saturada e fortificado com micropartículas coacervadas de proteína isolada de soja, inulina e óleo de peixe.

Avaliação dos Riscos e Benefícios:

Os riscos envolvidos no experimento serão minimizados.

Serão utilizados aditivos alimentares (citrato de potássio, ácido cítrico, glutamato monossódico e eritorbato de sódio) nos níveis permitidos em produtos cárneos (Brasil, 1998). Para o risco microbiológico, o produto será manipulado seguindo as normas de Boas Práticas de Fabricação e analisados microbiologicamente para os microrganismos patogênicos e sulfito redutores segundo a norma da ANVISA (Brasil, 2001). Os indivíduos recrutados deverão ser saudáveis, sem restrições alimentares (consumo de sódio ou gordura), livre de doenças (hipertensão, entre outras) e não apresentar alergia ou intolerâncias aos ingredientes e aditivos presentes na formulação.

Benefícios:

Os participantes não serão beneficiados diretamente com este experimento, mas os dados gerados por eles serão relevantes para caracterizar sensorialmente o produto e entender o comportamento do consumidor durante o consumo de hambúrguer bovino.

Comentários e Considerações sobre a Pesquisa:

O projeto está muito bem detalhado, levando-se em consideração os principais pontos que poderiam colocar o provador em situações de risco ou desconforto, como uso de quantidades de sal preconizadas pela ANVISA e análises microbiológicas também de acordo com a mesma; deixando claro também, a caracterização da população participante do estudo, a qual será composta por provadores voluntários, adultos acima de 18 anos, saudáveis, não fumantes, de ambos os sexos, estudantes e funcionários da ESALQ, que se declarem consumidores de hambúrguer e não possuam nenhuma relação direta com a pesquisa, e não apresentarem reações alérgicas ao consumo de qualquer ingrediente utilizado na formulação. Os voluntários serão convidados por meio da fixação de cartazes e nas redes sociais. O recrutamento será realizado pelos pesquisadores responsáveis do projeto, os mesmos estarão disponíveis para toda e qualquer eventual dúvida por parte dos participantes. Os indivíduos serão instruídos a lerem o TCLE antes de realizar qualquer atividade.

Considerações sobre os Termos de apresentação obrigatória:

Os termos obrigatórios estão presentes e adequados.

Recomendações:

As pendências foram atendidas conforme documentos inseridos na PB em 15/08.

Após a aprovação os pesquisadores devem atentar para a necessidade de envio de relatórios parciais de atividades (no mínimo um a cada 12 meses) e do relatório final de atividades (ao término da pesquisa). Destaca-se que o parecer consubstanciado é o documento oficial de aprovação do sistema CEP/CONEP.

Conclusões ou Pendências e Lista de Inadequações:

Pendência 1 (atendido): simplificar o TCLE, com termos mais acessíveis ao provador.

Pendência 2 (atendido): A Resolução 196/1996 foi revogada e substituída pela Res 466/2012. É necessário ajustar isto no TCLE, ou simplesmente, retirar do texto.

Considerações Finais a critério do CEP:

Após a aprovação os pesquisadores devem atentar para a necessidade de envio de relatórios parciais de atividades (no mínimo um a cada 12 meses) e do relatório final de atividades (ao término da pesquisa). Destaca-se que o parecer consubstanciado é o documento oficial de aprovação do sistema

CEP/CONEP.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Outros	TCLE_Pivot_profile_alterado.docx	15/08/2018 06:50:21	Sandra Helena da Cruz	Aceito
Outros	TCLE_DTSalterado.docx	15/08/2018 06:50:10	Sandra Helena da Cruz	Aceito
Outros	TCLE_CATAalterado.docx	15/08/2018 06:49:56	Sandra Helena da Cruz	Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1132191.pdf	09/07/2018 16:46:01		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_CATA.docx	14/06/2018 16:05:16	Carmen Josefina Contreras Castillo	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Pivot_profile.docx	14/06/2018 16:05:02	Carmen Josefina Contreras Castillo	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_DTS.docx	14/06/2018 16:04:52	Carmen Josefina Contreras Castillo	Aceito
Outros	CVJuanDarioRMera.pdf	22/05/2018 16:02:15	Sandra Helena da Cruz	Aceito
Outros	CVCarmenCCastillo.pdf	22/05/2018 16:01:28	Sandra Helena da Cruz	Aceito
Outros	CartaEncaminhamento.pdf	22/05/2018 16:01:00	Sandra Helena da Cruz	Aceito
Outros	Informacoes_relativas_ao_sujeito_da_pesquisa.doc	16/05/2018 13:14:15	Carmen Josefina Contreras Castillo	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_pesquisa_Doutorado_Juan_Dario_Rios_Mera.docx	16/05/2018 13:09:16	Carmen Josefina Contreras Castillo	Aceito
Orçamento	Orcamento_de_projeto_de_pesquisa_assinado.pdf	16/05/2018 13:08:53	Carmen Josefina Contreras Castillo	Aceito
Declaração de Pesquisadores	Declaracoes_dos_pesquisadores_assinado.pdf	16/05/2018 13:08:37	Carmen Josefina Contreras Castillo	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_de_infraestrutura_assinado.pdf	16/05/2018 13:08:23	Carmen Josefina Contreras Castillo	Aceito

Declaração de Instituição e Infraestrutura	Declaracoes_da_instituicao_assinado.pdf	16/05/2018 13:08:07	Carmen Josefina Contreras Castillo	Aceito
Folha de Rosto	Folha_de_rostro_assinado.pdf	16/05/2018 12:59:44	Carmen Josefina Contreras Castillo	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PIRACICABA, 15 de Agosto de 2018

Assinado por:
Sandra Helena da Cruz
(Coordenador)

Appendix B. Patent request.

INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

25/11/2019 870190123280 20:44

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Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2019 024837 8

Dados do Depositante (71)

Depositante 1 de 1

Nome ou Razão Social:	UNIVERSIDADE DE SÃO PAULO - USP
Tipo de Pessoa:	Pessoa Jurídica
CPF/CNPJ:	63025530000104
Nacionalidade:	Brasileira
Qualificação Jurídica:	Instituição de Ensino e Pesquisa
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Cidade:	São Paulo
Estado:	SP
CEP:	05508220
País:	Brasil
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Fax:	
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Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): COMPOSIÇÃO DE PARTÍCULAS ALIMENTÍCIAS PARA ENCAPSULAMENTO DE ÓLEO DE PEIXE E SEU USO

Resumo: A presente invenção refere-se a composição de partículas alimentícias obtidas por meio da técnica de coacervação complexa, compreendendo inulina e proteína isolada de soja ligadas, sendo que a inulina tem proporção minoritária em relação a proteína de soja, promovendo a encapsulação de óleo de peixe com elevada eficiência de encapsulação e rendimento adequado. Além disso, a adição da enzima transglutaminase como agente reticulante de proteína fornece elevada retenção de óleo de peixe em condições de estresse ao pH e tratamento térmico. Ditas partículas apresentam características de ingrediente funcional fonte de proteína, fibra e ácidos graxos poliinsaturados e podem ser adequadas para matrizes alimentares que recebem tratamento térmico.

Figura a publicar: 1

Dados do Inventor (72)

Inventor 1 de 3

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Qualificação Física: Engenheiro, arquiteto e afins
Endereço: [redacted]
Cidade: Piracicaba
Estado: SP
CEP: 13401-854
País: BRASIL
Telefone: (11) 309 11578
Fax:
Email: pidireto@usp.br

Documentos anexados

Tipo Anexo	Nome
Comprovante de pagamento de GRU 200	GRU Inulina.pdf
Esclarecimento	Esclarecimento Representante.pdf
Declaração de período de graça	Resumo_congresso.pdf
Relatório Descritivo	Relatório descritivo.pdf
Reivindicação	REIVINDICAÇÕES.pdf
Desenho	FIGURAS.pdf
Resumo	RESUMO.pdf

Acesso ao Patrimônio Genético

☑ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

Declaração de Divulgação Anterior Não Prejudicial

☑ Artigo 12 da LPI - Período de Graça.

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

14

[bb.com.br] - Boleto gerado pelo sistema MPAG. 08/08/2019 14:17:39

INSTRUÇÕES:

A data de vencimento não prevalece sobre o prazo legal. O pagamento deve ser efetuado antes do protocolo. Órgãos públicos que utilizam o sistema SIAFI devem utilizar o número da GRU no campo Número de Referência na emissão do pagamento. Serviço: 200-Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Clique aqui e pague este boleto através do Auto Atendimento Pessoa Física.
Clique aqui e pague este boleto através do Auto Atendimento Pessoa Jurídica.

