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**EXTENDERS DEVELOPED THROUGH THERMOPLASTIC EXTRUSION AND  
APPLICATION ON A RESTRUCTURED CHICKEN MEAT PRODUCT  
(HAMBURGER)**

**DESENVOLVIMENTO DE EXTENSORES ATRAVÉS DA EXTRUSÃO  
TERMOPLÁSTICA E SUA APLICAÇÃO EM PRODUTO CÁRNEO  
REESTRUTURADO (HAMBÚRGUER) DE FRANGO**

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Extenders developed through thermoplastic extrusion and application on a restructured chicken meat product (hamburger)

Desenvolvimento de extensores através da extrusão termoplástica e sua aplicação em produto cárneo reestruturado (hambúrguer) de frango

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*Quando o cara é do BEM...*

*...TUDO dá certo!*

*Andrei Ferreira de Araújo Lima*

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## RESUMO

Com as exigências atuais dos consumidores, novas opções de produtos cárneos que poderiam trazer benefícios à saúde precisam ser desenvolvidas, bem como trazer praticidade e longa vida de prateleira. Na industrialização de tais produtos, extensores são utilizados para gerar benefícios tecnológicos e o formato de pré-mistura pode resultar numa nova tendência de mercado. Assim, o objetivo deste trabalho foi desenvolver extensores proteicos a partir do glúten vital (VG) e de proteínas vegetais (soja, ervilha e grão-de-bico) processadas em extrusora dupla-rosca a 20% de umidade, avaliando-os conforme suas características tecnológicas e físico-químicas, sendo os resultados examinados com Análise de variância e Scott-Knott. Na sequência, as proteínas extrusadas e o VG foram aplicados (4%) como extensores em diferentes formulações (F1 – adição de isolado proteico de soja extrusado (SPIE); F2 – adição de concentrado proteico de ervilha extrusado (PPCE); F3 – adição de farinha de grão-de-bico extrusado (CPFE) e F4 – adição de VG) de produto cárneo tipo hambúrguer produzido com carne de frango liofilizada e no formato de pré-mistura. Uma amostra controle (CO) sem adição de extensor também foi elaborada e todos os ingredientes adicionados nas formulações foram em pó, havendo hidratação prévia à cocção e ao consumo do produto. Também foram realizadas análises físico-químicas e tecnológicas nas formulações do produto desenvolvido, finalizando com avaliações sensoriais (*Napping*, aceitação e *Check-All-That-Apply* (CATA)). Com a extrusão, notou-se o escurecimento das proteínas, provavelmente pelas reações de *Maillard*, sendo o menor valor de  $L^*$  ( $P < 0,05$ ) atribuído a PPCE; já a proteína de soja teve sua capacidade de hidratação em água e sua capacidade de absorção de óleo reduzidas ( $P < 0,05$ ), possuindo o maior resultado para este último; a capacidade emulsificante de todos os extrusados proteicos foi reduzida e a proteína de ervilha apresentou os menores valores antes e após o processo; VG teve a maior capacidade espumante ( $P < 0,05$ ) sem passar pela extrusão e a CPFE anulou essa propriedade com o processamento. As pré-misturas do hambúrguer apresentaram coloração amarelada e alta luminosidade ( $L^* > 75$ ), variando os valores de pH (5,83 – 6,04) dependendo da proteína extensora adicionada. A análise de atividade de água mostrou resultados inferiores a 0,25 para todas as formulações ( $P > 0,05$ ), apresentando a capacidade deste produto ser estável durante o armazenamento. A adição das proteínas vegetais extrusadas reduziu o índice de absorção de água quando comparadas com CO. O rendimento e encolhimento após o cozimento não foram afetados ( $P > 0,05$ ). Os avaliadores conseguiram diferenciar as formulações dos produtos com o teste *Napping*, sugerindo semelhanças entre F1 e F4; também atribuíram altas notas à F3 e F4 em relação à aceitabilidade, enquanto a análise de CATA tornou possível descrever os produtos através de 12 atributos significativos. Os resultados obtidos indicaram que um extensor substituto da soja deve ser escolhido de acordo com as necessidades do alimento no qual será aplicado, enquanto o produto produzido como uma pré-mistura foi desenvolvido com sucesso, possuindo alto aporte proteico, sem conservantes ou gordura adicionada, contribuindo como opção de alimento saudável, prático e estável.

**Palavras-chave:** leguminosas, proteínas, liofilização e análise sensorial

## ABSTRACT

With the current demands of consumers, new options for meat products that could bring health benefits need to be developed as well as bring practicality and long shelf life. In the industrialization of such products, extenders are used in order to generate technological benefits and the premix format may result in a new market trend. Thus, the objective of this work was to develop protein extenders from vital gluten (VG) and plant proteins (soy, pea, and chickpea) processed in a twin-screw extruder with 20% moisture, evaluating them according to their technological and physicochemical characteristics, and the results were evaluated with Analysis of Variance and Scott -Knott. In the sequence, extruded plant proteins and VG were applied (4%) as extenders in different formulations (F1 - addition of extruded soybean protein isolate (SPIE), F2 - addition of extruded pea protein concentrate (PPCE), F3 - addition of extruded chickpea flour (CPFE), and F4 - addition of vital gluten) of hamburger produced with freeze-dried chicken meat and having the form of premix. A control sample (CO) without extender incorporation was also elaborated, all added ingredients in the formulations were powdered, and there was previous hydration for cooking and consumption of the product. Physical-chemical and technological analyzes were also carried out in formulations of the developed product, ending with sensorial evaluations (Napping, acceptance, and Check-All-That-Apply (CATA)). With the extrusion, was observed protein darkening, probably by Maillard reactions, being the lowest value of  $L^*$  ( $P < 0.05$ ) attributed to PPCE; the soybean protein had its water hydration capacity and its oil absorption capacity decreased ( $P < 0.05$ ), with the highest result for the last one; the emulsifying capacity of all the protein extrudates was reduced and the pea protein showed the lowest values before and after the process; VG had the highest foaming capacity ( $P < 0.05$ ) without going through extrusion and CPFE annulled this property with the processing. The premixes showed yellowish color and high luminosity ( $L^* > 75$ ), varying the pH values (5.83 – 6.04) depending on the added extender protein. The water activity analysis showed values lower than 0.25 for all formulations ( $P < 0.05$ ), displaying a capacity of being stable during the storage. The incorporation of the extruded plant proteins reduced the water absorption index when compared to CO. The yield and shrinkage cooking were not affected ( $P > 0.05$ ). The assessors were able to differentiate the formulations of the products submitted to the Napping test, suggesting similarities between F1 and F4; also assigned high acceptability scores to F3 and F4, while the CATA analysis made it possible to describe the products through 12 significant attributes. The results indicated that a soybean substitute should be chosen according to the needs of the food system in which it will be applied, whereas the product produced as a premix has been successfully developed, having a high protein content, no chemical additives or fat, contributing as an option of healthy, practical, and stable food.

**Keywords:** legume, proteins, freeze-drying, and sensory analysis



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## **- CAPÍTULO 1 -**

### **Introdução Geral**

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## 1. INTRODUÇÃO GERAL

O maior acesso à informação, escassez de tempo e a procura por alimentos de rápido e fácil preparo traz exigências mais específicas sobre o alimento que os consumidores atuais procuram e preocupação com os possíveis problemas de saúde provenientes dos mesmos (WEISS et al., 2010). Desse modo, a principal busca está orientada em ingredientes que satisfaçam os requerimentos de proteína na nutrição humana, mantendo propriedades funcionais como capacidade de retenção de água, capacidade de formar géis e emulsões, que no processo de fabricação gerem o mínimo de resíduos para o meio ambiente e que sejam obtidos com o mínimo de custo (PÉREZ, MOLINA e VALENCIA, 2011; OLIVEIRA et al., 2013).

Embora as proteínas vegetais possuem qualidade nutricional e disponibilidade inferiores às proteínas animais (CAKMAK et al., 2016), leguminosas possuem alto valor nutricional e são utilizadas como ingrediente alternativo em novos produtos. A decisão de englobar ingredientes alternativos quando um novo produto é desenvolvido, que tragam ganhos econômicos ao fabricante, deve considerar a opinião do consumidor, além de não interferirem na saúde do mesmo e na qualidade do produto (VICTORINO, 2009). Proteínas vegetais tem um preço menor do que as proteínas de origem animal, como ocorre com o aumento da aceitação da proteína texturizada de soja e, conseqüentemente, podem reduzir o custo de produtos cárneos (SINGH et al., 2008).

Conhecidas como proteínas não cárneas, extensores ou substitutos de gordura em produtos cárneos emulsionados, as proteínas de origem vegetal vêm sendo estudadas nas suas mais diversas aplicações (YUN-SANG et al., 2009). Além de visar a diminuição de perdas durante o cozimento, esses ingredientes visam melhorar a capacidade emulsificante, a estabilidade da emulsão, a retenção de água, o valor nutritivo e as características de fatiamento (CORREIA, MITTAL e OSBORNE, 1991). Os benefícios tecnológicos produzidos dos extensores estão relacionados com as propriedades funcionais das proteínas, portanto, quanto maior o espectro destas propriedades, melhor será a proteína extensora (OLIVO e SHIMOKOMAKI, 2001).

A texturização através de extrusão com alta temperatura tem sido usada na conversão de proteínas vegetais em produtos funcionais, principalmente para aplicação como suplementos ou extensores em sistemas alimentares cárneos (HARPER, 1989). A maioria dos processos e produtos desenvolvidos sobre a texturização de proteínas vegetais tem sido

reportados para a soja (farinha ou concentrados proteicos), apresentando características úteis como textura e sabor semelhantes à carne e uma afinidade por gordura e água, servindo como carreadores de minerais, vitaminas e corantes (WANG, BHIRUD e TYLER, 1999).

O hambúrguer, o empanado e a almôndega se tornaram opções crescentes para as famílias devido ao pouco tempo de preparo (OLIVEIRA et al., 2013), logo, esses produtos precisam de alta qualidade e a carne de frango possui características nutricionais que a tornam a melhor escolha para dietas saudáveis (RIOVANTO et al., 2012; LI et al., 2016), podendo ser aplicada como matéria-prima principal desses produtos. A carne de frango é muito consumida, mas altamente perecível a contaminantes bacterianos devido a sua composição, alta atividade de água e alto pH final, limitando a vida de prateleira dos seus produtos (PETROU et al., 2012). Dessa maneira, o desenvolvimento de tecnologias de barreira efetivas que estendam a vida útil e mantenham a qualidade dos produtos durante longos períodos é a principal tarefa da indústria de produtos processados de frango (ZOUAGHI e CANTALLEJO, 2016), e a liofilização é um exemplo deste tipo de tecnologia. Tal processo traz vantagens para os consumidores, pois mantém 10 - 15% do peso original dos alimentos (ADAMS, 2004), economizando espaço para guardá-lo, agilizando o processo de reidratação (MARQUES e COSTA, 2015), podendo ser transportados e armazenados sob temperaturas ambientes por um longo período (ULLAH et al., 2017) e consumidos com mínima preparação (NOBRE e LIMA, 2011).

Os alimentos completamente desidratados através da liofilização são uma opção e solução para a falta de tempo e comodidade da vida atual, se tornando uma tendência global (MARQUES e COSTA, 2015), possuem uma longa vida de prateleira adicionando-se uma embalagem à vácuo e evitando luz durante o armazenamento, podendo ser mantidos em temperatura ambiente durante meses sem deterioração (MA et al., 2018). Esse processo produz alterações mínimas nos alimentos quanto aos aspectos nutricionais e sensoriais, pois retém 80% dos compostos voláteis e aroma, já que a água não contém estes constituintes (FELLOWS, 2006).

Outras técnicas de conservação associadas com o uso do frio, são largamente utilizadas em países desenvolvidos devido à manutenção da qualidade do produto. É um processo caro porque deve-se manter baixas temperaturas desde a produção até o consumo, obedecendo à chamada cadeia do frio (GAVA, SILVA e FRIAS, 2009). Esse método, de modo geral, apenas reduz reações químicas, enzimáticas e o crescimento microbiológico, não melhora a qualidade dos produtos, ou seja, apenas tecidos sadios e de qualidade devem ser refrigerados,

pois a temperatura baixa apenas diminui a atividade do patógeno (ORDÓÑEZ-PEREDA et al., 2005).

A ineficiência da indústria de alimentos na manutenção da cadeia do frio e a falta de informações sobre os procedimentos corretos para adequá-la à realidade industrial brasileira tornam as perdas econômicas significativas (NANTES e MACHADO, 2005), além disso, os custos de armazenagem e de distribuição são cerca de 30% maiores quando comparados a uma operação envolvendo produtos secos (BORRÉ e AGITO, 2005). Como consequência, a cadeia do frio é quebrada, prejudicando a qualidade e a inocuidade do alimento (PINTO e MORAIS, 2000).

Visando obter produtos que atendam à necessidade diária de nutrientes, práticos no preparo e que possam ser armazenados sob temperatura ambiente em comparação com os existentes atualmente no mercado, o objetivo deste trabalho foi desenvolver extensores proteicos através da extrusão termoplástica de proteínas vegetais e, posteriormente, aplicá-los em um produto cárneo reestruturado (hambúrguer) de frango de preparo rápido (pré-mistura). Não são observados relatos na literatura científica sobre este modo de aplicação, reforçando a importância desta pesquisa no meio científico e industrial, pois buscamos um substituto para a proteína de soja já utilizada comumente como extensor.

## **2. OBJETIVOS**

### **2.1 Objetivo Geral**

O objetivo do trabalho foi avaliar e caracterizar extensores alternativos desenvolvidos através da extrusão termoplástica de proteínas vegetais (isolado proteico de soja, concentrado proteico de ervilha e farinha de grão-de-bico), juntamente com o glúten vital não extrusado, e avaliar a aplicação desses extensores como ingrediente no produto cárneo tipo hambúrguer produzido como uma pré-mistura, sendo de preparo rápido e armazenamento sob temperatura ambiente.

### **2.2 Objetivos Específicos**

- Caracterizar e avaliar as principais matérias-primas do estudo (proteínas vegetais das leguminosas, glúten vital e carne liofilizada de frango) quanto as suas propriedades tecnológicas e físico-químicas;

- Produzir por extrusão termoplástica e avaliar físico-química e tecnologicamente os extensores proteicos, comparando com a proteína de soja comumente utilizada como extensor, a fim de encontrar um possível substituto;
- Desenvolver e avaliar as características físico-químicas e tecnológicas do produto cárneo tipo hambúrguer de frango em todas as suas formas (pré-mistura, hidratado pré e pós-cozção);
- Avaliar as características sensoriais através de duas técnicas inovadoras (*Napping* e CATA (*check-all-that-apply*)), além de testar a aceitação do produto cárneo desenvolvido.

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**- CAPÍTULO 2 -**

**Reformulations of poultry meat products with the use of functional vegetable  
ingredients – A review**

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Este capítulo foi submetido à revista *Trends in Food Science & Technology* (Anexo I).

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## Reformulations of poultry meat products with the use of functional vegetable ingredients – A review

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### Abstract

*Background:* Restructured **meat products** are associated with high fat and additive contents, and **chicken meat** is an option for the food industry to reduce this negative opinion, due to positive aspects as **healthy**, lower price, the absence of a cultural or religious effect, and sustainability.

*Scope and approach:* In this review, we have presented advances in the quality of restructured poultry meat products, with emphasis on the use of novel extenders as pea, chickpea, and gluten rather than soy protein which has been recognized by its numerous drawbacks, including allergic factors.

*Key findings and conclusions:* Since the plant protein sources can present anti-nutrients, which must be inactivated by **thermal processes**, the extrusion process under high temperature and pressure has been used and presented promising results, improving the possibilities of applying **plant proteins** as binders for poultry products.

**Keywords:** Meat products; Chicken meat; Healthy; Thermal processes; Plant proteins

## **1. Introduction**

Nowadays, poultry meat is the highest contributor to the animal protein supply for human consumption (Day, 2016), which may have variations in its composition by numerous interacting aspects including genetics, feeding, slaughter operations, chilling, processing, and a storage state (Milicevic et al., 2015).

While other meats are associated with an unhealthy nutritional profile as high fat, additives, and sodium chloride contents in meat products (Olmedilla-Alonso, Jiménez-Colmenero, & Sanchez-Muniz, 2013), the chicken meat gains space in the production of processed products, such as hamburgers, for having a pleasant flavor and a greater appeal of health and sustainability (Longato et al., 2017), which will be discussed in this review.

Despite the strong commercial appeal, the restructured meat products are products with high water activity and vulnerable to lipid oxidation, which can impair the sensorial acceptance by the consumer, therefore, antioxidants, dyes, and salt are used in order to improve the storage stability (Karre, Lopez, & Getty; Petracci, Bianchi, Mudalal, & Cavani, 2013).

Following tendencies for clean label and healthy products, the proposals to improve the quality of processed meat products are in the substitution of additives, salt, and fat reduction, and one of the strategies is the use of natural ingredients with great variations in the functional properties (the approach of this term is just from a technological viewpoint in this article) (Asgar et al., 2010; Correia, Mittal, & Osborne, 1991). Among them are the plant proteins, which have already been used in products as meat analogues and have good sensory acceptance

Therefore, the objective of this review paper is to present research to improve the healthiness of restructured poultry meat products with the use of protein ingredients.

## **2. Poultry meat market and nutritional composition**

Regarding chicken production, Brazil currently ranks second in the world ranking, with an average volume of around 12.82 million tons in the last five years, while the United States of America occupies the first position (USDA/ABPA, 2018). As can be seen in Table 1, the market shows stability in the last five years, which indicates that the sector is well organized and it may be the time for production and consumption expansions.

**Table 1.** Annual report on consumption, production, and export of Brazilian chicken meat. Source: ABPA (2018)

Year	Consumption per capita (kg/hab)	Production (million tons)	Exports	
			Volume (thousand tons)	Income (millions US\$)
2013	41.80	12.31	3,892.0	7,967.0
2014	42.78	12.69	4,099.0	8,085.0
2015	43.25	13.14	4,304.0	7,168.0
2016	41.10	12.90	4,384.0	6,848.0
2017	42.07	13.05	4,320.0	7,236.0

As the American population prefers to consume more chicken than other meats (beef and pork), some explanations can be made:

a) the healthful and beneficial concepts of poultry products, which have proteins of high biological value, as well as other meats, but they stand out for the lower fat (Table 2) and cholesterol contents (FAO, 2015);

b) As they are animals of average weight 6.3 kg, ready for slaughter in 47 days, they are more suitable for the industrial processing (National Chicken Council, 2018);

c) minor price contrasted with red meats (prime beef (tenderloin) costs, on average, R\$ 49.47/ kg, while the chicken meat (breast) costs R\$ 12.74/ kg (Caterwings, 2017));

d) non-appearance of cultural or religious effect (Cavani, Petracci, Trocino, & Xiccato, 2009), e.g., Indians do not eat beef and Muslims do not eat pork;

e) the technological aspects that satisfy the consumer are: poultry meat is more favorable for processing in comparison with other types of meats because it has a neutral flavor, consistent and good texture, and light color, which allow producers to impart desired flavor profiles (i.e. spicy vs. mild flavor) and textures according to market needs and consumers targeting (i.e. adult vs. children) (Barbut, 2012) and;

f) chicken farming presents greater sustainability, using lower volumes of water (3,900 L/kg) than cattle (15,500 L/kg) and pig (4,800 L/kg) for producing meat (Hoekstra et al, 2011).

**Table 2.** Average proximate composition (g/100 g) of lean beef and skinless chicken meat. Source: FAO (2015)

Product	Moisture	Protein	Fat	Ash
Skinless chicken	75.0	22.8	0.9	1.2
Lean beef	75.0	22.3	1.8	1.2

The consumption of animal fat has been replaced by vegetable fat due to the health appeal, since the vegetables have linoleic (18:2n-6, AL) and alpha-linolenic acids (18:3n-3, AAL) which are polyunsaturated fatty acids (PUFA) and required to maintain cell membranes, brains functions, nerve impulse transmission under normal conditions (Youdim, Martin, & Joseph, 2000), to transfer of atmospheric oxygen to blood plasma, hemoglobin synthesis, and cellular division, and are called essential because they are not synthesized by the organism (Yehuda, Rabinovitz, Carasso, & Mostofsky, 2002). Although popularly unknown, it is already known that chicken meat (breast, skinless, cooked) has a high concentration of PUFAs (0.6 g/ 100 g chicken) compared to red meat (beef, tenderloin, fat-free, grilled) (0.1 g/ 100 g beef). Following the comparison, only arachidonic polyunsaturated fatty acid is found in higher concentration in beef (0.01 g/ 100 g) than in chicken (0.005 g/ 100 g), while linoleic, linolenic, and timnodonic (more commonly known as eicosapentaenoic acid) acids are in higher concentrations on the chicken meat (0.53 g, 0.02 g, and 0.05 g per 100 g, respectively) compared to beef (0.07 g, 0.01 g, and lower than 0.01 g per 100 g, respectively) (TACO, 2011).

The nutritional benefits of chicken meat go far beyond the high availability of proteins and low-fat content, as they have PUFAs and low cholesterol (89 mg/ 100 g) (TACO, 2011). Furthermore, the anatomical localization and hemoglobin content define two chicken meat types: white meats (tender and breast fillet, lower hemoglobin content) having low-fat and high-protein content, and dark meats (drumstick and thigh, more hemoglobin content) have higher fat content (Bianchi et al., 2009). Due to the low iron content found in these white meats (0.3 mg per 100 g) (TACO, 2011), its consumption should be promoted through the dissemination of this data, since people with diseases related to excessive iron consumption, known as hemochromatosis (Bacon et al., 2011), can benefit from the intake of this meat type.

### **3. Restructured poultry meat products**

The term restructured is indicated for products which have been partially or completely comminuted (subdivided by mechanical means) and reconstituted, as examples, one can cite burgers, meatballs, steaks, and nuggets (Rocha et al., 2010). The selection of meat type for these products depends on their final products, with white meats having soft texture, light color, and commonly dedicated to producing premium meat products, while dark meats have darker color, harder texture, and stronger flavor, generally used to optimize the costs of production due to their lower economic value (Bianchi et al., 2009).

According to Brazilian regulatory instruction no. 20, of July 31<sup>st</sup>, 2000 (Brasil, 2000), hamburger is understood as the "industrialized meat product obtained from ground beef from butchered animals, added or not to adipose tissue and ingredients, molded and subjected to the appropriate technological process". It is a raw, semi-fried, fried, frozen or chilled product. Among the optional ingredients, the addition of intentional additives, the maximum addition of 4% of the non-meat protein in the aggregate form and the maximum addition of 30% mechanically separated meat (exclusively in a cooked burger) is allowed. Brazilian legislation requires a minimum percentage of protein (15 %) and a maximum of carbohydrates (3 %), the percentage of fat (23 %), and calcium content (0.1 %) (raw hamburger) and 0.45 % (cooked hamburger) on the dry basis.

Table 3 shows the occurrence of some developed studies looking for healthier hamburgers. One of the major problems encountered is in lipid oxidation usually found in chicken-based products as result of a high degree of lipid polyunsaturation that accelerates this process leading to the deterioration of flavor, color, texture, nutritional value of meat (Amensour et al., 2015), and can cause losses in its storage time. For this, the industry has used synthetic phenolic preservatives such as butylated hydroxyanisole (BHA), tertiary-butylhydroquinone (TBHQ), propyl gallate (PG), and butylated hydroxytoluene (BHT) are commonly allowed in various countries for being applied in meat processing, but the worry about the health exposure associated with daily consumption of these chemical elements are getting attention in the investigation for natural options (Rather et al., 2016), as examples shown in Table 3:

- Exchange of the meat type, the beef being replaced by poultry (Huber et al., 2016; Longato et al.; Pereira et al., 2017);
- Application of natural antioxidants (Trindade et al., 2010), and;
- Reduction/replacement of fat in meat products (Bastos et al., 2014).

