MIXTURES OF BEEF TRIPE, BEEF LIVER AND SOYBEANS APPLIED TO FOOD DEVELOPMENT

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ABSTRACT

A nutritive sausage-type product was developed with beef tripe, beef liver and soybeans as ingredients. A three-component mixture design was used to obtain seven different formulations (minimum of each main ingredient: 16.5%, maximum: 67.0%). Ingredients were ground, mixed and packed tightly with a polypropylene film to obtain a roll. Pieces were cooked in boiling water for 90 min. The composition of the obtained products varied within the following ranges: proteins 17.32–25.56 g/100 g, lipids 3.22–3.87 g/100 g, crude fiber 1.50–4.50 g/100 g, minerals 1.44–2.72 g/100 g. Total iron levels varied between 1.39 and 2.98 mg/100 g and calcium levels between 15.07 and 34.01 mg/100 g. Surface response analysis was applied to parameters obtained from texture profile analysis (hardness, adhesiveness, cohesiveness and elasticity). Products hardness increased when the soy content increased; on the contrary, formulations enriched in beef tripe were those of higher elasticity and cohesiveness. Color was mainly determined by the incorporation of liver. A nontrained panel was used to evaluate the acceptance of the different formulations. The most accepted one was that with equal proportions of the three main ingredients. Microbiological challenge testing showed that the thermal treatment was enough for assuring the product safeness even in samples with high initial microbial charge.

PRACTICAL APPLICATIONS

The application of different protein sources (beef tripe, beef liver and soybeans) in the formulation of a sausage-type product intended for nutritional

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support has been treated as a mixture problem. This type of approach allowed to obtain products of different composition, sensorial characteristics and acceptability. So, the use of non conventional, lesser cost protein sources could be adapted to the consumers' preferences. Even at a domestic scale, the heat treatment conditions for preparation assured a harmless product.

INTRODUCTION

An equilibrated diet must provide enough calories and nutrients in order to ensure the metabolic requirements. Among other consequences, the lack of enough nutrients is associated with poor growth and malnutrition. A way to prevent the lack of nutrients is to develop strategies concerning the availability and access to nutritive foods especially for groups at risk. According to the World Health Organization (WHO), these strategies should first address the production, processing, marketing and preservation of foods and also include feeding practices (WHO 2001).

Protein supply, particularly of high biological value, is one of the most important diet requirements because it is essential to growth and metabolic maintenance. The minimum requirement of protein for adults is 1 g for every kilogram of body weight per day to compensate tissues breakdown. Besides growth failure and loss of muscle mass, protein deficiency can cause a decreased immunity and adverse effects on all organs, including the brain (FNB 2005).

Insufficiency of animal proteins in diet is also associated with a decrease in the bioavailability of iron, making anemia a prevalent disease, particularly in developing countries. With respect to iron deficiency, the WHO remarks that "the efforts should be directed towards promoting the availability of, and access to, iron-rich foods. Examples include meat and organs from cattle, fowl, fish, and poultry; and non-animal foods such as legumes and green leafy vegetables" (WHO 2001).

In Argentina, beef tripe and beef liver are scarcely incorporated in usual domestic food preparations for several reasons (lack of information, cultural habits, etc.). However, these ingredients, of a lesser cost than the common beef cuts, are valuable from a nutritional point of view according to their protein amino acidic profile (Zarkadas *et al.* 1996; USDA-NDB 2005a,b). Tripe is an edible organ obtained from the stomach of various domestic animals. Beef tripe is typically made from the first three of cattle's four stomachs, the rumen, the reticulum and the omasum.

Liver can be obtained from different animals and is generally used as an ingredient in sausages and spreadable products like *pâté de foie gras*, *mousse de foie gras*, liverwurst, etc.

