Rev Chil Nutr 2020; 47(3): 463-469.

http://dx.doi.org/10.4067/S0717-75182020000300463

Artículo de Revisión / Review Article

Soy protein: a food allergen frequently used in the preparation of meat products

Proteína de soya: un alergeno alimentario altamente utilizado en la preparación de productos cárnicos

Cristiane Canan[1], Djonathan Adamante[1], Daneysa Kalschne[1], Marinês Paula Corso[1], Elciane Regina Zanatta[1].*

1. Departamento Acadêmico de Alimentos, Universidade Tecnológica Federal do Paraná (UTFPR), Medianeira, Paraná, Brazil.

*Corresponding author: Cristiane Canan
Avenida Brasil, 4232, Parque Independência, Medianeira, Paraná, Brazil.
E-mail: canan@utfpr.edu.br

Este trabajo fue recibido el 20 de enero de 2019.
Aceptado con modificaciones: 13 de diciembre de 2019.
Aceptado para ser publicado: 09 de enero de 2020.

ABSTRACT

The addition of soy proteins, currently classified as a food allergen, into meat products is a commonly used practice due to its functional properties and low cost. Its addition to meat products can cause health problems for individuals allergic to these proteins. Allergic individuals can be affected by the ingestion of low amounts of the allergen. In Brazil, limits are set for the addition of soy proteins in meat products in order to avoide fraud. Starting in 2015 reporting the name of the added component became mandatory for all food labelling. Some studies have reported that food processing can reduce the allergenicity, either by irreversible removal of allergens or by modifying the allergen structure. However, the technological approach to decrease allergenicity has largely been empirical. This review describes the use of soy protein in meat products and the health risk for allergic individuals and consumers of these products. Finally, appropriate methodologies for the detection and quantification of these proteins must be further explored and established to avoid fraud and to preserve consumer health.

Keywords: Allergenicity; Elisa; Epitopes; Food allergy; IgE-associated allergies.

RESUMEN

La adición de proteínas de soya, actualmente clasificadas como alergeno alimentario, en los productos cárnicos es una práctica comúnmente utilizada debido a sus propiedades funcionales y bajo costo. Su adición en productos cárnicos puede causar problemas de salud en personas alérgicas a estas proteínas. Las personas alérgicas pueden verse afectadas por la ingestión de cantidades diminutas de alérgeno. En Brasil, se establecen límites para la adición de proteínas de soya en los productos cárnicos con el objetivo de evitar el fraude. Solo en 2015 se hizo obligatoria la declaración en la etiqueta de todos los alimentos que indicaban la presencia de sustancias alérgicas, así como el nombre del componente. Algunos estudios se refieren al procesamiento de alimentos para reducir la alergenicidad, ya sea mediante la eliminación irreversible de alergenos o modificando la estructura del alergeno; sin embargo, el enfoque tecnológico hasta ahora para disminuir la alergenicidad ha sido en gran medida empírico. Esta revisión describe el uso de proteína de soya en los productos cárnicos y el riesgo que puede causar para la salud de las personas alérgicas y a los consumidores de estos productos. Finalmente, las metodologías apropiadas para la detección y cuantificación de estas proteínas deben explorarse en profundidad y establecerse para evitar el fraude y preservar la salud de los consumidores.

INTRODUCTION

The number of people affected by some type of food allergy has grown year by year, both in numbers of cases and in severity[1,2]. Food allergy is a serious health problem

that affects between 3% and 5% of adults, and 8% of children[3,4]. Additionally, the statistics in developed countries show that up to 20% of people suffer from some kind of food sensitivity caused by the consumption of foodstuffs containing chemical, microbiological, or pharmacologically active contaminants[5].

Food allergy occurs due to adverse reactions to food, caused by a particular individual intolerance towards commonly tolerated foods. Intolerance derived from an immunological mechanism is referred to as food allergy and the non-immunological form is called food intolerance[6]. It is important to emphasize that allergy can lead to death if people are not rescued immediately due to the great predisposition to develop an anaphylaxis state[7].

Due to the complexity of the subject, food manufacturers are now required to indicate the possible presence of potential allergens. Additionally, companies must ensure good control and traceability systems for processed foods in order to adequately inform the food composition[5]. Therefore, if the product contains ingredients classified as food allergens, it must be declared on the label describing also the name of the component that causes food allergy[8].

Food allergens are naturally occurring proteins of the allergenic foods. Small and specific regions of the protein molecule, called epitopes, are responsible for the immunoglobulin E (IgE)-mediated allergic response by acting as an antigen[9].

