QUANTITATIVE DETERMINATIONS OF COMMERCIAL SOY PROTEINS IN EMULSION-TYPE MEAT PRODUCTS USING AN ELISA ASSAY KIT.

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Background

Soy proteins are often added to meat products to improve texture, to act as binders, to aid in the retention of water and fat and to extend or substitute meat proteins (Kinsella, 1979). Soy protein products are cheaper than meat proteins. This economic standpoint clearly stimulated efforts to replace some of the meat by soy proteins. In Brazil, up to 4% soy proteins can be added to finely comminuted meat products such as frankfurter-type sausages and hot dogs, up to 2.5% to tradeless raw sausages; therefore, they cannot be added to frankfurters, wieners, portuguese and calabrian types raw sausages (BRASIL, 2000). Presently, there is no routine method used for the measurement of the amount of this protein. The ELISA-TEK assay kit employs the principle of enzyme immunoassay for soy proteins in the presence of other vegetable and meat proteins. The kit is intended to be used for soy protein at levels between 1 to 10% of the total wet weight of raw or processed mixed meat products. Samples containing levels of soy protein outside this range can be measured by altering the ratio of meat to buffer used to prepare the meat slurry (ELISA Technologies, 2000). This analysis, including sample preparation, can be completed within a workday, and immunoassay in less than 60 min (Rittenburg, 1987).

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, pasteurized (Lyoner sausage) and sterilized (canned conserve) emulsion-type meat products utilizing ELISA-TEK soy protein assay kit.

Methods

Meat formulations each weighting 8Kg consisted of 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 formulations with 0.5; 2.0; 4.0 and 6.0% 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) were adjusted altering beef, ice and backfat resulting in a relation moisture/protein 4.7 and 20% fat, approximately. Soy materials were incorporated in a form of powder or texturate (as received). 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 – water and vapor barrier, ∅ =60mm) for Lyoner-type sausage and cooked to 72°C internal temperature. A portion of each formulation was retained as raw (500g) and the remaining portion was sterilized under conditions of commercial canning at 121°C (~150g cylindrical cans, nominal process value F_o = 6.41). The ELISA-TEK soy protein assay kit was used according to the manufacturer's instructions (ELISA Technologies, 2000). This assay is an indirect competitive enzyme immunoassay. Meat samples are homogenized and then extracted (solubilized) in a urea-dithiothreitol buffer at 100°C followed by rapid renaturation in a cystine containing diluent. This assay is performed in plastic microwells that have been pre-coated with a purified preparation of soy protein. In the initial competition reaction, a fixed amount of the diluted extract of the meat sample is added into the soy protein coated microwell along with a fixed volume of specific rabbit anti-soy protein. With increasing concentrations of soy in the diluted extract, the amount of rabbit anti-soy protein binding to the soy protein attached to the microwell will decrease. The amount of rabbit anti-soy protein remaining to the soy protein coated microwell is determined by reacting a fixed amount of peroxidase conjugated swine anti-rabbit globulin. Bound peroxidase activity is determined by adding a fixed amount of TMB substrate which develops a blue color (subsequently changing to yellow) in the presence of peroxidase. Color development is inversely proportional to the original soy protein concentration in the diluted extract. The concentration of soy protein in the meat product can be determined by reading off a calibration curve derived from standards of known soy protein concentrations. The ELISA-TEK soy protein assay kit has been standardized using a soy protein isolate (Purina PP500E soy isolate). Other types of soy preparations may react somewhat differently in the assay. The kit manual recommends that if the type of soy preparation, which is being assayed for, is known, then a sample of that soy powder should be included in the assay and run in parallel to the soy protein control. The recovery factor obtained with the test soy preparation can then be applied as a correction factor to the test samples.

Results and Discussion

The kit assay utilizes 5 soy protein standards at concentrations of 3.5; 7.0; 15.0; 35.0 and 70.0 µg/mL. The response curve using soy protein standards exhibited a good linear response within the above concentration range (Y=0.645logx+1.342; R2=0.980). The assay performance was monitored through the internal control measurements of maximum binding, substrate blank, and soy protein control. The maximum binding microwell gave an absorbance value of 1.498 at 450 nm (should lie in the range of 1.4 to 2.0 absorbance units). The absorbance ratio of the 3.5µg/mL standard to the maximal binding microwell absorbance was 0.69 (it should lie between 0.50 and 0.70), indicating that assay components are performing within specifications (standard curve displacement). The response for soy protein kit control, SPT, SPTC and SPI was 74; 91; 75 and 88% respectively, indicating that the extraction procedure and the immunoassay were performed satisfactorily. The expected values given for the soy products in Table 1 are the recipe (formulation) values corrected for the response factors of the soy materials (SPT, SPTC and SPI) used in this particular experiment. Once these corrections have been made, the expected values and the determined values corresponded very closely. The recovery results for 6.0; 4.0; 2.0 and 0.5% soy material added to emulsion-type products ranged from 89 – 137%; 87 – 164%; 77 – 142% and 104 - 194% respectively. The samples without soy protein were characterized as containing no soy, or only insignificant amounts. Meat products added with SPT, SPTC and SPI showed intervals recovery of 89 - 142%, 112 – 164% and 77 – 121% respectively, excluding 0.5% soy added, which is out of the experimental spectrum predicted by the ELISA-TEK manual. In Figure 1, responses were linear for soy products with correlation coefficient of 0.95; 0.95 and 0.96 for SPT (y=0.97x+0.13), SPTC (y=1.33x+0.06) and SPI (y=1.08x-0.01) respectively. The observed levels of soy protein (y) agreed with expected soy proteins (x) in meat products within experimental error. The response for products with soy protein texturate concentrate (SPTC) was still linear but with greater slope; the observed results for all samples were found to be somewhat higher than the expected content. These results can be explained by the known high response of some soy ingredients, particularly texturates, that are highly denatured protein and have varying degrees of antigenic response depending upon its

