Chemical and physical characteristics of a soybean beverage with improved flavor by addition of ethylenediaminetetraacetic acid

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RESUMEN

Características químicas y físicas de un alimento líquido de soja con mejores cualidades organolépticas mediante adición de ácido etilendiaminetetraacético.

Se ha desarrollado un nuevo método para obtener un alimento líquido de soja (ALS) con mejores cualidades organolépticas mediante adición de ácido etilendiaminetetraacético (EDTA). En el producto obtenido se evaluaron el pH, la viscosidad y la densidad, así como también los contenidos de proteínas, grasas y cenizas, la composición en ácidos grasos y la actividad de lipoxigenasa. Se seleccionó una relación agua/soja de soja equivalente a 4.5:1 puesto que la misma produjo los más altos contenidos de proteínas (4.22 g 100 ml⁻¹) y sólidos totales (8.80 g 100 ml⁻¹). Se realizó una evaluación sensorial, mediante pruebas de aceptabilidad, del aroma y sabor del ALS y se compararon con los de una «leche de soja» comercial y un alimento líquido de soja sin agregado de EDTA. Las muestras de ALS presentaron las evaluaciones más bajas para sabores rancio y «afrijolado». Los resultados obtenidos indicaron que la adición de EDTA puede reducir sabores desagradables en productos de soja.

PALABRAS-CLAVE: EDTA - Leche de soja - Proteína - Sabor.

SUMMARY

Chemical and physical characteristics of a soybean beverage with improved flavor by addition of ethylenediaminetetraacetic acid.

A new method to obtain a soybean (SB) with improved flavor characteristics was developed by adding ethylenediaminetetraacetic acid (EDTA). The SB was evaluated for pH, viscosity and density, as well as for protein, oil and ash contents, fatty acid composition and lipoxigenase activity. A water/bean ratio of 4.5:1 was selected because it provided the best protein (4.22 g 100 ml⁻¹) and total solids (8.80 g 100 ml⁻¹) contents. Sensory ratings for flavor and aroma intensities were also determined and compared with those of a commercial soymilk and a soybean beverage without EDTA. Samples from SB had the lowest ratings for green/beany and rancid flavors. The results indicated that the addition of EDTA may reduce off-flavors in soybean products.

KEY-WORDS: EDTA - Flavor - Protein - Soymilk.

1. INTRODUCTION

At present, there is an increasing demand for oilseed beverages as alternative sources of dietary protein, especially in countries where dairy products are expensive or in limited supply. Extensive studies

have been carried out to improve the sensory attributes of beverages prepared from aqueous extracts of unroasted peanuts and soybeans (Rubico *et al.*, 1987; Chompreea *et al.*, 1989; Gálvez *et al.*, 1990; Lee and Beuchat, 1992; Hinds *et al.*, 1997).

Undesirable flavor associated with soybean products is an important factor limiting the use of soybeans in foods. There are two major interrelated causes for development of such flavors. One is the high proportion of unsaturated fatty acids, particularly linolenic acid, in the soybean oil fraction, and the other is the abundant presence of lipoxigenases (LOXs) in soybeans.

When soybeans are water-soaked and ground in the conventional manner of making soymilk, a rancid, green-beany flavor develops. Considerable evidence has implicated seed lipoxigenase as a major factor contributing to the oxidation of lipids during processing, thereby lowering product quality (Wolf, 1975; Rackis *et al.*, 1979). The hydroperoxides formed by lipoxigenase activity and their breakdown products are known to exhibit off-flavors (Kalbrener *et al.*, 1974; Rackis *et al.*, 1979). Furthermore, lipoxigenase inactivation by heat during processing of soybeans has been correlated with the improvement of sensory evaluation scores (Mustakas *et al.*, 1969). Heat treatments are widely used to inactivate lipoxigenase, but they are expensive, can produce undesirable alterations of the proteins, and themselves can add to the flavor profile of soy products (Davies *et al.*, 1987).

The present study was aimed to develop a new method to obtain a soybean beverage with improved flavor characteristics.

2. MATERIALS AND METHODS

2.1. Beverage preparation

A flow diagram for preparing the soybean beverage is shown in Fig. 1.

Soybeans of Asgrow 6404 cultivar were obtained from Estación Experimental Agropecuaria (EEA - INTA) of Manfredi, Córdoba province, Argentina.

Figure 1
Flow diagram for preparation of soybean beverage

Beverages using 4.5:1, 5:1 and 5.5:1 ratios of water to soybean were compared on the basis of composition and viscosity for the purpose of determining the optimum dilution to be used in the succeeding test.