Recibo do Pagador

BANCO DO BRASIL | **001-9** | **00190.00009 02940.916196 08438.974175 9 80040000007000**

Nome do Pagador/CPF/CNPJ/Endereço
UNIVERSIDADE DE SAO PAULO USP CPF/CNPJ: 63025530000104
RUA DA REITORIA 374 BUTANTA, SAO PAULO -SP CEP:05508220
Sacador/Avalista

Nosso-Número	Nr. Documento	Data de Vencimento	Valor do Documento	(=) Valor Pago
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Nome do Beneficiário/CPF/CNPJ/Endereço
INSTITUTO NACIONAL DA PROPRIEDADE INDUST CPF/CNPJ: 42.521.088/0001-37
RUA MAYRINK VEIGA 9 24 ANDAR ED WHITE MARTINS , RIO DE JANEIRO - RJ CEP: 20090910

Agência/Código do Beneficiário
2234-9 / 333028-1

Autenticação Mecânica

BANCO DO BRASIL | **001-9** | **00190.00009 02940.916196 08438.974175 9 80040000007000**

Local de Pagamento
PAGÁVEL EM QUALQUER BANCO ATÉ O VENCIMENTO

Data de Vencimento
06/09/2019

Nome do Beneficiário/CPF/CNPJ
INSTITUTO NACIONAL DA PROPRIEDADE INDUST CPF/CNPJ: 42.521.088/0001-37

Agência/Código do Beneficiário
2234-9 / 333028-1

Data do Documento	Nr. Documento	Espécie DOC	Aceite	Data do Processamento
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Nosso-Número
29409161908438974

(=) Valor do Documento
70,00

Informações de Responsabilidade do Beneficiário
A data de vencimento não prevalece sobre o prazo legal.
O pagamento deve ser efetuado antes do protocolo.
Órgãos públicos que utilizam o sistema SIAFI devem utilizar o número da GRU no campo Número de Referência na emissão do pagamento.
Serviço: 200-Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

(-) Desconto/Abatimento

(+) Juros/Multa

(=) Valor Cobrado

Nome do Pagador/CPF/CNPJ/Endereço
UNIVERSIDADE DE SAO PAULO USP CPF/CNPJ: 63025530000104
RUA DA REITORIA 374 BUTANTA,
SAO PAULO-SP CEP:05508220

Código de Baixa

Sacador/Avalista

Autenticação Mecânica - **Ficha de Compensação**

Visualização de arquivos

Auto-Atendimento
Comprovante – Arquivo

Agência débito: 1897-X
Conta débito: 139531-9
CPF/CNPJ: 63025530/0001-04 USPBO06092019091634508001LD470

Documento empresa:	201904064823
Data vencimento:	06/09/2019
Data pagamento:	06/09/2019
Valor pagamento:	70,00
Documento banco:	
Desconto:	0,00
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Valor título:	70,00
Acréscimo:	0,00
CNPJ sacado:	00000000388068
Nome sacado:	Banco do Brasil S/A
CNPJ cedente:	63025530000104
Nome cedente:	UNIVERSIDADE DE SAO PAULO
Autenticacão:	136C483993D6878B

ESCLARECIMENTOS

A Agência USP de Inovação é o Núcleo de Inovação Tecnológica da Universidade de São Paulo, responsável pela gestão das propriedades intelectuais geradas por seus pesquisadores, conforme definido na Resolução 5175, de 18 de fevereiro de 2005 e Resolução 7035 de 17 de dezembro de 2014.

Como a gestão é feita pela equipe interna de funcionários da própria Universidade, nos atos junto ao Instituto Nacional da Propriedade Industrial - INPI é apresentado o documento assinado pelo Magnífico Reitor que dá poderes a Sra. Maria Aparecida de Souza, funcionária da Instituição, sob número institucional RUSP 3081942, que atua como chefe técnica de propriedade intelectual da Agência USP de Inovação, para realizar os procedimentos necessários à proteção junto ao INPI.

Assim, para executar as ações necessárias à proteção junto ao INPI, conforme requisitos e procedimentos definidos pela legislação de propriedade industrial, possuímos *login* e senha que nos permitem representar e gerenciar os ativos de propriedade intelectual da Instituição.

São Paulo, 01 de novembro de 2017.

MARIA APARECIDA DE SOUZA
API 1833

GABINETE DO REITOR

PROCURAÇÃO

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Optimization and pH- and temperature-stability of the complex coacervation between soy protein isolate and inulin containing fish oil

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Abstract

Fish oil is an abundant source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, these bioactive compounds are quickly oxidized, causing loss of their functional value. To protect them, microencapsulation by complex coacervation can be applied. On the other hand, the application of microparticles in food matrices is a critical process, factors such as pH or heat treatment can be detrimental in terms of controlled release of the active encapsulated. The objectives of this study were to optimize the fish oil microencapsulation by complex coacervation, using soy protein isolate (SPI) and inulin (Inu); and to determine the effect of pH and temperature on the oil retention (%OR) in the microparticles. The coacervation between the biopolymer solutions was carried out at pH 4.0, then, the process yield (%PY) and the encapsulation efficiency (%EE) were determined. The optimized microparticles were visualized by optical microscopy. A central composite rotatable design was applied, having as variables the Inu:SPI ratio (0.5-1.5) and the amount of oil added (20%-80%). The results were analyzed through ANOVA, and the optimal conditions were estimated by regression analysis and response surface methodology. For the stability of the microparticles, three pH (5.5, 6.0, 6.5) and four temperature (Control, 50°C, 75°C, 100°C) levels were studied. Subsequently, the determination of %OR was performed. The results of %PY show that the maximum (55.60%) and the minimum (36.19%) %PY were obtained at Inu:SPI=0.5 and 80% oil, respectively. For %EE, the maximum (69.32%) and the minimum (36.13%) %EE were obtained at Inu:SPI=0.6 and 80% oil, respectively. In ANOVA, it was observed that the data of %EE presented significant lack of fit, discarding the optimization of %EE. The R2 for the %YP was 0.9234, and the response surfaces indicate that, the lower the amount of Inu used, the higher the %PY. In this regard, the optimized microparticles were produced at Inu:SPI=0.4 and 20% oil; then, %YP was 60.74±0.41% and %EE was 93.57±1.48%, and their morphology show oval and spherical shapes of various sizes. For %OR, there were differences between the pH values, ranging from 51.34% to 80.54% for pHs 6.0 and 5.5, respectively. The temperature significantly decreased the %OR at pH 5.5 (~34.33%-36.62%), while at pH 6.0 the %OR was significantly increased at 100°C (72.24±4.45%). The results of %YP and %EE could be dependent on some properties of Inu, such as the degree of polymerization (DP); that is, the greater the DP, the greater the interaction sites between Inu and SPI. For %OR, pH values above the pH of the coacervation (pH 4.0) and the temperature could decrease the electrostatic interaction between the biopolymers. However, other types of interactions may govern, such as hydrogen bonds at pH 5.5, and hydrophobic interactions induced by temperature at pH 6.0. Furthermore, the optimization of the complex coacervation was mainly dependent on the amount of Inu. On the other hand, the pH and temperature affected the %OR, which is an indicator to give better resistance to microparticles with additional treatments, such as the cross-linking.

Institutions

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COMPOSIÇÃO DE PARTÍCULAS ALIMENTÍCIAS PARA ENCAPSULAMENTO DE ÓLEO DE PEIXE E SEU USO

Campo da invenção:

[001] A presente invenção se insere no campo do micro encapsulamento de substâncias com o intuito de uso em matrizes alimentícias, utilizando principalmente polissacarídeos e um derivado proteico de um grão, sendo que o micro encapsulamento pode ser realizado por um processo de coacervação complexa devido à interação entre as cargas opostas das partículas.

Fundamentos da invenção:

[002] A tendência de consumo de alimentos saudáveis tem incitado as pesquisas da indústria de alimentos e da academia. Atualmente, o consumidor está cada vez mais informado sobre quais alimentos são bons ou ruins para sua saúde, o que incentiva a busca de estratégias de produção de alimentos mais saudáveis.

[003] Os alimentos saudáveis podem ser caracterizados por conter compostos bioativos benéficos para a saúde. As fibras, ácidos graxos poli-insaturados e proteínas de alto valor biológico são exemplos de compostos bioativos que vêm ganhando interesse pela indústria, devido à demanda de alimentos saudáveis, tais como os alimentos funcionais e nutracêuticos.