The papers (Table 3) presented hamburgers elaborated within conventional methods of production and conservation, with stability studies carried out in the research of Trindade et al. (2010), Longato et al. (2017), and Pereira et al. (2017), however, microbiological evaluations were not mentioned. Studies of the economic viability of added ingredients and/or used as a substitute were not performed and, although some ingredients such as banana peel and pulp, amaranth and pumpkin flours from their seeds, oregano and rosemary extracts were prepared, others were commercially selected, such as: apple fiber (Vitacel® AF 401), bamboo fiber

(Vitacel® BAF 200), pea fiber (Vitacel® EF 150), oat fiber (Vitacel® HF 200), potato fiber (Vitacel® KF 200), and wheat fiber (WF Vitacel®, 200), indicating a great diversity of carbohydrate sources ingredients that present limits of use in meat products.

The sensory evaluations of beef and chicken burgers were executed by Bastos et al. (2014), Huber et al. (2016), and Longato et al. (2017). They made tests such as acceptability with nine and seven points hedonic scales with consumers and quantitative descriptive analysis (QDA) with experienced panelists. However, most innovative evaluations were not carried out, such as those cited by Varela & Ares (2014): flash-profile, check-all-that-apply, projective mapping, and napping, which could be faster and give better information about the analyzed products. The applied tests have limitations for evaluation, as an example, the acceptance test using the seven points hedonic scale, since there is scientific evidence that the assessors do not use the extremities, there would be only five points left to evaluate the samples, being no effective as the nine points scales, as well as, longer scales tend to be more discriminating than shorter scales (Jones, Peryam, & Thurstone, 1955).

**Table 3.** Formulations, obtaining processes, and effects of alternative ingredients in hamburgers of chicken meat or beef

Reference	Goal	Formulations	Methodology	Effects
<b>TRINDADE et al., 2010</b>	To evaluate the effects of rosemary and oregano extracts as natural antioxidants against changes on fatty acid profile and lipid oxidation caused by irradiation process intentionally used in beef hamburgers	Beef (70g/100g); bovine fat (20 g/100g); iced water (8 g/10 g); salt (2 g/100g)	Control and 6 formulations with different combinations of antioxidants; rosemary (400 and 200 mg/kg); oregano (400 and 200 mg/kg)	The highest antioxidant capacity after lipid oxidation acceleration process by ionizing radiation was obtained from rosemary extract, however, this effect decreased with the irradiation intensity and during the storage time (90 days)
<b>BASTOS et al., 2014</b>	To develop a type of beef hamburger with the replacement of fat by oatmeal flour, green banana pulp flour, green banana peel flour, apple peel flour, and green banana pulp and to evaluate its physical characteristics and sensory acceptance	Beef (79 to 88 %); salt (1.5%); cold mineral water (10.5 %); added fat (9 %)	Conventional formulation, with (F1) and without (F2) fat, and experimental formulations with total replacement of fat (3%): oatmeal flour (F3), flour of green banana pulp (F4), flour of green banana peel (F5), flour of apple peel (F6), pulp of green banana (F7)	F4, F5, and F7 showed the highest-rated burgers in terms of water-holding capacity, yield, shrinkage and shear force, whereas F3, F4, and F5 greatest received scores of the sensory acceptance test suggest that these formulations can be used for total fat-substitution in a beef burger (3% of a substitute was used)
<b>HUBER et al., 2016</b>	To evaluate the technological, physicochemical and sensorial parameters of vegetable fibers application for total fat replacing in chicken burgers	Chicken breast (74.5%); chicken skin (10%); water (10%); salt (2%); textured soy protein (3%); sodium tripolyphosphate (0.2%); blend of spices (pepper, onion and garlic; 0.26%); sodium erythorbate (0.04%);	Control and a formulation with the replacement of the total chicken skin by a hydrated mix of vegetable fibers (bamboo, pea, wheat, potato, and oat)	Regarding color, all fibers could be used in chicken meat products, since they have light tonality. Bamboo, wheat and oat fibers showed the best emulsifying properties. The formulation containing the mix of 0.40% bamboo fiber, 1.60% wheat fiber and 1.60% pea fiber had the highest sensorial acceptance, besides presenting a higher tenderness compared to the control sample
<b>LONGATO et al., 2017</b>	To evaluate the effects of adding different levels of amaranth grains and pumpkin seeds on chicken burgers' quality properties (physicochemical, cooking, oxidation and sensorial properties) during storage	Chicken meat (breast;72%); pork backfat (28%); water/ice (18%); sodium chloride (1%)	Control, 1 and 2% ground amaranth, 1 and 2% ground pumpkin seeds	Amaranth and pumpkin seeds used in the manufacturing of chicken burgers improved their cooking characteristics, as well as lipid stability during storage when amaranth was added, and antioxidant properties of raw burgers when pumpkin seeds were added. The impact of these ingredients on sensory quality attributes of chicken burgers was not significant



<b>PEREIRA et al., 2017</b>	To determine the effects of adding rosemary lyophilized extract in chicken burgers, trying to avoid the lipid oxidation during cold storage (4°C)	6 kg of chicken meat (10% breast meat, 55% thigh meat and 15% fat from chicken skin); iced water (13.8%); hydrated textured soy protein (0.9%); isolated soy protein (1%); salt (1.4%); curing salts (0.5%); ground white pepper (0.2%); garlic powder (0.1%); aroma of onion (0.1%); cassava starch (2%)	Basic formulation divided into 3 lots: control without antioxidant addition (T1), adding butylated hydroxytoluene at a concentration of 0.01% (T2) and adding rosemary lyophilized extract at a concentration of 0.02% (T3)	Rosemary lyophilized extract had strong anti-oxidative effects in chicken burgers, probably due to the high antioxidant activity of the phenolic compounds (acid gallic, catechin, p-coumaric acid, ferulic acid, p-cinnamic acid, rutin, quercetin, and kaempferol) combined with storage in a refrigerated temperature. The rosemary extract provides good alternatives for consumer demand for healthy meat products
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In the meat industry, sodium sources are recognized by the use of sodium chloride or phosphate salts, which have antimicrobial activity, but with different technological functions. The sodium chloride improves water holding capacity and the texture by solubilization/extraction of the salt-soluble myofibrillar proteins in raw meat, promoting the taste, and complementing the flavors of the meat. In poultry meat formulations, a salt concentration is utilized in the range of 1.0% and 1.6%, however, Petracci, Rimini, Mulder, & Cavani (2013) consider that a concentration of 0.5% of sodium chloride may already be sufficient for poultry meat. Phosphate salts are used essentially in seafood and meat and are found in different chemical forms (pyrophosphates, tripolyphosphates, orthophosphates, polyphosphates) chosen according to the desired function in the final product as buffering agents, antioxidant, and water-binding (Feiner, 2006).

Brazilian legislation (Brazil, 2007) limits the use of 0.5% phosphate salts in meat products, since higher concentrations may impair the absorption of calcium and magnesium, resulting in an imbalance of these minerals and affecting the solidity of bones (Durand, 2002). These two salts (sodium and phosphate) are linked to the increase of the sodium content in processed meats, especially the emulsified ones, becoming a stimulus to reduce them through the use of natural ingredients like pectin, carrageen, wheat bran, waxy maize starch (Fellendorf, O'Sullivan, & Kerry, 2016), and yeast extracts (Santos, Campagnol, Morgano, & Pollonio, 2014).

Another category of functional ingredients involves a variety of compounds of distinct origins like binders (high-protein content able to bind fat and water), extenders (non-meat ingredients with great protein content), and fillers (plant substances with high carbohydrate content) (Petracci, Bianchi, Mudalal, & Cavani, 2013).

However, several studies have proposed that additives can contribute to harmful effects in the body and, because of increasing consumer concern about health and food safety, the industry desire to reduce their use in food products (Fernandes et al., 2015). Thus, many studies are carried out to identify natural alternatives for the substitution of synthetic additives or in combination with synthetic compounds, promoting the reduction of degenerative and inflammatory diseases risk (Hayes et al., 2011). As examples, Table 3 already showed this tendency to look for new options in order to reduce possible health problems caused by exaggerated absorption of sodium and phosphate salts, fat, preservatives, and stabilizers. This reduction or elimination is involved with the use of the term “clean label”, which is often

described as a decreasing of ingredients on labels, moreover, following actions of greater health and sustainability appeal, the use of vegetal ingredients with different functions in meat products has been investigated, mainly when used as binders or extenders.

#### **4. Meat extenders**

A meat extender is a product or a combination of ingredients such as fibers, gums, carrageenan, among others, which are part of the meat formulation, with the aim of reducing fat, protein, and salt, to obtain more economic products (Deng, Bhaduri, Ghatak, & Navder, 2011). In addition to minimizing losses during cooking, these ingredients aim to improve the emulsifying capacity, emulsion stability, water retention, nutritive value, and slicing characteristics (Correia, Mittal, & Osborne, 1991).

Table 3 shows the use of different types of fiber, but the use of proteins is limited to soybean derivatives, which has been used in concentrations of 2 to 4% according to the current legislation for each type of meat product. In Table 4, it is possible to verify the amino acid composition of the plant proteins compared to the daily amounts recommended by WHO/FAO (2007).

The physicochemical properties such as amino acid sequence and composition, size, shape, hydrophilicity/ hydrophobicity ratio, net charge, and load distribution, flexibility, primary, secondary, and tertiary structures, among others, can influence the performance of proteins in food systems. Moreover, protein interactions with other components within a food system like lipids, sugars, salts, polysaccharides, and others, promote changes in its properties (Ustunol, 2014).

Binders and extenders from protein origin when applied to meat products increase water retention since restructured products totally manufactured with comminuted meats tend to have an approximate shrinkage of 20% and weight loss 30% (Bastos et al., 2014), which increases the product cost to the consumer.

Habitually, the main sources of raw material for most commercially textured protein ingredients have been soy flour and soy protein concentrates. However, wheat gluten and other plant proteins can constitute different products and provide a wide range of textured vegetable protein as ingredients that can be used as meat extenders and meat analogues (Strahm, 2006). the main results of using these vegetable proteins and others in processed poultry products will be described in the next section.

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## 5. Soy

The functional properties from soy protein (foaming, water and fat binding, gelation, and emulsification) made this the most attractive plant protein source to perform as an ingredient in formulated foods (Day, 2016), due to its protein-water interaction which raise the viscosity and generate a gel matrix during warming (Moure et al., 2006), categorized as flours, concentrates, and isolates on the basis of their protein content in dry matter (50, 70, and 90%, respectively) as well as textured materials. Soy flour has less protein content (50%) and it could also improve the water binding capacity depending on the commercial forms (natural or full fat, defatted, and lecithinated soy flours), while soy isolates are mainly applied to increment the protein level, optimize the cost (by substitution the meat in emulsified and restructured meat products), reinforce the fat emulsification capacity and texture profile, and increase moisture retention (more juiciness and higher yield for products) (Feiner, 2006).

Protein concentrates can retain a high fiber content in the seed, which is positive in soybean used as meat analogues in the human meal (Rodrigues, Coelho, & Carvalho, 2012). In addition, soy protein concentrates are the most common ingredient applied as meat analogue or extender and was the pioneer in the restructuring process through extrusion (Stanley, 1989), producing a protein in its textured form that is available as flakes (< 2 mm), in a chopped shape (> 2 mm), and in the form of larger pieces (15 to 20 mm), which can absorb water 2.5 to 5 times the original weight (Riaz, 2004).

Although widely used and widespread in numerous foods, the application of soybeans, whether as flour or protein, textured or not, in chicken meat products is not commonly presented and scientifically discussed. Rare are the studies found, but Yeater, Casco, Miller, and Alvarado (2017) studied applications of 10, 20, 30, and 40% of textured soybean flour or textured soybean protein concentrate in chicken nuggets. The researchers observed that, compared to control formulations, there were reductions in losses during cooking and oil absorption during the product frying process, however, the technological benefits achieved in this study are unlikely to serve as a parameter to be followed, since the concentrations are well above the existing permissions in Brazilian legislation (2 to 4%).

The soy presents antinutritional factors as saponins and phytates, which interfere with the digestibility, absorption or utilization of nutrients and, if ingested in high concentrations, can have harmful effects on health (Benevides et al., 2011). Some of the effects are reduced biological availability of essential amino acids and minerals, causing irritations and lesions on

the gastrointestinal mucosa, interfering with the selectivity and efficiency of biological processes (Sgarbieri, 1987). Besides, isoflavones, saponins, and lipoxygenase generate astringent, bitter, bean, and grass off-flavors, respectively, associated with the application of soy proteins (Okubo et al., 1992). Moreover, there is a causative allergen known as 2S globulin, which is not very thermodesnaturalizable; and the trypsin inhibitor that also possesses an allergenic power. This intolerance poses a serious problem because soy concentrates were suggested to substitute milk proteins in the case of milk intolerance. Frequently, soy proteins constitute a “hidden antigen”, since they are incorporated into an ample quantity of food products (delicatessen, bakery, cooked foods, food stuffing, etc.) (Albert, 1979 apud Cheftel, Cuq, & Lorient, 1989).

Protein denaturation during extrusion decreases solubility, digestibility, and inactivates the antinutritional factors of soybean. In addition, the extrusion reduces the undesirable volatile compounds and the bitter taste related to this protein (Areas, 1992).

Currently, most soybean production has genetic modification (Costa et al., 2011), which represents a genetic transformation in the productive process, where the plant undergoes a modification in its structure and this gene change seeks to strengthen it against the undue actions of pests and make them resistant to the herbicides, related to the term transgenic or genetically modified (GM) (Fuscaldi, Medeiros, & Pantoja, 2011). The lack of knowledge about the influences that GM soy can cause to health and the environment leads to many problems since research on the subject is low and the advertisements encourage consumption and advocate as a healthy diet (Gavioli & Nunes, 2015). Several countries are conniving to the use of GM soy, whereas others, such as Australia and Europe, show great resistance towards GM soy proteins (Feiner, 2006).

The most widely used extenders are derived from soybeans (Feiner, 2006), however, due to the presence of many off-flavors (Okubo et al., 1992), antinutritional factors (Costa et al., 2011), allergic reactions (Albert, 1979 apud Cheftel, Cuq, & Lorient, 1989), and the modified genetics (Gavioli & Nunes, 2015), other options of protein not classified as major allergenic or obtained by non-genetically modified organism represent good alternatives. Proteins acquired by other sources as wheat gluten (Asgar et al., 2010) can represent an option since soy has been submitted to these judgments.

## 6. Wheat gluten

Another large plant protein source with economic importance is wheat gluten, a co-product in the recovery of wheat starch in the wet processing of wheat flour (Wang et al., 2006). Among cereals and other plant proteins, wheat gluten has a single ability to form a cohesive mix and other plant properties once it is plasticized. Gluten itself is not exactly the protein found in flour, but rather a blend of proteins stored in grains, such as barley, rye, and wheat (Balakireva & Zamyatin, 2016) which are present either as monomers or chains linked together by disulfide bonds, forming polymers (Wrigley & Bietz, 1988). It is subdivided into two similar arrangement based on their extractability (gliadin and glutenin) in aqueous alcohols (Singh & MacRitchie, 2001) and they are part of nearly 80% of the protein content in wheat seed (Shewry & Tatham, 1990).

The use of vital wheat gluten as a nutrient supplement, dough strengthener, formulation aid, thickener, processing aid, a surface finishing agent, texturizing agent, and the stabilizer is approved by Food and Drug Administration agency (Foster, 2006). Thus, in practice, the term gluten refers to proteins which have the role of conferring on the mass its capacity for viscosity, water absorption, elasticity, and cohesiveness (Wrigley & Bietz, 1988). The interaction between these proteins, mainly glutenins and gliadins, give the viscoelasticity of gluten. When hydrated and in its pure form, glutenin exhibits a rubbery and tough texture, while gliadin becomes extremely thick and offers minor resistance to extension (Foster, 2006).

When heated above 85°C, the hydrated gluten mass coagulates irreversibly without loss of its uncommon structural chain producing a moist, resilient, firm, and nonsticky gel (Kalin, 1979). These thermosetting properties of hydrated gluten make glutenin an option for seafood, poultry, and meat applications (Foster, 2006), is often used in processed meat products (Xiong et al., 2008), as extenders and analogue products (Orcutt et al., 2006).

However, one of the leading barriers for large use in food processing is the water insolubility of wheat gluten (Kong, Zhou, & Qian, 2007) and, although it can restrain the feasibility as a protein additive in meat products due to its impossibility of retaining water and reducing losses through cooking, a limited enzymatical hydrolysis by many commercially available proteases (Kong, Zhou, & Qian, 2007) increases gluten solubility (Agyare, Xiong, & Addo, 2008).

**Table 4.** Essential amino acids composition of textured soy protein, dried pea, chickpea flour, gluten, and the daily requirements of adults

Amino acids	Textured soy protein <sup>1</sup>	Dried pea <sup>2</sup>	Chickpea flour <sup>3</sup>	Gluten <sup>4</sup>	Adults <sup>5</sup>
Histidine <sup>#</sup>	29.8	11.7	33.0	41.0	15.0
Isoleucine	34.8	19.3	60.0	41.0	30.0
Leucine	76.7	33.3	100.0	72.0	59.0
Lysine	86.7	35.3	85.0	16.0	45.0
Methionine	18.7	11.5	21.0	56.0	22.0
Phenylalanine	96.8	36.2	79.0	95.0	38.0
Threonine	40.8	18.4	47.0	26.0	23.0
Tryptophan	n.d.*	4.28	n.d.*	n.d.*	6.0
Valine	36.5	21.6	57.0	49.0	39.0
<b>Total Essential</b>	420.9	191.6	482.0	396.0	277.0

<sup>1</sup> Pires et al., 2006 (mg/g protein); <sup>2</sup> Miranda & Viana, 2017 (mg/g protein); <sup>3</sup> Sánchez-Vioque et al., 1999 (mg/g protein);

<sup>4</sup> Rombouts et al., 2009 (mg/g protein); <sup>5</sup> WHO/FAO standard (2007): daily requirements of teenagers and adults (mg/g protein); <sup>#</sup> Essential amino acids only for children; \*n.d.: not determined.

Commercially, texturized wheat gluten manufactured in the extrusion process is available in distinct shapes, densities, sizes, textures, and colors; and it becomes popular. Furthermore, researchers aim to maintain technological properties of wheat varieties while developing options with a minimum amount of proteins that cause celiac disease (Hamer, 2010). In the extrusion process, wheat gluten can be applied in a blend of soy flours or soy concentrate or alone to develop extenders (Riaz, 2004), this association presents the nutritional benefit in relation to essential amino acids by complementing the deficiencies found for each one, since vital gluten does not meet the daily requirements of lysine in an adult's diet, soy has a concentration five times higher than this amino acid, on the other hand, gluten has a concentration three times higher than soybean over methionine (Table 4).

However, gluten presents limitations in relation to its application since it is the originator of the autoimmune disorder known as a celiac disease with a large part of the current population presenting such syndrome (Parzanese et al., 2017) and allergic reactions. In this way, other proteins appear as a choice, such as pea (Strahm, 2006) and chickpea (Serdaroglu, Yildiz-Turp, & Abrodimov, 2005), which will be discussed below, although they do not yet have commercial use of them in poultry meat products.

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## 7. Pea

The protein content of fresh peas is 6%, while the dry value increases to 22% (Food Ingredients Brasil, 2012). The content of the amino acid lysine present makes peas a good complement to cereals in nutritional terms (Oliveira et al., 2011). This non-oleaginous legume is rich in minerals such as potassium (330 mg/ 100 g cooked pea) and sodium (110 mg/ 100 g cooked pea), followed by phosphorus (68 mg/ 100 g cooked pea) and calcium (37 mg/ 100 g cooked pea), as well as carbohydrates, vitamins (B complex is the main) and protein (TCA, 2017).

In peas, the presence of legumitin (classified as albumin) and legumin (classified as globulin) is observed. While albumin has a solubility in water and coagulation by the action of heat, which distinguishes it from all other proteins, the globulins are practically insoluble in water (Food Ingredients Brasil, 2014).

The presence of antinutritional factors in the pea, such as stachyose, tannins, and phytic acid, which bind to proteins and some minerals, inhibiting their bioavailability (Canniatti-Brazaca, 2006), brings the need for processes to reduce these compounds, such as thermoplastic extrusion, already performed in some research. Beck, Knoerzer, and Arcot (2017) tested the effects of low moisture extrusion with pea isolated protein (81.5% protein on dry basis) on the molecular weight distribution, secondary structure, expansion, and solubility. Of the three major protein fractions (legumin, vicilin, and convicilin), the only legumin was altered by aggregation or proteolysis during extrusion process under the investigated conditions.

Osen et al. (2015) inspected the protein-protein interactions, amino acid composition, and molecular weight distribution of three pea protein isolates before and after extrusion. No amino acid losses or the formation of peptide bridges were observed in the extrudates. In controversy to findings in other studies, the authors believe in the importance of disulfide bonds as major participants compared to non-covalent interactions that remained unchanged during the process. In another study, the structure of the extruded pea was more influenced by the maximum temperature of the extruder cylinder than by the size of the particles or the origin of the peas, with higher temperatures modifying the flow profile of the melt (Osen et al., 2014).

In the literature, there are reports of the use of peas through the application of their fibers in chicken burgers in conjunction with bamboo and wheat fibers (Huber et al. (2016), however, yield studies and cooking losses were not performed, besides not having the application of the pea alone in some formulation. In addition, Pietrasik and Janz (2010) applied pea flour, fiber,



and starch in low-fat Bologna, but they did not observe differences between the formulations regarding the cooking losses comparing to control sample, thus, these ingredients can be promising for meat industry.

Although on the market is already possible to find options of food extenders using pea protein, as can be seen in the brands Axiom Foods and Roquette Nutralys® and pea protein isolates are suitable for being applied in processed meat products since they are soluble proteins with adequate water binding, emulsifying and gelling properties, it is still not traditional in flavor as soy proteins (Petracci, Bianchi, Mudalal, & Cavani, 2013) and it is not common in scientific studies to apply pea protein to chicken products in order to evaluate their technological qualities. Therefore, other additional alternatives to pea are proposed in this review as chickpea.

## **8. Chickpea**

Chickpea has been considered nutritionally better than other vegetables. It has become a functional food and the identification of bioactive compounds have exhibited useful effects as hypocholesterolemic and antioxidant activity (Yust et al., 2012). This legume can prevent type 2 diabetes, digestive tract diseases or cancer, and may be of great interest to the food industry. Most of these effects were attributed to non-protein components such as phytosterols, fiber, carotenoids, or starch, amylose (Jukanti et al., 2012).

Chickpeas are a source of protein (23%), carbohydrates (63.5%), minerals and vitamins, digestible fibers,  $\beta$ -carotene and health-promoting fatty acids (Knights & Robson, 2016), having carbohydrates and proteins as main components, constituting approximately 80% of the dry matter (Chibbar, Ambigaipalan, & Hoover, 2010).

Chickpea has not obtained recognition like other cereals such as wheat and barley, however, it presents functional and beneficial effects on human health (Jukanti et al., 2012). For example, when consumed (200 g/ day), this legume produces butyrate, a single-chain fatty acid (Fernando et al., 2010) which has been reported as a suppressor of cell proliferation and inducer of apoptosis, which may minimize the cancer risk (Mathers, 2002). Evaluating six varieties of chickpeas, Boye, Zare, and Pletch (2010) reported protein contents ranging from 20.9% to 25.27% and albumin, globulin, prolamin, and glutelin contents ranging from 8.39% to 12.31%, 53.44% to 60.29%, 3.12% to 6.89% and 19.38% to 24.40%, respectively.

The chickpea protein, in addition to good nutritional characteristics, exhibits minimal off-flavor and forms emulsions and stable gels. In a comparative study of protein extracts, this protein showed water and oil absorption capacities similar to bovine gelatin or white egg protein. Emulsifying capacity and foam stability of this protein were correlated to whey and soybean proteins, manifesting great interfacial characteristics (Aydemir & Yemenicioglu, 2013).

