Determination of soy proteins in *calabresa* sausage by densitometry on gel electrophoresis

Determinação de proteínas de soja em linguiça calabresa por densitometria em gel de eletroforese

RIALA6/1379

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Recebido: 14.04.2011 - Aceito para publicação 26.07.2011

ABSTRACT
Soy proteins are widely employed in meat products. However addition of non-meat proteins in *calabresa* sausages is not allowed according to the Brazilian legislation and in case of the non-declared addition of this foreign protein in consumed food**,** it may trigger allergic reactions in some consumers. Polyacrylamide gel electrophoresis (SDS-PAGE) was used for determining soy proteins in *calabresa* sausages. Fraud simulations were performed adding different concentrations (0%; 0.5%; 1%; 2%; 5%; 10%; 20% and 100%) of soy proteins in sausages. The qualitative analysis was not sensitive to detect the lowest concentrations of soy proteins. On the other hand, by using semi-quantitative analysis by means of densitometry of selected protein fractions from soy and porcine meat, the presence of soy proteins could be determined in the all of analyzed concentrations. This methodology could be implemented**,** without large investments, for conducting quality control of sausages.
Keywords. porcine meat, adulteration, soy proteins, SDS-PAGE, densitometry.

RESUMO
As proteínas da soja são amplamente utilizadas em produtos cárneos. No entanto, a adição de proteínas não cárneas em linguiças tipo calabresa não é permitida segundo a legislação brasileira e o consumo de alimento com o uso não declarado dessa proteína extrínseca, pode desencadear reações alérgicas em alguns consumidores. A eletroforese em gel de poliacrilamida (SDS-PAGE) foi utilizada para a determinação de proteínas de soja em linguiça calabresa. Foram realizadas simulações de fraude**,** adicionando-se diferentes concentrações (0%; 0,5%; 1%; 2%; 5%; 10%; 20% e 100%) de proteínas de soja nas linguiças. A análise qualitativa não apresentou sensibilidade suficiente para detectar as concentrações mais baixas de proteínas de soja. O emprego de análise semiquantitativa por densitometria de frações proteicas selecionadas de soja e de carne suína, possibilitou efetuar a detecção da presença de proteínas de soja em todas as concentrações avaliadas. Foi demonstrada que essa metodologia pode ser implantada, sem grandes investimentos, como ferramenta para realizar controle de qualidade de linguiças.
Palavras-chave: carne suína, adulteração, proteínas de soja, SDS-PAGE, densitometria.

INTRODUCTION

Meat industries have been investing in developing low fat products because of searching for current standards of beauty and prevention of diseases. Among the foreign proteins that are often added as non-meat extenders, soy proteins are the most used because they promote technological advantages and moreover, soy consumption is associated with reduction of cholesterol levels, menopausal symptoms and risk of emergence of several chronic diseases such as the cardiovascular ones, diabetes, osteoporosis and cancer[1,2].

According to Annex III of Normative Instruction nº 4 of 31/3/2000, which regulates the identity and quality of sausages in Brazil, the addition of foreign proteins is not allowed in *toscana*, *calabresa*, *portuguesa*, *colonial* and *blumenau* sausages. However there is no official method to assure the absence of soy proteins in these sausages[3].

The manufacturing of adulterated foods is generally carried out to increase the profit and is characterized as a public health problem. This attitude affects directly the consumers who buy products with different nutrition facts from those shown on the label[4,5]. The consumption of meat products containing soy proteins fraudulently can trigger allergic reactions in some individuals[6].

Many methods have been described in the literature to detect soy proteins in meat products, such as the microscopic, the immunochemical, the electrophoretic and the chromatographic as well as methods DNA-based[2,7]. Polyacrylamide gel electrophoresis (PAGE) using sodium dodecyl sulfate (SDS) is efficacious. Besides, it does not require special reagents, the presence of the analyst in some steps and it is the simplest and cheapest technique to analyze proteins. This technique promotes separation of protein fractions according to their molecular weight, without any influence of electric charges[4,8].

The aim of this study was to demonstrate the feasibility of using the method of polyacrylamide gel electrophoresis (SDS-PAGE) to detect soy protein fractions in *calabresa* sausages.