[004] O óleo de peixes de águas frias é uma fonte importante de ácido eicosapentaenóico (EPA) e ácido docosahexaenóico (DHA), ácidos graxos poli-insaturados (PUFAs) que têm benefícios a saúde clinicamente comprovados (SIMOPOULOS, 2016). O uso de óleo de peixe como ingrediente

em alimentos é limitado porque os PUFAs são muito suscetíveis à hidroperoxidação e subsequente formação de compostos orgânicos voláteis relacionados a sabores e odores desagradáveis. Para neutralizar esse problema, a tecnologia de microencapsulação pode ser aplicada para mascarar o gosto de peixe e prolongar a vida útil dos PUFAs (KARLSDOTTIR et al., 2014).

[005] Várias técnicas têm sido propostas para a encapsulação de óleo de peixe e outras fontes de ácidos graxos poli-insaturados. O método mais utilizado é a secagem por atomização, devido ao seu baixo custo, processo rápido e contínuo. A secagem por atomização utiliza altas temperaturas, mas em tempos muito curtos, o que garante uma baixa degradação térmica do ativo encapsulado.

[006] No entanto, existem outras técnicas de encapsulação que não aplicam temperaturas altas, permitindo maximizar a proteção de compostos sensíveis ao calor. A gelificação iônica é uma das técnicas de encapsulação que não aplica calor, baseada na gelificação de biopolímeros como o alginato na presença de íons cálcio durante um processo de extrusão (COMUNIAN e FAVARO-TRINDADE, 2016).

[007] Outra técnica é o processo de coacervação complexa, fenômeno de separação líquido-líquido que ocorre entre biopolímeros com carga oposta por meio da interação eletrostática (EGHBAL e CHOUDHARY, 2018). Embora ambos os métodos garantam estabilidade oxidativa de lipídios na produção de partículas, a principal diferença está no tamanho de partícula obtida. Enquanto o tamanho dos coacervados complexos é de 0,1 a 500 µm, na gelificação iônica o tamanho

da partícula está entre 1 a 10000 µm (COMUNIAN e FAVARO-TRINDADE, 2016), o que representa uma vantagem para a coacervação complexa devido a uma distribuição mais uniforme do tamanho de partícula.

[008] A gelatina e a goma arábica são os biopolímeros mais utilizados no processo de coacervação complexa (ERATTE et al., 2018). No entanto, devido ao surgimento de doenças derivadas de proteínas de origem animal, como as doenças de príons (CHOURPA et al., 2006), a busca de novos biopolímeros de origem vegetal compatíveis com o processo de coacervação complexa parece ser muito importante (MENDANHA et al., 2009; HUANG et al., 2012).

[009] Entre as fontes vegetais de proteína, a proteína isolada de soja vem sendo objeto de interesse de pesquisadores. Molina Ortiz et al. (2009) sugerem que a proteína isolada de soja possui propriedades funcionais para a encapsulação, como emulsificação, solubilidade, formação de filme e capacidade de ligação de água, além de apresentar alto valor nutricional (MOLINA ORTIZ et al., 2009). Para a encapsulação de lipídios, a propriedade emulsificante do biopolímero é importante, um requisito que a proteína isolada de soja cumpre, sendo ainda mais eficaz que outras fontes de proteínas, como a proteína isolada de soro de leite (KIM e MORR, 1996).

[010] Além disso, o uso de proteína isolada de soja foi otimizado em relação ao pH e materiais de parede, formando coacervados complexos com goma arábica, quitosana e pectina (De CONTO et al., 2013; HUANG et al., 2012; JUN-XIA et al., 2011; MENDANHA et al., 2009; NORI et al., 2011). No entanto,

não há relatos da formação de coacervados complexos com inulina para formação de uma partícula.

[011] Nesse contexto, pode se deduzir que existe o interesse de utilizar fibras prebióticas para formar complexos com proteína isolada de soja, com resultados que parecem ser promissores. Mendanha et al. (2009) observaram que utilizando 100% de pectina em relação à quantidade de proteína isolada de soja, obteve-se elevada eficiência de encapsulação de hidrolisado de caseína; enquanto que De Conto et al. (2013) obtiveram a maior eficiência de encapsulação de éster etílico de ômega 3 utilizando 67% de goma arábica em relação à quantidade de proteína isolada de soja. No entanto, a possível aplicação industrial de um sistema formado por esses componentes poderia estar comprometida pelo custo de produção das partículas. Enquanto que a proteína isolada de soja é um ingrediente barato, o custo das fibras mencionadas anteriormente é elevado. Desse modo, quanto mais fibra é adicionado no sistema mais caro pode ser o custo de produção das partículas.

[012] Desse modo, a inulina vem ganhando interesse dos consumidores, academia e indústria por ser uma fibra alimentar que apresenta efeitos prebióticos, além de ser versátil para uma ampla gama de aplicações farmacêuticas (MENSINK et al., 2015a). A inulina foi usada como agente encapsulante na técnica de secagem por atomização (BOTREL et al., 2014; PAIM et al., 2016; ZABOT et al., 2016). No entanto, o processo de atomização envolve a aplicação de temperaturas elevadas que poderiam prejudicar a estabilidade

oxidativa de compostos sensíveis ao calor, como os PUFAs presentes no óleo de peixe.

[013] Contudo, a quantidade de inulina para formar coacervados complexos com proteína isolada de soja é um fator que precisa ser determinado na tentativa de mostrar vantagens em relação à quantidade de outras fibras utilizadas para formar complexos com proteína isolada de soja, de modo que nas anterioridades, essa quantidade de fibra é muito elevada, inviabilizando a aplicação industrial de tais partículas.

[014] Outro aspecto considerado nesta invenção é a capacidade dos coacervados complexos de encapsular óleo de peixe; especificamente, é a primeira vez que o complexo inulina-proteína isolada de soja se utiliza como agente encapsulante pelo método de coacervação complexa. Portanto, um sistema formado por componentes de alto valor nutricional contendo proteína de alto valor biológico, fibra e ácidos graxos poli-insaturados, seria uma alternativa interessante para fortificar alimentos. Porém, em termos de aplicação em matrizes alimentícias, é necessário definir a estabilidade das partículas em termos de liberação controlada do ativo encapsulado, especialmente para aplicação de óleo de peixe em matrizes alimentícias, visando mascarar o sabor a peixe.

[015] Devido ao fato de que a coacervação complexa envolve interação eletrostática entre as proteínas e polissacarídeos, existe a possibilidade de serem dissociados em condições de pH e temperatura desfavoráveis (TIMILSENA et al., 2017). Porém, diversos autores sugerem que a instabilidade dos coacervados complexos pode ser resolvida com tratamentos como a reticulação das proteínas, baseado

nas ligações covalentes entre glutamina e lisina através de ligação isopeptídica. A transglutaminase é uma enzima de uso alimentar e é capaz de catalisar a reação entre glutamina e lisina nas proteínas (McKERCHAR et al., 2019), fornecendo resistência na formação de coacervados complexos (De CONTO et al., 2013, TIMILSENA et al., 2016).

[016] Em vista de todos os desenvolvimentos anteriores supracitados, o cerne da invenção foi estabelecer uma partícula altamente estável para encapsulação de óleo de peixe, com quantidade mínima de inulina, com o intuito de viabilizar a aplicação industrial das mesmas em produtos alimentícios.

Estado da técnica:

[017] Alguns documentos do estado da técnica descrevem processos e/ou métodos para obtenção de partículas alimentícias obtidas por coacervação complexa, nos quais ainda perduram algumas deficiências relativas a propriedade das partículas obtidas.

[018] No documento US4016098 revela-se um processo para produção de microcápsulas que compreende gotículas de óleo hidrofóbicas obtidas por coacervação complexa utilizando pelo menos dois coloides hidrofílicos com cargas elétricas opostas e sendo ionizáveis em água com pelo menos um dos coloides sendo gelificável, a melhoria compreendendo o endurecimento da parede de coacervados pela presença de um produto de oxidação de um polissacarídeo como agente endurecedor e ajustando o pH.

[019] Neste documento a inulina é citada como sendo um dos possíveis polissacarídeos cujos grupos alcoólicos

secundários podem ser oxidados em grupos aldeído, se enquadrando no grupo dos frutanos. Além disso, a faixa de pH da referida fase de endurecimento da microcápsula ocorre preferencialmente no pH 7, diferentemente da presente invenção que tem a formação ótima das partículas em pH 4,0-4,5.