One of the advantages over soybean is that chickpea has few antinutritional factors, such as protease and amylase inhibitors, which exhibit reduced cooking activities. These are sugars classified as  $\alpha$ -galactosides (oligosaccharides), which are neither hydrolyzed nor absorbed in the digestive system of humans but fermented by bacteria generating gases and flatulence (Kozłowska et al., 2001). This is because humans do not possess the  $\alpha$ -galactosidase enzyme, responsible for degrading its composition (Han & Baik, 2006).

Although Serdaroglu, Yildiz-Turp, and Abrodimov (2005) have applied chickpea flour as an extender in meatballs, the concentration used (10%) was higher than Brazilian legislation allows, but they obtained a cooking yield of 88.6% and a diameter reduction of 9.2 %. However, studies analyzing chicken meat products extended with chickpea protein or flour were not found, as well as the other proteins suggested as extensors.

Another proposal would be the processing of proteins prior to their use in food, since the majority of the native proteins do not present the desired functional properties of the food industry, and therefore they are subjected to modifications capable of increasing their nutritional value and their functional properties, especially in terms of solubility (Panyam & Kilara, 1996). So, modifications that aim to preclude degradation reactions, to remove toxic or inhibitors compounds, and to incorporate nutrients or additives through chemical, enzyme, and thermal treatments, presenting considerable advantage (Moure et al., 2006).

In spite of all the known benefits in legumes, the presence of several antinutritional factors has limited their application as an ingredient for food production. Thus, to enlarge nutritionally the legumes quality, it is possible to make the application of some typical pre-treatments such as thermal treatment, enzyme application, soaking, irradiation, and fermentation (Nadeem et al., 2010). Generally, the processing methods with heat are cooking at atmospheric pressure (Bressani, 1989), nevertheless, at high temperature and long time could cause undesirable physicochemical changes in proteins, starch, and other valuable heat-sensitive constituents of edible seeds (Li et al., 2014). In recent years, other techniques such as microwave, high-pressure

processing, and extrusion have been established in order to minimize the quantity of anti-nutritional factors (Zarei & Kafilzadeh, 2013).

Extrusion is considered to be a high-temperature-short-time (HTST) processing method, so, it is capable of preserving desirable food components, destroying microorganisms (Berrios, Camara, Torija, & Alonso, 2002), and, when used for processing vegetable proteins, it promotes modifications in the structures of these proteins (Shukla, 1998). The meat extenders have already been extruded from cottonseed, pea, and bean proteins (Strahm, 2006). Legume flours, such as black beans, chickpeas, and lentils, have slightly increased the meatball hardness and can be used as extenders with successful formulations of this product (Serdaroglu, Yildiz-Turp, & Abrodimov, 2005).

Research using plant proteins has hopeful results for healthier products with low-fat content. Thus, the proteins can maintain the nutritional value of meat products and be from different sources, which facilitate the inclusion of consumers with special diets. The most relevant is the absence of storage stability data, industrial scale pilot tests, economic feasibility studies, and faster and more effective sensory tests.

## **9. Future trends**

Previous scientific work has established that it is feasible to produce more attractive and healthier processed meat products for customers. However, it is unknown the capacity of the meat industry to face the challenges of functional food market with success, such as: focusing on the right market, satisfying the necessary regulations, and manufacturing functional meat products that are concomitantly flavorsome, safe, affordable, and easy to manufacture. Besides that, as poultry meat consumption continues to grow, there will be more need for highly trained food scientists and more opportunities to develop further processed products.

Challenges are derived from the necessity to reduce food additives application as salt and phosphate by natural options like oregano and rosemary extracts, legumes and fruits fiber, pulp, and peel, among others. Future prospects also must aim at using other alternative sources as protein extenders, for example, chickpea, pea, and wheat gluten, in order to avoid the allergic and antinutritional factors existing in soy protein, since studies of these proteins applied in chicken meat products are not common. Moreover, the use of these leguminous proteins as novel options is possible for improving or not modifying palatability in meat products.

Nutritional, sensorial, and technological qualities must be maintained after the use of these new ingredients in different food products while attending to consumer requirements.

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### **- CAPÍTULO 3 -**

#### **Food extenders obtained by twin-screw extrusion cooking**

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Foi depositado pedido de patente no Instituto Nacional da Propriedade Industrial (INPI) sob número do processo BR 10 2018 076620 1 (Anexo II).

Este capítulo será submetido ao periódico *LWT – Food Science and Technology*.

### Food extenders obtained by twin-screw extrusion cooking

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#### ABSTRACT

Proteins of plant origin are applied in food because they have a variety of possible sources and are used as extenders to increase technological characteristics in meat products, ice cream, baked foods, among others. Soybean is the most common extender; however, it has been recognized by its allergic and antinutritional factors, besides its modified genetic, being necessary to find novel extenders options. This way, soy protein isolate (SPI), pea protein concentrate (PPC), and chickpea flour (CPF) were extruded using a twin-screw extruder at 20% feed moisture. Before and after the processing, physicochemical and technological properties were evaluated, including the vital gluten (VG) in the analyses, which was not extruded, in order to find a feasible soy substitute for using as a food extender. The processing conditions favored the Maillard reactions, producing darker ( $P < 0.05$ ) extrudates than unprocessed materials. Changes in water hydration capacity also were noted for all extruded proteins, PPC and CPF increased their values, whereas SPI decreased by half than seen before extrusion. The oil absorption capacity decreased ( $P < 0.05$ ) after the processing, which could indicate an application in healthy foods that do not need to absorb oil. Without any processing, VG showed the highest foaming capacity (FC) ( $P < 0.05$ ); nevertheless, it is important to note the null FC value for chickpea flour after extrusion. All extruded proteins decreased their emulsifying capacity results after processing and PPC had the lowest value ( $P < 0.05$ ) both before and after extrusion. All results show that it is necessary to verify which type of food system is being made in order to choose the best food extender and soy substitute.

**Keywords:** Vegetable proteins, Technological analyses, Novel extenders, Soy substitute

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**HIGHLIGHTS:**

- The most common extenders that have been used nowadays are derived from soybeans.
- Soy protein contains off-flavors, allergens, and antinutritional factors.
- Pea and chickpea are examples of new options for applying as food extenders and as soy substitutes.
- Extrusion cooking is a cooking process that allows changing technological properties of vegetable proteins in order to use them in total or partial replacement of meat and its products.
- The antinutritional factors of legume proteins can be decreased by thermoplastic extrusion.

**1. INTRODUCTION**

Distinct amino acids that have essential functions such as tissue repair and replacement and human growth compose one of the principal macronutrients for the human body, also serving as an energy source (Day, 2016). Due to the diversification of possible sources, proteins originated from plants (oilseeds, legumes, cereals, and fungi) are a substitute to animal proteins for food applications (Asgar, Fazilah, Huda, Bhat, & Karim, 2010). They can be used as binders and extenders objectifying to increase water retention, (Asgar et al., 2010), slicing characteristics, nutritive value, emulsion stability, and emulsifying capacity (Correia, Mittal, & Osborne, 1991).

Extenders derived from soybeans are the most common to find in food composition (Feiner, 2006), because it possesses a satisfactory property for whipping or aerating agents, besides that, they are utilized functionally in the manufacture of ice cream, whipped topping, candy, frozen dessert, meat, and baked foods (Eldridge, Hall, & Wolf, 1963). However, due to the presence of antinutritional factors (Costa, Dias, Scheidegger, & Marin, 2011), many off-flavors (Okubo et al., 1992), allergic reactions (Albert, 1979 apud Cheftel, Cuq, & Lorient, 1989), and the modified genetic (Gavioli & Nunes, 2015), other alternatives of protein represent suitable options. Thus, pea (Strahm, 2006), chickpea (Serdaroglu, Yildiz-Turp, & Abrodimov, 2005), and wheat gluten (Asgar et al., 2010) are obtained by a non-genetically modified organism or not classified as primary allergenic, representing novel possibilities of proteins.

Chickpea has been becoming better than other vegetables, nutritionally evaluated as a functional food revealing bioactive compounds with advantageous effects as

hypcholesterolemic activity and antioxidant capacity (Yust et al., 2012). Thus, this protein also evidenced excellent interfacial characteristics (Aydemir & Yemenicioglu, 2013). Others soluble proteins with superior water binding, emulsification, and gelling properties are the pea protein isolates. Although this protein has not a common flavor as the soy proteins have, their properties make them favorable for using in processed meat products. (Petracci, Bianchi, Mudalal, & Cavani, 2013). Wheat gluten is singular among plant proteins due to its ability to form an adhesive blend and other properties when plasticized (Balakireva & Zamyatnin, 2016). Therefore, a range of textured vegetable protein can be applied as ingredients composed of wheat gluten such as meat analogue products and meat extenders (Orcutt et al., 2006). In addition, the extrusion process already was conducted to manufacture texturized products of wheat gluten (Riaz, 2004).

Extensive studies have examined the effects of extrusion cooking on properties of starch (Hashimoto et al., 2002) and proteins (Day & Swanson, 2013), but research directed toward comparisons among soy, pea, and chickpea proteins, looking for characterizing those proteins as extenders after a twin-screw extrusion processing are not found. The main difference between extrusion and other processing methods is the transformation with the raw material (Huber, 2001). The proteins have their secondary, tertiary, and quaternary structures modified during the process (Shukla, 1998), moreover, the improvement in protein digestibility due to denaturation is the main physicochemical modification and the decrease in lysine availability is the main chemical change that occurs (Cheftel, 1986). Extrusion is considered to be a high-temperature-short-time (HTST) processing method and is capable of preserving desirable food components (Berrios, Camara, Torija, & Alonso, 2002) and a processing at low moisture (< 35%) of vegetable proteins results in a product that can be used as meat extenders or analogues (Camire, 2000).

The objective of this study was therefore to investigate low moisture extrusion of three different materials (pea protein concentrate, chickpea flour and soy protein isolate) and vital gluten (not submitted to extrusion) with regard to the physicochemical and technological characteristics of them, in order to evaluate their applicability as food extenders to find a soy substitute.

## **2. MATERIALS AND METHODS**

### **2.1 Materials**

Soy protein isolate (SPI - SUPRO® 500E), pea protein concentrate (PPC), and vital gluten (VG) were obtained through donations from Solae (Esteio/RS), Obst Trade (Porto Alegre/RS) and Eurogerm (Itupeva/SP), respectively. The chickpea flour (CPF) was achieved from Farovitta® (São Paulo/SP).

### **2.2 Methods**

#### **2.2.1 Physicochemical characteristics of raw materials**

Proximate composition was determined following Association of Official Analytical Chemists (AOAC, 2006) methods: moisture content (method 925.09), ash content (method 923.03), protein content (method 960.52), and ethereal extract content (method 920.39). Carbohydrate content was obtained by difference and the results expressed in wet basis (g/100 g).

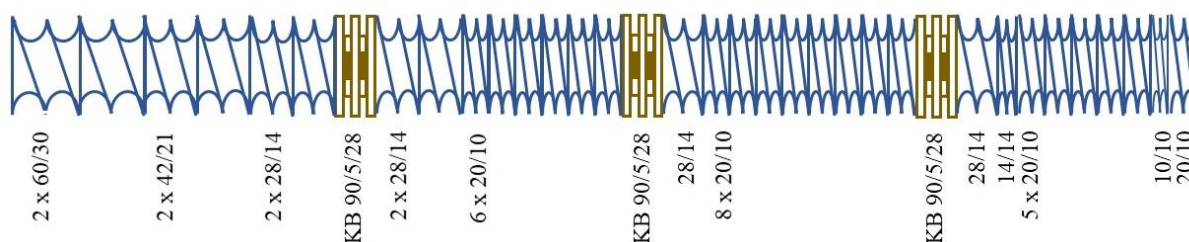
#### **2.2.2 Sample preparation and low moisture extrusion cooking**

The extrusion process of raw materials (SPI, PPC, and CPF) were performed in two days for a duplicate process in a co-rotating ZSK 30 twin-screw extruder (Werner & Pfleiderer Corporation, Chelton, USA) with a barrel diameter (D) 30 mm and barrel length (L) 872 mm ( $L/D = 29,07$ ). The screw configuration was operated as shown in Figure 1 and results from preliminary trials were used to select suitable extruder operating conditions. The barrel was separated in five heating zones; all the parameters were fixed and are shown in Table 1 for each vegetable protein. Feed rate (8.15 kg/h) and moisture content (20%) were fixed for all raw materials.

The extruded materials were immediately dried in a forced air oven TE-394/2 (Tecnal®, Piracicaba, BRA) at 85 °C until a moisture content of 5-7% was reached. Afterward, samples were ground to a powder using a blender OBL 10/2 (OXY, Santana de Parnaíba, BRA) at 35.000 rpm and stored in plastic bags at 20 °C until further analyses.



**Figure 1.** Screw configuration used for extrusion cooking performed in soy protein isolate, pea protein concentrate and chickpea flour



**Table 1.** Processing conditions of soy protein isolate (SPI), pea protein concentrate (PPC) and chickpea flour (CPF) used in extrusion cooking

Condition	SPI	PPC	CPF
Screw speed (rpm)	300	220	250
First zone (°C)	95	70	80
Second zone (°C)	90	80	90
Third zone (°C)	110	100	110
Fourth zone (°C)	140	120	140
Fifth zone* (°C)	150	135	145
Motor torque* (rpm)	50	25	35

\*Response from an extrusion process. It was not selected.

### 2.2.3 Physicochemical characteristics of raw and extruded materials

Instrumental color analysis was performed with a HunterLab Mini Scan 45/0-L (HunterLab Konica Minolta CR-400, Osaka, Japan) after standardization with a black and a white ceramic surfaces at 10° with a D-65 illuminant source. The color was recorded using CIE- $L^*$   $a^*$   $b^*$  uniform color space (CIE-Lab), where  $L^*$  indicates lightness,  $a^*$  indicates hue on a green (-) to the red (+) axis, and  $b^*$  indicates hue on a blue (-) to the yellow (+) axis.

The pH was measured potentiometrically following method 943.02 (AOAC, 2006) using a 10% (w/v) suspension of each sample at 25°C.

The water activity was analyzed using an Aqualab CX3 hygrometer (Decagon Devices Inc., Washington, USA) and the samples were displaced in specific capsules.

Scanning electron microscopy (SEM) was executed to obtain images from samples (TM 3000 Tabletop Microscope) with a magnification of 100x and 200x at 15 kV (Hitachi High Technologies, Japan). A small sample was placed on carbon tape, adhered to a stub, without coating.

### **2.2.4 Technological characterization of raw and extruded materials**

The water hydration capacity (WHC), also known as water absorption, water uptake, water holding or binding, was determined following method 56-30.01 (AACCI, 2010) as the highest volume of water that 1 g of material will absorb and retain under low-speed centrifugation. Since only enough water is added to saturate the sample and not to cause a liquid phase, solubility of the material do not affect the measurement.

To determine the oil absorption index (OAI), the method described by Wang et al. (2009) was followed.

Foaming capacity (FC) was determined following Hsu et al. (1982) and was calculated based on the total volume before and foam volume after whipping. Foam samples were allowed to stand in the cylinders at room temperature (22°C) for 30 min, and the volumes of the foam were recorded after this time. Foam stability (FS) was calculated according to Satterlee, Bembers, & Kendrick (1975).

Emulsifying capacity (EC) and emulsion stability (ES) were determined according to the method described by Yasumatsu et al. (1972) and modified by Wong & Cheung (2005).

## **3. STATISTICAL ANALYSIS**

The experimental data were generated from analyses conducted at least in triplicate. These data were assessed utilizing the analysis of variance (ANOVA) and the means compared by Scott-Knott test ( $P < 0.05$ ) in SISVAR software, version 5.6 (Federal University of Lavras, Lavras, MG, Brazil). The difference between raw and extruded materials was determined through the t-test and principal component analysis (PCA) was executed to visualize and to verify correlations between samples and analyses. The XLSTAT software, version 2018.1.49205 (Addinsoft, New York, United States), at a 5% significance level, was used to analyze these data.

## **4. RESULTS AND DISCUSSION**

### **4.1 Physicochemical characteristics of the raw material**

Table 2 shows the proximate composition of raw materials (VG, PPC, CPF, and SPI) establishing that SPI contains a higher amount of protein due to its isolated form, followed by

VG. Among the raw materials, the highest content of the ethereal extract was present in CPF and the lowest one was noted in SPI, which also showed the highest content of ash. Similarly, the carbohydrates were high in CPF. In general, those raw materials which demonstrated high levels of protein also contained low levels of fat, which means that it is inversely proportional. Moreover, the fat values were directly proportional to carbohydrates.

Among the studied samples, SPI protein content is not according to Brazilian legislation (RDC 268 - ANVISA/MS) which require a minimum protein content of 88% (Brasil, 2005), while VG and PPC are according (60 % and 40 %, respectively) to legislation, in dry basis. González-Rodríguez et al. (2016) evaluating the effects of partial replacement of fish meal with pea protein concentrate, have showed lower results for moisture (7.8 %), protein (52.2 %), and fat (1.8 %) contents, while carbohydrates (33 %) and ash (5.2 %) were higher, on wet basis.

**Table 2.** Proximate composition of raw samples

Proximate composition*	Sample <sup>2</sup>			
	VG	PPC	CPF	SPI
Moisture	5.43 ± 0.07	8.58 ± 0.06	8.48 ± 0.10	5.18 ± 0.36
Protein	66.23 ± 0.38	62.02 ± 0.53	13.15 ± 0.29	77.70 ± 2.87
Ethereal extract	2.12 ± 0.15	4.96 ± 0.19	7.42 ± 0.18	1.65 ± 0.15
Ash	0.83 ± 0.03	3.34 ± 0.11	2.89 ± 0.19	4.55 ± 0.13
Carbohydrates <sup>1</sup>	25.39 ± 0.13	21.10 ± 0.18	68.05 ± 1.52	10.92 ± 0.40

\* On wet weight basis (g/100 g); Values are mean ± standard deviation; <sup>1</sup> Carbohydrates were calculated by difference (100 - moisture - protein - ethereal extract - ash); <sup>2</sup> VG: vital gluten; PPC: pea protein concentrate; CPF: chickpea flour; SPI: soy protein isolate.

Oo, Ko, & Than (2017) looking for enhancement of protein isolation from chickpea flour and to determine the characteristics of chickpea protein isolate, observed a similar proximate composition in chickpea flour for moisture (8.21 %) and ash (3.01 %) contents, while fat and carbohydrates results were lower (5.76 % and 62.08 %, respectively). Furthermore, the authors have found higher values for protein content (19.95 %), on dry basis.

Schmiele et al. (2013) developed a free gluten pasta using soy protein isolate and presented, in wet basis, lower values of fat (0.37 %) and carbohydrates (0.18 %) in SPI, while ash content was similar (4.31 %) and protein and moisture contents were higher (88.38% and 6.75 %, respectively).

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## 4.2 Effects of extrusion cooking on the physicochemical and technological characteristics of vegetable samples

Pea protein concentrate (PPC), chickpea flour (CPF), and soy protein isolate (SPI) were extruded and become different from their previous status, making necessary to compare their results before and after extrusion. All physicochemical and technological analyses are showed in Table 3.

### 4.2.1 Physicochemical characteristics of extruded samples

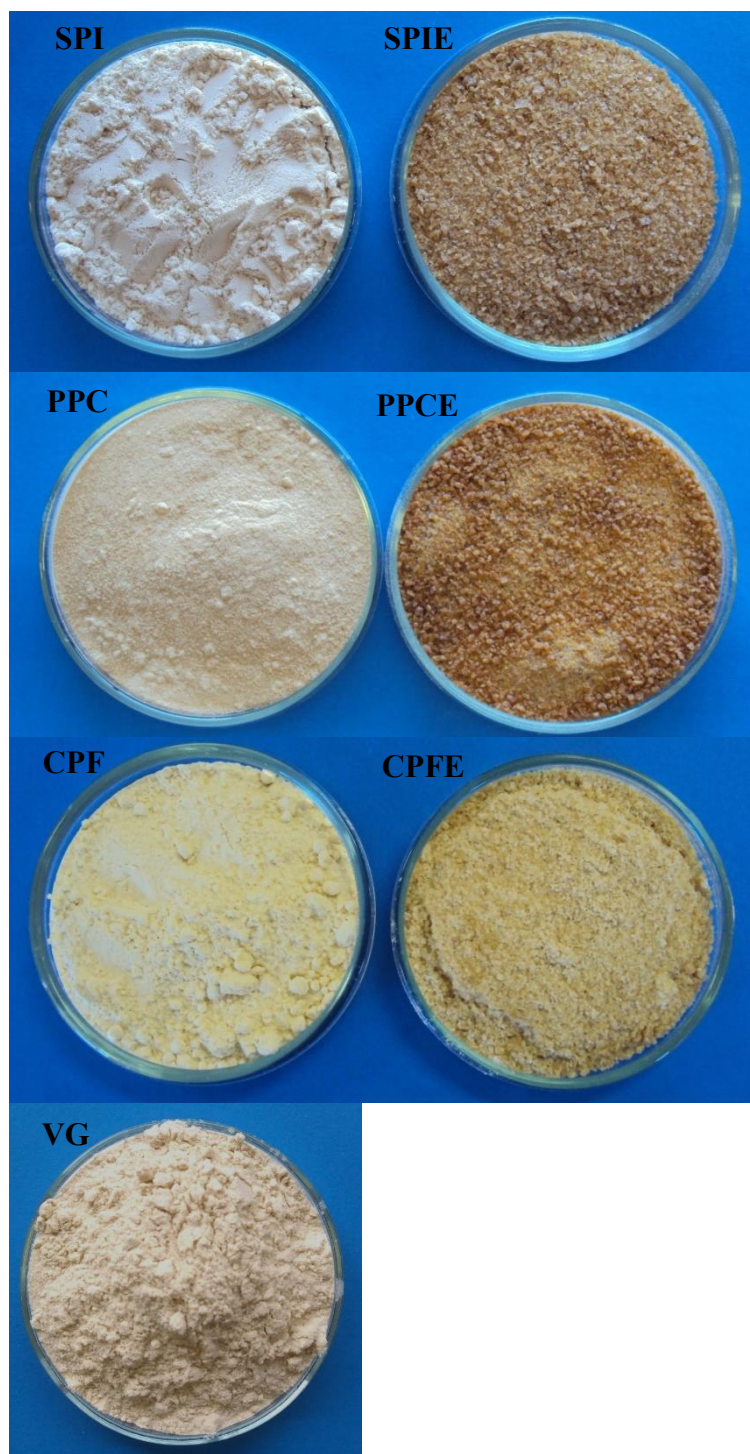
The extruded proteins (CPFE, PPCE, and SPIE) were analyzed for their proximate composition only in moisture and protein contents. Moisture content was higher in CPFE ( $6.17 \pm < 0.01$  g/100 g), followed by PPCE ( $5.88 \pm < 0.01$  g/100 g) and SPIE ( $4.28 \pm < 0.01$  g/100 g), while protein content (wet basis) was greater in SPIE ( $82.98 \pm 5.84$  g/100 g) followed by PPCE ( $68.19 \pm 5.14$  g/100 g) and CPFE ( $16.48 \pm 1.67$  g/100 g), as well as before processing.

All along extrusion, the moisture is a decisive variable that has numerous fractions in protein denaturation, starch gelatinization, barrel lubrication, and the final product quality (Rockey, 2000). It increments the mobility of proteins, cross-linking, and water absorption while reducing density (Holay & Harper, 1982). However, in this study, the moisture was low (20%) and fixed for all extruded proteins conditions.

Heat treatment of proteins can result in structural changes such as hydrolysis of peptide bonds, modification of the amino acid chains, and formation of new isopeptide covalent bonds, moreover, proteins are more resistant to heating when in low moisture content (Stanley, 1998). Extrusion process improves protein digestibility via denaturation, which exposes enzyme-access sites (Camire, 2001). The effects of extrusion on proteins are difficult to isolate since high protein concentrations are exposed to several processes simultaneously, but in this case, it is possible to note increasing in protein values comparing the results before and after the extrusion processing. The nutritional value of protein-containing food is dependent on the digestibility of the protein, as well as the availability of essential amino acids and their composition (Day & Swanson, 2013).