Soybeans (220 g) were rinsed three times with tap water, drained and then imbibed at reduced pressure (7.92 Kpa) for 90 min in 300 ml of distilled water containing 0.5 % (w/v) ethylenediaminetetraacetic acid (EDTA) disodium salt and 0.5% (w/v) sodium bicarbonate. After draining, seeds were washed with fresh tap water and blanched in 300 ml of distilled water at 100°C for 10 min. Finally, soybeans were ground in 1000 ml of distilled water at high speed in a waring blender. The homogenate was boiled for 20 min and then filtered through muslin cloth to obtain the soybean beverage (SIB). SB was then bottled, capped and sterilized at 120°C (150.5 KPa) for 20 min. Bottle-sterilized beverage was stored at 5°C until analyzed.

A control sample (SB1) was prepared, under the same conditions, without adding EDTA.

2.2. Physical and chemical analyses

The pH, viscosity and density were determined at 25°C following AOAC methods (1980). The total solids content was calculated by drying 50 ml samples at 60°C for 3 days. The protein, oil and ash contents were determined from dehydrated material using standard AOAC methods (AOAC, 1980):

protein content was evaluated by the micro-Kjeldahl procedure using a factor of 6.25 to calculate protein content; total lipid content (Soxhlet method) was determined by weight difference, and ash content by incineration at 600°C for 5 h. Carbohydrate content was calculated by difference (Wattanapat *et al.*, 1994). Fatty acid composition of total lipids was determined according to Maestri *et al.* (1998). Inhibition of lipoxygenase activity by EDTA was measured by spectrophotometric determination according to Pignata *et al.* (1984) on soybean beverage with and without EDTA (SB and SB1, respectively). Lipoxygenase activity was expressed as an optical density increase per mg protein⁻¹ per min⁻¹ ($\Delta OD \cdot mg \text{ prot}^{-1} \cdot \text{min}^{-1}$).

2.3. Sensory evaluation

Thirteen experienced panelists were selected to evaluate and compare the flavor and aroma intensity of SB, SB1 and a commercial soymilk (CSM). Additional training consisted of giving individual panelists a brief description of each attribute prior to sensory evaluation tests, using the standards listed in Table I.

Table I
Standards and intensity values assigned to standards used for evaluation of flavor and aroma attributes.

Attribute	Standard	Standard intensity
Flavor	Sweet	Sucrose (% w/v)
		10
		12.5
Bitter	Caffeine (% w/v)	15
		0.04
		0.07
Acid	Citric acid (% w/v)	0.10
		0.07
		0.10
Aroma	Green/beany Rancid Acetic acid Anisic aldehyde Benzaldehyde Ethyl acetate Vanillin	0.13
		uncooked soymilk ^a
		Aged soybean oil ^b

^a Uncooked soymilk was prepared at different water/soybean ratios (8:1, 5:1, 2:1, v/w) to provide light, moderate and strong green/beany flavors, respectively.

^b Crude oil samples were stored at 30°C for periods of 1, 2 and 3 weeks to provide light, moderate and strong rancid flavors, respectively.

To evaluate the flavor attributes, panel members were asked to assign intensity ratings to five flavor terms (sweet, bitter, acid, green/beany and rancid); descriptors for each attributes were light, moderate

and strong. To evaluate the aroma attribute, panelists were asked to recognize the aroma of the standard identified.

Each judge evaluated the samples in a partitioned booth. Samples were coded using three digit random numbers and were evaluated in random order. The judges were asked to rinse their mouths with water between samples.

3. RESULTS AND DISCUSSION

The proximate composition of the SB is showed in Table II. No differences were found between SB and SB1. The solids content of SB was similar to that of CSM, but higher than that observed for peanut milk (Lee and Beuchat, 1992) and lower than the average values for commercial whole cow's milk. All samples of SB had stable emulsions; no sediment or phase separations was observed after 45 days of quiescent standing.

Table II

Proximate (g 100 ml⁻¹) and fatty acid (% of total fatty acids) compositions, unsaturated fatty acid percentage (% US), and long-chain polyunsaturated fatty acids/saturated fatty acids ratio (PUFA/SFA) of soybean beverage (SB) and a commercial soymilk (CSM).
Mean values $\pm$ standard deviations ($s_{(n-1)}$), n=4