[020] Percebe-se, portanto, que o documento supracitado indica que o polissacarídeo deve ser oxidado antes de formar complexos com proteínas, uma condição que não é comum no processo de encapsulação por coacervação complexa e que modifica a composição das partículas para encapsulação, algo que não ocorre na presente criação. O referido processo de oxidação é oneroso e requer um controle acurado para que o processo de endurecimento e consolidação das microcápsulas ocorre de forma adequada, o que não é necessário na presente invenção, devido ao fato de não existir uma etapa de oxidação e não modificar a composição da partículas, pois o endurecimento ocorre somente pela adição de uma quantidade específica de enzima transglutaminase.

[021] Em artigo de revisão publicado por Warnakulasuriya e Nickerson (2018) menciona-se que a inulina é capaz de formar gel com proteína de aveia, através de pontes de hidrogênio e interações hidrofóbicas, sugerindo-se o potencial da inulina de formar complexos com proteínas, visto que foram obtidas propriedades de gel aprimoradas com a adição de inulina em pequenas quantidades, provavelmente devido às nanopartículas de inulina espalhadas homogeneamente na rede de gel.

[022] O artigo supracitado sugere que um tratamento de 100°C por 30 min deve ser aplicado para formar complexos com proteínas, outra condição que não é comum em coacervação complexa. Nesse sentido, a aplicação de temperaturas elevadas para encapsulação de compostos sensíveis ao calor muito provavelmente seria crítica do ponto de vista da estabilidade oxidativa. Ou seja, de acordo com a presente invenção a principal vantagem seria a falta de aplicação de temperaturas elevadas para a encapsulação de compostos de interesse. Desse modo, a presente invenção demonstra que, além da inulina formar complexo com proteína isolada de soja em temperatura inferior a 100°C, existe a possibilidade de encapsular compostos de interesse como o óleo de peixe.

[023] No artigo reportado em Habibi et. al. (2016) descreve-se um processo de preparação de microcápsulas compreendendo óleo de peixe por meio de coacervação complexa de gelatina e goma arábica em razão 1:1 e sua utilização para fortalecer o suco de romã com as microcápsulas. Os valores máximos de eficiência de encapsulamento e porcentagem de óleo de microcápsulas foram encontrados para a formulação contendo 1,5% de encapsulantes e 3% de óleo de peixe. Os autores discorrem ainda sobre a atuação da gelatina como um policátion e a goma arábica para um poliânion, que são um dos principais encapsulantes para este fim, devido à sua disponibilidade, biodegradabilidade e compatibilidade com o encapsulamento de uma ampla gama de materiais bio ativos.

[024] O referido estudo compreende a aplicação das microcápsulas liofilizadas em suco de romã. Os resultados

mostram que a adição das microcápsulas ao suco de romã levou a uma diminuição considerável na aceitabilidade sensorial, e que 14% do óleo de peixe foi liberado das microcápsulas adicionadas no suco de romã no 42° dia de armazenamento a 4°C. Desse modo, é possível que a liberação do óleo de peixe das microcápsulas tenha comprometido a aceitabilidade do suco de romã. Assim, tanto os resultados de eficiência de encapsulação quanto à aceitabilidade sensorial, sugerem a importância de fornecer maior resistência aos coacervados complexos com tratamentos adicionais e com o desenvolvimento de composições específicas com maior eficiência de encapsulação, tais como as desenvolvidas na presente invenção, principalmente considerando diversos fatores, tais como os custos de produção.

[025] Ou seja, em vista de todos os desenvolvimentos do estado da técnica, identifica-se que a utilização de enzima transglutaminase para reticulação de uma partícula compreendendo proteína isolada de soja e uma quantidade mínima de inulina, promoveu uma alta eficiência de encapsulação, algo que não era esperado devido à baixa quantidade de inulina utilizada, de modo que um técnico no assunto não seria capaz de identificar tal efeito com os documentos e estudos já conhecidos, de acordo com as proporções específicas encontradas.

Breve descrição da invenção:

[026] A presente invenção refere-se a composição de partículas alimentícias obtidas por meio da técnica de coacervação complexa, compreendendo inulina e proteína isolada de soja ligadas, sendo que a inulina tem proporção

minoritária em relação à proteína de soja, promovendo a encapsulação de óleo de peixe com elevada eficiência de encapsulação e rendimento adequado. Além disso, a adição da enzima transglutaminase como agente reticulante de proteína fornece elevada retenção de óleo de peixe em condições de estresse ao pH e tratamento térmico. Ditas partículas apresentam características de ingrediente funcional fonte de proteína, fibra e ácidos graxos poli-insaturados e podem ser adequadas para matrizes alimentares que recebem tratamento térmico.

Breve descrição das figuras:

[027] Para auxiliar na identificação das principais características deste processo e material utilizado para o desenvolvimento de partículas alimentícias, são apresentadas as figuras às quais se faz referências, conforme se segue:

[028] Na Figura 1 apresenta-se um fluxograma de obtenção das partículas com a composição pretendida compreendendo as principais etapas para sua obtenção.

[029] Na Figura 2 ilustra-se a formação de partículas de proteína isolada de soja e inulina produzidas por coacervação complexa, representada pelo precipitado em solução aquosa (1).

[030] Na Figura 3 apresenta-se a superfície de resposta dos efeitos da razão inulina:proteína isolada de soja e quantidade de óleo de peixe, no rendimento do processo de obtenção das partículas.

[031] Na Figura 4 apresenta-se a comparação entre a retenção do óleo de peixe nas partículas de proteína isolada de soja e inulina sem (1) e com (2) reticulação com enzima

transglutaminase, em condições de estresse ao pH (5,5-6,5) e temperatura (Controle [25°C], 50, 75 e 100 °C)

[032] Na Figura 5 apresentam-se: a) micrografias a 10x de magnificação das partículas úmidas compreendendo a borda formada pela interação entre proteína isolada de soja e inulina (1) e o óleo de peixe encapsulado (2); (b) micrografias das partículas secas obtidas com aumento de 1500 vezes no Microscópio eletrônico de varredura (MEV).

Descrição detalhada da invenção:

[033] A presente invenção se refere a uma composição compreendendo proteína isolada de soja e inulina como encapsulantes, um óleo de peixe atuando como encapsulado e um agente reticulante; em que a razão inulina/proteína isolada de soja é menor do que 0,5; em que a concentração de óleo de peixe encapsulado é preferencialmente de 20% em relação à soma das massas de proteína isolada de soja e inulina; em que o agente reticulante é preferencialmente a enzima transglutaminase.

[034] Para cada componente na formação das partículas foram considerados os seguintes parâmetros preferenciais: concentração da proteína isolada de soja de 93%; grau de polimerização da inulina entre 10 e 60 resíduos de β-D-frutossilo; para a reticulação das partículas utiliza-se enzima transglutaminase, em concentração de 10 U/g proteína.

[035] As partículas são obtidas por um processo de coacervação complexa conforme as etapas:

A) Preparo de soluções aquosas de proteína isolada (p. ex. 2,5% p/v) de soja e inulina (p. ex. 1,5%-3,75%) em concentrações específicas;

B) Ajuste de pH da solução de proteína isolada de soja a pH a 8,0, com uso de solução alcalina;

C) Emulsão entre a solução de proteína isolada de soja e o ativo a ser encapsulado com uso de ultraturrax, preferencialmente a 14000 rpm por 2 minutos;

D) Mistura da emulsão com a solução de inulina a 40°C em constante agitação com uso de agitador magnético (preferencialmente a 400 rpm);

E) Ajuste de pH entre 4,0 e 4,5, com o uso de solução ácida;

F) Decantação das partículas (coacervados);

G) Drenagem com o uso de uma peneira micrométrica;

H) Opcionalmente, desidratação por técnicas de secagem por atomização ou liofilização.

[036] O processo de formação de partículas contendo óleo de peixe foi sujeito à otimização do rendimento do processo e a eficiência de encapsulação. Os fatores de estudo foram: razão inulina:proteína isolada de soja (0,5-1,5); e concentração de óleo de peixe adicionado em função da soma das massas de proteína isolada de soja e inulina (20-80%).

[037] Os resultados aqui apresentados demonstraram que as menores quantidades de inulina (razão inulina:proteína isolada de soja < 0,5) e de óleo de peixe (20%) apresentam maior eficiência de encapsulação. Além disso, a estabilidade das partículas relativas a retenção de óleo de peixe em condições de estresse ao pH e temperatura também foi avaliada.