Therefore, the increase in protein content after extrusion can indicate an evolution in its digestibility and some amino acids may have modified their availability due to changes in protein conformation, which allowed higher nitrogen values in the method utilized for the

protein content measurement. Previously, such proteins probably had lower digestibility, which makes impossible to find them through the method utilized.



**Figure 2.** Illustrative image of raw materials (soy protein isolate (SPI), pea protein concentrate (PPC), chickpea flour (CPF), and vital gluten (VG)) and their extruded flours (SPIE, PPCE, and CPFE) obtained from twin-screw extruder

The nature of the raw materials implied in changes in the color of extruded. Furthermore, the processing settings adopted in extrusion cooking (high temperature and low water content) are known to facilitate the Maillard reaction, in which the first step involves a reaction between free amine groups and reducing sugars. Repercussions are both in the appearance of products (formation of colored compounds) and nutritional (losses of available lysine) (Camire, 2001).

The lightness ( $L^*$ ) was different ( $P < 0.05$ ) after extrusion cooking for all extruded samples, producing darker (lower  $L^*$ ) extrudates than unprocessed materials (Figure 2). CPF had the highest lightness while PPC had the lowest, and even after reducing the  $L^*$  values with the extrusion cooking, CPFE and SPIE maintained the highest and the lowest results, respectively. An indication of Maillard reactions is the dark color of the extruded material, due to the formation of melanoidins and other reaction products; however, darker color is not necessarily due to the imperative presence of these materials (Arêas, Rocha-Olivieri, & Marques, 2016). Another suggestion for the reduction in  $L^*$  values would be the different grain size visibly found in the extruded samples after grounded to powder, since the materials with greater granulometry (SPIE and PPCE) showed darker stains.

Before the process, PPC had the greatest  $a^*$  and  $b^*$  value, while SPI had the lowest ones. These results in extrudates were higher ( $P < 0.05$ ) than in raw materials, but PPCE maintained its reddish, whereas CPFE becomes more yellow and less red. Negative  $b^*$  values are indicative of a blue color, which means that no sample showed bluish instrumental color.

In general, expanding protein concentrations in the formulation enhance the yellowness (increase  $b^*$  values) of extrudates (Brncic et al., 2011), however, in this study, the only flour (CPF) which contains less protein content also demonstrated the greatest yellow color after extrusion. Yellowness also increases with rising moisture contents, probably revealing less browning reaction (Faller, Klein, & Faller, 1999), and it may be the reason for the yellowish color of CPFE, which also showed higher values of moisture after extrusion cooking.

The present study is according to Wang, Bhurud, & Tyler (1999) who reported decreasing in  $L^*$  values and increasing in  $a^*$  and  $b^*$  measurements after extrusion cooking. Yu, Ramaswamy, & Boye (2013) evaluated the effects of processing parameters on the quality of protein-rich extruded products prepared from soy protein isolate and corn flour blends, and showed that an increasing in SPI concentrations can result in darker colors (decreased  $L^*$  values), and in this study, the soy protein extruded showed the lowest  $L^*$  value, may be due to its high protein content.

**Table 3.** Instrumental color, pH, water activity (Aw), water hydration capacity (WHC), oil absorption index (OAI), foaming capacity (FC), foam stability (FS), emulsifying capacity (EC), and emulsion stability (ES) analyzed in raw and extruded samples

Samples	RAW				EXTRUDED		
Analysis <sup>+</sup>	VG	PPC	CPF	SPI	PPCE	CPFE	SPIE
<i>L</i> *	87.02 ± 0.29 <sup>c</sup>	81.85 ± 0.02 <sup>d#</sup>	91.31 ± 1.05 <sup>a#</sup>	88.12 ± 0.09 <sup>b#</sup>	65.67 ± 1.31 <sup>B</sup>	70.37 ± 0.45 <sup>A</sup>	58.63 ± 1.12 <sup>C</sup>
<i>a</i> *	1.84 ± 0.10 <sup>b</sup>	5.53 ± 0.06 <sup>a</sup>	1.48 ± 0.05 <sup>c</sup>	0.87 ± 0.02 <sup>d</sup>	8.35 ± 0.77 <sup>A#</sup>	6.94 ± 0.12 <sup>B#</sup>	7.87 ± 0.23 <sup>A#</sup>
<i>b</i> *	18.78 ± 0.97 <sup>c</sup>	23.40 ± 0.25 <sup>a</sup>	21.52 ± 0.36 <sup>b</sup>	17.81 ± 0.10 <sup>c</sup>	27.83 ± 0.88 <sup>C#</sup>	29.85 ± 0.23 <sup>A#</sup>	28.99 ± 0.43 <sup>B#</sup>
<b>pH</b>	6.38 ± 0.01 <sup>c</sup>	5.71 ± < 0.01 <sup>d</sup>	6.40 ± < 0.01 <sup>b#</sup>	7.39 ± 0.01 <sup>a</sup>	6.15 ± 0.15 <sup>C#</sup>	6.38 ± 0.01 <sup>B</sup>	7.56 ± 0.06 <sup>A#</sup>
<b>Aw</b>	0.45 ± < 0.01 <sup>c</sup>	0.60 ± < 0.01 <sup>a#</sup>	0.47 ± < 0.01 <sup>b#</sup>	0.32 ± 0.02 <sup>d#</sup>	0.39 ± 0.01 <sup>A</sup>	0.40 ± 0.03 <sup>A</sup>	0.21 ± 0.03 <sup>B</sup>
<b>WHC (mL/g)</b>	1.60 ± 0.04 <sup>b</sup>	3.08 ± 0.40 <sup>b</sup>	1.62 ± 0.20 <sup>b</sup>	15.50 ± 3.53 <sup>a#</sup>	3.23 ± 0.19 <sup>C</sup>	7.46 ± 0.46 <sup>A#</sup>	6.15 ± 0.43 <sup>B</sup>
<b>OAI (g/g)</b>	2.03 ± 0.03 <sup>d</sup>	2.13 ± 0.02 <sup>c</sup>	2.21 ± 0.02 <sup>b#</sup>	2.38 ± 0.02 <sup>a#</sup>	1.87 ± 0.14 <sup>B</sup>	1.91 ± 0.09 <sup>B</sup>	2.15 ± 0.08 <sup>A</sup>
<b>FC (%)</b>	333.04 ± 28.83 <sup>a</sup>	42.48 ± 4.42 <sup>b#</sup>	5.88 ± 0.98 <sup>b#</sup>	30.67 ± 2.23 <sup>b</sup>	22.55 ± 1.52 <sup>B</sup>	nd	47.39 ± 4.32 <sup>A#</sup>
<b>FS (%)</b>	70.38 ± 3.36 <sup>c</sup>	76.71 ± 2.21 <sup>b</sup>	100.00 ± < 0.01 <sup>a#</sup>	72.45 ± 2.82 <sup>c#</sup>	78.23 ± 3.08 <sup>A</sup>	nd	62.24 ± 1.79 <sup>A</sup>
<b>EC (%)</b>	39.50 ± 2.28 <sup>a</sup>	30.77 ± 0.37 <sup>b#</sup>	39.26 ± 2.05 <sup>a#</sup>	42.66 ± 3.13 <sup>a#</sup>	1.77 ± 0.03 <sup>C</sup>	3.58 ± 0.05 <sup>B</sup>	36.29 ± 1.65 <sup>A</sup>
<b>ES (%)</b>	98.68 ± 2.63	100.00 ± < 0.01	95.43 ± 4.37	92.83 ± 9.50	100.00 ± < 0.01 <sup>A</sup>	100.00 ± < 0.01 <sup>A#</sup>	78.91 ± 3.60 <sup>B#</sup>

All the values are mean ± standard deviation; *L*\*: lightness; *a*\*: redness; *b*\*: yellowness; VG: vital gluten; PPC: pea protein concentrate; PPCE: pea protein concentrate extruded; CPF: chickpea flour; CPFE: chickpea flour extruded; SPI: soy protein isolate; SPIE: soy protein isolate extruded; <sup>a-d</sup> Means with different lowercase superscript letters in the same row are significantly different at *P* < 0.05 between raw materials; <sup>A-C</sup> Means with different uppercase superscript letters in the same row are significantly different at *P* < 0.05 between extruded samples; <sup>#</sup> Means followed by the number sign in the same row are significantly different (*P* < 0.05) at test T-student between raw materials and extruded samples; <sup>+</sup> Three replicates; **nd**: not detected.

It was observed that all pH values differed statistically from each other and increased with extrusion processing, except for CPF. Moreover, all raw materials and extruded samples were significantly different ( $P < 0.05$ ) among them. The highest and the lowest values were for SPI and PPC, respectively, either before or after extrusion cooking.

The solubility of a protein is influenced by the pH (minimal at the isoelectric point), temperature, and ionic strength (Bolontrade, Scilingo, & Añón, 2013). The isoelectric point (pI) of a protein is the pH where there is no electrostatic repulsion keeping them apart and they tend to aggregate and precipitate. Each protein has a distinct pI because of its amino acid sequences and thus, they can be separated by regulating the pH of a solution (Sforza, Tedeschi, & Wierenga, 2016).

The effect of pH on soy protein solubility gives a curve with u-form, where the highest solubility is presented to be on both sides of the isoelectric point (4.5), with a high solubility above the pI and a low solubility below the pI (Freitas, Albano, & Telis, 2017). Pea proteins also show the same curve form as a function of pH, with a high solubility above the pI (5.0), and a moderate solubility below the pI (Adebiyi & Aluko, 2011). Sánchez-Vioque et al. (1999) determined the isoelectric point of chickpea flour in pH 4.3, while Majzoobi & Abedi (2014) found the gluten's pI around pH 6.2. Soy protein pH in the present work is more away from its pI than the other proteins.

According to Machado et al. (2007), in general, proteins are more soluble at low (acidic) or high (alkaline) pH values due to excessive charges, producing repulsion among the molecules. In food applications, insoluble proteins are not good, thus, it is important to control the denaturation so that the protein solubility will be not affected in a negative way (Raikos, Campbell, & Euston, 2007).

The  $A_w$  provides information on the expected microbiological contamination level as well as the degree of fat deterioration in foods (Pathania, Kaur, & Sachdev, 2017), since lower  $A_w$  values are better. It is evident that the extrusion cooking reduced water activity in both samples ( $P < 0.05$ ). PPC was significantly higher and SPI was the lowest, but PPC was the one that further decreased its result. After processing, CPFE and PPCE did not present significant difference anymore. The reduction of microbial contamination and enzymes inactivation turn the extrusion, which is an HTST process, the main method of preservation of both hot and cold extruded foods by the low water activity of product (0.1 - 0.4) (Bordoloi & Ganguly, 2014).

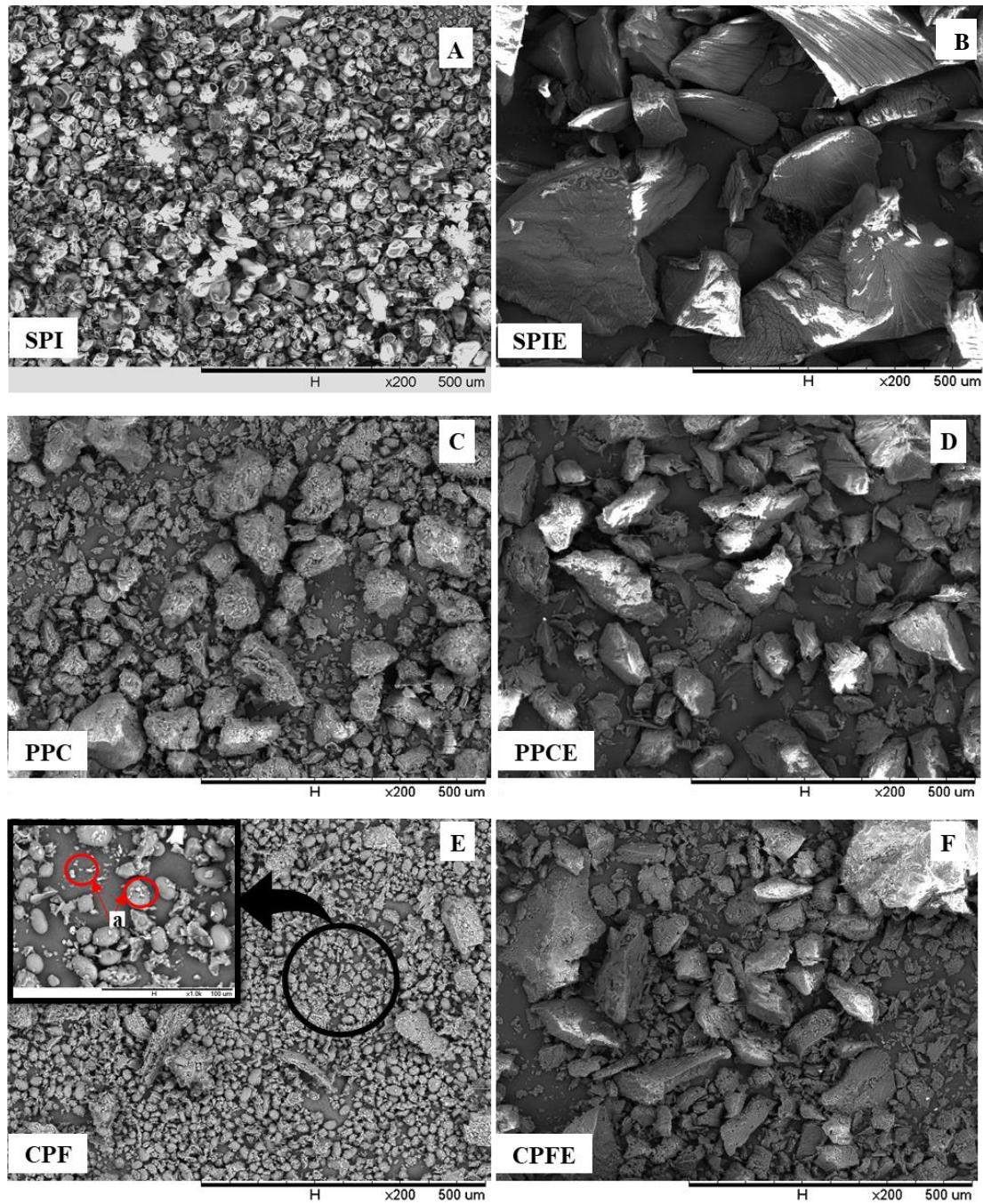


Besides, *Aw* is one critical factor that causes another deterioration as non-enzymatic browning, lipid deterioration, and overall stability of foods (Roos, 2000).

#### **4.2.2 SEM from raw and extruded materials**

Figure 3 presents SEM micrographs of the raw materials and their extruded samples after a twin-screw extrusion process where is possible to see the different particle size and some small disperse starch granules (Fig. 3E - letter a) at higher magnification. Although all the raw samples were nearly spherical, SPI (Fig. 3A) contained particles more homogeneous in diameter with a smooth surface containing invaginations, while PPC (Fig. 3C) and CPF (Fig. 3E) have particles of heterogeneous sizes, possibly because some particles are aggregates of smaller ones.

The processing modified particle size distribution and surface characteristics of the samples. SPIE (Fig. 3B) had the largest particles between the extruded samples and CPFE (Fig. 3F) contained the surfaces of the particles rougher than processed soy and pea, however, all samples had non-spherical shapes after extrusion. Therefore, it is possible to note that the higher protein content of the sample, the more homogeneous are the distribution of the particles both before and after the extrusion process.



**Figure 3.** Scanning electron micrographs of raw (SPI, PPC, and CPF) and extruded (SPIE, PPCE, and CPFE) materials

#### 4.2.3 Technological characteristics of extruded vegetable proteins

The extrusion cooking has caused changes in WHC for all extruded proteins. Before the processing, SPI had the highest WHC ( $P < 0.05$ ), however, this value decreased by half after the extrusion, while PPC and CPF increased their values and CPFE demonstrated the highest

WHC. It may be due to high starch content in flours which, after a heating process, gelatinized, favoring the hydrogen bonding sites to interact with more water. According to these data, extruded chickpea flour evidenced a great upgrade and has the capacity to compose products that require hydration.

Proteins have the ability to avoid the water releasing or expelling from their three-dimensional structure, known as water holding capacity. A selection of proteins with convenient WHC is crucial in food formulation, mainly because it plays a meaningful role in developing food texture (specifically in comminuted meat products), since proteins with very high WHC may dehydrate other ingredients and with low WHC can be more susceptible to storage humidity. Nevertheless, numerous factors affect the WHC of proteins as extrinsic (temperature) and intrinsic (pH and ionic strength). At the isoelectric point, a protein generally has the lowest WHC, besides that, a change in the pH of a protein solution modifies its conformation (Zayas, 1997a), exposing previously enclosed amino acid side chains, thereby making them available to interact with water (Abu-Tarboush, Ahmed, & Al-Kahatani, 1997). As the extrusion process denatures the proteins, the conformational and structural changes that occur modify the hydrophilic/hydrophobic balance of the same, and may contribute to the increasing or decreasing of water absorption (Alvim, Sgarbieri, & Chang, 2002).

Wang, Bhirud, & Tyler (1999) aimed to analyze an extruded air-classified pea protein and also reported that extrusion improves WHC of the protein (from 1.03 to 2.08 g H<sub>2</sub>O/ g). Oo, Ko, & Than (2017) after removing fat, fiber, and starch from chickpea flour showed a similar water absorption capacity of chickpea protein isolate (1.65 mL/g), demonstrating that different protein content from the same source (chickpea in this case) does not change WHC values, since chickpea flour has lower protein content than its protein isolate.

The OAI values showed statistically significant differences by materials and by extrusion effects. For unprocessed materials, the SPI mean was the highest, whereas VG was the lowest. After extrusion cooking, soy protein maintained the highest result ( $P < 0.05$ ) and the values of CPFE and PPCE were not different between them. The thermal processing decreased ( $P < 0.05$ ) oil absorption index as well as reported and concluded by Wang, Bhirud, & Tyler (1999), since amino acids containing nonpolar side chains may have been hidden with the processing and the size of the particles seen in Figure 3 shows that SPIE presented the largest sizes, also influencing the highest values found in OAI, exposing more nonpolar amino acids.

Oo, Ko, & Than (2017) studied chickpea protein isolate and have founded an oil absorption index of 1.72 mL/g, very similar to chickpea flour studied in this study. However, Kaur & Singh (2007) reported higher OAIs (2.08 – 3.96 g/g) for protein isolates prepared from chickpea and the results for the protein isolates were significantly higher than those of the corresponding flours (1.05 – 1.24 g/g), which were lower than chickpea flour in this research.

Regarding healthy foods, low oil absorption values are desirable, thus, SPIE could be replaced by CPFE or PPCE. However, the OAI is an important functional property because it upgrades mouthfeel and flavor retention (Porrás-Saavedra et al., 2013). In this work, the pH values seem to be positively correlated to OAI results, as can be seen clearly in Figure 4.

In food formulations, the ability of proteins to retain fat and to interact with them in food systems, mainly in emulsions, is essential. Processing conditions, protein-amino acid composition, protein type, temperature, and size of fat particles affect the fat absorption by proteins (Zayas, 1997b). Differences in functional properties might come from variations in vegetable protein composition due to differences in climate, growth conditions, and cultivars (Aydemir & Yemenicioglu, 2013).

When proteins disclose to generate an interfacial skin that keeps air bubbles in suspension and prevents their collapse, foams are developed to be applied in foods such as beverages, mousses, and whipped toppings (Boye, Zare, & Pletch, 2010). In this study, VG evidently showed the highest foaming capacity ( $P < 0.05$ ) even without any processing. However, only SPIE showed improvement in FC value and it is important to note the null FC for chickpea flour after extrusion, although its foam stability before processing was the highest. The extrusion caused increasing in FS only for PPCE and between SPIE and PPCE have not a significant difference.

Oo, Ko, & Than (2017) reported a foaming capacity of 63.64 % for chickpea protein isolate, reinforcing the data on this study that concentrates or isolate proteins have more capacity to form foam than flours, which have lower protein content. Fernandez-Quintela et al. (1997) and Boye, Zare, & Pletch (2010) reported FS of 94 % for pea protein isolate and of 93 % for SPI, respectively, without any cooking process. The individual conditions used for the foaming tests as well as differences in the protein purity could cause variations in the results from distinct studies (Boye, Zare & Pletch, 2010).

Machado et al. (2007) suggest that foams formed by proteins are more stable at near-isoelectric pH, provided they remain soluble. VG and PPCE were the proteins with higher FS

results and with pH values closer to their isoelectric points. The same authors believe that insoluble proteins are not adsorbed at the gas-liquid interface, which reduces the formation of foams, however, may contribute to the stability of the foam after its formation.

Shearing also affects the stability of proteins, since many proteins may denature and/or precipitate when subjected to specific agitation conditions, although this process is important for the formation of foams. The excessive mechanical shear generated by agitation, beating, kneading etc, can cause the denaturation of the proteins reducing their ability to form foams (Sikorski & Pokorny, 2010).

Emulsifying capacity is an assessment of how much oil a protein can emulsify per unit protein (Boye, Zare, & Pletch, 2010) and when the results of this work are verified, raw materials had no difference among them, except for PPC which showed the lowest value ( $P < 0.05$ ). Moreover, all extruded proteins had their EC results reduced after the processing. The ES is a measure of the stability of the emulsion over a certain period (Boye, Zare, & Pletch (2010), and regarding its results, native materials were not different ( $P > 0.05$ ) between them, but, after processing, SPIE had the lowest result and it was the only one that reduced its values by extrusion. Wang, Bhirud, and Tyler (1999) reported different results for pea protein, reducing its ES in 56.3 % after the extrusion process. Emulsion stability of CPF and CPFE was higher than chickpea protein isolate studied by Oo, Ko, & Than (2017) which was 40.12 %.

The presence of hydrophilic and hydrophobic amino acids makes the proteins good emulsifiers and emulsions stabilizers (Lam & Nickerson, 2013). The stability of the emulsion is related to the ability of the protein to maintain the homogeneous water /oil mixture. However, this stability can be affected by particle size, quantity, and type of protein, temperature, and pH. Therefore, protein denaturation impairs the formation of the oil/water interface (Sgarbieri, 1996). Thus, soybean was the only protein that showed a reduction in its emulsion stability result, probably this fact is related to a higher degree of protein denaturation due to its higher amount of protein because it is an isolate. Furthermore, since the emulsifying properties depend on the hydrophilic-lipophilic balance of the protein in particular (Wang et al., 2007), the emulsifying capacity values of all proteins reduced after being extruded because the processing did not favor this balance in the extrudates.