The high population growth promoted an increase in the demand for protein foods. In this context, the high biological value of animal proteins is indisputable. However, due to its high cost, the meat industry began to use vegetable protein in the elaboration of its products, especially soy protein. The use of soy proteins associated with new meat production technologies enabled the development of low cost food formulations with excellent nutritional quality[10,11].

Soy proteins are classified as food allergens and have been used in meat product preparation due its good functional properties. It is important to highlight the gel formation and high water retention capacities, contribution to texture improvement, flavor characteristics and increased product stability. However, the addition of soy protein has been considered a food safety risk for many consumers who suffer with food allergies. Addition of soy protein may vary according to the processed meat product[10].

Processed meat products

The consumption of processed meat products is expanding in the Brazilian market. One of the results of this increase is the growing diversity of products developed in the last decade[12]. In the United States, the European Union and other developed countries, processed meat intake constituted 22% of the total meat consumed from either red meat or poultry categories[13].

Fresh meat processing aims to reduce the problems of perishability, transport, storage, and costs, while meeting consumer expectations by using appropriate physical, chemical and/or biological treatments[10]. For consumers, the most important attributes of a food product are its sensory characteristics[14] and the nutritional composition of a food is often forgotten.

Meat products are prepared wholly or partially with meat, fish or fat, edible by-products from slaughter animals, or from other species, followed or not by the addition of authorized additives, seasonings, spices and/or ingredients from animal or vegetable origin[15]. Vegetable proteins and polysaccharides are used in the preparation of meat products because they form complexes with good functional properties[16]. Soy protein acts by encapsulating fat droplets and forming a continuous protein-gel network through the aqueous phase, in addition to providing increased water absorption, gelling, cohesiveness or tackiness, emulsification and fat absorption[17]. However, Tsao et al.[18] reported that soy protein used as a food binder has lower adhesive strength in restructured meat processing compared to myofibrillar proteins, gluten, blood plasma and egg white albumin. However, because of its high availability and low cost, it is widely used in meat product formulations.

Food processing can reduce allergenicity, either by irreversible removal of allergens or by modifying the allergen structure[19], but it should be stressed that processing does not completely abolish the allergenic potential of allergens[20]. However, the technological approach to decrease allergenicity has largely been empirical[21]. The three-dimensional structure of food proteins undergo alterations by heating, acid treatment, and hydrolytic or other enzyme activity during food processing or cooking. In contrast, the primary structure of proteins is cleaved through the actions of digestive enzymes (proteases) such as pepsin, trypsin, and chymotrypsin in the digestion process[20]. The change in structure of the epitope decreases its IgE antibody binding ability, and the attenuation of an allergic reaction due to decreased IgE antibody binding ability is termed hypoallergenicity[22]. However, studies evaluating the effect on soy protein caused by its addition in meat products have not yet been carried out.

In Brazil, maximum limits of soy protein addition ranging from 1 to 4% are established as an aggregate protein, depending on the type of meat product. Generally, in mortadella, hot dog sausage, meatballs, nuggets, hamburger and kibe up to 4% of soy protein is allowed; 3% for pâté; 2.5% for thick milled sausage; 2-2.5% for different types of cooked ham; 2% for bacon, loin, and cooked pork swede; 1% for tender ham and, for marinated chicken, it is allowed for use as an optional ingredient without specifying limits. Additionally, soy protein addition has some restrictions for use in some specific products within some above-cited meat products[23,24].

However, these limits were established in order to prevent fraud, without consideration of consumer health. It should be noted that soy protein detection and quantification in meat products is still a problem for supervision, due to the shortage of appropriate analytical methodologies for determining this ingredient in this complex matrix[17].

Food allergy

There are more than 160 foods that can cause an allergy and 90% of cases of allergic reactions are connected with eight foods (milk, eggs, fish, crustacean shellfish, nuts, peanuts, wheat and soy). These eight foods, and any ingredients that contain proteins derived from one or more of them, are the main food allergens[25].

Adverse reactions to foods, aside from those considered toxic, are caused by individual intolerance towards commonly tolerated foods. Intolerance derived from an immunological mechanism is referred to as food allergy, this term should be used only for immune mediated reactions; non-immune mediated reactions and non-immunological form is called food intolerance[6]. The most common mechanism of food allergy is IgE-mediated reactions, which cause immediate reactions within 2 h after the exposure to food allergens. Non-IgE mediated reaction is a food allergic reaction that occurs independent of IgE[22]. IgE-mediated food allergy is the most common and dangerous type of adverse food reaction. Generally, regardless of the dose consumed, the development of symptoms and serious clinical manifestations may occur within minutes or hours after ingestion, and the individual should be attended immediately by a specialized health service, otherwise the person may die, if the reaction reaches the anaphylactic stage[2,26]. In Brazil, food allergy is classified as the second most common cause of anaphylaxis[26].

