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Chemical characterization of commercial soybean products

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Some commercial soybean products—soybean protein isolate, soybean flour, textured soybean, whole soybeans, and soybean dairy-like products (liquid and powdered milks, shake, yogurt, and infant formulas)—have been analysed for their content in solids, ash, pH, acidity, protein, fat, phosphorus, and some metal ions (calcium, copper, iron, potassium and zinc). The differences found in the protein, phosphorus, and metal ion content and other chemical properties of these products are discussed, taking into account the procedures used to produce the above derivatives.

INTRODUCTION

Soybean is a legume increasingly consumed for economical and nutritional reasons (Steinke, 1992; Henley *et al.*, 1993; García *et al.*, 1997*a*). In fact, soybean products are an important low-cost source of proteins, minerals, phosphorus and vitamins. Furthermore, soybean products play an important role in health (Messina and Barnes, 1991; Messina, 1995; Sirtori *et al.*, 1995). The intake of soybean is not only suitable for people with allergenic reactions caused by animal milk, but it is also recommended to prevent heart disease, obesity, hypercholesterolemia, cancer, diabetes, kidney disease, and osteoporosis. These reasons have promoted the recent appearance of numerous products derived from soybean such as soybean flour, textured soybean, soybean dairy-like products, meat, bakery products prepared with soybean etc., in order to facilitate its consumption and to improve its flavour (Wang and Ascheri, 1991; Ishii and Yamagucho, 1992; Ladodo and Borovik, 1992). However, there is not much information about the nutritional value of soybean once it has been transformed into one of these products. The process that soybean undergoes in order to obtain these products may alter its properties. Some of these industrial processes are the following:

1. Soybean flakes are made by a process consisting of: cleaning, heating, and cracking the seed, dehulling by aspiration, flaking to 0.25–0.30 mm thickness, and removing the oil with hexane. This is the starting material for most commercial soybean products with the exception of full-fat flour or grits, in which case flakes are obtained without removing the oil (Soy Protein Council, 1987; Lusas and Riaz, 1995).
2. Soybean flour and grits are prepared by grinding the flakes either after or before removing the fat to pass through a US 100–325 mesh (in the case of the flour) or a 10–80 one (in the case of the grits). Afterwards, flour and grits are submitted to a controlled moist heat treatment to provide products with different nitrogen solubility indices (white, cooked or toasted) (Soy Protein Council, 1987; Lusas and Riaz, 1995).
3. Extrusion cooking is a heating process at high temperatures during a short time in which soybean flour changes into textured soybean. During this process, quaternary structures of proteins open due to the moisture and high temperatures, proteins polymerize and reorientate, and intermolecular bonds are set up. At the same time, enzymes such as urease (which reduces the useful life-time of the product) and lipoxygenase (which produces off-flavours due to the oxidation of the soybean oil) and the trypsin inhibitor (which reduces the digestibility of the protein) are destroyed (Fellows, 1994). This process improves the biological value and modifies the functional properties of the product (Horváth *et al.*, 1989). Nevertheless, extrusion cooking needs suitable

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control because underprocessing of the product causes gastro-intestinal disorder and extraprocessing damages the nutritional and functional properties of the protein (Narayan *et al.*, 1995).

4. Soybean protein isolates are made from white flakes or from flour milled to US 200 mesh by removing most of the non-protein components. Protein from flakes or flour is solubilized at pH 6.8–10 using an alkaline agent and separated by centrifugation or filtration from insoluble fibrous residues. The resulting supernatant is acidified (pH 4.5) to precipitate protein as a curd and separated from soluble oligosacharides by centrifugation. Then, the protein is spray-dried at its precipitating pH or it could be neutralized to pH 6.5–7.0 as sodium or potassium proteinates to make it more soluble and functional (Soy Protein Council, 1987; Lusas and Riaz, 1995).
5. Soybean milk is an aqueous extract of whole soybeans (Soy Protein Council, 1987). During the process, whole soybeans are soaked in distilled water, washed, drained, mixed with distilled water, ground, and centrifuged to separate the soybean milk from the insoluble residue. This insoluble residue is commercialized as Okara (Byun *et al.*, 1995).
6. Powdered soybean milk can be produced from soybean protein isolates or by drying soybean milk. Sometimes calcium or magnesium salts are added (Lusas and Riaz, 1995).
7. Soybean infant formulas are made from soybean protein isolates in which other nutrients such as vitamins, minerals or amino acids (generally methionine) are added (Soy Protein Council, 1987; Anderson and Wolf, 1995).
8. Soybean yogurt results from the fermentation of soybean milk (Shirai *et al.*, 1992*a*,*b*).