MATERIAL AND METHODS

Calabresa sausages were made from porcine *longissimus dorsi muscle* and others specific ingredients. A soy protein-based product, named Provesol PE503 (Olvebra S/A, Porto Alegre, Brazil), containing about 52% (w/w) of protein, was purchased. Then sausages were ground in a processor (Walita, model R17625, Varginha, Brazil) and some simulations of frauds were prepared consisting of different levels of Provesol PE503 (0%, 0.5%, 1%, 2%, 5%, 10%, 20 % and 100%). Samples were washed with acetone in the ratio 1/10, shaken and centrifuged at 3775 x g for 15 min (Sigma centrifuge, model 2K15, Berlim, Germany) to degrease them. Then the proteins were transferred to an Eppendorff tube, dissolved in solubilizing buffer (0.0625 M Tris.HCl pH 6.8, 3% SDS, 2% β-mercaptoethanol, 0.02% bromophenol blue) to make the final protein concentration approximately 4 mg mL^{-1}, agitated for 3 min, heated in boiling water bath for 15 min and centrifuged at 12.500 x g for 20 min.

Molecular weight markers (Amersham Biosciences, calibration kit for SDS electrophoresis, Little Chalfont, UK) containing phosphorylase b (97 KDa), albumin (66 KDa), ovalbumin (45 KDa) and carbonic anhydrase (30 KDa), which were lyophilized, were resuspended in 1.0 mL of the same sample solubilizing buffer and electrophoreticly resolved together to the samples.

Electrophoresis was performed under reducing conditions[9], in vertical slab system (Sigma Chemical Co, model Z35280-2, St. Louis, USA) and the whole gel consisted of two portions: a stacking gel (2.92% acrylamide, 0.08% bis-acrylamide) and a running gel (9.73% acrylamide, 0.27% bis-acrylamide). Samples and markers (20 µL) were applied to each well in the stacking gel with a microsyringe (Hamilton, model 710N, Reno, USA). Potential difference was promoted by power supply (Amersham Pharmacia Biotech, model EPS301, Canton, USA).

Immediately after ending electrophoresis, gels were removed from the plates and placed in a fixative solution containing 40% (v/v) methanol and 20% (w/v) trichloroacetic acid. After 30 min, the fixative solution was replaced by a staining solution containing 0.1% (w/v) coomassie blue R-250, 40% (v/v) methanol and 10% (v/v) acetic acid where gels were left for approximately 12 h. Destaining of gels was performed for 3 h (50% v/v methanol and 20% v/v acetic acid). Afterwards, they were dried using the method described by Alfenas et al.[10].

Semi-quantitative analyses were performed by densitometric scanning, using 16-bit TIFF images of

the gels, produced by HP scanjet (Hewlett-Packard, model 2.400, Loveland, USA). Then they were sent to ImageQuant TL (Amersham Biosciences, version 2005, Little Chalfont, UK), which calculates retention factor (Rf), area and height of each peak in the densitograms[11].

RESULTS AND DISCUSSION

Typical stacking gel SDS-acrylamide of porcine meat, soy and mixtures of meat- soy were obtained. There were no significant differences of intensity between porcine meat bands protein and soy bands protein. This result contradicts those reported by Janssen et al.[12], who described it is extremely difficult to detect the presence of bands from non-meat proteins in meat products. According them, non-meat bands are always of minor intensity compared to bands originated from the meat proteins.

Molecular weight of each protein fraction in the gel was determined using the follow calibration curve: $y = 27.908\ x^{-0.849}$ ($R^2 = 0.9945$), which related the retention factor with the known molecular weight of the markers. Thus, it was possible to identify some protein fractions (table 1): myosin, α-actinin and actin which are proteins from meat and α', α, β subunits of β-conglycinin (7S) and acidic subunits of glycinin (11S) which are proteins from soy [13,14].

In densitograms (Picture 1), it is possible to see only three soy peaks with different retention factor comparing to meat peaks. These peaks were named

Table 1. Estimated molecular weights for the major meat and soy proteins from SDS-PAGE technique, compared with the reported molecular weights in literature

Protein	Estimated molecular weight by SDS-PAGE (kDa)[a]	Reported molecular weight (kDa)[b]
Myosin	213.25 ± 6.52	205
α-actinin	98.13 ± 1.73	100
α`- subunit β-conglicinin	76.80 ± 1.10	72
α- subunit β-conglicinin	69.12 ± 1.28	68
β- subunit β-conglicinin	48.32 ± 0.40	52
Actin	43.76 ± 0.22	45
Acidic subunits glicinin	36.83 ± 0.15	35

[a]*Average ± standard deviation for 8 identical protein fractions*
[b]*Reported molecular weights from: Mccord et al.[15] and Mujoo et al.[14]*