The symptoms of food allergy include: (1) gastrointestinal reactions (abdominal pain, nausea, vomiting, diarrhea, gastrointestinal bleeding, protein-losing enteropathy, oral, and pharyngeal pruritus); (2) skin reactions (urticaria, angioedema, eczema, erythema, itching, and redness; (3) respiratory (rhinitis, asthma, cough, laryngeal edema, Heiner's syndrome, and narrowing of the airways); (4) systemic (anaphylaxis, hypotension, and dysrhythmia); and (5) controversial or unproven (behavioral disorders, stress-fatigue syndrome, hyperactivity disorder and attention deficit, otitis media, psychiatric disorders, neurological disorders, musculoskeletal disorders, and migraines)[2].

Food intolerance is mainly caused by enzymatic defects in the digestive system, as is the case with lactose intolerance, but may also result from pharmacological effects of vasoactive amines present in foods (e.g. histamine). Prevention and treatment are based on avoiding these foods[6]. Currently, the only method available to avoid any allergic reaction is the complete dietary exclusion of the offending foods[21].

Main food allergens

Environmental and genetic factors contribute to the atopic predisposition of individuals and determine susceptibility to develop allergic immune responses. In atopic people who have a predisposition toward developing IgE-associated allergies, encounters with allergens are activated after processing by antigen-presenting cells (e.g., macrophages, dendritic cells and B cells) and allergen-specific T-helper 2 (Th2) cells, which produce cytokines such as interleukin (IL)4 and IL13. These cytokines induce class switching and production of allergen-specific IgE[27]. Thus, a specific IgE antibody recognizes a specific site of these structures (epitope) for binding. In proteins, epitopes are equivalent to a sequence of approximately 5 to 7 amino acids[28], which are recognized sequentially in the primary structure, together with their spatial position in the tertiary or quaternary structure. Among the multiple proteins composing any food, the protein molecules with allergenicity (IgE antibody binding ability) are called the allergenic component of the food[22].

According to the Brazilian Association of Allergy and Immunopathology, the form of induction of the allergic reaction to food can be classified as: class I (ingestion, ingested proteins) and class II allergens (proteins inhaled through the respiratory tract, pollen, cross-reactivity with homologous epitopes of plant-derived foods)[1].

Among the main food allergens are some foods that provide more routine notifications in Brazilian health centers. For example, cow's milk that contain proteins with high reactivity rates and eggs that contain protein in albumen, which may delay reaction in the body, making it difficult to identify and assimilate the symptoms. The food most frequently related to allergy cases of anaphylaxis are crustaceans and fish, due to the high reactivity of their proteins in people allergic to this food[1].

Matsuo, Yokooji and Taogoshi[29] have described that foodborne allergies are increasing worldwide. In Japan, the highest number of food-borne allergy cases are chicken eggs with 38.2%, cow's milk with 15.9% and wheat with 8%. However, in the United States, peanuts have the highest number of reports of allergy with 25.2%, followed by cow's milk with 21.1%, shellfish with 17.2%, and chestnuts with 13.1%. On the European continent, cow's milk, wheat, chicken eggs, fish, and soybeans are the main causes of food allergy. It has been observed that the foods associated with food allergies and their proportions differ by region. However, what should be taken into account is the number of cases, which increase more than 30% each year in the world.

Soy protein

The origin of the soybean was described about 4000-5000 years ago, originating in the north and central regions of China and introduced in the United States in the mid-18th century, with official large-scale production occurring after the 20th century[11,30]. Some trials to cultivate soybeans have been known in France and England since the 18th century, but were not developed further[30]. The United States Department of Agriculture (USDA) estimates that world soybean production for the 2018/2019 crop is 367.50 million tons and, compared to the 2017/2018 crop, there will be an increase of 8.54% in production. The United States is still the largest producer of soybeans (34% of world production), followed by Brazil (32.79%)[31].

Soybeans are composed of proteins (35%), carbohydrates (31%), oil (17%), water (13%) and ash (4%). It is important to emphasize that the protein and lipid contents are the components of soybeans that are of greatest commercial interest[32]. By removing oil at lower temperatures, soy protein is obtained, and is widely used in the food industry[30].