The addition of other components to some soybean products can also change soybean physicochemical properties.

In this work, the nutritional differences found by experimental means among different products from soybean are discussed in relation to the industrial process used for their preparation as well as the presence of additives in their composition. Also, products from different brands (soybean infant formulas and powdered soybean milks) were compared. The soybean products used, with the exception of soybean protein isolate, were obtained from local markets or pharmacies for direct human consumption.

MATERIALS AND METHODS

Apparatus

The following instrumentation was used for the analysis: an oven (Jouan, Saint Herblain, France) to determine moisture and solids; a shaking water bath with temperature control (1024 Shaking Water Bath, Jouan, Saint Herblain, France); a digestion system (1007 Digestor, Tecator, Höganäs, Sweden) to hydrolyse the sample for the analysis of its protein and mineral content; a Kjeltec system (1026 Distilling Unit, Tecator, Höganäs, Sweden) to determine protein content; a Soxtec system (1043 Extraction Unit, Tecator, Höganäs, Sweden) to determine fat in whole soybeans, soybean flour, textured soybean and soybean protein isolate; a spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan) to determine phosphorus; a muffle furnace (M-525 Serie II, J. M. Ney Company, USA) to estimate ash; and a pH-meter (PHM 93 Reference pH-meter) with a combined electrode, both from Radiometer Copenhagen (Radiometer Analytical A/S, Bagsvaerd, Denmark) to estimate pH and acidity. The atomic absorption spectrophotometer (model 2380) and the hollow cathode lamps of calcium, copper, iron, potassium, and zinc used to estimate the metal ion content were from Perkin Elmer (Perkin Elmer, Norwalk, Connecticut, USA).

Reagents

Standards of calcium, copper, iron, potassium, and zinc were from Carlo Erba (Farmitalia Carlo Erba, Milano, Italy). Lanthanum (III) chloride heptahydrated and ammonium metavanadate were acquired from Merck (Merck, Darmstadt, Germany). Potassium phosphate (dibasic) and ammonium molybdate (VI) were from Sigma (Sigma Chemical Company, St Louis, MO, USA). All reagents were of analytical grade.

Soybean protein isolate was purchased from ICN (ICN Biomedicals, Aurora, Ohio, USA) and soybean flour, textured soybean, whole soybeans, soybean milk, powdered soybean milks, soybean shake, soybean yogurt and soybean infant formulas were acquired in local markets or pharmacies in Alcalá de Henares, Madrid, Spain.

All samples and standards were stored at 7°C until use.

Procedure

For sample preparation, whole soybeans and textured soybean were milled with an electric grinder before analysis. The remaining samples were analysed without further treatment. The analytical methods used were generally chosen among AOAC (Association of Official Analytical Chemists) methods. In some cases, in which a suitable AOAC method could not be found, other official methods were used: norms were of the International Dairy Federation (IDF), Spanish norms (UNE) and other methods established by Spanish authorities.

For soybean protein isolate, soybean flour, textured soybean and whole soybeans, the specific AOAC methods for soybean flour were used. When methods for soybean flour do not exist for a specific analysis, methods used for similar samples were utilized. For soybean dairy-like products, AOAC methods for dairy products were used, because specific methods for soybean dairy-like products do not exist.

All the determinations were done in quintuplicate.

Moisture was determined in soybean protein isolate, soybean flour, textured soybean, whole soybeans (AOAC, 1990, method 925.10), and in powdered soybean milk B, as an example of a powdered soybean milk (Métodos Oficiales de Análisis, 1993, Tomo IV). The results were reported as total solids content.

The total solids content in soybean milk, soybean shake, and soybean yogurt was determined in accordance with Norm IDF-21 (1962) (Métodos Oficiales de Análisis, 1993, Tomo IV).

Ash was estimated in soybean protein isolate, soybean flour, textured soybean, and whole soybeans by drying at 550°C for 2 h (AOAC, 1990, method 923.03). A Spanish official analysis method (Métodos Oficiales de Análisis, 1993, Tomo IV) was used in the case of soybean milk, soybean shake, powdered soybean milk B, and soybean yogurt.

An AOAC, 1990 (method 943.02) was used for pH determination in soybean flour, textured soybean, and whole soybeans. In the case of soybean protein isolate the pH could not be estimated by this method, due to the viscosity of the sample solution.

Titratable acidity was estimated by titration with an alkaline solution and expressed as per cent of lactic acid (AOAC, 1990 method 947.05) in soybean milk, soybean shake, and powdered soybean milk B, in which case a solution of approximately 12 g of sample in 250 ml was prepared. The acidity of the yogurt could not be determined by this method, because the sample solution was not homogeneous.