[038] O rendimento do processo foi calculado a partir da quantidade de partículas coletadas após peneiramento em peneira micrométrica (63 µm). As partículas úmidas foram desidratadas a 100°C por 24 h, e o rendimento (%) foi

calculado pela quantidade de massa após desidratação em relação à quantidade de proteína, inulina e óleo de peixe, utilizados na produção de partículas (EQUAÇÃO 1):

$$\%Rendimento = \frac{\text{Partículas desidratadas (g)}}{\text{Massa inicial de proteína, inulina e óleo de peixe (g)}} \times 100$$

[039] A eficiência de encapsulação refere-se à quantidade de óleo encapsulado em relação à quantidade inicial de óleo adicionado na produção de partículas. O óleo contido nas partículas foi liberado adicionando uma solução alcalina para desestabilizar a interação entre os materiais da parede (inulina e proteína isolada de soja). Assim, 3% de solução de citrato de sódio (p/v) foi adicionada a 5 g de partículas (TELLO et al., 2015). Em seguida, a quantificação do óleo foi realizada de acordo com a metodologia de Bligh e Dyer (1959). Posteriormente, a eficiência de encapsulação (%) foi determinada pela EQUAÇÃO 2:

$$\%\text{Eficiência de encapsulação} = \frac{\text{Óleo total nas partículas (g)}}{\text{Óleo inicial (g)}} \times 100$$

[040] Utilizou-se um Delineamento Central Composto Rotacional (DCCR) de acordo com Saldaña et al. (2018) para otimizar o rendimento e a eficiência de encapsulação. Utilizou-se o delineamento fatorial 2^2, incluindo quatro ensaios nas pontos axiais e três repetições no ponto central, totalizando 11 ensaios (TABELA 1).

[041] Os resultados do DCCR foram ajustados para um modelo polinomial de segunda ordem e os coeficientes de regressão foram obtidos. Os modelos foram analisados pela ANOVA de uma via, e a falta de ajuste e o coeficiente de determinação (R^2) foram considerados para a subsequente

otimização do rendimento e eficiência de encapsulação. Os fatores não significativos na ANOVA (P < 0,05) foram excluídos do modelo e as condições ótimas foram determinadas pela metodologia de superfície de resposta. A análise dos dados foi realizada no software Statistica (versão 12.0, StatSoft, EUA). A verificação do modelo preditivo foi realizada produzindo as partículas otimizadas em triplicado.

[042] Os valores codificados e decodificados e os resultados experimentais do DCCR são mostrados na TABELA 1. Os resultados mostram que o maior rendimento (55,60%) foi obtido quando foi usada a menor quantidade de inulina (experimento 5) e o menor rendimento foi obtido em altas quantidades de inulina e óleo de peixe (experimento 4). Para a eficiência de encapsulação, o comportamento foi semelhante. A máxima eficiência de encapsulação (69,32%) foi obtida com uma baixa concentração de inulina, independentemente da concentração de óleo de peixe (experimento 3), enquanto a menor eficiência de encapsulação foi obtida com altas quantidades de inulina e óleo (experimento 4).

Experimento	**Valores codificados**		**Valores decodificados**		**Rendimento do processo (%)**	**Eficiência de encapsulação (%)**
	X1	X2	Inulina:proteína isolada de soja	% Oleo		
1	-1	-1	0,6	28,8	44,64	43,33
2	+1	-1	1,4	28,8	41,41	64,07
3	-1	+1	0,6	71,2	52,42	69,32
4	+1	+1	1,4	71,2	36,19	36,13
5	-1,41	0	0,5	50,0	55,60	63,34

6	+1,41	0	1,5	50,0	38,48	57,77
7	0	-1,41	1,0	20,0	40,31	65,47
8	0	+1,41	1,0	80,0	43,47	58,90
9	0	0	1,0	50,0	42,94	55,62
10	0	0	1,0	50,0	42,87	54,93
11	0	0	1,0	50,0	44,31	56,44

X1: razão inulina:proteína isolada de soja; X2: % óleo de peixe.

[043] As análises de variância (ANOVAs) para o rendimento eficiência de encapsulação são mostrados na TABELA 2. A otimização da eficiência de encapsulação foi descartada por falta de ajuste. O rendimento foi afetado principalmente pela quantidade de inulina adicionada. Considerando apenas os fatores significativos, o rendimento foi otimizado pelo seguinte modelo: %Rendimento = 70,0812 – 41,3168X_1 + 13,2401X_1^2 + 0,01X_1X_2 (R^2 = 0,9234). Este modelo foi representado na FIGURA 2, indicando que quanto menor a quantidade de inulina utilizada, maior o rendimento do processo.

Fator	Suma quadrática	Graus de liberdade	Quadrado médio	F calculado	*P* valor
%Rendimento					
X_1	234,3213	1	234,3213	355,4452	0,002802
X_1^2	13,3949	1	13,3949	20,3189	0,045857
X_2	6,1767	1	6,1767	9,3696	0,092205
X_2^2	6,7461	1	6,7461	10,2332	0,085390
X_1X_2	42,2500	1	42,2500	64,0896	0,015247
Falta de ajuste	10,3009	3	3,4336	5,2085	0,165283
Erro puro	1,3185	2	0,6592		
Total	320,2661	10			
%Eficiência de encapsulação					
X_1	52,8905	1	52,8905	92,558	0,010632
X_1^2	0,6690	1	0,6690	1,171	0,392357

X_2	15,8025	1	15,8025	27,654	0,034311
X_2^2	6,1757	1	6,1757	10,807	0,081392
X_1X_2	727,1112	1	727,1112	1272,434	0,000785
Falta de ajuste	141,4134	3	47,1378	82,490	0,012001
Erro puro	1,1429	2	0,5714		
Total	946,5788	10			

X1: razão inulina: proteína isolada de soja; X2: % óleo de peixe.

[044] De acordo com o gráfico de superfície de resposta para o rendimento, o óleo de peixe pode ser adicionado em qualquer faixa estudada se a quantidade de inulina for baixa. No entanto, isso pode comprometer a eficiência de encapsulação. Vários autores relatam que um excesso de material encapsulado diminui a eficiência de encapsulação (JUN-XIA et al., 2011; MENDANHA et al., 2009; NORI et al., 2011).

[045] Nesse sentido, de acordo com a FIGURA 2, as partículas otimizadas foram produzidas com a razão inulina:proteína isolada de soja = 0,4, ou seja, 40% de inulina em relação à quantidade de proteína isolada de soja; e 20% óleo de peixe. Assim, de acordo ao modelo matemático o rendimento foi 55,75%, mas o rendimento experimental (real) foi 60,74 ± 0,41%. Além disso, nas condições otimizadas, obteve-se uma alta eficiência de encapsulação (93,57 ± 1,48%). Portanto, de acordo aos resultados, as partículas devem ser produzidas preferivelmente a 1,0% inulina e 20% óleo de peixe.

[046] Além disso, a estabilidade das partículas foi avaliada pela retenção de óleo de peixe sob condições de estresse (pH e temperatura). Soluções aquosas de pH 5,5, 6,0 e 6,5 foram preparadas (10 mL cada solução). Em seguida, as partículas foram homogeneizadas nessas soluções (1:1 p/v) a

2400 rpm por 5 min usando vórtex, e levadas para banho-maria a 50, 75 e 100°C por 10 min, ou sem tratamento de temperatura (temperatura ambiente ou tratamento de controle, 25°C).

[047] Posteriormente, as partículas foram filtradas usando papel de filtro qualitativo (porosidade 15 µm) e enxaguadas com água deionizada e etanol absoluto. A estabilidade das partículas foi determinada em função do pH e da temperatura pelo método de Bligh e Dyer (1959). Previamente, o óleo foi liberado das partículas adicionando 3% de solução de citrato de sódio (p/v) (TELLO et al., 2015). A retenção de óleo foi calculada usando a EQUAÇÃO 3:

$$\%\text{Retenção de óleo} = \frac{\text{Óleo final nas partículas (g)}}{\text{Óleo inicial nas partículas (g)}} \times 100$$

[048] O teste foi realizado em duplicado e foi utilizado um delineamento inteiramente casualizado, com arranjo fatorial 3 x 4, considerando três níveis de pH (pH 5,5, 6,0 e 6,5) e quatro níveis de temperatura (controle [25°C], 50 ° C, 75 ° C e 100 ° C). Os dados foram analisados pela ANOVA e teste de Tukey com 5% de significância.

[049] Houve diferenças entre os valores de pH no tratamento controle, variando de 51,34% a 80,54% para os pH 6,0 e 5,5, respectivamente (FIGURA 3a). A temperatura diminuiu significativamente a retenção de óleo a pH 5,5 (~ 34,33% a 36,62%), enquanto que a pH 6,0 aumentou significativamente a 100°C (72,24 ± 4,45%) (FIGURA 3a). Pathak et al. (2017) relatam que o aumento da solubilidade dos biopolímeros induzidos pela temperatura diminui a eficiência da formação de coacervados complexos. Da mesma forma, Jaramillo, Roberts e Coupland (2011) observaram que

a solubilidade da proteína isolada de soja aumenta a partir do pH 5,0 e, com tratamento térmico (90°C/30 min), o comportamento foi semelhante.