Soy proteins are obtained through debarking, baking and degreasing by hexane extraction, followed by milling to obtain defatted flours or flours with approximately 50 to 54% protein. For the production of protein concentrates with 65 to 70% protein, carbohydrates and compounds responsible for the flavor are eliminated by extraction with ethanol or acid. Soybean flours can also be subjected to extraction with alkali, centrifugation in order to remove the fibers, reprecipitation and drying to obtain protein isolates containing 90% protein[33,34].

Soy proteins are a mixture of macromolecules of different sizes, charge densities and structures. The molecular weight of soy proteins range from 8 to 600 kDa, with a higher concentration in the range of 110 to 350 kDa. The main protein fractions of soybean are at 2, 7, 11 and 15S (S stands for *Svedberg* unit, and is a sedimentation constant). Larger Svedberg numbers indicate a protein with higher molecular weight. The 2S fraction predominates during seed development and represents about 20% of proteins, being composed of biologically active proteins, trypsin inhibitors, and cytochrome C. The 7S represents a little more than a third of the soluble proteins, about 37% of the soy protein and is composed of the enzymes β-amylase and lipoxygenase, conglycinin, and hemagglutinin. The 11S corresponds to 31% of total proteins, also known as glycinin, is a reserve protein, whereas the 15S fraction (15S globulin) represents only one tenth of the total protein and may be a polymer of other proteins[11,32].

Consumption of soy protein products mainly occurs in the form of derivatives or ingredients that are used in the manufacturing of various food products, such as dairy products, meat products, bakery products, breakfast cereals, baby foods and beverages, with only a small amount consumed directly in the human diet[35].

The specificity of soy allergens is variable and complex. A total of 28 different soy proteins were recognized as being able to bind IgE in soy-allergic patients. However, only a few of these proteins are considered major allergens, about 50%[36]. The major storage proteins β-conglycinin (Gly m 5) and glycinin (Gly m 6) represent 70% of the whole soybean protein and have been related to severe allergic reactions[21,37]. Additionally, various IgE-binding soy proteins, considered potential soy allergens, have been described. However, for some of these IgE-binding proteins, the clinical relevance is unclear. The time to tolerance acquisition for soy protein depends on the allergen proteins recognized by specific IgE antibodies and antibody binding sites (epitope)[37].

According Ladics et al.[38] new varieties of soybean should be analyzed. Eight of the potential allergens listed in the Organization of Economic Cooperation and Development (OECD)[39] soybean consensus document (Gly m 3, Gly m 4, Gly m Bd28K, Gly m Bd30K, Gly m 5, Gly m 6, Gly m 8, and Kunitz trypsin inhibitor) have both appropriate supporting clinical data and sufficient sequence information to be evaluated in comparative endogenous soybean allergen studies (Table 1). The remaining seven proteins (Gly m 1, Gly m 2, unknown 50 kDa protein, unknown 39 kDa protein, P-22-25, lipoxygenases and lectin) lack sufficient data for clear classification as confirmed allergens and/or available sequence information.

Table 1. Allergenic soybean proteins listed in the Organization of Economic Cooperation and Development (OECD) soybean consensus document.

Allergen	Protein Family	MD (SDS-Page) (kDa)	Length (amino acids)	Reference
Gly m 3	Profilin	14	131	40
Gly m 4	Bet v 1-like protein	17	158	40
Gly m Bd28K	Cupin (7S-vicilin; β-conglycin, α-subunit)	60-80	473	40
Gly m Bd30K	Maturing seed protein (peptidase C1-like; papain-like)	9	122	40
Gly m 5	Cupin (7S-vicilin; β-conglycin, α-subunit)	140-170	543-559	40,41
Gly m 6	Glycinin (11S-legumin)	320-360	495-562	40,41
Gly m 8	Napin-type (2S seed storage albumins)	8	158	38,42
Kunitz trypsin inhibitor	Soybean trypsin inhibitor (Kunitz) protease inhibitors	21	170 -200	43

It is important to emphasize that in soy-based products such as soy flour, texturized soy protein, soy protein concentrates, soy protein isolates, protein hydrolysates and fermented products the proteins can be modified, which influences the study of allergenicity. Comprehensive allergenicity assessment has only been conducted for soybean oil and lecithin[20,21].

