QUANTITATIVE DETERMINATIONS OF COMMERCIAL SOY PROTEINS IN EMULSION-TYPE MEAT PRODUCTS BY MODIFIED OFFICIAL ELISA PROCEDURE

DELLA TORRE, Jussara C.M.[1]; BARBOSA, Sônia F.C.[1]; LICHTIG, Jaim[1]; FERRACIOLI, Viviane R.[1]; ZENEBON, Odair[1]; BERAQUET; Nelson J.[2]

[1]Instituto Adolfo Lutz / São Paulo/ SP / Brazil / jussarat@ial.sp.gov.br; [2]Meat Technology Centre of Institute of Food Technology / Campinas / SP / Brazil

Background

The utilization of soy proteins in various meat products optimizes functional parameters such as water-binding, fat-binding, emulsification capability, texture and contribute to improving economy. These soy proteins can be used as meat extenders (part of the meat can be replaced by adding nonmeat protein and water) representing more than 88% of the weight of isolates (dry basis), 68% of concentrates, and 50% of texturates (BRASIL, 1978). An important chemical property of soy proteins is their amino acid composition, which determines the nutritional value of the proteins. Soy proteins contain significant amounts of all amino acids commonly found in proteins; except for methionine, that is the first limiting amino acid in soy proteins (WOLF, 1970), followed by triptophan (ZARKADAS et al., 1994). This limitation must be considered when the proteins are added for nutritional purposes rather than simply for functionality (WOLF, 1970). The increasing use of soy proteins by the food industry has been followed by increasing demands for effective methods of both detection and quantification of these extenders. Many approaches for their determination were investigated, such as stereological technique, electrophoresis, immunoassays, peptide analysis, and indirect methods based on determination of particular metals, carbohydrates, sterols or phytates. Presently, a very attractive technique is the enzyme-linked immunosorbent assay (ELISA). The official ELISA (AOAC, 1997) steps for meat extraction employ a large quantity of solvents, making the assay long and laborious: at least several days are needed to prepare reagents and samples in order to perform the assay; the fibrous nature of the raw product acetone-dried powder (prepared to eliminate any fat interference) results in homogeneity problems. RITTENBURG et al. (1987) described a rapid and simplified sample extraction procedure with urea-dithiothreitol buffer suitable for assay that complements the rapid ELISA immunoassay.

Objectives

Quantification of soy protein isolate (SPI), texturate concentrate (SPTC) and texturate (SPT) at the levels of 0.5; 2.0; 4.0 and 6.0% of the total wet weight added to raw (fresh), pasteurized (Lyoner sausage) and sterilized (canned conserve) emulsion-type meat products following the official ELISA procedure modified by sample extraction.

Methods

Thirteen emulsion-type formulations, each weighting 8kg were prepared by typical production methods. The control composition was 56.2% beef, 12.5% mechanically deboned poultry meat, 17.25% pork backfat, 9.18% crushed ice, 1.5% salt, 0.015% sodium nitrite, 0.3% sodium tripolyphosphate, 0.05% sodium erythorbate, 1% spices and 2% manioc starch. The types of soy products used were: soy protein isolate (Supro 500E, natural color powder), texturate concentrate (Proteimax TR-120, natural color texturate) or texturate (Maxten E-100, powder coloured with erythrosine). The formulations added with 0.5; 2.0; 4.0 and 6.0% of SPI, SPTC and SPT were adjusted altering beef, ice and backfat to provide constant moisture/protein relation (4.7) and 20% fat. Soy materials were incorporated as receveid (powder or texturate). The products were prepared in the Meat Technology Centre of the Institute of Food Technology according to industrial standards. To determine the effect of thermal processing, the emulsion-type formulation was divided into three lots. One lot remained raw, one was filled into water-vapour impermeable casing (K plus - CaseTech – size 60mm) and normally pasteurized (72°C internal temperature), and one was canned and retorted at 121°C (~150g cylindrical cans, F_o=6.41). The official method for soy protein determination in raw and heat-processed meat products (AOAC, 1995) was followed, except for the preparation of meat sample, that was performed according to Rittenburg et al. (1987). Meat samples were homogenized and extracted (solubilized) in a urea-dithiothreitol [13.3M urea, 18.8mM dithiothreitol (DTT), 0.05M Tris, pH 8.6] buffer at 100°C followed by rapid renaturation in a cystine containing diluent [7.5mM cystine, 0.06M NaCl, pH 9.0]. The cystine serves to remove the excess DTT and also provides the oxidizing conditions that are necessary for the reformation of disulfide linkages. Commercial protein isolate (Purina - supro 500E) extracted according to AOAC (1995) was used as reference standard. The complete ELISA assay (since sample dilution) was repeated for three days.

