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

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

[1]Instituto Adolfo Lutz / São Paulo/ SP / Brazil / jussaratorre@uol.com.br [2]Meat Technology Centre of Institute of Food Technology / Campinas / SP / Brazil

Background

Soy proteins as flour, texturate, concentrate, texturate concentrate or isolate are being applied extensively in meat products that may be raw, cooked, canned or dried as a partial replacement for animal proteins. The increasing use of soy proteins demands adequate control of their levels, which can be a problem to the analyst. Nowadays in Brazil, whether or not any of these proteins is illegally used remains obscure because adequate analytical methods to detect them in meat products are scarce. The applicability and performance of method for analyzing soy proteins in meat products depend on various factors, the most important of them being the type of soy used; soy protein rate in the meat product; type and composition of the meat product; the processing of the final meat product, especially its heat treatment. The most desirable analyte for determination of soy in meat products is the protein itself. Soybeans contain 2 principal storage proteins, glycinin (11S protein) and β-conglycinin (7S protein). The fraction 7S appears to be most antigenic after renaturation (BERKOWITZ & WEBERT, 1987). Hitchcock and co-workers (1981) developed a competitive ELISA with polyclonal antibodies. This assay was subjected to collaborative studies by CRIMES et al (1984) and OLSMAN et al. (1985) and endorsed by the Association of Official Analytical Chemists (AOAC, 1995) as an official method.

Objectives

Quantification of soy protein isolate (SPI), texturate concentrate (SPTC) and texturate (SPT) at levels 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 utilizing AOAC official procedure.

Methods

Three types of soy products were used in the preparing of emulsion-type meat products: 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). Thirteen emulsion-type formulations (8Kg each) were prepared; control ingredient compositions are summarized in: 56,2% beef, 12.5% mechanically deboned poultry meat, 17.25% pork back fat, 9.18% crushed ice, 1.5% salt, 0.015% sodium nitrite, 0.3% sodium tripoliphosphate, 0.05% sodium erythorbate, 1% spices and 2% manioc starch. The formulations with 0.5; 2.0; 4.0 and 6.0% SPI, SPTC or SPT were adjusted altering beef, ice and pork fat resulting in a relation moisture/protein 4.7 and 20% fat, approximately. The soy products were incorporated as received in a powder or texturate form. The products were prepared in the Meat Technology Centre of the Institute of Food Technology according to industrial standards, i.e. chopped until an emulsion was formed, stuffed into casing (K plus - CaseTech – gases and steam barrier, ∅=60mm) and cooked to 72°C internal temperature. A portion of each formulation was retained as raw (500g) and another (1.5Kg) sterilized under conditions of commercial canning at 121.1°C (F_o = 6.41, ~150g cylindrical cans). Thus, the test samples to be analyzed were 13 raw, 13 cooked and 13 sterilized (total, 39 products). The protocol of the AOAC (1997) procedure was followed. The samples were macerated in a sequence of organic solvents. The observed level of total protein (N x 6,25) in the acetone powder was used to calculate an appropriate weight to be taken for the determination of soy protein. The acetone powders of the samples were solubilised in hot aqueous urea solution. After dilution, the "renatured" protein was analyzed by ELISA. An inhibition mode of ELISA was applied in which the soy protein analyte (antigen) reacted with a fixed volume of appropriate antiserum (rabbit antiserum to soy protein - Sigma-Aldrich S-2519) in excess, while the unreacted antibody was determined after isolation on an immunosorbent (F96 Maxisorp 442404 – Nunc Immuno Plate); in this case, the inside surface of a sensitized plasticmicrowell onto which antigen (Purina Supro 500E soy protein isolate) has been passively immobilized. The captured antibody was determined after adding a second antibody to which an enzyme had been covalently attached (goat anti-rabbit IgG – alkaline phosphatase conjugate – Sigma-aldrich A-7539). The captured enzyme was determined by adding chromogenic substrate (p-nitrophenyl phosphate – Sigma-Aldrich 104-105). The absorbance at 405nm of the solution in each microwell was measured and recorded using an automatic ELISA Reader (Multiskan Ascent - Labsystems). Washing steps (Wellwash 4 - Labsystems) were incorporated after each interaction stage to remove any non-immobilized species. ELISA protocol was designed with four blanks (substrate, conjugate, antibody-positive and antibody-negative) as quality checks to determine nonspecific color formation of enzyme substrate attributed to nonspecific binding of first or second antibodies adsorbed directly onto the solid phase. Total soy protein in meat product could be determined directly from the equation of the line derived from the calibration curve on a semi-logarithmic scale. The complete ELISA assay (since sample dilution) was repeated for three days. The cross-reactivity of anti-soy protein with whole milk protein powder, wheat flour and egg albumin was also determined.