Soy derivatives have their identity and quality standards established by Brazilian Resolution RDC No. 268 of 2005[44] and, for quantity of allowed limits for use in meat products, the Technical Regulations of Identity is used for meatball, hams, hamburger, kibbe, mortadela, hot dog sausage, and thick milled sausage[23,24]. More recently, the Brazilian Health Regulatory Agency (ANVISA) published Resolution DC No. 26 in 2015[45] in order to establish the requirements for mandatory labeling of the main foods that cause food allergies. The cited legislation is complementary to Resolution RDC No. 259 of 2002[46], which approves the technical regulation for the labeling of packaged foods and their updates. In the newest resolution, food allergens are defined as any protein, including modified proteins and protein fractions, derived from the main foods that cause food allergies. As allergens can accidentally end up in foods because of contamination during transport, storage, or food processing[3], Resolution DC No. 26 of 2015 also created an Allergens Control Program. The main purpose of program is to identify and control the major foods that cause food allergies and to prevent cross-contamination at any stage of their manufacturing process, from primary production to packaging and trade[45].

Undeclared food allergens account for between 30 and 40% of food recalls in the United States. The compliance with ingredient labelling regulations and the implementation of effective manufacturing allergen control plans require the use of reliable methods for allergen detection and quantitation in complex food products[4].

Undeclared food allergens account for 30-40% of food recalls in the United States. Compliance with ingredient labeling regulations and the implementation of effective manufacturing allergen control plans require the use of reliable methods for allergen detection and quantitation in complex food products[4].

Determination of soybean protein in processed meat products

Considering consumer health and the risk of consumption of a food that can lead to a food allergy, adequate controls and analytical methods for the detection and quantification of soy protein in meat products are necessary[5,17,47]. However, until now, quantification techniques have been mainly aimed at avoiding fraud. For decades, methods have been proposed to quantify and control the content of vegetable protein added to meat products. The methods currently used for the detection of allergens in foods target allergen markers (i.e., proteins, peptides, DNA) to indicate the presence of allergenic ingredients[4]. The effect of processing is an important aspect to consider when detecting allergens in food because it usually induces denaturation and aggregation of the target protein which makes it difficult to analyze[48].

One of the techniques first proposed for quantification of soy protein in fresh and cooked meat products was electrophoresis in acrylamide gel with sodium dodecyl sulfate (SDS)[49]. Another alternative was indirect determination of soy protein content using the quantification of the phytic acid content, which is bound to plant proteins[50]. Studies describing the use of immunological analysis for the detection and quantification of plant proteins in meat products have also been reported[51,52,53,54,55].

However, due to the high specificity of the antibody produced, sensitivity of this reaction and ease of application, the immunoassay was applied in the food area and recognized as an official method by the Association of Official Analytical Chemists (AOAC). Thus, the use of Enzyme Linked Immunosorbent assay (ELISA) was proposed for soy addition control in meat products (Method 988.10)[56]. ELISA is a safe method for determining soybean protein content in foods. It has a detection limit (LOD) of 0.016 mg kg^{-1}, while soybean has an observed adverse effect level (LOAEL) of 88 - 522 mg of protein[41].

More recently, several authors propose for analysis of soy proteins in foods in general using modern methodologies such as Image Analysis[57], High Performance Liquid Chromatography (HPLC), HPLC-MS/MS (LOD= 4 mg kg^{-1} soy in meat product)[58,59], Polymerase Chain Reaction (PCR) (LOD= 10 mg kg^{-1} soy in meat product)[60], Western blotting, and Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (LOD= 200 mg kg^{-1} soy in meat product)[61] as an alternative to ELISA. New proposals for methodologies of soy determination have been described because ELISA is considered, by many researchers, to have limited accuracy, to be time consuming, costly and complex[62].

The development of appropriate detection methods for allergens in general is still a challenge, in part due to the inherent complexity of the food matrix[4,63]. Nevertheless, and despite the huge efforts, the problem is still not solved, and new methods are necessary to control and prevent the misuse of additives/ingredients in meat products. The food industry should be responsible for the declaration of soy proteins in food labels. Moreover, additional difficulties with the analysis of soybean proteins in meat products may arise due to the use of new processing techniques and the addition of genetically modified variants of soybean[17].

CONCLUSION

The economic, nutritional and technological importance of soy protein is indisputable, but it is necessary to make the meat industry and its consumers aware of the problems associated with the consumption of soy protein due its allergen potential. Soy proteins added to meat products should be identified on nutritional labels. In order to control the addition of soy protein to meat products, it is necessary to develop analytical methodologies for the detection and

quantification of soy proteins to prevent fraud and preserve consumer health.

Conflict of Interest. The authors declare that they have no conflict of interest or benefit arising from the direct applications of this manuscript.