Protein content was calculated by determining the nitrogen content by a Kjeldahl procedure. Depending on the nature of the sample (solid, liquid or semi-liquid), different AOAC (1990) methods were followed to determine nitrogen content: 920.87 for soybean protein isolate, soybean flour, textured soybean, and whole soybeans and 920.105 for dairy-like products (soybean milk, soybean shake, two powdered soybean milks, soybean yogurt, and five soybean infant formulas). The protein content was calculated by multiplying the nitrogen content by 6.25.

Fat in soybean protein isolate, soybean flour, textured soybean and whole soybeans was determined using a Soxtec system extractor following the AOAC (1990) method 945.39. Fat in powdered soybean milk, soybean milk, soybean shake and soybean yogurt was determined using Norm IDF-1A (1969) (Métodos Oficiales de Análisis, 1993, Tomo IV).

The determination of phosphorus was achieved by Molecular Absorption Spectrometry at 430 nm, according to the norm UNE 64.017 (Métodos Oficiales de Análisis, 1993, Tomo I).

Calcium, copper, iron, potassium, and zinc were determined by air–acetylene Flame Atomic Absorption Spectrometry (FAAS) and by Flame Atomic Emission Spectrometry (FAES) in soybean protein isolate, soybean flour, textured soybean, whole soybeans, and powdered soybean milk B. For that purpose, approximately 1 g of sample was acid-digested (with nitric acid and some drops of hydrogen peroxide (30% w/v)) until the remaining volume was approximately 5 ml. This volume was diluted up to 100 ml with Milli-Q water and the resulting solution was used for determination of metal ions. The digestion was carried out five times for every sample and results were corrected with a blank. Simultaneously, the moisture was determined in triplicate. Analyses were performed in duplicate.

Data analysis

When appropriate, data were analysed by one-way analysis of variance (ANOVA) using Fisher's Least Significant Difference test (Miller and Miller, 1993). A Univariate Linear Calibration program was used for treating the results obtained by Atomic Absorption Spectrometry (Blanco *et al.*, 1994).

RESULTS AND DISCUSSION

Discussion of the results obtained in this work has been arranged to present first of all, those soybean chemical components most important from a nutritional viewpoint: protein and minerals. Other chemical parameters of samples are also provided.

Protein content

Total protein content was determined in whole soybeans and 13 soybean derivatives, of which 10 were dairy-like products. Table 1 shows the results obtained in this study. The highest content of protein corresponds to soybean protein isolate due to the manufacturing process of protein concentration. Comparing the protein content of the soybean flour (46.49%, as is basis) and the textured soybean (46.70%, as is basis), it is possible to deduce that the extrusion cooking process seems not to affect the protein content of the final product obtained from the flour (textured soybean).

Dairy-like products have lower protein contents than the previous ones. The highest proportion of protein is found in powdered soybean milks due to the concentration of the sample. Soybean infant formulas present a very similar protein content and liquid and semi-liquid dairy-like products (soybean milk and soybean shake and yogurt, respectively) always have less protein. These quantities are established by the formulation of all of the products. In addition, the values of protein content found in soybean shake and soybean yogurt are not significantly different ($p < 0.05$).

All these products seem to be good sources of proteins for human consumption. In fact, soybean

Table 1. Protein content (%), determined by the Kjeldahl procedure, of different soybean products[a]

	Mean	SD[b]
Protein isolate	79.9	0.74
Flour	46.5	0.91
Textured	46.7	0.39
Whole soybeans	23.4	0.43
Milk	4.51	0.02
Powdered milk		
A	24.7[c]	0.40
B	34.2	0.19
Shake	3.47	0.06
Yogurt	3.11	0.04
Infant formulas		
A	14.2	0.26
B	12.8	0.42
C	13.8	0.07
D	13.8	0.03
E	15.6	0.44

From García *et al.* (1997*b*).
[a]Results (mean of five determinations) are expressed 'as is' basis.
[b]SD: standard deviation.
[c]Mean of seven determinations.

products contain all the essential amino acids required by humans, as shown in Table 2. Besides, digestibility of soybean products is comparable to meat, fish, milk or egg digestibility (Soy Protein Council, 1987).

Contents of phosphorus and metal ions

The content of phosphorus and some metal ions of nutritional interest were determined in some of the products studied in this work. The results obtained are grouped in Table 3.