[050] Por outro lado, a solubilidade da inulina aumenta linearmente com a temperatura (MENSINK et al., 2015b). Portanto, a diminuição da retenção de óleo pode estar relacionada à solubilidade do complexo proteína isolada de soja-inulina nos pHs e temperaturas testados. No entanto, a 100°C, pode ser observado um aumento na retenção de óleo nos pHs 6,0 e 6,5. Jaramillo et al. (2011) relataram que o complexo proteína isolada de soja-pectina é altamente solúvel em pH 4-5, mas que em pH 6-7 a solubilidade com o tratamento térmico diminui. Esse fenômeno também pode estar relacionado ao observado nesta proposta de ingrediente funcional.

[051] Como a retenção de óleo de peixe foi baixa em algumas condições específicas de estresse (FIGURA 3a), tratamentos adicionais devem ser aplicados para proporcionar melhor resistência às partículas. Por esse motivo, a enzima transglutaminase foi usada como agente de reticulação na produção das partículas. O parâmetro utilizado para obter a máxima eficiência de encapsulação foi 10 U de transglutaminase por grama de proteína. A transglutaminase foi diluída em 100 mL de água deionizada em pH 4,0, e foi adicionada após ajuste de pH (pH 4,0-4,5) na produção das partículas, deixando reagir por 5 a 10 min.

[052] Foi observado que os tratamentos com e sem reticulação apresentaram diferenças significativas, de acordo a teste t-Student ($P < 0,05$). Os valores de retenção

de óleo foram de 91,32 ± 4,23% e 49,15 ± 13,74% para partículas reticuladas e não reticuladas, respectivamente. O tratamento com transglutaminase manteve acima de 81% a retenção de óleo, com efeitos insignificantes do pH e da temperatura (FIGURA 3b).

[053] Morfologicamente, as partículas úmidas apresentaram formas arredondadas a irregulares, com presença de óleo no interior e algumas aglomerações. O perímetro é bem marcado e com tamanho aproximado menor de 100 µm (FIGURA 4a). Por outro lado, as partículas desidratadas (liofilizadas) apresentam a mesma configuração das partículas úmidas (FIGURA 4b), mas o tamanho diminuiu, o qual pode estar relacionado com a liberação de água devido à desidratação.

[054] Em suma, os resultados apensos revelaram que as partículas apresentam elevada eficiência de encapsulação (~94%) e rendimento de processo adequado (~61%) quando utilizados baixas quantidades de inulina e óleo de peixe, algo não evidenciado pelo estado da técnica. Além disso, as partículas mostram-se resistentes à liberação do ativo encapsulado em condições de estresse ao pH e tratamento térmico, quando usado enzima transglutaminase comparado às partículas sem reticulação. As partículas podem ser adequadas para matrizes alimentares com a faixa de pH avaliada nesta proposta e que recebem tratamento térmico.

[055] Os versados na arte valorizarão os conhecimentos aqui apresentados e poderão reproduzir a invenção nas modalidades apresentadas e em outras variantes, abrangidas no escopo das reivindicações anexas.

REIVINDICAÇÕES

1) COMPOSIÇÃO DE PARTÍCULAS ALIMENTÍCIAS obtidas por meio de um processo de coacervação complexa preferencialmente em faixas de pH de 4,0 a 4,5 **caracterizada por** compreender proteína isolada de soja e inulina como encapsulantes, um óleo de peixe atuando como encapsulado e um agente reticulante;

em que a razão inulina/proteína isolada de soja é igual ou menor do que 0,4;

em que a concentração de óleo de peixe encapsulado é preferencialmente de 20% em relação a soma das massas de proteína isolada de soja e inulina;

em que o agente reticulante é preferencialmente a enzima transglutaminase.

2) COMPOSIÇÃO, de acordo com a reivindicação 1, **caracterizado** pelo fato de a concentração de proteína isolada de soja ser preferencialmente de 93%;

3) COMPOSIÇÃO, de acordo com a reivindicação 1, **caracterizado** pelo fato de o grau de polimerização da inulina ser preferencialmente entre 10 e 60 resíduos de β-D-frutossilo;

4) COMPOSIÇÃO, de acordo com a reivindicação 1, **caracterizado** pelo fato de a enzima transglutaminase estar preferencialmente na concentração de 10 U/g de proteína.

5) COMPOSIÇÃO, de acordo com a reivindicação 1, **caracterizado** pelo fato de a eficiência de encapsulação ser de pelo menos 94%.

6) COMPOSIÇÃO, de acordo com a reivindicação 1, **caracterizado** pelo fato de o rendimento de obtenção ser de pelo menos 61%.

7) USO DA COMPOSIÇÃO ALIMENTÍCIA, conforme definida nas reivindicações de 1 a 5, **caracterizada** pelo fato de ser na preparação de matrizes alimentares nutricionalmente fortificadas, que recebem tratamento térmico e tem faixa de pH entre 5,5 e 6,5.

8) USO DA COMPOSIÇÃO ALIMENTÍCIA, de acordo com a reivindicação 6, **caracterizada** pelo fato de ser preferencialmente na preparação de produtos cárneos atuando como substituto de gordura.

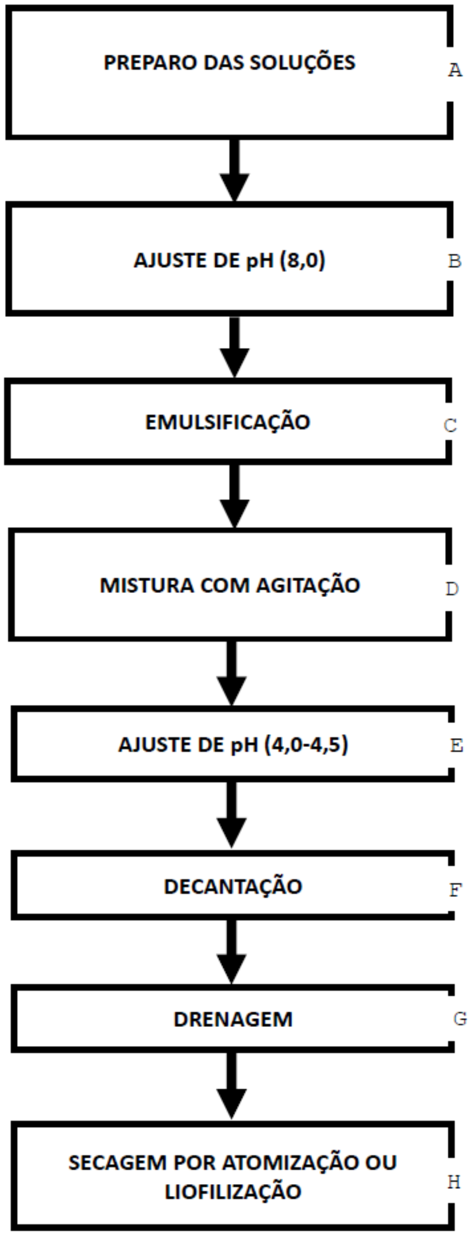
PREPARO DAS SOLUÇÕES
A
AJUSTE DE pH (8,0)
B
EMULSIFICAÇÃO
C
MISTURA COM AGITAÇÃO
D
AJUSTE DE pH (4,0-4,5)
E
DECANTAÇÃO
F
DRENAGEM
G
SECAGEM POR ATOMIZAÇÃO OU LIOFILIZAÇÃO
H