Acknowledgements. The authors would like to thank CNPq (Grant: 443261/2016-4) for financial support.

REFERENCES

1. *Solé D, Silva LR, Cocco RR, Ferreira CT, Sarni RO, Oliveira LC, et al. Brazilian Consensus on Food Allergy: 2018 - Part 1 - Etiopathogenesis, clinical features, and diagnosis. Joint position paper of the Brazilian Society of Pediatrics and the Brazilian Association of Allergy and Immunology. Arq Asma, Alerg e Imunol 2018; 2: 7-38.*
2. *Mahan L. Krause's Food, Nutrition & Diet Therapy Elsevier, São Paulo, 2006.*
3. *Blom WM, Michelsen-Huisman AD, van Os-Medendorp H, van Duijn G, de Zeeuw-Brouwer M lène, Versluis A, et al. Accidental food allergy reactions: Products and undeclared ingredients. J Allergy Clin Immunol 2018; 142: 865-875.*
4. *Parker CH, Khuda SE, Pereira M, Ross MM, Fu TJ, Fan X, et al. Multi-allergen quantitation and the impact of thermal treatment in industry-processed baked goods by ELISA and liquid chromatography-tandem mass spectrometry. J Agric Food Chem 2015; 63: 10669-10680.*
5. *Jankovic V, Matekalo-Sverak V, Lakicevic B, Spiric D, Petronijevic R. Soybean and gluten in meat products - Consumer protection strategy. Procedia Food Sci 2015; 5: 121-124.*
6. *Ortolani C, Pastorello EA. Food allergies and food intolerances. Best Pract Res Clin Gastroenterol 2006; 20: 467-483.*
7. *L'Hocine L, Boye JI, Munyana C. Detection and quantification of soy allergens in food: Study of two commercial enzyme-linked immunosorbent assays. J Food Sci 2007; 72: 145-153.*
8. *Guzek D, Głąbska D, Brodowska M, Godziszewska J, Górska-Horczyczak E, Pogorzelska E, et al. The sensory quality of allergen-controlled, fat-reduced, salt-reduced pork-ostrich sausages during storage. J Sci Food Agric 2017; 97: 5327-5334.*
9. *Taylor SL, Hefle SL. Food allergies and other food sensitivities. Food Technol 2001; 55: 68-83.*
10. *Shimokomaki M. News on meat science and technology, Varela, São Paulo, 2006.*
11. *Liu K. Soybeans: Chemistry, Technology and Utilization. 1st ed. Springer US, Boston, 1997.*
12. *Oliveira JF, Silva UR, Pastore VAA, Azevedo EC, Campos GM, Silva FCG, Raghiante F, Martins OA. Spectrophotometric determination of nitrite in cured meat products. Rev Bras Hig Sanid Anim 2017; 11: 19-31.*
13. *Daniel CR, Cross AJ, Koebnick C, Sinha R. Trends in meat consumption in the United States. Public Heal Nutr 2010; 14: 575-83.*
14 *Nagarajarao RC. Recent advances in processing and packaging of fishery products: A review. Aquat Procedia 2016; 7: 201-213.*
15 *Ordóñez JA. Food Technology: Animal origin foods - Vol 2. 1st ed. Artmed, Porto Alegre, 2004.*
16. *Garti N, Benichou A, Aserin A. Protein-polysaccharide interactions for stabilization of food emulsions. J Dispers Sci Technol 2002; 23: 93-123.*
17. *Belloque J, García MC, Torre M, Marina ML. Analysis of soyabean proteins in meat products: A review. Crit Rev Food Sci Nutr 2002; 42: 507-532.*
18. *Tsao CY, Kao YC, Hsieh JF, Jiang ST. Use of soy protein and microbial transglutaminase as a binder in low-sodium restructured meats. J Food Sci 2002; 67: 3502-3506.*