Component	SB	CSM
Moisture	91.2 $\pm$ 0.18	91.7 $\pm$ 0.2
Proteins	4.22 $\pm$ 0.05	3.03 $\pm$ 0.1
Lipids	1.53 $\pm$ 0.04	1.70 $\pm$ 0.04
Ash	0.67 $\pm$ 0.03	0.60 $\pm$ 0.02
Carbohydrates	2.37 $\pm$ 0.04	3.00 $\pm$ 0.05
Total solids	8.80 $\pm$ 0.09	8.27 $\pm$ 0.07
Palmitic acid	11.0 $\pm$ 0.2	11.2 $\pm$ 0.2
Stearic acid	4.30 $\pm$ 0.3	4.14 $\pm$ 0.2
Oleic acid	18.9 $\pm$ 0.4	21.1 $\pm$ 0.3
Linoleic acid	56.2 $\pm$ 0.6	56.0 $\pm$ 0.6
Linolenic acid	9.60 $\pm$ 0.2	7.51 $\pm$ 0.2
% US	84.7 $\pm$ 0.6	84.6 $\pm$ 0.7
PUFA/SFA	4.30 $\pm$ 0.5	4.13 $\pm$ 0.5

The United Nations Protein Advisory Group (PAG, 1972) recommended that imitation milk should contain 3.5% (w/w) fat, 3.5% (w/w) protein and 5.0% (w/w) carbohydrate. Although fat and carbohydrate contents in SB did not reach those values, the protein content was greater not only than the minimum recommended but also than those of the

CSM and peanut milk (Rubico *et al.*, 1987; Lee and Beuchat, 1992).

A key factor in determining the quality and cost of soymilk is its richness or thickness, which depends largely on the weight of water added per weight of dry soybeans used (W/S ratio). The selection of W/S ratio for preparing the SB was based on solid and protein contents. A W/S ratio of 4.5 was selected because it provided the best protein and solids scores. In general, soymilk is classified into three basic categories based on proximate composition (Liu, 1997). According to protein and total solids contents, SB could be classified as rich soymilk, although fat content did not reach the values required in the standard.

Fatty acid composition of the lipid fraction is showed in Table II. The main fatty acids were palmitic (11.0%), oleic (18.9%) and linoleic (56.2%), these representing more than 86 % of the total fatty acid content. The total unsaturated fatty acids was almost 85%, whereas the polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) ratio (4.3) was well above the recommended minimum intake for humans.

In addition to protein yield and chemical composition, genetic variability of soybeans also affects other characteristics of the finished product, including color, flavor and aroma. A comparison of sensory scores for aroma and flavor characteristics of SB, SB1 and CSM, is showed in Fig. 2. The panelists were similar in response to aroma of SB and CSM, whereas SB1 was qualified as unacceptable. On the other hand, when SB, SB1 and CSM were evaluated for flavor, the former was chosen by more than 76% of the panelists. Samples from SB had the lowest panel ratings for green/beany and rancid flavors.

Figure 2

Sensory scores for aroma and flavor attributes (as percentage of the panelist who chose the product) of soybean beverage with addition of EDTA (SB), commercial soymilk (CSM) and soybean beverage without EDTA (SB1)

Lipoxygenase is at least partially responsible for the development of off-flavors and it has been suggested that it is inhibited by primary (chain-breaking) and secondary (metal-complexing) antioxidants, i.e. ethylenediaminetetraacetic acid (Peterman and Siedow, 1983). This compound, accepted by the Código Alimentario Argentino (CAA, 1980) is widely used in margarines and dressings (Lindsay, 1993; Nabrzyski, 1997) because of its strong antioxidative efficiency attributed to the metal-chelating action. Lipoxygenase activity in SB without EDTA was $11.5 \Delta\text{OD} \cdot \text{mg} \cdot \text{prot}^{-1} \cdot \text{min}^{-1}$, whereas it was reduced to $4.5 \Delta\text{OD} \cdot \text{mg} \cdot \text{prot}^{-1} \cdot \text{min}^{-1}$ when EDTA was added.

The pH of SB (Table III) was lower than that of CSM and very similar to that of cows'milk (6.8 to 6.9). It is considered the optimum pH to be used as a substrate for fermentation by lactic cultures. Based on this characteristic, SB made from soybeans soaked in water containing 0.5% NaHCO_3 would be considered the most suitable for that purpose. The viscosity and density of SB (Table III) were higher than those of CSM. The addition of EDTA did not affect pH, viscosity and density. No differences were observed in these parameters between SB and SB1.

Table III
pH, viscosity and density at 25°C of soybean beverage (SB) and a commercial soymilk (CSM). Mean values $\pm$ standard deviations ($s_{(n-1)}$), $n=4$.

	SB	CSM
pH	6.84 ± 0.001	6.95 ± 0.002
Viscosity (Pa.s)	5.04 ± 0.002	4.80 ± 0.004
Density (gml^{-1})	1.02 ± 0.005	0.97 ± 0.004

In summary, the results obtained showed that the processing conditions for preparing SB produced a very stable emulsion with high solids and protein contents. The addition of EDTA to SB improved the flavor attributes as comparing them with those of a commercial soymilk and soybean beverage without EDTA.

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