Phosphorus was determined in soybean protein isolate, soybean flour, textured soybean, whole soybeans, soybean milk, powdered soybean milk B, soybean shake and soybean yogurt. The highest level was found in the powdered soybean milk B as opposed to other soybean derivatives, especially dairy-like products. This could be because the powdered soybean milk used was made from soybean protein isolate and because calcium phosphate was included in the formulation of the product. Since the phosphorus contents of soybean flour, textured soybean and soybean protein isolate were very similar and much higher than that of whole soybeans, it could be deduced that the process to which soybean is submitted to obtain soybean flour has concentrated the proportion of this element, and also that the process to make textured soybean and soybean protein isolate from soybean flour has not affected this content. With the exception of powdered soybean milk B, the remaining dairy-like products have less phosphorus content than solid soybean products, probably because these products are less concentrated (liquid or semi-liquid derivatives).

Table 2. Essential amino acid contents of some soybean products (mg amino acid per g protein)

	Defatted soybean flour/grits[a]	Soybean isolate[b]
Histidine	26	28
Isoleucine	46	49
Leucine	78	82
Lysine	64	64
Total sulfur amino acid (methionine + cystine)	26	26
Total aromatic amino acid (phenylalanine + tyrosine)	88	92
Threonine	39	38
Tryptophan	14	14
Valine	46	50

[a]From Cavins *et al.* (1972).
[b]From Kolar *et al.* (1985).

As for metal ions, copper contents are minimal for all soybean products ($\leq$2.00 mg/100 g). From the metal ions studied, the highest concentration is obtained for potassium ($\geq$234 mg/100 g), its content in soybean protein isolate being much lower than in whole soybeans, soybean flour, textured soybean and powdered soybean milk B. As expected, the calcium content in powdered soybean milk B is very high in comparison with other soybean products due to the formulation of the product, in which calcium phosphate was added.

The concentrations of phosphorus, copper, iron and zinc are closely related to the amount of protein in soybean products. In fact, as determined by regression analysis, the concentration of phosphorus, zinc, total iron and total iron + zinc, and the protein contents of soybean protein isolate, soybean flour, textured soybean and whole soybeans, are highly correlated. Zinc ($r^2 = 0.97$) and iron ($r^2 = 0.96$) have the highest partial correlation coefficient, although phosphorus ($r^2 = 0.85$), iron + zinc ($r^2 = 0.88$) and the protein content of these samples are also well correlated. This behaviour has already been observed for samples of similar nature (Champagne and Phillippy, 1989; Miles, 1989).

The contents found for the metal ions studied in soybean products (Table 3) may suggest that the intake of moderate quantities of them generally would provide the required amounts of copper, iron and zinc necessary for a healthy diet according to Table 4, which shows the recommended daily requirements of some minerals for humans of different ages (Negretti *et al.*, 1995). Table 4 shows that soybean products constitute an important source of calcium, which makes them an interesting alternative to cow's milk (120 mg/ml) (Cervera, 1994), with the advantage of them being lower in fat and richer in phosphorus, potassium, copper and iron than cow's milk.

Other chemical characteristics

Total solids, ash, pH, acidity, and fat were evaluated in eight soybean samples and the results obtained are presented in Table 5.

Table 3. Metal ions and phosphorus contents (mg/100 g) of some soybean products[a]

	Phosphorus		Calcium[b]		Copper[b]		Iron[b]		Potassium[b]		Zinc[b]	
	Mean	SD[c]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Protein isolate	743	4.75	238	18.4	1.32	0.07	6.98[d]	0.43	235	13.8	4.39	0.41
Flour	736	17.9	345	28.2	1.76	0.10	4.26[d]	0.28	2358	104	5.03	0.22
Textured	741	17.0	744	65	2.00	0.03	3.05[d]	0.55	2686	38.9	5.36	0.26
Whole soybeans	443	18.6	125	6.20	0.33[d]	0.04	≤1.89	0.08	1316	66.4	3.50[d]	0.23
Milk	57	1.81	—	—	—	—	—	—	—	—	—	—
Powdered milk B	1168	9.36	1818	44	0.66	0.04	1.74	0.13	2357[d]	60.8	5.11	0.25
Shake	50	1.14	—	—	—	—	—	—	—	—	—	—
Yogurt	100	2.88	—	—	—	—	—	—	—	—	—	—

[a]All values, except those indicated, are mean of five determinations.
[b]Results, except for liquid and semi-liquid products, are expressed as dry basis.
[c]SD: standard deviation.
[d]Mean of four determinations.