19. *Li H, Zhu K, Zhou H, Peng W. Effects of high hydrostatic pressure treatment on allergenicity and structural properties of soybean protein isolate for infant formula. Food Chem 2012; 132: 808-814.*
20. *Verhoeckx KCM, Vissers YM, Baumert JL, Faludi R, Feys M, Flanagan S, et al. Food processing and allergenicity. Food Chem Toxicol 2015; 80: 223-240.*
21. *Meinlschmidt P, Schweiggert-Weisz U, Eisner P. Soy protein hydrolysates fermentation: Effect of debittering and degradation of major soy allergens. LWT - Food Sci Technol 2016; 71: 202-212.*
22. *Ebisawa M; Ito K; Fujisawa T. Japanese guidelines for food allergy 2017. Allergol Int. 2017; 66(2): 248-264.*
23. *Brazil (Ministry of Agriculture Livestock and Food Supply). Normative Instruction No. 4 from 31 Mar 2000. Technical Regulations for the Identity and Quality of Mechanically Separated Meat, Mortadela, Thick Milled and Hot Dog Sausage. Available at: http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=7778.*
24. *Brazil (Ministry of Agriculture Livestock and Food Supply). Normative Instruction No. 20 from 31 Jul 2000. Technical Regulations for the Identity and Quality of Meatballs, some Ham types, Hamburger, Kibbe. Available at: http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=1681.*
25. *FDA - Food and Drug Administration. What you need to know about food Allergies. 2018. Available from: https://www.fda.gov/food/buy-store-serve-safe-food/what-you-need-know-about-food-allergies.*
26. *Sarinho E, Lins M das GM. Severe forms of food allergy. J Pediatr 2017; 93: 53-59.*
27. *Valenta R, Hochwallner H, Linhart B, Pahr S. Food allergies: The basics. Gastroenterology 2015; 148: 1120-1131.*
28. *Gazzaz SS, Rasco BA, Dong FM. Application of immunochemical assays to food analysis. Crit Rev Food Sci Nutr 1992; 32: 197-229.*
29. *Matsuo H, Yokooji T, Taogoshi T. Allergology International common food allergens and their IgE-binding epitopes. Allergol Int 2015; 64: 332-343.*
30. *Nishinari K, Fang Y, Guo S, Phillips GO. Food Hydrocolloids Soy proteins: A review on composition, aggregation and emulsification. Food Hydrocoll 2014; 39: 301-318.*
31. *CONAB. National Supply Company. Monthly analysis - soybean. 2019. p. 2018-20. Available from: file:///F:/NaoApagar/Downloads/SojaZ-ZAnliseZMensalZ-ZDezembroZ2019.pdf*
32. *Norman AG. Soybean Physiology, Agronomy, and Utilization. 1st ed. Academic Press, New York, 1978.*
33. *Mattos EC de, Atui MB, Silva AM da, Ferreira AR, Nogueira MD, Soares J dos S, et al. Study on the histological identity of soy products (Glycine max L.). Rev Inst Adolfo Lutz 2015; 74: 104–110.*
34. *Lusas, E W, Riaz MN. Soy protein products: processing and use. J Nutr 1995; 125:573S-580S.*
35. *Liu Z, Cheng F, Zhang G. Grain phytic acid content in japonica rice as affected by cultivar and environment and its relation to protein content. Food Chem 2005; 89: 49–52.*
36. *Kattan, Jacob D; Cocco, Renata R; Järvinen KM. Milk and Soy Allergy. Pediatr Clin North Am 2011; 58: 407–426.*