Results and Discussions

Studies on optimum binding conditions revealed antibody and anti-globulin phosphatase conjugate dilutions of 1:3000 both. Antibody to soy protein showed no cross-reactivity or detectable binding with spices and other protein species (beef, pork, chicken, whole milk powder, wheat and egg albumin). False positives resulting from nonspecific binding of immunochemicals were not observed in this assay. The assay utilized soy protein standards at concentrations of 0.41; 1.23; 3.70; 11.11; 33.33; 100.00 and 300.00µg/mL. Assay precision determined as the coefficient of variation for absorbance values of the antigen standard curve (Supro 500E), over nine separate standard curves runned simultaneously, was good (coefficients of variation, 2.4 – 5.8%); the regression equation and correlation was as follows: Y= -0.543 logx + 1.3871; R2=0.980. The assay exhibited a good linear response within a wide concentration range. The maximum binding well gave absorbance value of 2.456 at 405 nm. Between-assay repeatability (four different days) had a CV of 27.2 – 36.5% for absorbance values. Common to all ELISA systems, our assay exhibits a day-to-day variation, which makes daily measurement of standards necessary. The protein contents (N*6.25) ranged from 11.9 – 13.8% for 39 meat products and 69.3 – 78.0% for respective acetone powders. 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 sample was characterized as containing no soy, or only insignificant amounts. The observed recovery results for emulsion-type products with 6.0; 4.0; 2.0; and 0.5% soy material were respectively 15 - 80%; 34 – 100%; 63 – 124% and 74 – 325% higher than the expected content. The expected values and the determined values for 0.5% soy products corresponded not closely for ELISA results; discrepancies in small concentrations result from greater relative error. The meat products added with SPT, SPTC and SPI showed intervals recovery of 143

- 197% (351), 115 – 206% (425) and 115 – 224% (394) respectively (results for 0.5% soy added between brackets). In Figure 1, the data showed linearity for soy products with correlation coefficient of 0.98; 0.85 and 0.85 for SPT (y=1.40x+0.38), SPTC (y=1.17x+0.93) and SPI (y=1.23x+1.01) respectively. Considering heat treatments (Table 1), the intervals recovery were 121 – 224% (425) for raw, 124 – 203% (401) for pasteurized and 115 – 192% (302) for sterilized emulsion-type meat products. In Figure 2, the responses were linear when considering heat treatments with correlation coefficient of 0.92; 0.93 and 0.94 respectively for raw (y=1.51x+0.50), pasteurized (y=1.31x+0.68) and sterilized (y=1.13x+0.59) emulsion-type meat products. The best correlation coefficients were for meat products with soy protein texturate (SPT) and sterilized, indicating minor results variability. The observed results for all samples were found to be somewhat higher than the expected content, principally for raw products (greater slope). High values (>100%) would indicate high levels of 7S protein or the exposure of extra antigenic sites in the sample. The fibrous nature of the raw product acetone powder was noted, as well as some problem of homogeneity when acetone powders were sampled. The quantity of solvents and labor time required to prepare each acetone powder make the assay long and laborious. Sample extraction merits further investigation.