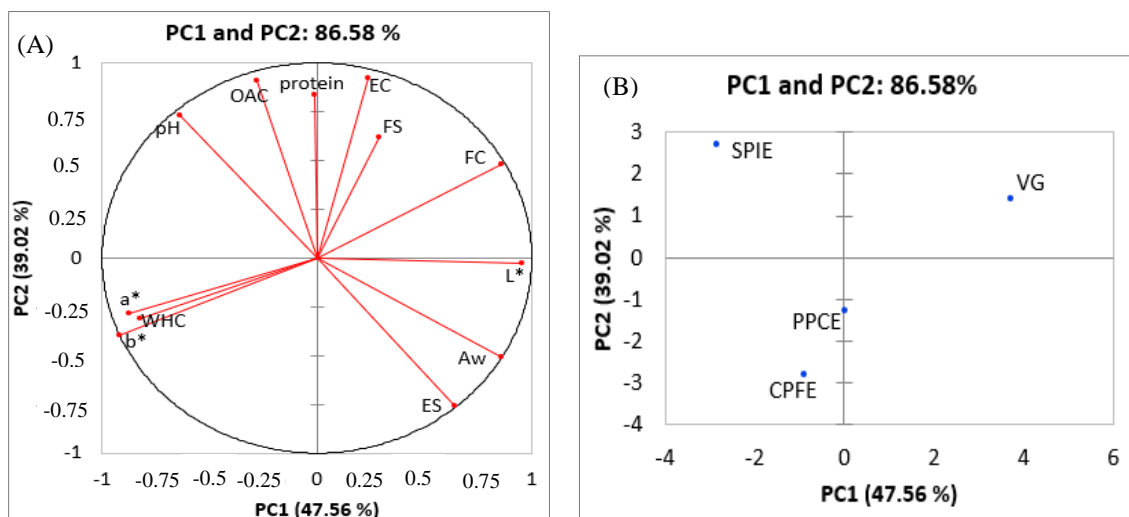
Soybean protein is commonly accepted as an ingredient that gives desirable functional properties to the final food products (Wolf, 1970) such as the capacity for whipping or aerating agents and its application in the manufacturing of whipped topping, ice cream, meat, and baked

foods (Eldridge, Hall, & Wolf, 1963). However, after the extrusion process, only the foaming capacity of the soybean presented an increase in value (from 30.67 to 47.39%), while the other functional characteristics reduced.

#### 4.2.4 Principal component analysis (PCA)

PCA is an exploratory method that investigates differences or similarities among samples in a given dataset and permits obtaining of information about regular features from the samples based on studied variables, thus simplifying to understand the dataset (Cruz et al., 2013). Therefore, the principal component analysis was conducted to provide easy visualization of the relationships between physicochemical and technological analyses with the vegetable proteins studied.

The first two dimensions derived from PCA explain 86.58% of the original variance on data (Figure 4). The first principal component (PC1) described 47.56% of the variation between the data. This component is correlated with color parameter ( $L^*$ ),  $A_w$ , and FC variables along the positive axis; color parameters ( $a^*$ ,  $b^*$ ) and WHC along the negative axis; whereas PC2 explained 39.02% of the remaining data set. Differently, PC2 was defined by protein content, pH, EC, FS, OAC along the positive axis; and ES along the negative axis (Figure 4A).



WHC: water hydration capacity; OAC: oil absorption capacity; EC: emulsifying capacity; ES: emulsion stability; FC: foaming capacity; FS: foam stability;  $A_w$ : water activity; VG: vital gluten; SPIE: soy protein isolate extruded; CPFE: chickpea flour extruded; PPCE: pea protein concentrate extruded.

**Figure 4.** Principal Component Analysis (PCA) representing (A) physico-chemical and technological analyses; (B) the special representation of the extruded proteins

The samples showed distinct distribution where VG and SPIE were located along PC1 and samples PPCE and CPFE were located along PC2. FC and color parameter  $L^*$  correlated with VG, whereas SPIE sample was described by pH and OAC. The samples CPFE and PPCE does not appear to be associated with any analysis.

VG demonstrated more variance in the characteristics of the materials, pointed out by its distancing from the other vegetable proteins as can be seen in Figure 4B. In addition, CPFE and PPCE are close in the graph, and even one being protein concentrate and the other flour, both showed similar behaviors in the analyzes performed, which could mean a possible replacement of protein concentrate with flour, making a product more economically viable when using flour, keeping the same characteristics.

Therefore, PCA showed that the most important characteristics to differentiate the samples were pH, OAC, and FC.

## 5. CONCLUSION

All proteins submitted to extrusion cooking (PPC, CPF, and SPI) were successfully texturized using a twin-screw extruder with the established conditions. This processing changes the physicochemical and technological characteristics of the samples.

The extrusion cooking has produced darkish, reddish, and yellowish samples. Changes in pH after the extrusion process resulted in approximations and distancing from the isoelectric point of each protein, causing different modifications in water holding capacity, oil absorption index, emulsifying capacity, and foaming capacity, depending on the type of protein analyzed. Each raw material had a unique behavior in the extrusion, showing results that did not follow a specific pattern, which means that occurred increases and decreases in the results of the analyses carried out, probably due to the protein content of each sample.

For applications as food extenders and soy substitute, it is necessary to verify what kind of food system is manufactured in order to choose the best protein option with its unique physicochemical and technological characteristics. A study with these proteins in a specific food system with different formulations could provide information about protein interactions and its acceptability by consumers. In addition, mixing two of the studied materials could improve the characteristics of the food extender, as it would aggregate the features presented by each one separately.



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## **- CAPÍTULO 4 -**

### **Evaluation of chicken hamburger developed as a premix extended with different vegetable proteins**

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Este capítulo será submetido ao periódico *Innovative Food Science & Emerging Technologies*

Foi depositado pedido de patente no Instituto Nacional da Propriedade Industrial (INPI) sob número do processo BR 10 2018 076620 1 (Anexo II).

O parecer do comitê de ética para a realização da análise sensorial se encontra no Anexo III.

O Termo de Consentimento apresentado previamente à realização dos testes sensoriais e as fichas preenchidas pelos consumidores podem ser vistas nos Apêndices I e II.

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## **Evaluation of chicken hamburger developed as a premix extended with different vegetable proteins**

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### **ABSTRACT**

Chicken meat products that are quickly prepared, manufactured as dried premixes, solve current problems of transport, storage, shelf life, and excessive use of additives and fat, having a healthy appeal. Such a product proposal is still unexplored, both scientifically and technologically. Thus, the purpose of this study was to investigate the effect of extenders applied to chicken hamburgers developed as dried premixes. The products were extended with extruded soy protein isolate (SPIE – F1), extruded pea protein concentrate (PPCE – F2), extruded chickpea flour (CPFE – F3), and vital gluten (VG – F4) at a level of 4%, totalizing five samples, being one control formulation (CO) without extender. Physicochemical and technological characteristics were evaluated in dried premixes and in raw and cooked hydrated hamburgers, moreover, texture profile analysis (TPA) and sensory tests were conducted. Regarding dried premixes, they have presented pH values between 5.83 and 6.04, depending on the type of extender, and yellowish color with water activity results below 0.25 in all formulations, indicating the possibility of storage under room temperature without deterioration even over long periods. Control formulation's water absorption index reduced with protein application, from 3.22 mL/g to 2.92 – 3.18 mL/g, as well as oil absorption index values decreased more with VG incorporation (from 2.90 to 2.32 g/g). After cooking, the color differences ( $\Delta E$ ) between control and each product extended with vegetable proteins got higher, where F1 was the most distinct from control ( $p < 0.05$ ) with an  $\Delta E$  of 16.71. Contrastingly, yield and shrinkage analyses have shown no difference, but F4 displayed the greatest result for yield (74.88 %), while F3 (10.46 %) decreased more the shrinkage from CO (12.10 %). Protein extenders influenced all textural properties in cooked products and CPFE reduced by half the hardness compared to control sample (122.95 N) and was the lowest one (65.72 N), as well as for springiness (4.17 mm) and chewiness (56.60 N.mm). According to sensory evaluation

results, the use of different extenders led to formulations where the consumers distinguished sensorial attributes and positioned the hamburger samples in the obtained biplot by the Napping test. With reference to the acceptance test, all the products with the protein extenders presented different or better scores in relation to the control product without any type of extender. Besides that, it is important to emphasize that F3 and F4 formulations, containing CPFE and VG respectively, had the highest overall impression (6.66 and 6.78), appearance (6.83 and 6.90), and texture (5.69 and 6.08) attributes, and the aroma received the greatest score (7.08), which was the same for both. Analyzing the Check-All-That-Apply test, it was possible the sensorial description of the product as being homogeneous, hamburger consistency, pasty, pale, ragged, crisp, firm, aftertaste, soft, dry, chicken flavor, and juicy. One can conclude that it is possible to obtain a premix chicken hamburger with good characteristics, potential shelf life, and sensory acceptance. This work contributes to the novel and new products with shorter preparation time and smaller storage space, portion control, room temperature storage, and healthy appeal.

**Keywords:** chickpea, pea, soybean, freeze-dried chicken, sensory evaluation

## 1. INTRODUCTION

Non-meat proteins are often used as alternative ingredients in meat products to boost the yield and texture by elevating water-binding properties (Pietrasik, Jarmoluk, & Shand, 2007), known as extenders. Legume flours, such as chickpeas and lentils, were applied in low-fat meatballs formulations successfully (Serdaroglu, Yildiz-Turp, & Abrodimov, 2005), besides, cottonseed, pea, and bean proteins have been already extruded in order to be extenders (Strahm, 2006).

The demand for industrialized foods of easy and quick preparation becomes increasingly evident due to the fast pace of the current population's lifestyle. In this context, consumers are also concerned about the health problems that such food choices could bring to their lives, prompting the industrial and scientific communities to seek food that can nurture and promote well-being. Meat products are options that do not require time to prepare, making them attractive to consumers. In this way, the hamburger comes as a result of its nutrients that feed and quench hunger quickly, being consumed by all popular classes (Oliveira et al., 2013). However, formulations currently found in the market have excess fat, additives, and sodium chloride, which can cause health problems when consumed frequently.

Another common problem in the meat products industry is the maintenance and high costs related to the cold chain. It is often broken by lack of information, transportation and storage costs, damaging the quality and safety of the food. With this, new forms of processing and conservation arise to extend shelf life by maintaining the quality of food for long periods. Freeze-drying appears as a process that brings advantages in space saving when storing the products, agility in the rehydration of the products (Marques & Costa, 2015), transport under long-term ambient temperatures (Arshadullah et al., 2017). In addition, few nutritional and sensory changes occur, because 80% of the volatile compounds and aroma are retained since evaporated water does not contain such attributes (Fellows, 2006).

In view of the above, the present work proposes the development of a hamburger as an alternative to the existing formulations, trying to solve the health, refrigeration, and storage problems mentioned, through the use of freeze-dried chicken meat and manufacturing it as a premix format with different extruded plant proteins and vital gluten applied as protein extenders.

## **2. MATERIALS AND METHODS**

### **2.1 Materials**

The soy isolate (SPI - SUPRO ® 500E), pea concentrate (PPC), and vital gluten (VG) proteins were supplied by Solae (Esteio/RS), Obst Trade (Porto Alegre/RS), and Eurogerm (Itupeva/SP), respectively, through donations. The freeze-dried chicken meat was achieved from Lio Foods (Itupeva / SP) and the chickpea flour (CPF) from Farovitta® (São Paulo / SP).

All plant proteins, except VG, were previously submitted to thermoplastic extrusion for later application as extenders in the hamburger formulations. After preliminary testing, feed moisture content (20%) and feed rate (8.15 kg/h) were fixed. The temperatures of the five different extruder zones and screw speed were variable among the proteins, as follow: SPI – 95, 90, 110, 140, and 150 °C, and 300 rpm; PPC – 70, 80, 100, 120, and 135°C, and 220 rpm; CPF – 80, 90, 110, 140, and 145 °C, and 250 rpm. Thus, the extruded soy protein isolate (SPIE), the extruded pea protein concentrate (PPCE), and the extruded chickpea flour (CPFE) were developed.

In addition, spices were used, all in their powder form, being: salt, onion, garlic, smoked paprika, and urucum, acquired from local commerce.



## 2.2 Methods

### 2.2.1 Development of the dried premix

The formulations were developed as a premix where all the ingredients were powdered and dehydrated, while the ingredients and their amounts were adapted from the study by Huber et al. (2016), as described in Table 1. The variation between the formulations consisted of the application of different plant proteins as extenders: F1 - SPIE, F2 - PPCE, F3 – CPFE, and F4 - VG, with no addition of preservatives or stabilizers. The control formulation (CO) was characterized by the absence of extender.

After weighing and mixing the dry ingredients, the premix was obtained and the formulations were stored under vacuum in specific packages with the aluminum layer to prevent the entry of light and oxygen until the analyzes were performed.

**Table 1.** Experimental formulations developed for the production of chicken hamburger dried premix

Ingredients (%)	Formulations				
	CO	F1	F2	F3	F4
Freeze-dried chicken meat <sup>1</sup>	19.4	19.0	19.0	19.0	19.0
Water*	77.6	74.0	74.0	74.0	74.0
Salt	1.5	1.5	1.5	1.5	1.5
Onion powder	0.6	0.6	0.6	0.6	0.6
Garlic powder	0.3	0.3	0.3	0.3	0.3
Paprika powder	0.3	0.3	0.3	0.3	0.3
Urucum	0.3	0.3	0.3	0.3	0.3
SPIE <sup>2</sup>	-	4.0	-	-	-
PPCE <sup>3</sup>	-	-	4.0	-	-
CPFE <sup>4</sup>	-	-	-	4.0	-
VG <sup>5</sup>	-	-	-	-	4.0

\*Water was added only in the hydration step of the formulations; CO: control without extender; <sup>1</sup> moisture content:  $1.79 \pm 0.06$  g/100 g, ash content:  $4.58 \pm 0.12$  g/100g, ethereal extract content:  $4.87 \pm 1.06$  g/100 g, protein content:  $82.70 \pm 0.59$  g/100 g, carbohydrates by difference: 6.06 g/100 g; <sup>2</sup> SPIE: soy protein isolate extruded, moisture content:  $4.28 \pm 0.00$  g/100 g, protein content:  $82.98 \pm 5.84$  g/100 g; <sup>3</sup> PPCE: pea protein concentrate extruded, moisture content:  $5.88 \pm 0.00$  g/100 g, protein content:  $68.19 \pm 5.14$  g/100 g; <sup>4</sup> CPFE: chickpea flour extruded, moisture content:  $6.17 \pm 0.00$  g/100 g, protein content:  $16.48 \pm 1.67$  g/100 g; <sup>5</sup> VG: vital gluten, moisture content:  $5.43 \pm 0.07$  g/100 g, protein content:  $66.23 \pm 0.38$  g/100 g

### 2.2.2 Physicochemical and technological evaluation of the dried premix

The formulations were evaluated by the following analyses according to AOAC (2006): moisture (method 925.09), ash (method 923.03), protein (method 960.52), and ethereal extract

(method 920.39). Carbohydrate content was calculated by difference. All the results were expressed in wet basis (g/100 g).

Color parameters were obtained in the CIELab system ( $L^*$ ,  $a^*$ , and  $b^*$ ) using a colorimeter CR-400 (HunterLab Konica Minolta, Osaka, Japan) with illuminant D65 and 10° standard observer angle. The total color difference ( $\Delta E$ ) was calculated following the methodology described by Mokrzycki and Tatol (2011).

The pH (method 943.02) of the samples was determined according to AOAC (2006). Water activity ( $A_w$ ) was determined using an Aqualab CX3 hygrometer (Decagon Devices Inc., Washington, United States) and specific capsules at room temperature (25°C).

The water absorption index (WAI) was evaluated according to Anderson (1982), however, the samples were centrifuged at 596.3 x g and the oil absorption index (OAI) was determined as described by Wang et al. (2009).

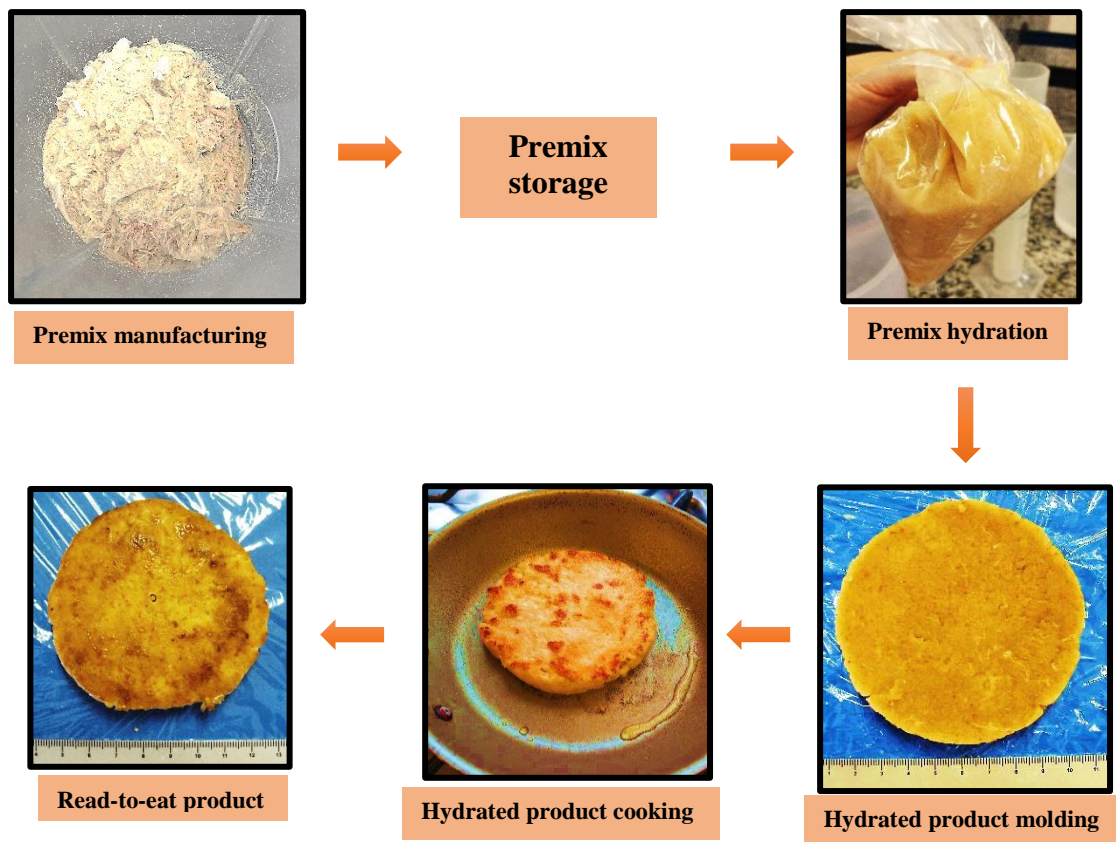
### **2.2.3 Premix hydration, hamburger manufacturing, and cooking procedure**

After premix manufacturing, the water was added after a storage of 15 days to the ingredients within the packaging used for storage and remained so for 15 minutes in order to hydrate properly. For the production of hamburgers, the hydrated premix was molded by hand into a Petri plate (9 x 1.5 cm). After that, the burgers were cooked on a preheat skillet to 163 °C for about 10 min (turned every 2 min to prevent sticking and excess surface crust formation) to achieve an internal end-point temperature of 71 °C (AMSA, 2015) which was monitored using a portable spit thermometer (Alla Brasil, IM-910.0150E). Figure 1 presents a schematic illustration of hamburger manufacturing.

### **2.2.4 Color evaluation and cooking characteristics of hydrated hamburgers**

The color parameters were determined on the surface of the product, before and after cooking, as described in section 2.2.2. The cooking yield was measured according to El-Magoli, Laroia, & Hansen (1996) with calculations of weight differences before and after cooking procedures, being expressed as a percentage (%).

The hamburger shrinkage percentage due to thermal treatment was determined following the method proposed by Adams (1994), through thickness and diameter measurements before and after the cooking process.



**Figure 1.** Schematic illustration of hamburger manufacturing

### 2.2.5 Texture profile analysis (TPA)

TPA of chicken hamburgers was performed after cooking process with a texture analyzer (TA-XT2i, Stable Systems, Haslemere, England) previously calibrated with a cylindrical weight of 5 kg following the procedures suggested by AMSA (2015), and the parameters of the profile obtained through the deformation curves were determined using the available computer software and interpreted following Bourne (2002), as follows: hardness, springiness, cohesiveness, and chewiness. After cooking, the samples were refrigerated to 4 °C, nine cylindrical samples (2.6 cm in diameter) were cut from each formulation and subjected to a double compression speed of 100 mm/min. The samples were compressed twice to 70% of their height using a cylindrical probe (P75) of 7.5 cm diameter and a 25 kg load cell.

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### 2.2.6 Sensory evaluation

The sensorial analyses were performed in the Sensory Analyses Laboratory of the Department of Food Technology in an individual cabin under white light. The samples were prepared following the cooking procedure (Section 2.2.3) and submitted to three different tests (Napping, Check-all-that-apply (CATA), and acceptance), all with untrained consumers participation. Approval for the study was obtained from the Ethics Committee of the University of Campinas (CAAE 84450318.3.0000.5404), and all volunteers gave written consent before starting the test. After the testing, consumers received a token incentive for their participation (chocolate).

#### 2.2.6.1 Napping

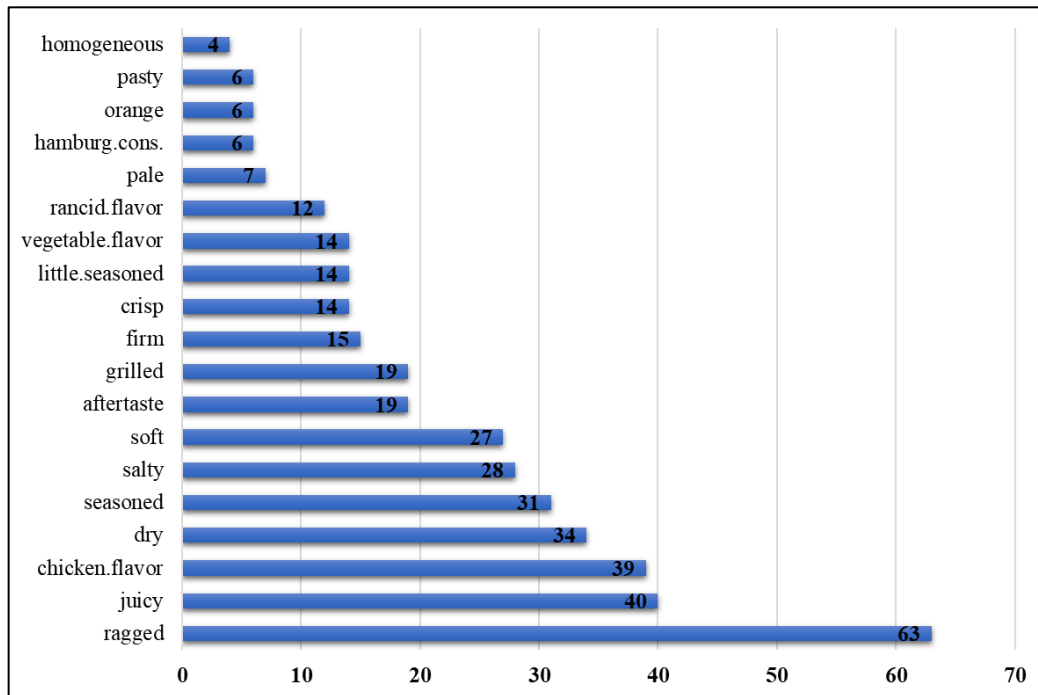
Projective mapping performed as global Napping is a rapid alternative to conventional descriptive profiling. It is fast to perform and hence saves resources such as assessor and panel leader man-hours (Valentin et al., 2012). The assessor designs their own system for product separation and this technique is most often used to highlight the main differences between products (Dehlholm, 2014).

Napping was performed based on the protocol proposed by Pagès (2003). For the panel, all the six hamburger samples were simultaneously presented to each assessor (25 in total) in plastic plates codified with three-digit numbers. The control sample – without extender – was served as blind duplicates to investigate the performance of the panel, so six samples in total were used for this sensory test. Water was available to clean the palate.

Initially, an oral and printed description of the task was provided to the consumers but had no further training. In the first test step, they were provided with an A3 paper sheet (29.7 x 42 cm) and were instructed to place samples perceived as similar close to each other and samples perceived to be more different further apart. Each assessor was free to create their own positioning criteria, tasting how many times they wanted and during the time that they would best understand to perform a good test. After placing the samples on the sheet, the panel was asked to describe them individually by directly writing on the sheet up to five words, as a means to describe the differences or sensory characteristics.

Upon completion of this sensory test, the X and Y coordinates on the paper were measured for each sample relative to the left bottom corner of the sheet. The nominal data were constituted by the terms assigned to each sample, forming a contingency table containing the

frequency of citing them. Such terms have gone through processes of lemmatization and categorization in order to choose the most appropriate, and from 61 terms originally generated, a final list of 19 different terms were obtained for statistical analysis, as seen in figure 2. In addition, it is possible to note that the most cited attributes (more than 25 times) as present in the samples evaluated were: soft, salty, seasoned, dry, chicken flavor, juicy, and ragged.