Results and Discussions

Studies on optimum binding conditions revealed antibody (Sigma-Aldrich S-2519) and anti-globulin phophatase conjugate (Sigma-aldrich A-7539) dilutions of 1:3000 both. The expected values given for the soy sample concentrations in Table 1 are the recipe (formulation) values corrected for soy protein content of the products used. The control product, which contained no soy protein, showed only insignificant amounts. The observed recovery results for emulsion-type products with 6.0% soy materials were 7 to 32% higher than the expected content and agreed well with the expected content. For 4.0; 2.0; and 0.5% soy material added, the results were respectively 13 - 77%, 25 - 107% and 8% (minor) - 205% higher than the expected content. In general, values under 100% would occur when the soy protein does not interact quantitatively with the antibodies under test conditions; this could be caused by the epitopes being rendered unavailable during processing. The results showed good agreements for raw and sterilized products with 0.5% soy texturate protein (SPT). Discrepancies in small concentrations result from greater relative error. Meat products added with SPT, SPTC and SPI showed intervals recovery of 92 – 180 (196), 109 – 182% (194) and 107 – 207% (305) respectively (results for 0.5% soy added between brackets). In Figure 1, the data showed somewhat linearity for soy products with correlation coefficients of 0.95; 0.88 and 0.87 for SPT (y=1.23x+0.15), SPTC (y=1.10x+0.54) and SPI (y=1.06x+0.78) respectively. The addition of SPT to comminuted meat products showed minor results variability (greater correlation coefficient). It is evident that the solubilisation-renaturation procedure does not always convert the soy protein in all samples to quite the same antigenic form. Considering heat treatments (Table 1), the intervals recovery were 107 – 164% (211) for raw, 113 – 207% (305) for pasteurized and 107 – 169% (194) for sterilized emulsion-type products. In Figure 2, the responses were linear when considering heat treatments with correlation coefficient of 0.95; 0.91 and 0.94 respectively for raw (y=1.14x+0.29), pasteurized (y=1.27x+0.54) and sterilized (y=1.08x+0.29) products. The minor correlation coefficient for pasteurized meat products, indicate greater results variability. The observed results were found to be somewhat higher than the expected content, mainly for pasteurized products (greater slope). A shorter sample preparation procedure according to RITTENBURG et al. (1987) showed to be suitable for a quantitative ELISA. The authors explain that the performance of an immunoassay largely depends on the

characteristics of the antibody and antigen used. By immunizing rabbits with soy protein that has been denatured by a combination of heat, urea and DTT, the antibodies obtained can recognize the modified form of soy protein. Thus, when various types of meat samples containing soy are solubilized using heat, urea and DTT, similar forms of modified soy protein are produced, helping to normalize the analysis of different types of samples.

Conclusions

The procedure described offers a simple and rapid extraction of proteins for ELISA to quantify soy protein in cooked and uncooked emulsion-type products. The methodology allowed rapid aqueous extraction of meat samples into a liquid form suitable for assay. Since the soy antibody presents no cross-reactivity with sausage ingredients, this assay can be used to detect soy proteins in these mixtures. The results showed good agreements for 6.0% SPT, SPTC or SPI added. The assay was applicable to raw, cooked and sterilized meat emulsions. The results indicated that no strong dependence on different types of soy ingredients exists. It is necessary to continue the study for bettering the quantitative aspects of the methodology to perform the method.

References

A.O.A.C. Official Methods of Analysis. Association of Official Analytical Chemists. Washington D.C., Chapter 39, p.16-19, 1995.

BRASIL. Resolução nº14/78. Diário Oficial, Brasília, 28 de junho de 1978. Seção 1, p.9896-9900.

RITTENBURG, J.H.; ADAMS, A; PALMER, J.; ALLEN, J.C. Improved Enzyme-Linked Immunosorbent Assay for Determination of Soy Protein in Meat Products. J. Assoc. Off. Anal. Chem. 70 (3):582-587, 1987.