Among vegetable products, soybean (*Glycine max*) is a good source of proteins and iron. The amino acidic profile of these proteins (USDA-NDB

2005c) is good enough to consider soybean a convenient ingredient in supplementary food formulations. Besides, soy is valuated due to the presence of other components like isoflavones, phospholipids and fiber that are claimed to have beneficial effects on health (Anderson 2004; Messina 2004). Soy culture has had an outstanding increase in Argentina during the last years (Cuniberti *et al.* 2004); however, it is not a traditional ingredient.

The inclusion of the aforementioned protein sources (beef, liver and soy) in the usual diet could be facilitated if they were incorporated in mixtures with acceptable attributes. A sausage-type product can be adapted to the preferences of consumers; besides, it is easy to manipulate and divide into portions. A characteristic of food preparation at domestic scale is that ingredients proportions may vary according to availability, costs, measuring procedure (by volume, by weight), etc. So, the formulation of a product prepared from different protein sources could be approached as a mixture problem. In this way, the sensorial attributes of a product as well as the variations in composition can be evaluated within a wide range.

The mixture design approach (Cornell 1990) has been widely used to obtain product formulations. In this type of experiments, two or more ingredients are blended in different proportions and the resulting mixture characteristics are analyzed. Nowadays, mixture design experiments are considered a useful technological tool to optimize product formulations and processes, to accelerate product development, to reduce costs, to improve the transfer of research results and to solve production problems (Graf and Saguy 1991). Other authors like Castro *et al.* (2000) used the response surface methodology for developing protein mixtures (hydrolyzed gelatin, wheat gluten and soybean protein isolate) demonstrating that this is a useful tool for optimization of the nutritional quality of protein mixtures. This methodology has been used also for starch mixtures (Karam *et al.* 2005, 2006) as a useful method for the evaluation of textural attributes.

The objectives of the present work are:

(1) to obtain an acceptable formulation of a sausage-type food including as protein sources beef tripe, beef liver and soybeans;
(2) to determine the proximate composition of the obtained product: proteins, lipids, carbohydrates, fiber and minerals (particularly iron and calcium);
(3) to determine the organoleptic characteristics of this product (color and texture) by instrumental techniques and relate these results with the degree of acceptability of the product; and
(4) to evaluate the safeness of the preparation process by means of microbiological assays.

MATERIALS AND METHODS

Mixture Components

Three basic ingredients were used in the different formulations: beef tripe, beef liver and soybean seed (*G. max*).

According to USDA-NDB (2005a,b,c), beef tripe (raw) has 12.07% protein, iron (0.59 mg/100 g) and calcium (69 mg/100 g); beef liver (raw) has 20.36% protein and it is an important source of iron (4.90 mg/100 g); soybeans (mature seeds, raw) have 36.49% proteins and 15.70 mg/100 g iron.

Both beef liver and beef tripe were acquired in a local market. Before it was ground, the beef tripe was washed and cut into small pieces (2×2 cm). Small bile ducts were separated from the liver and were also cut in 2×2 cm pieces. Commercial soybeans were macerated overnight and boiled 45 min before grinding. Even though ground soybeans would be submitted to a thermal treatment when blended with the other ingredients, cooked soybean seeds are easier to convert into a paste. The total time of thermal treatment (before grinding and after blending with the other ingredients) was quite enough to ensure the inactivation of the tripsine inhibitor (Witte 1995).

Wheat flour (Molinos Río de la Plata S.A., Buenos Aires, Argentina) and eggs were used as binding agents. Wheat flour (9 g/100 g proteins, 1 g/100 g lipids, 3 g/100 g fiber, by weight) was an enriched type as stipulated by the local laws (iron: 7 mg/100 g, folic acid: 0.22 mg/100 g, niacin: 1.3 mg/100 g, vitamin B1: 0.63 mg/100 g, vitamin B2: 0.13 mg/100 g) (data provided by manufacturer).

Small amounts of spices and salt (NaCl) were used as flavoring agents.