Conclusions

The ELISA showed a high detection level (0.41μg/mL of soy protein), precision (low intra-assay CV), being applicable even to severely heat-processed meat products. The ELISA was highly specific for soy; no interference by other sausage ingredients was founded; the assay can be used to detect soy proteins in meat products. The ELISA procedure gave somewhat high responses, principally for raw products, with low agreements for all treatments with 0.5% soy proteins. In general, the values obtained for 4.0 and 6.0% soy protein added to sterilize comminuted meat products were consistent within the limits expected for this type of assay. The results indicate that no strong dependence on different types of soy ingredients exists. This study demonstrated that significant progress has been made in the difficult area of the quantitative determination of soy proteins in meat products. ELISA AOAC Official method, that offers high sensitivity, specificity and large sample throughput is worth further refinement to make it fully acceptable for general product surveillance purposes.

References

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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	0.01	0.00	-	0.00	0.01	-	0.01	0.01	-
SPT	0.5	0.25	0.49	0.07	194	0.89	0.27	351	0.44	0.06	174
	2.0	1.02	1.92	0.30	188	2.01	0.41	197	1.86	0.29	182
	4.0	2.04	3.21	0.52	157	3.48	0.78	171	3.30	0.66	162
	6.0	3.06	4.94	1.22	162	4.38	0.39	143	4.38	0.93	143
SPTC	0.5	0.32	1.34	0.35	425	1.27	0.09	401	0.95	0.24	302
	2.0	1.26	2.61	0.74	206	2.57	0.36	203	2.31	0.58	183
	4.0	2.53	4.03	0.87	160	4.66	0.95	184	3.39	0.77	134
	6.0	3.79	4.58	0.45	121	6.83	1.67	180	4.34	0.30	115
SPI	0.5	0.41	1.60	0.25	394	1.35	0.60	332	0.92	0.13	225
	2.0	1.63	3.64	0.80	224	2.65	0.88	163	3.12	0.75	192
	4.0	3.25	6.51	1.53	200	4.78	2.01	147	4.79	1.14	147
	6.0	4.88	8.53	2.04	175	6.07	1.20	124	5.60	1.32	115

[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

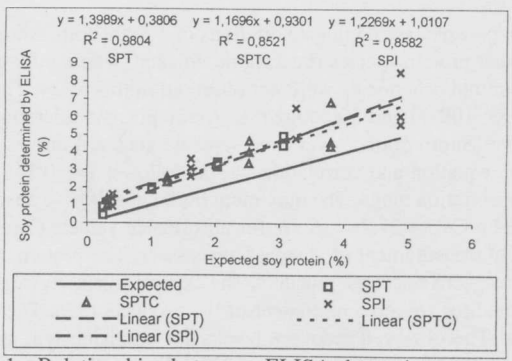

Figure 1. Relationship between ELISA-determinate percent soy protein and expected values of texturate (SPT), texturate concentrate (SPTC) and isolate (SPI) soy protein in emulsion-type meat products.

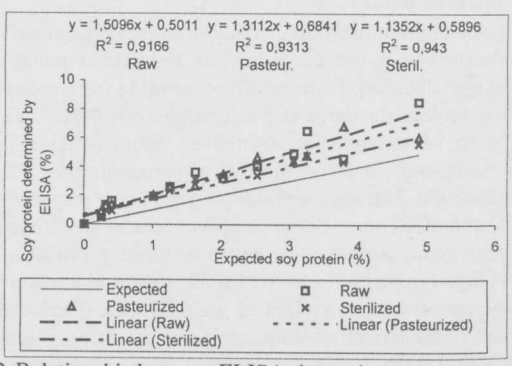

Figure 2. Relationship between ELISA-determinate percent soy protein and expected values of raw, pasteurized and sterilized emulsion-type meat products.