ZARKADAS, C.G.; YU, Z.; VOLDENG, H.D.; HOPE, H.J.; MINERO-AMADOR, A.; ROCHEMONT, J.A. Comparision of the protein-bound free amino acid contents of two northern adapted soybean cultivars. J. Agric. Food Chem. 42(1):21-33, 1994.

WOLF, W.J. Soybean proteins: their functional, chemical and physical properties. J. Agr. Food Chem., 18(6):969-976, 1970.

Acknowledgements

This work was supported by FAPESP project nº 01/03499-9.

Table 1. Percentage of soy proteins in emulsion-type meat products determined with ELISA

Type of soy product	Soy product added / recipe values (%)	Soya protein content / expected value[a] (%)	Raw			Pasteurized at 72°C			Sterilized at 121°C		
			Soy protein determined[b] (%)	S.D.	Percent of soy protein content	Soy protein determined[b] (%)	S.D.	Percent of soy protein content	Soy protein determined[b] (%)	S.D.	Percent of soy protein content
	0.0	0.00	0.02	0.02	-	0.02	0.02	-	0.00	0.01	-
SPT	0.5	0.25	0.29	0.05	113	0.50	0.10	196	0.23	0.03	92
	2.0	1.02	1.50	0.10	147	1.83	0.07	180	1.27	0.22	125
	4.0	2.04	2.60	0.37	127	3.38	0.25	166	2.31	0.88	113
	6.0	3.06	4.00	0.34	131	3.97	0.56	130	3.54	0.27	116
SPTC	0.5	0.32	0.51	0.02	160	0.60	0.15	187	0.61	0.15	194
	2.0	1.26	1.58	0.10	125	2.30	0.50	182	2.08	0.38	165
	4.0	2.53	3.50	0.39	138	4.46	0.40	177	4.27	0.18	169
	6.0	3.79	4.14	0.58	109	4.26	1.10	113	4.26	0.49	112
SPI	0.5	0.41	0.86	0.11	211	1.24	0.08	305	0.75	0.26	185
	2.0	1.63	2.67	0.16	164	3.37	0.23	207	2.07	0.30	127
	4.0	3.25	4.80	0.47	148	5.69	0.66	175	3.69	0.79	113
	6.0	4.88	5.20	0.33	107	6.43	1.21	132	5.20	1.36	107

[a] Soy protein texturate (SPT), soy protein texturate concentrate (SPTC) and soy protein isolate (SPI) contained 50.92; 63.15 and 81.30% of protein by Kjeldahl analysis (Nx6.25), respectively. [b]Averages of three different days are recorded relative to SPI (Supro 500E) SD = Standard deviation

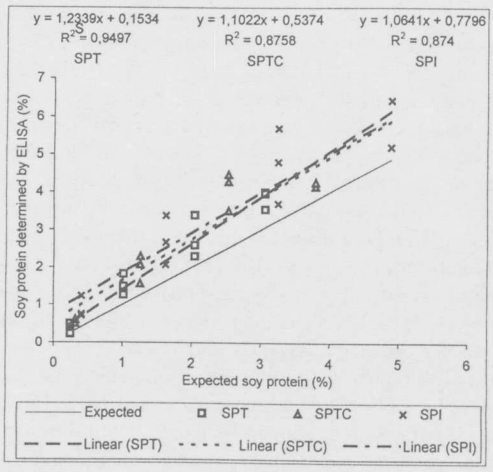

Figure 1. Relationship between ELISA-determinate percent soy protein and expected values of texturate (SPT), texturate concentrate (SPTC) and isolate (SPI) soy protein in emulsion-type meat products.

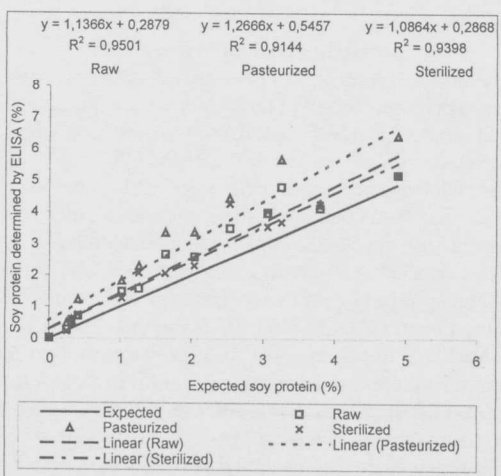

Figure 2. Relationship between ELISA-determinate percent soy protein and expected values of raw, pasteurized and sterilized emulsion-type meat products.