Figura 1

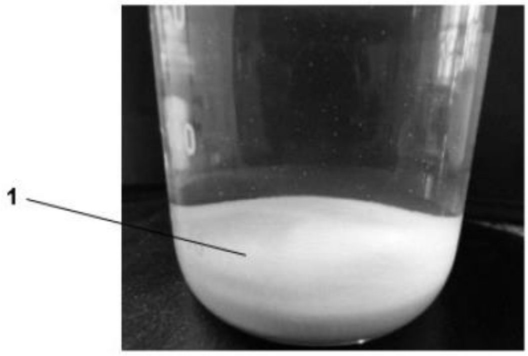

Figura 2

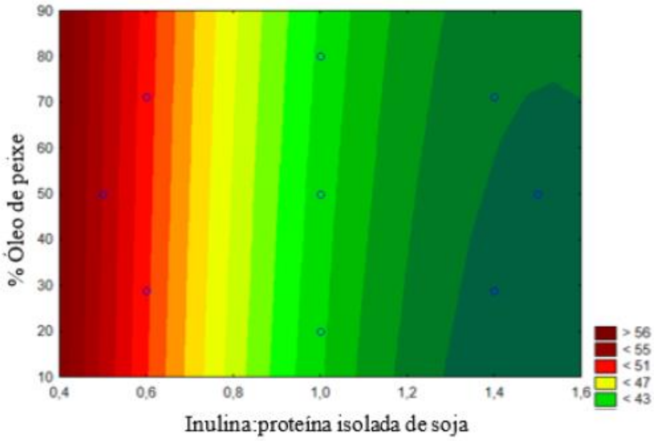

Figura 3

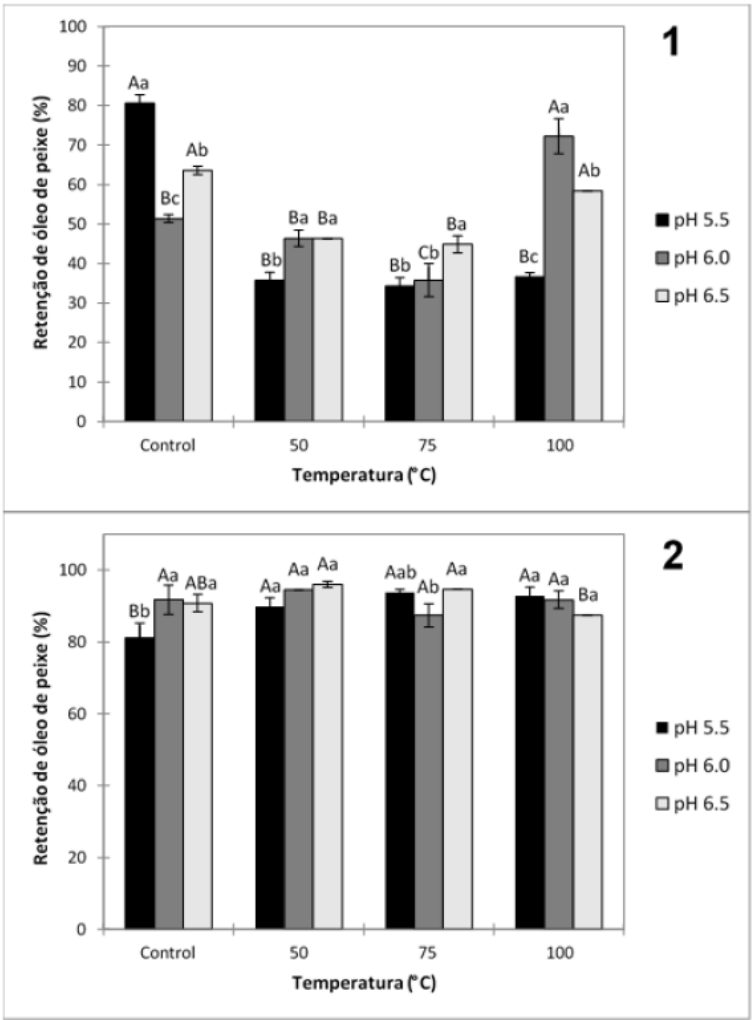
1
Retenção de óleo de peixe (%)
100
90
80
70
60
50
40
30
20
10
0
Aa
Bc
Ab
Bb
Ba
Ba
Bb
Cb
Ba
Bc
Aa
Ab
Control
50
75
100
Temperatura (°C)
pH 5.5
pH 6.0
pH 6.5
2
Retenção de óleo de peixe (%)
100
80
60
40
20
0
Bb
Aa
ABa
Aa
Aa
Aa
Aab
Ab
Aa
Aa
Aa
Ba
Control
50
75
100
Temperatura (°C)
pH 5.5
pH 6.0
pH 6.5

Figura 4

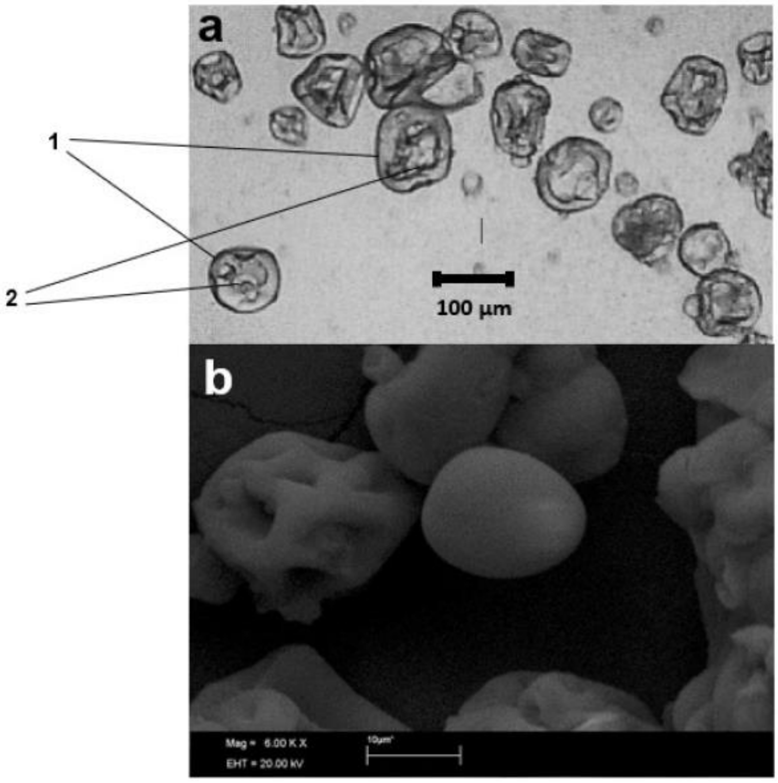

Figura 5

RESUMO

COMPOSIÇÃO DE PARTÍCULAS ALIMENTÍCIAS PARA ENCAPSULAMENTO DE ÓLEO DE PEIXE E SEU USO

A presente invenção refere-se a composição de partículas alimentícias obtidas por meio da técnica de coacervação complexa, compreendendo inulina e proteína isolada de soja ligadas, sendo que a inulina tem proporção minoritária em relação a proteína de soja, promovendo a encapsulação de óleo de peixe com elevada eficiência de encapsulação e rendimento adequado. Além disso, a adição da enzima transglutaminase como agente reticulante de proteína fornece elevada retenção de óleo de peixe em condições de estresse ao pH e tratamento térmico. Ditas partículas apresentam características de ingrediente funcional fonte de proteína, fibra e ácidos graxos poli-insaturados e podem ser adequadas para matrizes alimentares que recebem tratamento térmico.

INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

15/01/2020 870200006760
12:19
29409161913044199

Outras petições

Número do Processo: BR 10 2019 024837 8

Dados do Depositante (71)

Depositante 1 de 1

Nome ou Razão Social: UNIVERSIDADE DE SÃO PAULO - USP
Tipo de Pessoa: Pessoa Jurídica
CPF/CNPJ: 63025530000104
Nacionalidade: Brasileira
Qualificação Jurídica: Instituição de Ensino e Pesquisa
Endereço: Rua da Reitoria, 374 - Butantã
Cidade: São Paulo
Estado: SP
CEP: 05508220
País: Brasil
Telefone: (11) 3091.4474
Fax:
Email: pidireto@usp.br

Referência Petição

Pedido : BR102019024837-8

Documentos anexados

Tipo Anexo	Nome
Comprovante de pagamento	GRU - Complemento BR 10 2019 024837-8.pdf
Esclarecimento	Esclarecimento complemento de documentos BR 10 2019 024837-8.pdf
Autorizações dos inventores	Autorizações Inventores BR 10 2019 024837-8.pdf
Decl. div. não prejudicial	Declaração de Divulgação não prejudicial BR 10 2019 024837-8.pdf

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 15/01/2020 às 12:19, Petição 870200006760

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

[bb.com.br] - Boleto gerado pelo sistema MPAG. 27/11/2019 09:02:41

INSTRUÇÕES:

A data de vencimento não prevalece sobre o prazo legal. O pagamento deve ser efetuado antes do protocolo. Órgãos públicos que utilizam o sistema SIAFI devem utilizar o número da GRU no campo Número de Referência na emissão do pagamento. Processo: 1020190248378 Serviço: 260-Outras petições

Clique aqui e pague este boleto através do Auto Atendimento Pessoa Física.
Clique aqui e pague este boleto através do Auto Atendimento Pessoa Jurídica.