**Figure 2.** Citation frequency of attributes by consumers on samples of chicken hamburger

#### 2.2.6.2 Acceptability and Check-all-that-apply (CATA)

A total of 120 consumers between 18 and 50 years old were recruited (66 females and 54 males) at random in the surroundings of School of Food Engineering, University of Campinas (Campinas, SP, Brazil). Among these consumers, 45.83% eat meat-based products once a week, 42.50% eat a hamburger, 65.83% at dinner. The preferred consumption manner cited by consumers was fried (49.17%).

The five samples of chicken hamburger (CO, F1, F2, F3, and F4) were monadically served in 10g samples, in plastic cups coded with three-digit random numbers following a complete randomized block design. The affective acceptance test was carried out with the consumers using a 9-point structured hedonic scale (1 = “I dislike it extremely”; 5 = “neither like, nor dislike”; and 9 = “I liked it extremely”) (Lawless & Heymann, 2010). The parameters

analyzed were: overall impression, appearance, aroma, taste, and texture. Then, the experimental group was presented with a list of 19 attributes and asked to indicate which words appropriately described their experience with the sample being evaluated. These sensory terms placed as options were chosen considering the descriptors elicited by the Napping test and the order in which the CATA terms were listed was balanced for each sample and assessor in order to avoid any mistake related to the order (Ares & Jaeger, 2013).

### 3. STATISTICAL ANALYSIS

All physical and chemical analyses were conducted in triplicate at least. The experimental data were evaluated using the analysis of variance (ANOVA) and the Scott-Knott test was used to verify the significance of differences between averages ( $P < 0.05$ ) in SISVAR software, version 5.6 (UFLA, Lavras, Brazil).

For the Napping results, a Multiple Factor Analysis (MFA) was carried out using the FactoMineR package (Lê, Josse, & Husson, 2008) in the statistical software R 1.1.453. The data from affective acceptance test were subjected to an ANOVA and Tukey's test for the means comparisons, used the SISVAR software version 5.6 (UFLA, Lavras, Brazil), considering a significance level of 10%. CATA results were treated through Cochran's Q test and correspondence analysis using the feature CATA analysis ( $P < 0.10$ ) on XLStat, Version 2018.1.49205 (Addinsoft, New York, USA), considering chi-square distances, as recommended by Vidal et al. (2015).

## 4. RESULTS AND DISCUSSION

### 4.1 Physicochemical and technological analyses of dried premix

Table 2 shows the proximate composition of the dried premix of all formulations, including total carbohydrates calculated by difference. All formulations had protein as the major component ( $> 60$  g/100g), unlike other previous studies with processed meat products in which the moisture content always had the highest means ( $\geq 60\%$ ) (Aleson-Carbonell et al.; Serdaroglu, Yildiz-Turp, & Abrodimov, 2005; Santhi & Kalaikannan, 2014). Values for moisture content ranged from 2.46 to 3.36 g/100g and the CO formulation presented the lowest mean, whereas F4 had the highest one, moreover, the last also contained the highest value of ethereal extract content. It is possible to note that, since the VG did not undergo extrusion process, its moisture was not reduced as the other proteins used as extenders. F1 and F3 had

great results for protein and carbohydrates contents, probably due to the incorporation of SPIE and CPFE in these formulations as extenders, respectively.

**Table 2.** Proximate composition of dried premix

Proximate composition*	Samples				
	CO	F1	F2	F3	F4
Moisture	2.46 ± 0.27	3.13 ± 0.21	3.21 ± 0.30	3.05 ± 0.37	3.36 ± 0.25
Ash	9.03 ± 0.60	8.63 ± 0.65	9.15 ± 0.22	6.28 ± 0.44	8.71 ± 0.14
Ethereal extract	3.01 ± 0.12	2.56 ± 0.08	2.58 ± 0.10	2.45 ± 0.16	6.14 ± 0.84
Protein	84.08 ± 6.25	85.03 ± 0.70	84.33 ± 1.10	64.23 ± 3.66	80.14 ± 5.04
Carbohydrates <sup>1</sup>	1.42	0.65	0.73	19.99	1.65

Values are mean ± standard deviation; \*On wet weight basis (g/100 g); <sup>1</sup> Carbohydrates were calculated by difference (100-moisture-protein-fat-ash); CO: control without extender; F1: with soy protein isolate extruded (SPIE); F2: with pea protein concentrate extruded (PPCE); F3: with chickpea flour extruded (CPFE); F4: with vital gluten (VG).

The color parameters are shown in Table 3 with  $L^*$ ,  $a^*$ , and  $b^*$  values, furthermore, the total color difference ( $\Delta E$ ) also was verified for analyzing if this difference is perceived by observers without a colorimeter. It was observed that all formulations differed statistically from each other in  $L^*$  values, however, all of them characterize yellowish coloration and luminosity ( $L^*$ ) above 75.

F2 was darker (lower  $L^*$ ) with significant differences and even the non-experienced evaluators are able to distinguish this difference, whereas F4 was significantly lighter (higher  $L^*$ ), less red and yellow (lower  $a^*$  and  $b^*$ ), and two different colors can be noticed, which can be explained by VG in this formulation, which was not extruded and consequently was not affected by Maillard reactions, which causes a darker color to appear in the processed material (Arêas, Rocha-Olivieri, & Marques, 2016), as perceived in the extruded pea protein added in F2. These differences in color follow the study's parameters below performed by Mokrzycki and Tatol (2011):

- 0 <  $\Delta E$  < 1 - assessor does not note the difference;
- 1 <  $\Delta E$  < 2 - only experienced assessor can notice the difference;
- 2 <  $\Delta E$  < 3.5 - unexperienced also notes the difference;
- 3.5 <  $\Delta E$  < 5 - clear difference in color is noticed;
- 5 <  $\Delta E$  - assessor observes two different colors.

Therefore, since F3 presented a minimal color difference (< 1.0) with the addition of CPFE, which is not observed with the naked eye, we can indicate that the use of this extender would be the best option to maintain the CO color. However, by analyzing the darker yellow

coloration of freeze-dried chicken meat ( $L^* = 66.18 \pm 2.10$ ,  $a^* = 6.52 \pm 0.54$ ,  $b^* = 23.83 \pm 1.07$ ), the addition of spices and extender proteins to produce the formulations altered the color which it held.

**Table 3.** Physico-chemical and technological analyses of dried premix

Analysis	Formulation				
	CO	F1	F2	F3	F4
$L^*$	$77.85 \pm 0.66^b$	$76.22 \pm 1.03^c$	$75.22 \pm 0.58^c$	$77.07 \pm 0.69^b$	$83.45 \pm 0.90^a$
$a^*$	$8.52 \pm 0.94^a$	$8.26 \pm 0.97^a$	$8.34 \pm 1.24^a$	$8.28 \pm 0.92^a$	$5.19 \pm 1.01^b$
$b^*$	$28.96 \pm 0.95^a$	$28.76 \pm 1.53^a$	$28.88 \pm 1.14^a$	$29.22 \pm 1.11^a$	$24.37 \pm 1.35^b$
$\Delta E$	nd	1.66	2.64	0.85	7.97
pH	$5.91 \pm 0.03^c$	$6.04 \pm 0.03^a$	$5.83 \pm 0.03^d$	$5.90 \pm 0.01^c$	$5.96 \pm 0.01^b$
Aw	$0.24 \pm 0.02$	$0.23 \pm 0.02$	$0.23 \pm 0.01$	$0.24 \pm 0.02$	$0.23 \pm 0.01$
WAI (mL/g)	$3.22 \pm 0.12$	$3.15 \pm 0.17$	$3.18 \pm 0.07$	$3.07 \pm 0.04$	$2.92 \pm 0.04$
OAI (g/g)	$2.90 \pm 0.15^a$	$2.86 \pm 0.07^a$	$2.84 \pm 0.06^a$	$2.88 \pm 0.05^a$	$2.32 \pm 0.04^b$

Values are the mean  $\pm$  standard deviation.  $L^*$ : lightness;  $a^*$ : redness;  $b^*$ : yellowness;  $\Delta E$ : difference of color; Aw: water activity; WAI: water absorption index; OAI: oil absorption index; <sup>a-d</sup> Means with different lowercase superscript letters in the same row are significantly different at  $p < 0.05$ ; CO: control without extender; F1: with soy protein isolate extruded (SPIE); F2: with pea protein concentrate extruded (PPCE); F3: with chickpea flour extruded (CPFE); F4: with vital gluten (VG); nd: not determined.

The objective of pH determination in food is the possible evaluation of the predominant microbiota, the potential and likely nature of the deterioration processes that it may suffer, as well as the type, intensity, and parameters of the thermal processing to which it should be submitted (Silva, 2000). The formulations showed differences ( $P < 0.05$ ) in pH evaluation, except between CO and F3, although results were very close. The observed changes in pH values were dependent on the type of vegetable protein added to the formulation, since F1 (highest mean) and F4, containing SPIE and VG, presented higher results, while F2 (lower average) and F3, containing PPCE and CPFE, resulted in lower values when compared to the CO sample. The freeze-dried chicken meat had a pH of  $6.00 \pm 0.01$ , considered a little higher than the unmodified chicken breast in natura, where Milicevic et al. (2015) found mean values close to 5.76.

Low acidity foods ( $pH > 4.5$ ) are the most susceptible to microbial multiplication, either pathogenic or deteriorating species (Franco & Landgraf, 2005), however, the results of the water activity analysis show a stable product. The study of the amount of water present in food is important to predict undesirable enzymatic and chemical reactions, as well as the development of microorganisms (Silva et al., 2010). According to Park, Bin, & Brod (2001), it is possible to establish a close relationship between the free water content in the food and its



conservation, in which the free water content is expressed by the water activity which, when reduced, increases the stability and food safety. Among the formulations studied there was no significant difference ( $P > 0.05$ ) in the  $A_w$  results and all values were below 0.25, being in the low water activity range (0.1 - 0.4) where the food stability is the highest (Bordoloi & Ganguly, 2014).

High water absorption capacity results are important for helping to maintain moisture content in products (Wang et al., 2006). Moisture loss unfavorably influences the yield and quality attributes of meat products and thus can result in economic losses for industries (Ordóñez-Pereda et al., 2005). The WAI of the formulations showed no difference ( $P > 0.05$ ) among them and the value found for freeze-dried chicken meat ( $3.61 \pm 0.05$  mL/g) was higher than the indices found in the formulations.

The addition of vital gluten as protein extender in F4 reduced the OAI value ( $P < 0.05$ ) since the control formulation presented a higher result, as well as freeze-dried chicken meat ( $3.33 \pm 0.05$  g/g). Among the other formulations, it was not possible to verify significant differences. Lower OAI values may be related to products of lower caloric content, due to the low absorption of oil, especially if the product is subjected to frying. However, the oil absorption capacity is a relevant functional property because it upgrades mouthfeel and retention of flavor molecules (Ma et al., 2011).

## 4.2 Color evaluation of raw and cooked hydrated hamburgers and cooking characteristics

After premix hydration and before cooking procedures, the products were evaluated for their instrumental color and  $\Delta E$  (Table 4). The F1 formulation containing SPIE was the darkest ( $P < 0.05$ ), while CO did not differ from F2, as well as F3 did not differ from F4. Generally, data show that after hydration and cooking all formulations changed their colors, starting to have a darker (lower  $L^*$ ), reddish (higher  $a^*$ ), and yellowish (higher  $b^*$ ) color. So, it can be seen that hydration made the products more similar to those already found in the control formulations of other studies with chicken hamburger (Aleson-Carbonell et al., 2005; Sáyago-Ayerdi, Brenes, & Goñi, 2009), but remained more yellowish and reddish.

With cooking, the formulations presented no difference ( $P > 0.05$ ) neither in the values of  $L^*$  nor in  $a^*$ , while F1 was significantly less yellow (lower  $b^*$ ), also showing a higher  $\Delta E$ . However, all samples resulted in differences clearly seen by consumers ( $\Delta E > 3.5$ ) both before and after cooking.

**Table 4.** Instrumental color, total color difference ( $\Delta E$ ), yield cooking, and shrinkage of chicken hamburgers

Analysis	Product				
	CO	F1	F2	F3	F4
<b>Raw burger</b>					
$L^*$	$51.86 \pm 0.63^b$	$50.34 \pm 1.20^c$	$52.73 \pm 1.45^b$	$56.98 \pm 0.66^a$	$56.73 \pm 0.81^a$
$a^*$	$18.40 \pm 0.48^a$	$16.37 \pm 1.19^b$	$15.07 \pm 1.07^b$	$16.10 \pm 0.48^b$	$15.86 \pm 0.92^b$
$b^*$	$35.30 \pm 1.32$	$31.70 \pm 1.04$	$32.75 \pm 1.05$	$34.00 \pm 0.56$	$33.29 \pm 1.37$
$\Delta E$	nd	4.40	4.28	5.77	5.85
<b>Cooked burger</b>					
$L^*$	$55.49 \pm 8.14$	$56.44 \pm 3.15$	$51.71 \pm 5.43$	$58.56 \pm 2.99$	$55.98 \pm 1.84$
$a^*$	$20.02 \pm 4.48$	$13.87 \pm 0.58$	$17.70 \pm 1.63$	$17.86 \pm 1.62$	$18.18 \pm 0.85$
$b^*$	$38.88 \pm 4.87^a$	$23.37 \pm 2.60^b$	$31.45 \pm 5.37^a$	$36.86 \pm 5.22^a$	$35.85 \pm 1.42^a$
$\Delta E$	nd	16.71	8.65	4.26	3.58
Yield cooking (%)	$67.97 \pm 2.49$	$72.95 \pm 3.96$	$69.82 \pm 3.80$	$73.23 \pm 2.13$	$74.88 \pm 1.86$
Shrinkage (%)	$12.10 \pm 0.64$	$14.03 \pm 1.71$	$12.64 \pm 1.25$	$10.46 \pm 1.97$	$12.10 \pm 3.65$

Values are the mean  $\pm$  standard deviation.  $L^*$ : lightness;  $a^*$ : redness;  $b^*$ : yellowness; <sup>a-c</sup> Means with different lowercase superscript letters in the same row are significantly different at  $p < 0.05$ ; CO: control without extender; F1: with soy protein isolate extruded (SPIE); F2: with pea protein concentrate extruded (PPCE); F3: with chickpea flour extruded (CPFE); F4: with vital gluten (VG); nd: not determined.

The formulations with extenders did not differ significantly from control sample. In fact, it was expected that the CO sample would have lower results than extended formulations, since extenders are ingredients used to increase water retention and minimize losses during cooking in meat products (Asgar et al., 2010; Deng et al., 2011). Asgar et al. (2010) reported that, in poultry rolls, wheat gluten could reduce cooking losses due to its binding ability, keeping moisture and fat in the matrix.

The results from shrinkage analysis showed no differences ( $P > 0.05$ ) among the samples, however, the decline in diameter is the result of the denaturation of meat proteins with the loss of water and fat (Bastos et al., 2014).

### 4.3 Texture profile analysis (TPA) of cooked hydrated hamburgers

Table 5 shows that all textural properties investigated of chicken burgers were influenced by protein extenders incorporation. It is important to observe that hamburgers with added soy and pea were harder than CO ( $P < 0.05$ ), while a different trend was recorded for chickpea flour which reduced almost by half the hardness of control sample and showed the lowest values for all the textural parameters. On the contrary, vital gluten increased springiness and had the

highest value, moreover, chewiness also was raised by VG addition in the formulation, but F1 obtained the greatest mean.

Vital gluten probably coagulated irreversibly when heated above 85°C, generating a firm and resilient gel, as mentioned by Kalin (1979), which made the hardness, springiness, and chewiness higher than CO, while cohesiveness did not differ between these two products (F4 and CO) and F1.

**Table 5.** Texture profile analysis of cooked chicken burgers.

Samples*	Texture profile analysis			
	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N x mm)
<b>CO</b>	122.95 ± 9.43 <sup>c</sup>	6.30 ± 0.51 <sup>c</sup>	0.39 ± 0.03 <sup>a</sup>	300.42 ± 22.96 <sup>c</sup>
<b>F1</b>	161.45 ± 11.68 <sup>a</sup>	7.28 ± 0.53 <sup>b</sup>	0.38 ± 0.02 <sup>a</sup>	448.82 ± 26.77 <sup>a</sup>
<b>F2</b>	134.75 ± 10.64 <sup>b</sup>	6.77 ± 0.67 <sup>c</sup>	0.34 ± 0.03 <sup>b</sup>	310.32 ± 35.15 <sup>c</sup>
<b>F3</b>	65.72 ± 7.37 <sup>d</sup>	4.17 ± 0.74 <sup>d</sup>	0.21 ± 0.02 <sup>c</sup>	56.60 ± 7.30 <sup>d</sup>
<b>F4</b>	125.71 ± 11.18 <sup>c</sup>	7.97 ± 0.82 <sup>a</sup>	0.37 ± 0.02 <sup>a</sup>	371.40 ± 28.59 <sup>b</sup>

Values are the mean ± standard deviation. <sup>a-d</sup> Means with different lowercase superscript letters in the same column are significantly different at  $p < 0.05$ . \*CO: control without extender; F1: with soy protein isolate extruded; F2: with pea protein concentrate extruded; F3: with chickpea protein extruded; F4: with vital gluten

Consumers nowadays wish burgers that are less springy and cohesive, not hard, and easy to chew considering that harder and chewy burgers mean more time wasted in masticating and completing a burger meal (Akwetey & Knipe, 2012), thus, F3 was the best formulation in order to follow these preferences, probably as a result of its high yield cooking value, which increases moisture inside the product and reduces water loss. Moreover, high protein concentrations appear to influence the hardness, where chickpea flour extruded showed less protein content and, consequently, the lowest hardness result.

## 4.4 Sensory evaluation

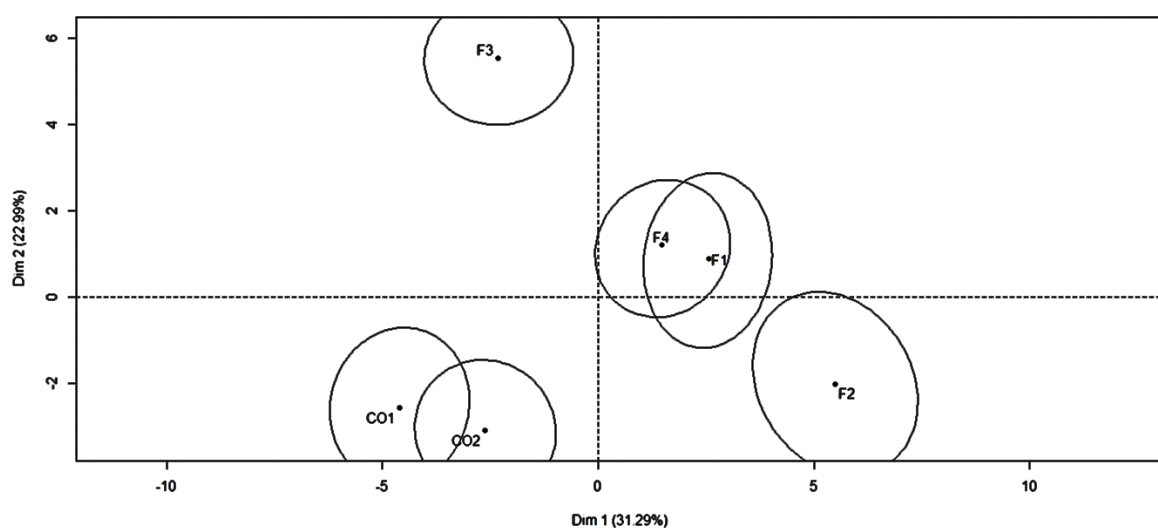
### 4.4.1 Napping

The similarities of the outcomes by the Napping test can be studied by analyzing the biplot obtained that represent the sensory spaces generated by the panel where 95% confidence ellipses were applied (Figure 3). Once the positioning of blind duplicates (sample control - CO1 and CO2) was considered as a way to check the assessors' discriminative ability, it was observed a high level of accuracy, because those duplicated samples were close to each other in the biplot and the most ellipses are not overlapped with each other.

Observing the positioning of the hamburger samples in this plot, it can be seen that the sample F1 and F4 (with soy protein and vital gluten, respectively) also were close to each other, which show similarities between these formulations according to consumers. The sample F3 (with chickpea flour) is clearly separate from the others samples and there are no overlaps, showing that it was the easiest to be distinguished from the others.

Figure 4 shows the biplot obtained by MFA of the Napping data, which showed the first two dimensions accounted for 54.28% of the explained variance (31.29% and 22.99%, respectively). It is possible to note that non-trained assessors used a more varied and spontaneous vocabulary and made more references about flavor and texture.

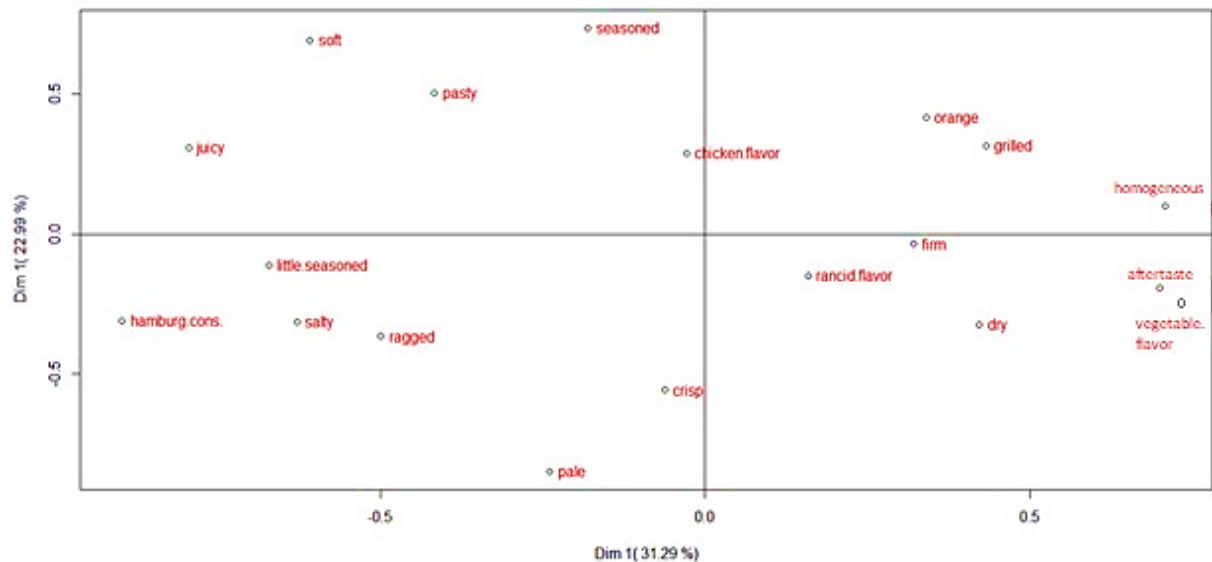
Liu et al. (2016) performed three different approaches of Napping with nine assessors: one based on the classical Napping (the same as played in this present study) and two others including training sessions prior to the Napping. They observed different values of variability obtained by MFA and an increase of the total explained variability was observed in Napping with training strategies. Compared to the classical Napping (44%), the total variance of the Napping with method training increased to 56%, and the Napping with product training showed a larger increase with an overall 67% variance explained in the first two dimensions. However, in our research, we observed a similar variability (54.28%) to Liu et al. (2016)'s Napping training on method even without conducting that training, which can demonstrate reliability in our results.