processing treatment. In this case, the recovery results would be minor (25%) if no correction factor was used. Considering heat treatments (Table 1), the intervals recovery were 87 – 148% for raw, 95 – 164% for pasteurized and 77 – 157% for sterilized emulsion-type meat products. In Figure 2, the responses were still linear when considering heat treatments with correlation coefficient of 0.95; 0.95 and 0.91 respectively for raw (y=1.11x+0.08), pasteurized (y=1.14x+0.09) and sterilized (y=1.01x+0.15) emulsion-type meat products. The best slope was for sterilized products however with greater results variability (minor correlation coefficient). The recovery of sterilized products with soy protein isolate (SPI) was somewhat underestimate. Since legal limits of soy proteins are based on total weight of sausage product, it is practical to analyze and measure soy protein on a wet basis. Directed analysis of wet samples eliminated the need for determination of total protein or total nitrogen from which the amount of soy protein is extrapolated.

Conclusions

The ELISA-TEK soy assay kit offers a simple and rapid extraction of soy proteins in meat products. There was good agreement between expected and determined levels of texturate, texturate concentrate and isolate soy proteins. The assay was suitable for use with raw, pasteurized and sterilized emulsion-type meat products. Unfortunately, there are differences in responses from different soy texturates, texturate concentrates or isolates to the same ELISA procedure and soy standard. This is one of the inherent weaknesses of the method in the present form. The procedure is in fact a quantitative method if the type and protein content of the added soy protein are known (must be declared on the product label) and especially if a sample of the specific soy type is available for calibration. The method was easy to work. Because of assay's high cost, its use is quite limited in developing countries.

References

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Table 1. Percentage of soy protein in emulsion-type meat products determined with ELISA-TEK kit

Type of soy product	Soy product added[a] (%)	Soy protein content[b] (%)	Expected value of soy protein[c] (%)	Raw		Pasteurized at 72ºC		Sterilised at 121ºC	
				Soy protein determined (%)	Percent of expected value	Soy protein determined (%)	Percent of expected value	Soy protein determined (%)	Percent of expected value
	0.00	0.00	0.00	<0.35	-	<0.35	-	<0.35	-
Soy protein texturate (SPT)	0.50	0.25	0.23	0.24	104	0.28	122	0.33	143
	2.00	1.02	0.92	1.08	117	0.97	105	1.31	142
	4.00	2.04	1.85	1.84	99	2.03	110	1.93	104
	6.00	3.06	2.77	3.30	119	2.64	95	2.47	89
Soy protein texturate concentrate (SPTC)	0.50	0.32	0.24	0.36	150	0.26	108	0.31	129
	2.00	1.26	0.94	1.30	138	1.09	116	1.22	130
	4.00	2.53	1.89	2.80	148	3.10	164	2.97	157
	6.00	3.79	2.83	3.17	112	3.87	137	3.76	133
Soy protein isolate (SPI)	0.50	0.41	0.36	0.55	153	0.70	194	0.38	106
	2.00	1.63	1.43	1.62	113	1.55	108	1.10	77
	4.00	3.25	2.86	2.49	87	3.26	114	2.66	93
	6.00	4.88	4.29	5.19	121	4.89	114	4.33	101

[a] Recipe values [b] SPT, SPTC and SPI contained 50.92, 63.15 and 81.30% of protein by Kjeldahl analysis (Nx 6.25), respectively. [c] Soy protein after correction for response to standard / Response of SPT, SPTC and SPI to soy Kit ELISA-TEK standard (Purina PP500E soy isolate) was 90.6; 74.7 and 88.0%.

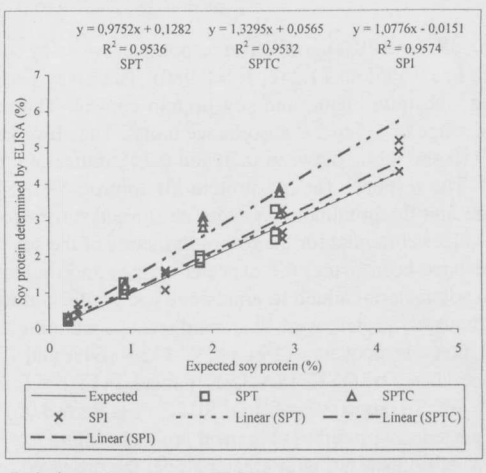

Figure 1. Relationship between ELISA-determinate percent soy protein and expected values of texturate (SPT), texturate concentrate (SPTC) and isolate (SPI) soy protein in emulsion-type meat products.

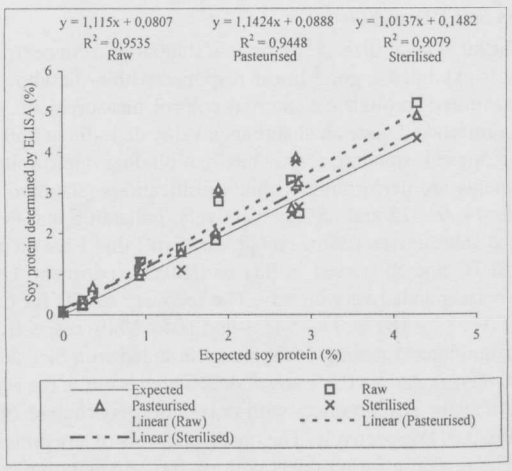

Figure 2. Relationship between ELISA-determinate percent soy protein and expected values of raw, pasteurized and sterilized emulsion-type meat products.