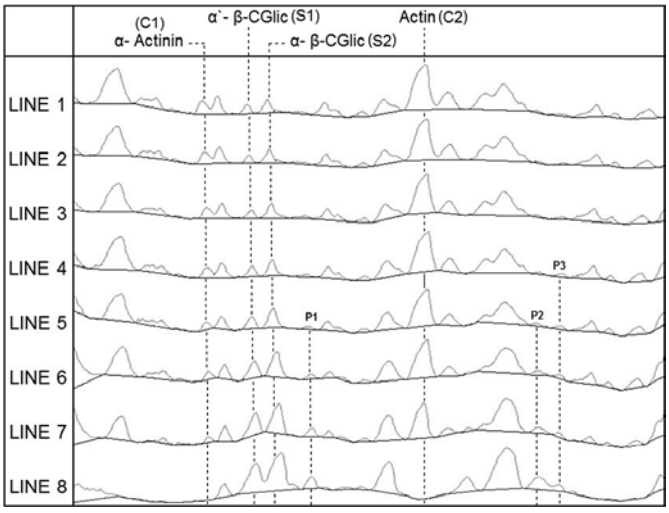

Figure 1. Densitograms of samples: (Line 1) – *calabresa* sausage; (Line 2-7) - *calabresa* sausage with 0.5%, 1%, 2%, 5%, 10% and 20% of Provesol PE503 respectively; (Line 8) – Provesol PE503

of P_1 (~60.7 KDa), P_2 (~34.8 KDa) and P_3 (~33.7 KDa). Although they were not expressive, they can be used in qualitative detection from frauds between 2% to 5% adding of Provesol PE503. Daguer et al.[16] used the basic subunits of glycinin (19 kDa) and tested whether qualitative SDS-PAGE method would be sensitive to detect 1.5% of soy protein in pork loin, but their analysis did not sensitive in this concentration.

Application of this separate technique resulted in high resolution between porcine meat and soy protein components (Figure 1) allowing to carry out the semi-quantitative analysis by densitometric scanning. Two protein fractions from soy and two from meat were selected as protein markers: α'- and α- β-conglycinin (S_1 and S_2), α-actinin (C_1) and actin (C_2). Height and area of soy protein peaks are directly proportional to the increase concentration of soy proteins whereas the ones referred to meat are indirectly proportional. Therefore, different analytical signals were tested to constructed calibration curves using ratios of soy protein and meat markers (n=9): S_1 and/or S_2 (numerator) and C_1 and/or C_2 (denominator).

According to Lee et al.[17], due to frequent variations in the background and in the peaks, the use of soy protein/meat protein ratio improves the linearity. This study compared two curves constructed with peak area: the first one just with basic subunits of glycinin isolated and the second one, which it was the best, with the ratio of glycinin/actin.

In the present work, the best calibration curves (in a total of 18) contained at least one sum of the

Table 2. Values of height and area of best responses obtained in the determination of soy protein in *calabresa* sausage

Concentration of Provesol PE503 (%)	Analytical signals					
	S1+S2/C1		S1+S2/C2		S1+S2/C1+C2	
	Height(a)	Area(b)	Height (c)	Area(d)	Height (e)	Area(f)
0	1,702	1,909	0,498	0,976	0,385	0,646
0,5	1,852	1,954	0,518	1,000	0,402	0,661
1	2,192	2,045	0,608	1,046	0,476	0,692
2	2,581	2,350	0,662	1,093	0,527	0,746
5	4,065	2,754	0,865	1,238	0,713	0,838
10	5,201	3,200	1,093	1,600	0,903	1,067
20	8,478	4,375	1,481	1,842	1,261	1,296

(a): $y = 0,3349x + 1,8855$, $R^2 = 0,9907$.

(b): $y = 0,1217x + 1,9860$, $R^2 = 0,9874$.

(c): $y = 0,0489x + 0,5492$, $R^2 = 0,979$.

(d): $y = 0,0452x + 1,0082$, $R^2 = 0,9608$.

(e): $y = 0,0435x + 0,4275$, $R^2 = 0,9807$.

(f): $y = 0,0332x + 0,6668$, $R^2 = 0,9806$.

peaks. This alternative was already used previously and it also contributed to increase the correlation of the results[18].

The combination S_1+S_2/C_1, presented the highest correlations, especially the curve from the peak height: $y = 0.3349x + 1, 8855$ ($R^2 = 0.9907$). Woychik et al.[19], who quantified soy proteins into German sausages, related that values height have less variation than the values area. The difficulty of establishing the beginning and the end of peaks may be one of the main causes of these bigger variations[20].