Table 4. Recommended daily requirements of some minerals (mg) by humans of different ages

	Youth	Adults	Elderly	Pregnant
Calcium	1200	900–1000	800	1200
Copper	1.50–3.00	1.50–1.30	1.50–3.00	1.50–3.00
Iron	1.50–1.20	1.50–1.00	1.00	3.00
Zinc	1.50–1.20	1.50–1.20	1.50–1.20	1.50

From Negretti *et al.* (1995).

The highest solids content is naturally yielded in the solid samples. While the soybean protein isolate, soybean flour, textured soybean and powdered soybean milk yield similar solids contents, it is the product submitted to the simplest manufacturing process (whole soybeans), which has the highest content of water. As with liquid and semi-liquid products, the highest solids content is found in the yogurt, followed by the shake and milk. The solids content decreases with decrease in the viscosity of the sample, as expected.

Ash represents the inorganic part of a sample. Based on the results obtained, it can be observed that the ash content increases as the complexity of the industrial process increases. Soybean protein isolate and the powdered soybean milk B here have the highest ashes, their contents being very similar (values are not significantly different, $p < 0.05$), whereas soybean milk, soybean shake and soybean yogurt have the lowest ash contents.

Industrial processes seem to affect values for the three samples in which measurement was possible. In fact, the pH was significantly different (Miller and Miller, 1993) ($p < 0.05$) for whole soybeans, soybean flour, and textured soybean and pH values increased in that order.

Acidity was determined in soybean milk, soybean shake, and powdered soybean milk B. The highest acidity was found in liquid soybean products (soybean milk and soybean shake) whereas powdered soybean milk B had the lowest acidity.

With respect to the fat content, differences were also found between dairy and non dairy-like products. While soybean protein isolate, soybean flour, textured soybean, and whole soybeans have less than 1% fat, soybean milk, soybean shake, powdered soybean milk B,

Table 5. Other chemical parameters of some soybean products[a]

	Total solids		Ash		pH		Acidity		Fat	
	Mean	SD[b]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Protein isolate	94.20[c]	0.06	9.92	0.32	—	—	—	—	0.28[g]	0.08
Flour	93.26[c]	0.16	6.81	0.12	6.79	0.02	—	—	0.84	0.05
Textured	93.12[c]	0.06	7.35	0.21	6.97	0.06	—	—	0.42[g]	0.03
Whole soybeans	88.42[c]	0.06	5.23	0.30	6.65	0.05	—	—	0.54[g]	0.01
Milk	9.08[d]	0.03	0.56	0.01	—	—	0.18	0	2.28	0.02
Powdered milk B	95.55[e]	0.03	9.80	0.04	—	—	0.04	0	3.00	0.11
Shake	12.73[d]	0.20	0.47	0.01	—	—	0.17	0.01	2.20	0.09
Yogurt	15.99[d,f]	0.13	0.68	0.02	—	—	—	—	2.18	0.03

[a]Results (mean of five determinations) are expressed as % 'as is' basis.
[b]SD: standard deviation.
[c]Total solid content given as an indirect measurement from the determination of the moisture content in the samples (AOAC, 1990, method 925.10).
[d]Total solid content according to the Norm IDF-21 (1962) (Métodos Oficiales de Análisis, 1993, Tomo IV).
[e]Total solid content given as an indirect measurement from the determination of the moisture content in the sample (Métodos Oficiales de Análisis, 1993, Tomo IV)
[f]Mean of eight determinations.
[g]Mean of four determinations.

and soybean yogurt have more than 2% fat. This could be due to the fact that lecithin (98% soybean fat) and other vegetable fats are usually added to soybean dairy-like products to increase the nutritional properties of the product or to enhance its functionality. Fat content in soybean milk, soybean shake and soybean yogurt are not statistically different ($p < 0.05$).

When a comparison of the results obtained in this work with those available in the literature for some soybean derivatives was possible, good agreement was found in most cases (Soy Protein Council, 1987; Guerriero and Ruggeri, 1992, 1996; Narayan *et al.*, 1995). An exception was the content of fat found for whole soybeans. While (in the literature) it usually appears close to 20%, in our case it was 0.54% (which is in good agreement with the fat content given by the manufacturer), probably due to differences in the type of soybean studied, green soybean in our case.

Conclusions

Soybean can be found commercially as a wide variety of derivatives, but the contents of their nutrients are not the same. The protein, phosphorus and mineral contents, as well as other chemical parameters, seem to depend on the industrial process used, the addition of other nutrients (lecithin, minerals, vitamins, etc.), and the original soybean product used. The formulation of the product is another factor to take into account. As well as protein, soybean products also contain important quantities of minerals (copper, iron, potassium, calcium and zinc) that provide, in most cases, the recommended daily requirements and that are even higher than those found in cow's milk.

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