37. Holzhauser T, Wackermann O, Ballmer-Weber BK, Bindslev-Jensen C, Scibilia J, Perono-Garoffo L, et al. Soybean (Glycine max) allergy in Europe: Gly m 5 (β-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. J Allergy Clin Immunol 2009; 452-458.
38. Ladics GS, Budziszewski GJ, Herman RA, et al. Measurement of endogenous allergens in genetically modified soybeans - Short communication. Regul Toxicol Pharmacol. 2014; 70:75-79.
39. OECD. Organization of Economic Cooperation and Development. Revised consensus document on compositional considerations for new varieties of soybean [Glycine max (L.) Merr.]: key food and feed nutrients, antinutrients, toxicants and allergens. Series on the Safety of Novel Foods and Feeds No. 25; 2012.
40. Nollet LML, van Hengel AJ. Food Allergens: Analysis Instrumentation and Methods. 1st ed. (CRC Press, ed.). Boca Raton: Taylor & Francis; 2016.
41. Alvarez PA, Boye JI. Food Production and Processing Considerations of Allergenic Food Ingredients: A Review. J Allergy 2012:1-14.
42. UniProtKB. UniProtKB - P19594 (2SS_SOYBN); 2019. Available from: https://www.uniprot.org/uniprot/P19594#names_and_taxonomy.
43. UniProtKB. UniProtKB - Q8RXD5 (KTI4_ARATH); 2019. Available from: https://www.uniprot.org/uniprot/Q8RXD5.
44. Brazil. (Health Ministry). Collegiate Board of Directors Resolution No. 268 from 22 Sep 2005. Establishes the identity and the minimum quality characteristics that must be obeyed the vegetal products of vegetal origin. Available at: https://www.saude.rj.gov.br/comum/code/MostrarArquivo.php?C=MjI0Nw%2C%2C.
45. Brazil (Health Ministry). Collegiate Board of Directors Resolution No. 26 from 02 Jul 2015. Provides for the requirements for mandatory labeling of the main foods that cause food allergies. Available at: http://portal.anvisa.gov.br/documents/10181/2694583/RDC_26_2015_.pdf/b0a1e89b-e23d-452f-b029-a7bea26a698c.
46. Brazil (Health Ministry). Collegiate Board of Directors Resolution No. 259 from 20 Sep 2002. Approves the Technical Regulation on Packaged Food Labeling. Available at: https://lcqa.farmacia.ufg.br/up/912/o/resoluo_rdc_n_259_2002_-_rotulagem_em_geral.pdf.
47. Cellerino K, Binaghi MJ, Cagnasso CE, Docena G, López LB. Comparison of SDS-PAGE and immunochemical methods for the detection of soy proteins in raw and cooked meat products. Rev Chil Nutr 2012; 39: 52–57.
48. Segura-Gil I, Nicolau-Lapeña I, Galán-Malo P, Mata L, Calvo M, Sánchez L, et al. Development of two ELISA formats to determine glycinin. Application to detect soy in model and commercial processed food. Food Control 2018; 93: 32–39.
49. Lee YB, Rickansrud DA, Hagberg EC, Briskey EJ, Greaser ML. Quantitative determination of soybean protein in in fresh and cooked meat-soy blends 1975; 40: 38–41.
50. Kloczko I RA. Studies on usability of phytic acid determination for estimation of soybean protein content in meat products. Acta Aliment Pol 1979; 5: 331-335.
51. Cellerino K, Lopez LB. Soy Protein Detection in Raw and Cooked Meat Products Using Different ELISA Kits. J Food Nutr Sci 2016; 4:170-174.
52. Macedo-Silva A, Barbosa SFC, Alkmin MGA, Vaz AJ, Shimokomaki M. Hamburger meat identification by dot-ELISA. Meat Sci 2000; 56: 189-192.
53. Medina MB. Extraction and Quantitation of Soy Protein in Sausages by ELISA. J Agric Food Chem 1988; (1980): 766-771.
54. Ravestein P, Driedonks RA. Quantitative immunoassay for soya protein in raw and sterilized meat products. J Food Tech 1986; 19-32.
55. Griffiths NM, Billington MJ, Crimes AA, Hitchock CHS. An assessment of commercially available reagents for an enzyme-linked immunosorbent assay (ELISA) of soya protein in meat products. J Sci Food Agric 1984; 1255-1260.
56. Association of Official Agricultural Chemists. Official Methods of Analysis of the AOAC. 18th ed. The Association, Gaithersburg, 2005. pp. 1-24.
57. Randulova Z, Tremlová B, Řezáčová-Lukášková Z, Pospiech M, Straka I. Determination of soya protein in model meat products using image analysis. Czech J Food Sci 2011; 29: 318-321.
58. Jira W, Münch S. A sensitive HPLC-MS/MS screening method for the simultaneous detection of barley, maize, oats, rice, rye and wheat proteins in meat products. Food Chem 2019; 275: 214-223.
59. Hoffmann B, Münch S, Schwägele F, Neusüß C, Jira W. A sensitive HPLC-MS/MS screening method for the simultaneous detection of lupine, pea, and soy proteins in meat products. Food Control 2017; 71: 200-209.
60. Safdar M, Abasiyanik MF. Development of fast multiplex real-time PCR assays based on EvaGreen fluorescence dye for identification of beef and soybean origins in processed sausages. Food Res Int 2013; 54: 1652-1656.
61. Yang WW, de Mejia EG, Zheng H, Lee Y. Soybean Allergens: Presence, Detection and Methods for Mitigation. In: El-Shemy H, ed. Soybean and Health. InTech; 2011: 502.
62. Ducatti R, Paes J, Nogueira DA, Márcia M, Sartori P, Ducatti C. Quantification of soy protein using the isotope method (δ 13 C and δ 15 N) for commercial brands of beef hamburger. Meat Sci 2016; 122: 97-100.
63. Cagnasso CE, Martín ME, Cellerino K, Audisio F, Docena G, Polenta G, et al. Electrophoretic and immunochemical methods for the detection of milk, soy and egg proteins in commercial mix products. Rev Chil Nutr 2014; 41: 412-419.