**Figure 3.** MFA product biplot of the Napping data

The control samples (CO1 and CO2) were related to the characteristics little seasoned, ragged, crisp, pale, hamburger consistency, and salty. Formulas F1 and F4 are characterized by

being homogeneous and orange, having grilled flavor and aroma. The F2 sample is related to the attributes: aftertaste, rancid, and vegetable flavors, firm texture, and dry. Finally, the formulation F3, outstanding of the others, has the terms soft, juicy, pasty, seasoned, and chicken flavor as the main characteristics that define it.



**Figure 4.** Graphical representation of terms assigned to samples in the first two dimensions of the MFA

#### 4.4.2 Acceptability and Check-all-that-apply (CATA)

Results of the acceptance testing performed by consumers are summarized in Table 6. The samples containing vital gluten (F4) received the highest scores ( $p < 0.10$ ) for all attributes evaluated (overall impression, appearance, aroma, taste, and texture), whereas F3 and F4 did not show a statistical difference between them in all categories studied, as well as between F1 and F2. The highest score among all attributes and samples was associated with the products containing vital gluten and CPFE in the aroma feature, related to the response “like moderately”.

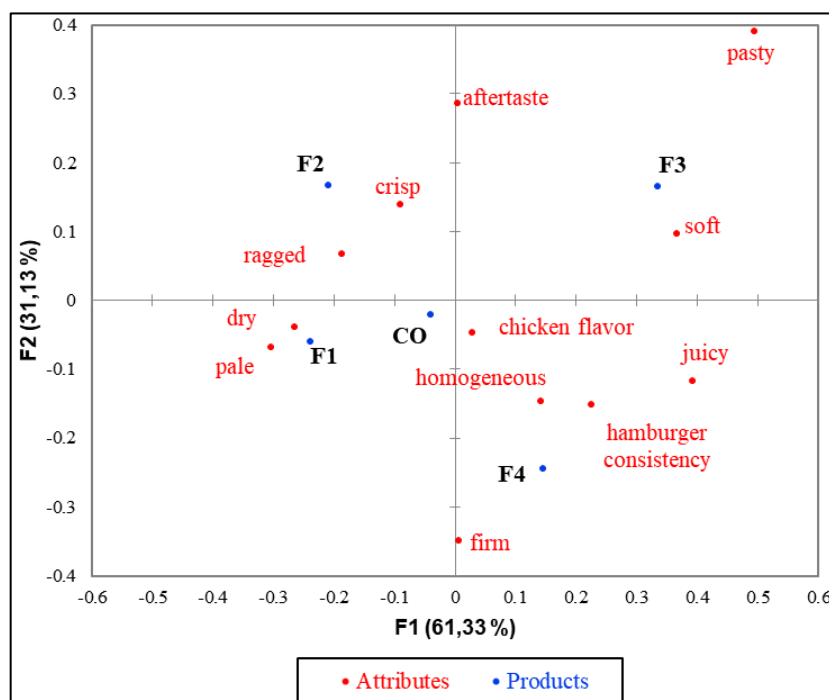
Additionally, the aroma attribute presented the highest average (6.85) of the scores attributed to all products, while the lowest was for the texture attribute (5.52), which was also the only attribute with a mean value below 6.0, standing in the scale response referring to “neither like nor dislike”.

**Table 6.** Means of the attributes evaluated in the sensory acceptance test for a chicken hamburger with different formulations.

Samples*	Overall impression	Appearance	Aroma	Taste	Texture
CO	6.55 ± 1.54 <sup>ab</sup>	6.24 ± 1.78 <sup>b</sup>	6.97 ± 1.47 <sup>b</sup>	6.65 ± 1.67 <sup>a</sup>	5.59 ± 1.84 <sup>ab</sup>
F1	6.25 ± 1.57 <sup>b</sup>	6.45 ± 1.73 <sup>ab</sup>	6.48 ± 1.53 <sup>ab</sup>	6.11 ± 1.72 <sup>ab</sup>	5.32 ± 1.76 <sup>bc</sup>
F2	6.21 ± 1.63 <sup>b</sup>	6.26 ± 1.68 <sup>b</sup>	6.65 ± 1.69 <sup>b</sup>	6.05 ± 1.92 <sup>b</sup>	4.94 ± 1.92 <sup>c</sup>
F3	6.66 ± 1.53 <sup>ab</sup>	6.83 ± 1.54 <sup>a</sup>	7.08 ± 1.45 <sup>a</sup>	6.24 ± 1.86 <sup>ab</sup>	5.69 ± 2.01 <sup>ab</sup>
F4	6.78 ± 1.43 <sup>a</sup>	6.90 ± 1.66 <sup>a</sup>	7.08 ± 1.43 <sup>a</sup>	6.65 ± 1.62 <sup>a</sup>	6.08 ± 1.87 <sup>a</sup>

Values are the mean ± standard deviation. <sup>abc</sup> Means with different lowercase superscript letters in the same column are significantly different at  $p < 0.10$ . \*CO: control without extender; F1: with soy protein isolate extruded (SPIE); F2: with pea protein concentrate extruded (PPCE); F3: with chickpea flour extruded (CPFE); F4: with vital gluten (VG)

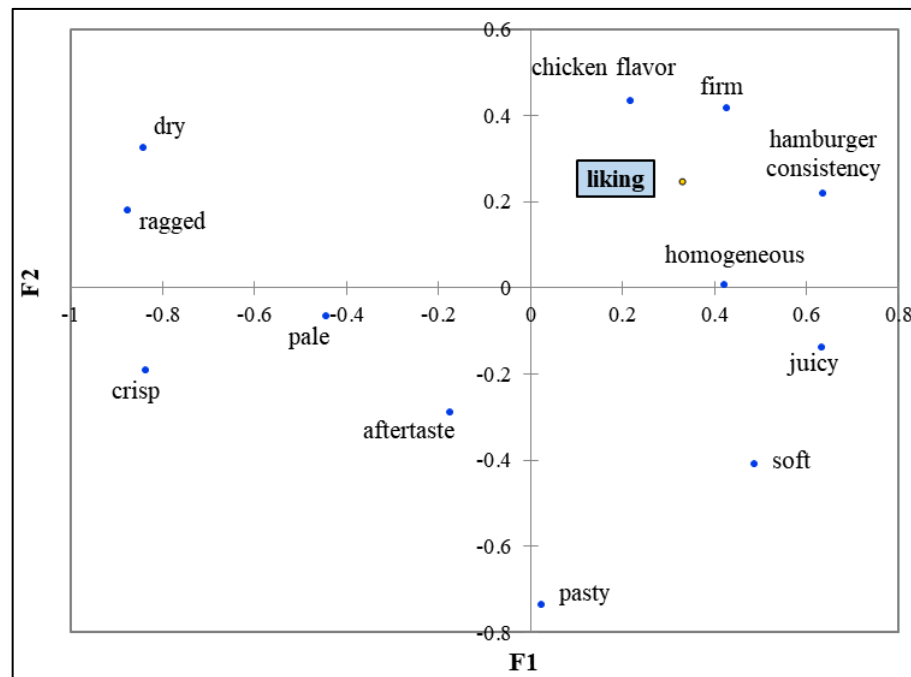
Analyzing the CATA data, 12 of the 19 CATA terms showed association with the hamburger samples ( $P \leq 0.10$ ) according to the Cochran's Q test and then only these terms, namely homogeneous, hamburger consistency, pasty, pale, ragged, crisp, firm, aftertaste, soft, dry, chicken flavor, and juicy were used in correspondence analysis (Figure 5). Therefore, from a sensory viewpoint, the texture is the most important characteristic of a chicken hamburger valued by consumers. The first two dimensions accounted for by 92.46% of the variance in the experimental data, with 61.33% and 31.13% for the first and second dimensions, respectively.



**Figure 5.** Perceptual map resulting from correspondence analysis on significant CATA terms. CO: control without extender; F1: with SPIE; F2: with PPCE; F3: with CPFE; F4: with VG.

The consumers were able to perceive real differences among the products in terms of their sensory profiles because results from correspondence analysis using Chi-square distance showed a p-value lower than the significance level (0.10) and the samples are in distinct locations around the graphic. CO, F1, and F2 were similarly perceived as ragged, crisp, and with chicken flavor, which made them stand out from the rest, mainly because F3 was more associated with the term soft and F4 with the term firm. Moreover, the control product was more perceived as pale, the product F1 was identified as dry, F2 showed association with the aftertaste, F3 was correlated with pasty and juicy, whereas the product F4 was characterized as homogeneous with hamburger consistency. Thus, F3 data are corresponding to its high yield cooking characteristic (Table 4) that, consequently, maintains great moisture inside the product, reduces water loss, and results in juiciness perceived by the final consumer. In addition, the extruded chickpea flour applied in F3 has a higher concentration of starch and allows the occurrence of gelatinization, also influencing the sensorial characteristics perceived by the assessors, since the starch molecules in the presence of water and heat break down its intermolecular bonds allowing the hydrogen bonding sites to engage more water and swell. This irreversibility dissolves the starch granule in water while maintaining the juiciness of the developed product (Liu et al., 2009).

Figure 6 shows the results of principal coordinate analysis between significant CATA terms and liking scores (overall impression scores from the acceptance test) where the first two dimensions explain 51.83% of the variation. According to the map, a product with the highest liking scores should have positive correlations with chicken flavor, hamburger consistency, juicy, soft, firm, and homogeneous attributes. On the other hand, it should not be relatively dry, crisp, ragged, pale, aftertaste, and pasty. For the foregoing, results (Figure 5 and 6) suggest that F4 seems to be the product that consumers liked more, as well as in acceptance test, once this sample was associated with the positive terms close to liking scores.

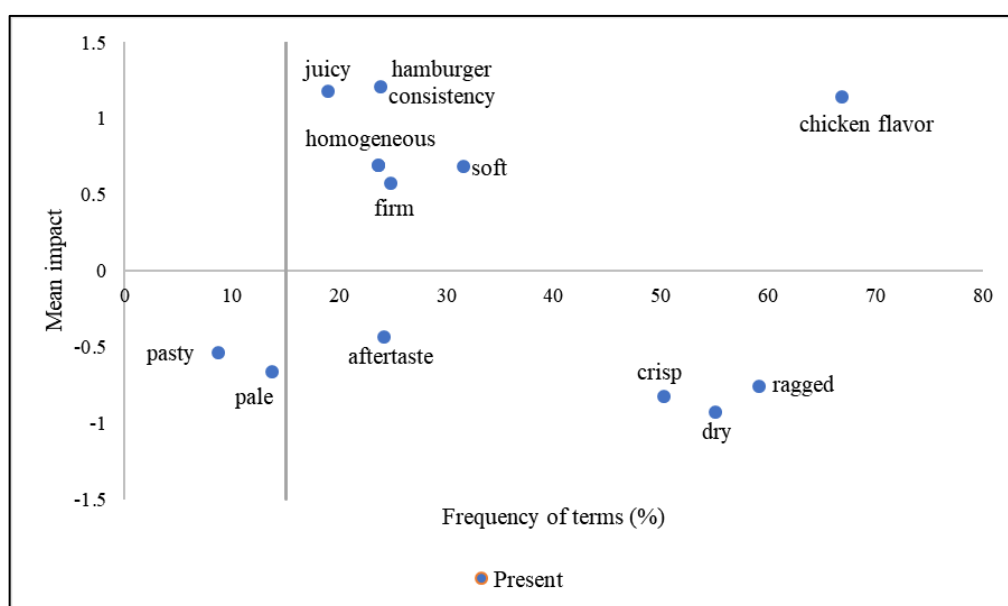


**Figure 6.** Principal Coordinate Analysis between significant CATA terms and liking scores (overall impression)

Figure 7 allows to clearly identifying attributes with a significant mean impact. The Y-axis represents the means that increase and decrease depending on the CATA term, while the X-axis corresponds to percentage of responses including a check of the product for a given attribute, thus, attributes that are correlated to high coordinates on both the X and Y axes appear to be “must haves” and those linked to low coordinates are “must not haves” (XLSTAT, 2017).

Then, the findings presented herein are disclosing that juicy, hamburger consistency, homogeneous, firm, soft, and chicken flavor are characteristics that appear as “must have” and increased the mean in 0.5 to almost 1.5 points; whereas pasty, pale, aftertaste, crisp, dry, and ragged are “must not haves” which decreased the mean in 0.5 to 1.0 points. Finally, the terms chicken flavor, ragged, dry, and crisp were the most associated to the products with a frequency ranged from 50.25% (crisp) to 66.89% (chicken flavor).





**Figure 7.** Mean impact versus the frequency (%) of terms cited in CATA analysis

## 5. CONCLUSIONS

It was possible to develop a hamburger as a premix, also, this work suggests that extruded vegetable materials (soy protein isolate, pea protein concentrate, and chickpea flour) and vital gluten can be successfully incorporated in chicken hamburger formulations as extenders at the level of 4%, obtaining a premix and hydrated product with good characteristics, healthy composition, potential shelf life, and acceptable. Overall, among the samples, that containing chickpea flour was found to be the best in almost all analyses performed, highlighting the CATA and acceptance sensory analysis in which F3 had high scores; and juicy and soft as its terms that increased these scores, as well as F4 with similar performance.

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**- CAPÍTULO 5 -**

**DISCUSSÃO GERAL**

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## 1. DISCUSSÃO GERAL

As emulsões e os produtos reestruturados cárneos são conhecidos como danosos à saúde devido ao seu alto conteúdo de lipídios e de sódio (FELISBERTO et al., 2015), tornando-se fundamental a busca por alternativas focadas em melhorar as características nutricionais, possibilitando o consumo destes produtos como prato principal sem prejuízos à saúde (ABDEL-NAEEM e MOHAMED, 2016). O aumento global da população e a manutenção do atual consumo de proteína torna perceptível a necessidade de produzi-la mais, contudo, a extração proteica de vegetais e de grãos pode ser uma opção sem prejuízos ao meio ambiente (JONES, 2016), sendo também necessários tratamentos térmicos que possam reduzir os anti-nutrientes encontrados em diversas fontes vegetais de proteína, estando a extrusão termoplástica como uma opção amplamente utilizada e conhecida.

Inúmeros estudos mostram a possibilidade de produzir produtos cárneos mais atrativos e saudáveis para os consumidores atuais através do uso da carne de frango como substituta da carne bovina ou suína (BASTOS et al., 2014), uso de antioxidantes naturais para substituir aditivos químicos (TRINDADE et al., 2010; PEREIRA et al., 2017) e aplicação de fibras, polpas e cascas para melhorar a composição e características tecnológicas de hambúrgueres (HUBER et al., 2016; LONGATO et al., 2017). Ingredientes proteicos provenientes de algumas leguminosas como soja, ervilha e grão-de-bico também já são utilizadas, em maior ou menor escala, em produtos cárneos reestruturados, normalmente como fibras, a fim de melhorar suas propriedades funcionais e reduzir ou substituir a adição de gordura nas formulações, contudo, as proteínas extraídas (isolada ou concentrada) desses vegetais não são comumente submetidas ao processo de extrusão termoplástica para melhorar suas características e reduzir seus fatores antinutricionais antes de serem aplicadas nos produtos desenvolvidos. Todavia, deve-se considerar que as qualidades nutricional e sensorial não podem ser alteradas com o uso de ingredientes inovadores substitutos da soja, uma vez que as necessidades dos consumidores precisam ser verificadas e mantidas.

O processo de extrusão termoplástica com 20% de umidade utilizado para produzir proteínas vegetais extrusadas (SPIE, PPCE e CPFE) foi eficiente, uma vez que tais proteínas apresentaram características diferentes dependentes da leguminosa de origem, possibilitando aplicá-las como extensores em diversos sistemas alimentares, atingindo o objetivo do trabalho. Quanto aos resultados verificados, o conteúdo de proteína aumentou após o processamento das amostras, podendo indicar uma evolução na digestibilidade proteica e mudança conformacional

de alguns aminoácidos, permitindo a exposição de sítios anteriormente escondidos (DAY e SWANSON, 2013). O glúten vital não foi extrusado, mas também foi analisado para comparação com as demais proteínas, apresentando o menor índice de absorção de óleo (2,03 g/g), característica conveniente em produtos com baixo valor calórico, demonstrando também a maior capacidade espumante (333,04%) e, juntamente com a soja antes da extrusão (42,66%), a maior capacidade emulsificante (39,50%), propriedade importante na fabricação de processados cárneos. A capacidade de hidratação em água foi intensamente alterada com o processo de extrusão, tendo reduzido pela metade os resultados apresentados para o isolado proteico de soja (de 15,50 para 6,15 mL/g) e aumentado para a farinha de grão-de-bico (de 1,62 para 7,46 mL/g), evidenciando a possibilidade de aplicação em produtos que necessitam de hidratação prévia ao consumo.

Na análise microscópica de varredura, foi possível identificar que a SPI apresentou partículas de tamanho mais homogêneo com pequenas invaginações na sua superfície e, embora a farinha de grão-de-bico e a proteína concentrada de ervilha também tenham partículas arredondadas, sua distribuição foi mais heterogênea quanto ao tamanho das mesmas. Entretanto, após a extrusão, as proteínas foram modificadas e pôde-se perceber que quanto mais proteica for a amostra, mais homogênea se torna a distribuição das suas partículas, uma vez que todas resultaram em superfícies irregulares e formatos não esféricos.

De maneira geral, todas as análises executadas e os resultados encontrados demonstraram que, primeiramente, deve-se verificar qual o sistema alimentar está sendo trabalhado para definir a melhor proteína vegetal que poderá ser aplicada para atingir as características nutricionais e tecnológicas desejadas. Além disso, sugere-se que, dentro de uma formulação, as interações com os demais ingredientes adicionados, como carboidratos e lipídeos, poderiam modificar os resultados aqui apresentados, visto que as proteínas foram avaliadas separadamente.

Mantendo o foco de atender às exigências dos consumidores atuais como: menor tempo para preparação e consumo de alimentos, possibilidade de porcioná-los, redução do espaço de armazenamento, independência da cadeia do frio e saudabilidade, foi proposto um novo formato de hambúrguer de frango produzido como uma pré-mistura, aplicando-se todas as proteínas extrusadas anteriormente, bem como o glúten vital. As formulações foram avaliadas antes e após a hidratação e pós cocção. Na composição centesimal, todas tiveram a proteína como seu componente majoritário (> 60 g/100 g), apresentando conteúdos de umidade



(2,46 – 3,36 g/ 100 g) e  $A_w$  (0,23) baixos, mostrando a fabricação de um produto estável (BORDOLOI e GANGULY, 2014), com pouca suscetibilidade à ocorrência de reações químicas e enzimáticas indesejáveis, bem como ao desenvolvimento de micro-organismos (SILVA et al., 2010). Determinar o pH tem como objetivo avaliar a possível microbiota predominante e os processos de deterioração do alimento (SILVA, 2000), entretanto, as variações nos resultados foram provenientes do tipo de proteína extensora aplicada à formulação, onde a proteína de soja e de ervilha apresentaram o maior (6,04) e o menor (5,83) valor, respectivamente, com valores acima de 4,5, o que poderia tornar o alimento mais susceptível à multiplicação microbiana (FRANCO e LANDGRAF, 2005), contudo, os resultados de  $A_w$  citados não permitem tal acontecimento.

Após passarem pelo processo de hidratação, os produtos se tornaram evidentemente ( $\Delta E > 3,5$ ) escurecidos, avermelhados e amarelados, todavia, apresentaram valores similares aos resultados de estudos com hambúrguer de frango fabricado sob técnicas convencionais (ALESÓN-CARBONELL et al., 2005; SÁYAGO-AYERDI, BRENES e GOÑI, 2009). Com a cocção, os produtos desenvolvidos não apresentaram diferenças significativas quanto ao rendimento e encolhimento, entretanto, o maior valor de perda no cozimento ocorreu com a formulação controle, apresentando um rendimento de 67,97 %, uma vez que os extensores possuem como objetivo aumentar a retenção de água e minimizar as perdas durante o cozimento (DENG et al., 2011). Além disso, é importante notar que o glúten vital foi o ingrediente adicionado que mostrou ser o melhor em termos de rendimento (74,88 %) e, apesar da soja ser comumente utilizada como fonte de proteína aplicada como extensor, o maior valor (14,03 %) de encolhimento do hambúrguer encontrado foi na formulação contendo essa leguminosa. Quando a análise de perfil de textura foi realizada, o produto contendo grão-de-bico mostrou resultados satisfatórios aos consumidores, uma vez que os mesmos preferem hambúrgueres que sejam fáceis de mastigar e não duros, menos elásticos e coesos (AKWETEY e KNIPE, 2012).

Na primeira análise sensorial executada com a metodologia de *Napping* (duas primeiras dimensões explicaram 54,28 % da variância), os consumidores foram capazes de diferenciar as amostras e localizá-las sobre a folha de papel em branco de acordo com seus próprios parâmetros. Desse modo, F1 e F4 foram situadas muito próximas uma da outra, bem como as duas amostras controle fornecidas para verificar a habilidade discriminatória dos provadores e, uma vez que substituir a soja foi um dos objetivos propostos, o produto contendo glúten vital apresentou características sensoriais muito semelhantes ao produto contendo SPIE.

No teste de aceitação, as maiores notas para todos os atributos avaliados foram atribuídas ao produto contendo VG, o qual não diferiu ( $P > 0,10$ ) de F3 em nenhuma dessas categorias estudadas e apresentaram a mesma e maior nota para o atributo aroma (7,08). Exceto para a textura de F2, todas as notas atribuídas aos produtos ficaram com a média acima de 5,0 (nem gostei nem desgostei), estando a maior parte das notas situadas na escala referente à resposta “gostei ligeiramente” ( $> 6,0$ ). Analisando os dados gerados pelo CATA, 12 dos 19 termos foram significativamente associados com as amostras, sendo eles: homogênea, consistência de hambúrguer, pastosa, pálida, esfarelenta, quebradiça, firme, sabor residual, macia, seca, sabor de frango e succulenta; com isso, do ponto de vista sensorial, a textura foi o atributo do hambúrguer mais valorizado pelos avaliadores. Com as amostras situadas em diferentes localizações nos gráficos gerados, percebe-se que, assim como no *Napping*, os consumidores foram capazes de perceber diferenças reais entre os produtos. Também se notou que os escores mais altos atribuídos à impressão global (*liking*) demonstraram correlações positivas com os termos “sabor de frango”, “consistência de hambúrguer”, “succulenta”, “macia”, “firme” e “homogênea”, aumentando a nota em 0,5 a 1,5 pontos.

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**- CAPÍTULO 6 -**

**CONCLUSÃO GERAL**

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## CONCLUSÃO GERAL

Os estudos encontrados através da revisão bibliográfica demonstraram que há a necessidade do desenvolvimento de novas formulações para os produtos cárneos convencionais, visto que os consumidores atuais possuem exigências diferenciadas e é possível tornar esses produtos mais atrativos e saudáveis, de forma que possuam altos conteúdos de proteína, baixos valores de gordura e de aditivos químicos. A aplicação de ingredientes de origem vegetal processadas termicamente pode trazer benefícios nutricionais (redução de fatores antinutricionais) e sensoriais aos produtos, bem como auxiliar na melhoria das propriedades tecnológicas (propriedades emulsificantes e espumantes, capacidade de hidratação em água) necessárias para o desenvolvimento de produtos cárneos em geral, mas principalmente, o hambúrguer, pois se destaca no alto consumo pelas classes populares.

A extrusão das proteínas de soja e de ervilha e da farinha de grão-de-bico resultou em extensores com características diferentes daquelas encontradas nas proteínas sem processamento, uma vez que as altas temperaturas e pressão da extrusora modificam a conformação proteica e, conseqüentemente, o comportamento destas durante a realização das análises propostas. Contudo, pode-se concluir que cada material possui características únicas e demonstrou propriedades distintas, não havendo outra proteína que possuísse resultados semelhantes aos da SPIE para que pudesse servir de substituto da mesma. Entretanto, foi proposto que os extrusados fossem aplicados em algum sistema alimentar para avaliá-los melhor, visto que a interação com os demais ingredientes de uma formulação torna as proteínas diferentes de quando analisadas isoladamente.