Recibo do Pagador

BANCO DO BRASIL | **001-9** | **00190.00009 02940.916196 13044.199175 1 81150000003600**

Nome do Pagador/CPF/CNPJ/Endereço
UNIVERSIDADE DE SAO PAULO USP CPF/CNPJ: 63025530000104
RUA DA REITORIA 374 BUTANTA, SAO PAULO -SP CEP:05508220
Sacador/Avalista

Nosso-Número	Nr. Documento	Data de Vencimento	Valor do Documento	(=) Valor Pago
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Nome do Beneficiário/CPF/CNPJ/Endereço
INSTITUTO NACIONAL DA PROPRIEDADE INDUST CPF/CNPJ: 42.521.088/0001-37
RUA MAYRINK VEIGA 9 24 ANDAR ED WHITE MARTINS , RIO DE JANEIRO - RJ CEP: 20090910

Agência/Código do Beneficiário
2234-9 / 333028-1

Autenticação Mecânica

BANCO DO BRASIL | **001-9** | **00190.00009 02940.916196 13044.199175 1 81150000003600**

Local de Pagamento
PAGÁVEL EM QUALQUER BANCO ATÉ O VENCIMENTO

Data de Vencimento
26/12/2019

Nome do Beneficiário/CPF/CNPJ
INSTITUTO NACIONAL DA PROPRIEDADE INDUST CPF/CNPJ: 42.521.088/0001-37

Agência/Código do Beneficiário
2234-9 / 333028-1

Data do Documento	Nr. Documento	Espécie DOC	Aceite	Data do Processamento	Nosso-Número
27/11/2019	29409161913044199	DS	N	27/11/2019	29409161913044199
Uso do Banco	**Carteira**	**Espécie**	**Quantidade**	**xValor**	**(=) Valor do Documento**
29409161913044199	17	R$			36,00

Informações de Responsabilidade do Beneficiário
A data de vencimento não prevalece sobre o prazo legal.
O pagamento deve ser efetuado antes do protocolo.
Órgãos públicos que utilizam o sistema SIAFI devem utilizar o número da GRU n
o campo Número de Referência na emissão do pagamento.
Processo: 1020190248378
Serviço: 260-Outras petições

(-) Desconto/Abatimento

(+) Juros/Multa

(=) Valor Cobrado

Nome do Pagador/CPF/CNPJ/Endereço
UNIVERSIDADE DE SAO PAULO USP CPF/CNPJ: 63025530000104
RUA DA REITORIA 374 BUTANTA,
SAO PAULO-SP CEP:05508220
Sacador/Avalista

Código de Baixa
Autenticação Mecânica - **Ficha de Compensação**

Visualização de arquivos

Auto-Atendimento
Comprovante – Arquivo

Agência débito:	1897-X
Conta débito:	139531-9
CPF/CNPJ:	63025530/0001-04 USPBO26122019142322704001LD470

Documento empresa:	201905951220
Data vencimento:	26/12/2019
Data pagamento:	26/12/2019
Valor pagamento:	36,00
Documento banco:	
Desconto:	0,00
Linha digitável:	00190.00009 02940.916196 13044.199175 1 81150000003600
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Acréscimo:	0,00
CNPJ sacado:	00000000388068
Nome sacado:	Banco do Brasil S/A
CNPJ cedente:	63025530000104
Nome cedente:	UNIVERSIDADE DE SAO PAULO
Autenticacão:	E4F5E51B4FB7F0EB

ESCLARECIMENTOS

Depositante: UNIVERSIDADE DE SÃO PAULO - USP

Número pedido: BR 10 2019 024837-8

Data de depósito: 25/11/2019

Para que possa ser complementado o depósito do pedido em referência, são apresentados em anexo os documentos de autorizações dos inventores e declaração de divulgação não prejudicial.

Assim sendo, a Depositante solicita respeitosamente seu aceite juntamente com os demais documentos apresentados na data do depósito.

São Paulo, 15 de Janeiro de 2020.

MARIA APARECIDA DE SOUZA
Procuradora
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AUTORIZAÇÃO DO INVENTOR

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Piracicaba, 10 de janeiro de 2020.

JUAN DARIO RIOS MERA

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Piracicaba, 10 de Janeiro de 2020.

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Piracicaba, 10 de Janeiro de 2020.

CARMEN JOSEFINA CONTRERAS CASTILLO

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DECLARAÇÃO

Eu, JUAN DARIO RIOS MERA, peruano, solteiro, engenheiro, portador do RG nº , CPF nº , residente e domiciliado à Rua leão XIII, 428, Piracicaba - SP, CEP: 13418-110, abaixo assinado, na qualidade de inventor(a), declaro para os devidos fins e efeitos dos incisos I, II e III do artigo 12 da Lei da Propriedade Industrial - LPI Nº 9.279 de 14.05.96 e de acordo com os itens 2; 2.1; 2.2 e 2.3 das Instruções Normativas - INPI Nºs 30 e 31 de 04.12.13 que, em 23/11/2018, houve a divulgação de parte do conteúdo na forma de resumo em congresso, correspondente ao título **"OPTIMIZATION AND PH- AND TEMPERATURE-STABILITY OF THE COMPLEX COACERVATION BETWEEN SOY PROTEIN ISOLATE AND INULIN CONTAINING FISH OIL"**, conforme anexo, o qual originou o pedido de patente de invenção, ora depositado sob o título **"COMPOSIÇÃO DE PARTÍCULAS ALIMENTÍCIAS PARA ENCAPSULAMENTO DE ÓLEO DE PEIXE E SEU USO"**.

Piracicaba, 10 de janeiro de 2020.

JUAN DARIO RIOS MERA

≡ ICBC 2018

Optimization and pH- and temperature-stability of the complex coacervation between soy protein isolate and inulin containing fish oil

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Abstract

Fish oil is an abundant source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, these bioactive compounds are quickly oxidized, causing loss of their functional value. To protect them, microencapsulation by complex coacervation can be applied. On the other hand, the application of microparticles in food matrices is a critical process, factors such as pH or heat treatment can be detrimental in terms of controlled release of the active encapsulated. The objectives of this study were to optimize the fish oil microencapsulation by complex coacervation, using soy protein isolate (SPI) and inulin (Inu); and to determine the effect of pH and temperature on the oil retention (%OR) in the microparticles. The coacervation between the biopolymer solutions was carried out at pH 4.0, then, the process yield (%PY) and the encapsulation efficiency (%EE) were determined. The optimized microparticles were visualized by optical microscopy. A central composite rotatable design was applied, having as variables the Inu:SPI ratio (0.5-1.5) and the amount of oil added (20%-80%). The results were analyzed through ANOVA, and the optimal conditions were estimated by regression analysis and response surface methodology. For the stability of the microparticles, three pH (5.5, 6.0, 6.5) and four temperature (Control, 50°C, 75°C, 100°C) levels were studied. Subsequently, the determination of %OR was performed. The results of %PY show that the maximum (55.60%) and the minimum (36.19%) %PY were obtained at Inu:SPI=0.5 and 80% oil, respectively. For %EE, the maximum (69.32%) and the minimum (36.13%) %EE were obtained at Inu:SPI=0.6 and 80% oil, respectively. In ANOVA, it was observed that the data of %EE presented significant lack of fit, discarding the optimization of %EE. The R2 for the %YP was 0.9234, and the response surfaces indicate that, the lower the amount of Inu used, the higher the %PY. In this regard, the optimized microparticles were produced at Inu:SPI=0.4 and 20% oil; then, %YP was 60.74±0.41% and %EE was 93.57±1.48%, and their morphology show oval and spherical shapes of various sizes. For %OR, there were differences between the pH values, ranging from 51.34% to 80.54% for pHs 6.0 and 5.5, respectively. The temperature significantly decreased the %OR at pH 5.5 (~34.33%-36.62%), while at pH 6.0 the %OR was significantly increased at 100°C (72.24±4.45%). The results of %YP and %EE could be dependent on some properties of Inu, such as the degree of polymerization (DP); that is, the greater the DP, the greater the interaction sites between Inu and SPI. For %OR, pH values above the pH of the coacervation (pH 4.0) and the temperature could decrease the electrostatic interaction between the biopolymers. However, other types of interactions may govern, such as hydrogen bonds at pH 5.5, and hydrophobic interactions induced by temperature at pH 6.0. Furthermore, the optimization of the complex coacervation was mainly dependent on the amount of Inu. On the other hand, the pH and temperature affected the %OR, which is an indicator to give better resistance to microparticles with additional treatments, such as the cross-linking.

Institutions

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Keywords

PUFAs

EPA and DHA

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