CONCLUSION

Bands were clearly defined and application of this SDS-PAGE slab gel procedure resulted in an enhanced degree of electrophoretic resolution. Qualitative detection just could be sensitive in concentrations above 2%-5% of soy protein and the peaks used were not expressive. Semi-quantitative analysis, using densitometry of gel bands, could determine fraud from the lowest concentration employed of 0.5% of soy protein.

This method, since it is simple, inexpensive and efficacious, can be easily introduced as a tool to assure absence of soy protein in meat products. The main way to prevent allergic reactions caused by adulterated *calabresa* sausages containing soy proteins should be to promote supervision actions.

REFERENCES

1. Castro F, García MC, Rodríguez R, Rodríguez J, Marina ML. Determination of soybean proteins in commercial heat-processed meat products prepared with chicken, beef or complex mixtures of meats from diferent species. Food Chem. 2007;100(2):468-76.
2. Espiñeira M, Herrero B, Vieites J, Santaclara F. Validation of end-point and real-time PCR methods for the rapid detection of soy allergen in processed products. Food Addit Contam. 2010;27(4):426-32.
3. Brasil. Instrução Normativa nº 4, de 31 de março de 2000. Regulamentos Técnicos de Identidade e Qualidade de Carne Mecanicamente Separada, de Mortadela, de Linguiça e de Salsicha – Anexo III: Regulamento Técnico de Identidade e Qualidade de Lingüiça. Diário Oficial [da] República Federativa do Brasil, Brasília, DF, 5 abr. 2000. Seção 1, p. 6.
4. Andrade JM, Furtado MAM, Santos LO, Marques FM, Simões AS. Utilização de eletroforese para caracterização de leite UHT, soro de queijo e suas misturas. Rev Inst Lat Cândido Tostes. 2007;62:423-9.
5. Kartheek M, Smith AA, Muthu AK, Manavalan R. Determination of Adulterants in Food: A Review. J Chem Pharm Res. 2011;3(2):629-36.
6. Savage JH, Kaeding AJ, Matsui EC, Wood RA. The natural history of soy allergy. J Allergy Clin Immunol. 2010;125(3):683-6.
7. Belloque J, García MC, Torre M, Marina L. Analysis of soybean protein in meat products: A review. Crit Rev Food Sci Nutr. 2002;42(5):507-32.
8. Silva JGJ. Eletroforese de Proteínas: Guia teórico e prático. Rio de Janeiro: Editora Interciência; 2001.
9. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680-5.
10. Alfenas AC, Peters I, Brune W, Passador GC. Eletroforese de proteínas e isoenzimas de fungos e essências florestais, Universidade Federal de Viçosa - UFV: Editora Viçosa; 1991.

11. Choi H, Selpides PJI, Nowell MM, Rourke BC. Functional overload in ground squirrel plantaris muscle fails to induce myosin isoform shifts. Am J Physiol Regul Integr Comp Physiol. 2009;297:R578-86.

12. Janssen FW, Voortman G, Baaij JA. Detection of wheat gluten, whey protein, casein, ovalbumin, and soy protein in heated meat products by electrophoresis, blotting and immunoperoxidase staining. J Agric Food Chem. 1987;35(4):563–7.

13. Claeys E, Uytterhaegen L, Buts B, Demeye D. Quantification of beef myofibrillar proteins by SDS-PAGE. Meat Sci. 1995;39(2):177-93.

14. Mujoo R, Trinh DT, Ng PKW. Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. Food Chem. 2003;82(2):265-73.

15. Mccord A, Smyth AB, O'Neill EE. Heat-induced gelation properties of salt-soluble muscle proteins as affected by non-meat proteins. J Food Sci. 1998;63(4):580-3.

16. Daguer H, Stephan MP, Bersot LS. Perfil eletroforético de lombo suíno adicionado de proteínas não cárneas. Cienc Rural. 2010;40(2):434-40.

17. Lee YB, Rickansrud DA, Hagberg EC, Briskey EJ. Quantitative determination of soybean protein in fresh and cooked meat-soy blends. J Food Sci. 1975;40:380–3.

18. López LB, Greco CB, Ferrer PR, Valencia ME. Identificación de proteínas extrínsecas en jamones cocidos por SDS-PAGE: Nível de detección en sistemas modelo. Arch Latinoam Nutr. 2006;56(3):282-7.

19. Woychik JH, Happich MC, Trinh H, Seilers R. Quantitation of soy protein in frankfurters by electrophoresis. J Food Sci. 1987;52(6):1532-4.

20. United States of Pharmacopeia. 30ª ed. NF 25. Rockville (MD): USP Convention; 2007.