Conseqüentemente, após o desenvolvimento de um hambúrguer de frango no formato de pré-mistura com diferentes formulações incorporadas das proteínas vegetais extrusadas e do glúten vital, obteve-se sucesso na aplicação como extensores proteicos e fabricação do produto, apresentando boas características para estabilidade no armazenamento sob temperatura ambiente, composição saudável e aceitação sensorial. Tal proposta enfrenta as dificuldades encontradas no mercado atual de produtos cárneos convencionais e acredita-se que há a capacidade de tornar-se a nova direção da cadeia deste tipo de produto. Além disso, a facilidade de fabricação em conjunto com todos os resultados apresentados gerou um depósito de patente do trabalho proposto, evidenciando ainda mais que a pesquisa foi inédita e segue uma tendência, tanto no processo de extrusão quanto na elaboração do novo produto.

**- CAPÍTULO 7 -**

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**- CAPÍTULO 8 -**

**APÊNDICES E ANEXOS**

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## Apêndice I: Termo de Consentimento Livre e Esclarecido (TCLE)

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)	
<p><b>Título da pesquisa:</b> DESENVOLVIMENTO DE EXTENSORES PROTEICOS ATRAVÉS DA EXTRUSÃO TERMOPLÁSTICA E SUA APLICAÇÃO EM PRODUTO CÁRNEO REESTRUTURADO (HAMBÚRGUER) DE FRANGO</p> <p><b>Nome do(s) responsável(is):</b> Mestranda: Ana Carina Matos Hamerski Orientadora: Profª Drª Maria Teresa Pedrosa Silva Clerici</p> <p><b>Número do CAAE:</b> 84450318.3.0000.5404</p>	
<p>Você está sendo convidado a participar como voluntário de uma pesquisa. Este documento, chamado Termo de Consentimento Livre e Esclarecido, visa assegurar seus direitos como participante e é elaborado em <u>duas vias, uma que deverá ficar com você e outra com o pesquisador</u>.</p> <p>Por favor, leia com atenção e calma, aproveitando para esclarecer suas dúvidas. Se houver perguntas antes ou mesmo depois de assiná-lo, você poderá esclarecê-las com o pesquisador. Se preferir, pode levar este Termo para casa e consultar seus familiares ou outras pessoas antes de decidir participar. <u>Se você não quiser participar ou retirar sua autorização, a qualquer momento, não haverá nenhum tipo de penalização ou prejuízo.</u></p> <p><b>Justificativa e objetivos:</b> Essa pesquisa tem como objetivo selecionar sensorialmente a melhor formulação de hambúrgueres de frango elaborados com extensores proteicos extrusados. A proposta do trabalho visa substituir a soja comumente utilizada no mercado de extensores por proteínas de ervilha e de grão-de-bico, buscando uma constituição de nutrientes adequada do produto e também desenvolvendo um produto em pó, em formato de pré-mistura, o qual necessitará apenas de hidratação para sua preparação, viabilizando transporte, personalização da formulação, viagens, entre outros, sem refrigeração. A carne de frango foi escolhida sendo liofilizada para permitir o objetivo de retirada da cadeia do frio para armazenamento desse produto.</p> <p><b>Procedimentos:</b> Participando do estudo você está sendo convidado a experimentar seis diferentes amostras de hambúrgueres de frango e receber fichas sensoriais onde poderá preencher sua frequência de consumo de produtos cárneos e expressar o que acha do produto através das semelhanças e diferenças existentes entre as amostras, caracterizando-as com palavras de sentimentos ou sensações, e também avaliar as amostras quanto à aceitação global através de uma escala.</p> <p><b>Desconfortos e riscos:</b> Você <u>não</u> deve participar deste estudo se:</p> <ul style="list-style-type: none"> <li>• Possuir intolerância ou alergia ao glúten (celíacos), pois há uma formulação fornecida para consumo que possui esse ingrediente, o que poderá gerar dores agudas intestinais, náuseas e reações alérgicas devido à ingestão deste ingrediente.</li> </ul> <p><b>Benefícios:</b> A participação nesta análise não resulta em pagamentos ou bonificação direta aos provadores voluntários. Os resultados da pesquisa contribuirão para o desenvolvimento de hambúrguer de frango no formato de pré-mistura (em pó) e seleção do (s) extensor (es) proteico (s) que não gerou (aram) alterações perceptíveis na análise sensorial. Também contribuirá para valorização da retirada da cadeia do frio nesse tipo de produto e ingestão de menores quantidades de soja. Não haverá benefícios diretos para o participante da pesquisa.</p> <p><b>Ressarcimento:</b> A participação nesta análise não gerará nenhum custo previsto aos provadores. Portanto não está prevista qualquer forma de pagamento e/ou reembolso aos voluntários, entretanto, o participante tem direito à indenização em casos de danos decorrentes da pesquisa.</p>	
<p><b>Acompanhamento e assistência:</b> Os pesquisadores envolvidos estarão presentes ao longo do estudo, podendo esclarecer qualquer tipo de dúvida sobre a composição do produto, também explicando os procedimentos para a realização da análise sensorial.</p> <p><b>Sigilo e privacidade:</b> Você tem a garantia de que <u>sua identidade será mantida em sigilo e nenhuma informação será dada a outras pessoas que não façam parte da equipe de pesquisadores</u>. Na divulgação dos resultados desse estudo, seu nome ou qualquer outra informação coletada não será divulgada.</p> <p><b>Contato:</b> Em caso de dúvidas sobre a pesquisa, você poderá entrar em contato com os pesquisadores da Universidade Estadual de Campinas – Unicamp, Faculdade de Engenharia de Alimentos – FEA, Rua Monteiro Lobato, 80 – Cidade Universitária, Campinas – SP, 13083-862, Departamento de Tecnologia de Alimentos, Laboratório de Cereais, Raízes e Tubérculos. Ana Carina Matos Hamerski, ana.hamerski.vet@gmail.com, (19) 999710545 Dra. Maria Teresa Pedrosa Silva Clerici, mclerici@fea.unicamp.br, (19)3521-4000 (sala). Em caso de denúncias ou reclamações sobre sua participação e sobre questões éticas do estudo, você poderá entrar em contato com a secretaria do Comitê de Ética em Pesquisa (CEP) da UNICAMP das 08:30hs às 11:30hs e das 13:00hs às 17:00hs na Rua: Tessália Vieira de Camargo, 126; CEP 13083-887 Campinas – SP; telefone (19) 3521-8936 ou (19) 3521-7187; e-mail: <a href="mailto:cep@fcm.unicamp.br">cep@fcm.unicamp.br</a>.</p> <p><b>Consentimento livre e esclarecido:</b> Após ter recebido esclarecimentos sobre a natureza da pesquisa, seus objetivos, métodos, benefícios previstos, potenciais riscos e o incômodo que esta possa acarretar, aceito participar:</p> <p>Nome do (a) participante: _____</p> <p>_____ Data: ____/____/____ (Assinatura do participante ou nome e assinatura do seu RESPONSÁVEL LEGAL)</p> <p><b>Responsabilidade do Pesquisador:</b> Asseguro ter cumprido as exigências da resolução 466/2012 CNS/MS e complementares na elaboração do protocolo e na obtenção deste Termo de Consentimento Livre e Esclarecido. Asseguro, também, ter explicado e fornecido uma via deste documento ao participante. Informo que o estudo foi aprovado pelo CEP perante o qual o projeto foi apresentado e pela CONEP, quando pertinente. Comprometo-me a utilizar o material e os dados obtidos nesta pesquisa exclusivamente para as finalidades previstas neste documento ou conforme o consentimento dado pelo participante.</p> <p>_____ Data: _____ (Assinatura do pesquisador)</p>	
Rúbrica do pesquisador: _____	Rúbrica do participante: _____
Rúbrica do pesquisador: _____	Rúbrica do participante: _____

## Apêndice II: Ficha Sensorial para teste de aceitação e CATA

Avaliação sensorial de hambúrguer de frango	Avaliação sensorial de hambúrguer de frango																																			
<p>Nome: _____ Idade: _____</p> <p>Sexo: F ( ) M ( ) Data: ____/____/____</p> <p>Por favor, assinale a opção que melhor corresponde ao seu hábito de consumo:</p> <p>1. Quantas vezes na semana você consome (em média)?</p> <p><input type="checkbox"/> Nenhuma ( )</p> <p><input type="checkbox"/> Uma vez ( )</p> <p><input type="checkbox"/> Duas vezes ( )</p> <p><input type="checkbox"/> Três vezes ( )</p> <p><input type="checkbox"/> Quatro vezes ou mais ( )</p> <p>2. Em qual refeição você tem o hábito de consumir?</p> <p><input type="checkbox"/> Café da manhã      <input type="checkbox"/> Jantar</p> <p><input type="checkbox"/> Lanche da manhã      <input type="checkbox"/> Ceia</p> <p><input type="checkbox"/> Almoço      <input type="checkbox"/> Não se aplica</p> <p><input type="checkbox"/> Lanche da tarde</p> <p>3. Qual tipo de produto cárneo você consome mais?</p> <p>( ) nuggets ( ) salsicha ( ) hambúrguer ( ) almôndega</p> <p>( ) embutidos (presunto, mortadela, peito de peru, entre outros)</p> <p>( ) outro. Especifique: _____</p> <p>4. De que forma você costuma consumi-los?</p> <p>( ) Frito (com ou sem óleo) ( ) Assado ( ) Cozido</p> <p>( ) Outro. Qual? _____</p>	<p>Você está recebendo uma amostra de hambúrguer de frango, por favor, prove e avalie a amostra codificada usando a escala abaixo para indicar o quanto você gostou ou desgostou dos seguintes atributos:</p> <p>(9) Gostei muitíssimo</p> <p>(8) Gostei muito</p> <p>(7) Gostei regularmente</p> <p>(6) Gostei ligeiramente</p> <p>(5) Indiferente</p> <p>(4) Desgostei ligeiramente</p> <p>(3) Desgostei regularmente</p> <p>(2) Desgostei muito</p> <p>(1) Desgostei muitíssimo</p> <table style="width: 100%; margin-top: 10px;"> <thead> <tr> <th colspan="2" style="text-align: center;">AMOSTRA _____</th> </tr> <tr> <th style="text-align: center;">ATRIBUTO</th> <th style="text-align: center;">NOTA</th> </tr> </thead> <tbody> <tr> <td>Impressão global</td> <td>_____</td> </tr> <tr> <td>Aparência</td> <td>_____</td> </tr> <tr> <td>Aroma</td> <td>_____</td> </tr> <tr> <td>Gosto</td> <td>_____</td> </tr> <tr> <td>Textura</td> <td>_____</td> </tr> </tbody> </table> <p>Agora, para a segunda etapa do teste, por favor, assinale abaixo todas as opções que você relaciona com a amostra:</p> <table style="width: 100%; margin-top: 10px;"> <tbody> <tr> <td><input type="checkbox"/> Homogêneo</td> <td><input type="checkbox"/> sabor de legume</td> <td><input type="checkbox"/> macio</td> </tr> <tr> <td><input type="checkbox"/> consistência de hambúrguer</td> <td><input type="checkbox"/> quebradiço</td> <td><input type="checkbox"/> salgado</td> </tr> <tr> <td><input type="checkbox"/> textura pastosa</td> <td><input type="checkbox"/> pouco condimentado</td> <td><input type="checkbox"/> condimentado</td> </tr> <tr> <td><input type="checkbox"/> cor alaranjada</td> <td><input type="checkbox"/> firme</td> <td><input type="checkbox"/> seco</td> </tr> <tr> <td><input type="checkbox"/> pálido</td> <td><input type="checkbox"/> sabor residual</td> <td><input type="checkbox"/> sabor de frango</td> </tr> <tr> <td><input type="checkbox"/> sabor de ranço</td> <td><input type="checkbox"/> sabor grelhado</td> <td><input type="checkbox"/> suculenta</td> </tr> <tr> <td><input type="checkbox"/> esfarelento</td> <td></td> <td></td> </tr> </tbody> </table>	AMOSTRA _____		ATRIBUTO	NOTA	Impressão global	_____	Aparência	_____	Aroma	_____	Gosto	_____	Textura	_____	<input type="checkbox"/> Homogêneo	<input type="checkbox"/> sabor de legume	<input type="checkbox"/> macio	<input type="checkbox"/> consistência de hambúrguer	<input type="checkbox"/> quebradiço	<input type="checkbox"/> salgado	<input type="checkbox"/> textura pastosa	<input type="checkbox"/> pouco condimentado	<input type="checkbox"/> condimentado	<input type="checkbox"/> cor alaranjada	<input type="checkbox"/> firme	<input type="checkbox"/> seco	<input type="checkbox"/> pálido	<input type="checkbox"/> sabor residual	<input type="checkbox"/> sabor de frango	<input type="checkbox"/> sabor de ranço	<input type="checkbox"/> sabor grelhado	<input type="checkbox"/> suculenta	<input type="checkbox"/> esfarelento		
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**Anexo I: Submissão do artigo à revista Trends in Food Science & Technology**

Successfully received: submission Reformulations of poultry meat products with the use of functional vegetable ingredients – A review for Trends in Food Science & Technology  

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Journal: Trends in Food Science & Technology

Dear Mrs. Hamerski,

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Trends in Food Science & Technology



## Anexo II: Pedido Nacional de Invenção

<div data-bbox="174 338 519 414">  <b>INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL</b> </div> <div data-bbox="680 338 972 443"> 10/12/2018 870180165519 18:44    29409161807174378 </div> <div data-bbox="197 469 1059 539"> <b>Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT</b> </div> <div data-bbox="237 568 719 596"> <b>Número do Processo:</b> BR 10 2018 076620 1 </div> <div data-bbox="163 639 432 667"> <b>Dados do Depositante (71)</b> </div> <div data-bbox="163 699 338 724"> <b>Depositante 1 de 1</b> </div> <div data-bbox="259 759 990 785"> <b>Nome ou Razão Social:</b> UNIVERSIDADE ESTADUAL DE CAMPINAS - UNICAMP </div> <div data-bbox="324 799 627 825"> <b>Tipo de Pessoa:</b> Pessoa Jurídica </div> <div data-bbox="362 839 640 865"> <b>CPF/CNPJ:</b> 46068425000133 </div> <div data-bbox="336 880 568 904"> <b>Nacionalidade:</b> Brasileira </div> <div data-bbox="277 922 611 951"> <b>Qualificação Jurídica:</b> Órgão Público </div> <div data-bbox="376 963 781 994"> <b>Endereço:</b> Cidade Universitária Zeferino Vaz </div> <div data-bbox="398 1007 575 1032"> <b>Cidade:</b> Campinas </div> <div data-bbox="398 1046 515 1072"> <b>Estado:</b> SP </div> <div data-bbox="418 1090 580 1115"> <b>CEP:</b> 13084-971 </div> <div data-bbox="418 1129 537 1155"> <b>País:</b> Brasil </div> <div data-bbox="383 1169 479 1197"> <b>Telefone:</b> </div> <div data-bbox="425 1211 479 1236"> <b>Fax:</b> </div> <div data-bbox="409 1252 732 1281"> <b>Email:</b> patentes@inova.unicamp.br </div>	<div data-bbox="1113 343 1283 368"> <b>Dados do Pedido</b> </div> <div data-bbox="1247 399 1671 426"> <b>Natureza Patente:</b> 10 - Patente de Invenção (PI) </div> <div data-bbox="1126 437 1933 464"> <b>Título da Invenção ou Modelo de Utilidade (54):</b> PROCESSO DE EXTRUSÃO DE PROTEÍNAS E PRODUTO </div> <div data-bbox="1281 462 1910 488"> <b>Resumo:</b> CÂRNEO COMPREENDENDO PROTEÍNA EXTRUSADA </div> <div data-bbox="1326 485 1998 713"> A presente invenção refere-se à processo de modificação de extensores proteicos a partir de extrusão termoplástica e a um produto cárneo desidratado contendo tais extensores, em que o produto é isento de conservantes e estabilizantes. O produto pode ser usado por pacientes com doenças de baixa imunidade, hipertensos e em controle de glicemia e ingestão de gordura, pessoas que realizam atividade em ambiente externo, por empresas que fabricam sopas e caldos em pó ou processados cârneos, pois não apresentam adição de amido e gordura, somente necessitando de água para reidratação. </div> <div data-bbox="1252 707 1435 732"> <b>Figura a publicar:</b> 2 </div> <div data-bbox="1113 759 1321 785"> <b>Dados do Procurador</b> </div> <div data-bbox="1113 815 1223 839"> <b>Procurador:</b> </div> <div data-bbox="1198 853 1655 880"> <b>Nome ou Razão Social:</b> Patricia Franco Leal Gestic </div> <div data-bbox="1279 893 1417 919"> <b>Numero OAB:</b> </div> <div data-bbox="1288 932 1417 957"> <b>Numero API:</b> </div> <div data-bbox="1299 971 1545 999"> <b>CPF/CNPJ:</b> 21927410819 </div> <div data-bbox="1310 1013 1957 1040"> <b>Endereço:</b> Rua Roxo Moreira 1831, Caixa Postal 6131 - INOVA UNICAMP </div> <div data-bbox="1330 1054 1514 1082"> <b>Cidade:</b> Campinas </div> <div data-bbox="1330 1094 1451 1120"> <b>Estado:</b> SP </div> <div data-bbox="1350 1134 1518 1160"> <b>CEP:</b> 13083-970 </div> <div data-bbox="1317 1173 1417 1198"> <b>Telefone:</b> </div> <div data-bbox="1357 1214 1417 1238"> <b>Fax:</b> </div> <div data-bbox="1344 1252 1664 1279"> <b>Email:</b> patentes@inova.unicamp.br </div>
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**Documentos anexados**

Tipo Anexo	Nome
Comprovante de pagamento de GRU 200	1343_GRU.pdf
Procuração	PROCURACAO_140318.pdf
Relatório Descritivo	1343_RELATORIO DESCRITIVO_191218.pdf
Reivindicação	1343_REIVINDICACOES_191218.pdf
Desenho	1343_FIGURAS_191218.pdf
Resumo	1343_RESUMO_191218.pdf



**Acesso ao Patrimônio Genético**

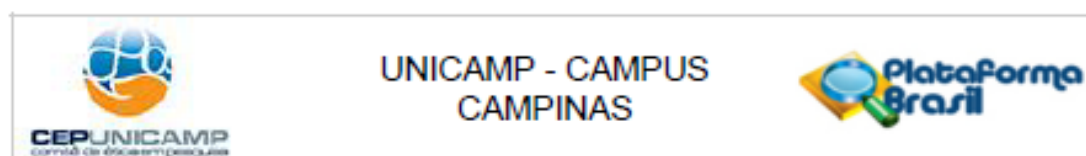
- ☒ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

**Declaração de veracidade**

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

### Anexo III: Parecer do Comitê de Ética e Pesquisa (CEP)

 <b>CEP UNICAMP</b> <small>comitê de ética em pesquisa</small>	<b>UNICAMP - CAMPUS CAMPINAS</b>									
<b>PARECER CONSUBSTANCIADO DO CEP</b>										
<b>DADOS DO PROJETO DE PESQUISA</b>										
<b>Título da Pesquisa:</b> DESENVOLVIMENTO DE EXTENSORES PROTEICOS ATRAVÉS DA EXTRUSÃO TERMOPLÁSTICA E SUA APLICAÇÃO EM PRODUTO CARNEO REESTRUTURADO (HAMBÚRGUER) DE FRANGO										
<b>Pesquisador:</b> Ana Carina Matos Hamerski										
<b>Área Temática:</b>										
<b>Versão:</b> 1										
<b>CAAE:</b> 84450318.3.0000.5404										
<b>Instituição Proponente:</b> Faculdade de Engenharia de Alimentos										
<b>Patrocinador Principal:</b> Financiamento Próprio										
<b>DADOS DO PARECER</b>										
<b>Número do Parecer:</b> 2.617.682										
<b>Apresentação do Projeto:</b>										
<b>Introdução:</b>										
<p>Quando comparados com as gerações anteriores, os consumidores atuais possuem maior acesso à informação e exigências mais específicas sobre o alimento que procuram. No entanto, a escassez de tempo e a procura por alimentos de rápido e fácil preparo traz a preocupação com os problemas de saúde que esses alimentos podem ocasionar. Desse modo, a principal busca está orientada em ingredientes que satisfaçam os requerimentos de proteína na nutrição humana, que contenham propriedades funcionais como capacidade de retenção de água, capacidade de formar géis e emulsões, que no processo de fabricação gerem o mínimo de resíduos para o meio ambiente e que sejam obtidos com o mínimo de custo (PÉREZ, MOLINA e VALENCIA, 2011; OLIVEIRA et al., 2013). Conhecidas como proteínas não cámeas, extensores ou substitutos de gordura em produtos cámeos emulsionados, as proteínas de origem vegetal vêm sendo estudadas nas suas mais diversas aplicações (YUN-SANG et al., 2009). As proteínas vegetais possuem qualidade nutricional e disponibilidade inferiores às proteínas animais, as quais são consideradas proteínas completas devido ao seu balanço de aminoácidos essenciais (CAKMAK et al., 2016). Leguminosas possuem melhor valor nutricional e são usadas como um ingrediente alternativo em novos produtos. A decisão de englobar ingredientes alternativos quando um novo produto é desenvolvido, que tragam ganhos econômicos ao fabricante, deve considerar a opinião do</p>										
<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td colspan="2"><b>Endereço:</b> Rua Tessália Vieira de Camargo, 126</td> </tr> <tr> <td><b>Bairro:</b> Barão Geraldo</td> <td><b>CEP:</b> 13.083-887</td> </tr> <tr> <td><b>UF:</b> SP</td> <td><b>Município:</b> CAMPINAS</td> </tr> <tr> <td><b>Telefone:</b> (19)3521-8936</td> <td><b>Fax:</b> (19)3521-7187      <b>E-mail:</b> cep@fcm.unicamp.br</td> </tr> </table>			<b>Endereço:</b> Rua Tessália Vieira de Camargo, 126		<b>Bairro:</b> Barão Geraldo	<b>CEP:</b> 13.083-887	<b>UF:</b> SP	<b>Município:</b> CAMPINAS	<b>Telefone:</b> (19)3521-8936	<b>Fax:</b> (19)3521-7187 <b>E-mail:</b> cep@fcm.unicamp.br
<b>Endereço:</b> Rua Tessália Vieira de Camargo, 126										
<b>Bairro:</b> Barão Geraldo	<b>CEP:</b> 13.083-887									
<b>UF:</b> SP	<b>Município:</b> CAMPINAS									
<b>Telefone:</b> (19)3521-8936	<b>Fax:</b> (19)3521-7187 <b>E-mail:</b> cep@fcm.unicamp.br									



Continuação do Parecer: 2.617.682

apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento".

-O pesquisador deve manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda e responsabilidade, por um período de 5 anos após o término da pesquisa.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1065476.pdf	21/02/2018 15:40:28		Aceito
Projeto Detalhado / Brochura Investigador	BROCHURA_PESQUISA.pdf	21/02/2018 15:38:51	Ana Carina Matos Hamerski	Aceito
Outros	Anexoll_aceitacao_global.pdf	21/02/2018 15:37:08	Ana Carina Matos Hamerski	Aceito
Outros	Anexol_questionario_consumo.pdf	21/02/2018 15:36:45	Ana Carina Matos Hamerski	Aceito
Outros	declaracao_colaboradora.pdf	21/02/2018 15:35:00	Ana Carina Matos Hamerski	Aceito
Outros	Comprovacao_vinculo_Unicamp.pdf	21/02/2018 15:33:41	Ana Carina Matos Hamerski	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	21/02/2018 15:32:57	Ana Carina Matos Hamerski	Aceito
Cronograma	CRONOGRAMA.pdf	21/02/2018 15:32:30	Ana Carina Matos Hamerski	Aceito
Folha de Rosto	folha_de_rosto.pdf	21/02/2018 15:24:16	Ana Carina Matos Hamerski	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Endereço: Rua Tessália Vieira de Camargo, 126  
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