Experimental Design for Product Formulation

Blends of the three protein sources (beef tripe, beef liver and soybean seeds) were prepared with the proportions defined by the simplex-centroid experimental design for mixtures of three components expanded with internal points, with constraints, according to the software Statistica 5.1 (StatSoft, Tulsa, OK). In an experimental design without restrictions, each triangle vertex corresponded to a pure component. In order to obtain a product where the three ingredients were always present, the mixture formulation was restricted. A minimum (on the mixture total) of 16.5 g/100 g and a maximum of 67.0 g/100 g values were imposed as restrictions for each ingredient in the experimental design. The new design can be visualized as a smaller triangle included in the major one corresponding to seven different proportions of the three protein sources as shown in Table 1.

For all the formulations, the minor ingredients were added in the same quantity. Samples were prepared according to the elaboration methodology

TABLE 1.
PROPORTIONS OF THE THREE MAIN INGREDIENTS IN THE SEVEN FORMULATIONS (g/100 g)

Formulation	Beef tripe	Soybean	Beef liver
1	16.5	16.5	67.0
2	67.0	16.5	16.5
3	16.5	67.0	16.5
4	16.8	41.6	41.6
5	41.6	16.8	41.6
6	41.6	41.6	16.8
7	33.3	33.3	33.3

described in the product preparation section. Each sample was submitted to different assays to determine the composition and the sensorial attributes. To adjust the experimental textural attributes, the Scheffé equation was used (Cornell 1990):

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3$$

where Y is the studied response, β_1, β_2, β_3, β_{12}, β_{13}, β_{23} and β_{123} are the regression coefficients and X_1, X_2, X_3 are the different levels of each component in the blend. Positive β-values are related to a synergistic effect while negative values indicate an antagonist effect among components.

Triaxial diagrams were obtained from polynomial equations for each attribute using the software Statistica for Windows 5.1 (StatSoft).

Product Preparation

Elaboration process was designed according to usual domestic practices because this type of product was intended to be an easy-to-prepare supplement. Main rich protein ingredients (beef tripe, beef liver and soybean) were weighed and ground in a cutter-type processor. Macerated and cooked soybeans were processed until a fine particle paste was obtained. These three ingredients were then mixed according to the proportions shown in Table 1. The total mixture of protein-rich ingredients weighed 500 g. The other minor components (binding agents and spices) were added the same for each formulation in the following proportions: one egg (55–65 g), 50-g flour, 5-g NaCl, 4-g spices and flavor agents, so the seven obtained formulations only differed from each other in the proportion of the main ingredients. In all cases, a uniform paste was obtained. For packaging, a plastic film of polypropylene, resistant to heat treatment was used. Film thickness was 28 to 50 µm. Portions of paste were wrapped forming a roll (mean weight = 616 g; diameter = 9 cm; large = 20 cm).

Rolls were tightened with a thin cord to avoid desegregation during cooking. Thermal treatment was performed for 90 min in boiling water. Each piece was then immersed in cool water for 20 min. Thermal history was registered with T-type thermocouples (DAS TC/B, Keithley, Cleveland, OH). Samples were stored overnight at 4C before analyzing their composition and sensorial attributes.

Product Analysis

The water content of each formulation was determined by drying samples up to a constant weight in an oven at 105C (METTLER PE 300, Mettler-Toledo, Greifensee, Switzerland) (AOAC 1984, method 31.006).

Proteins were quantified according to the reference method of Kjeldahl–Arnold–Gunning (AOAC 1984, methods 2.061-2.062). Factor 6.25 was applied to convert total nitrogen to protein. Fat was extracted by a semicontinuous procedure using a Soxhlet device and ethyl ether as the extraction solvent. For this determination, samples were previously dried at 105C to allow an effective solvent action. Eight extraction cycles were performed.

Ash content was determined by calcination at 550C in an oven (INDEF, Córdoba, Argentina) upon modification of the AOAC method 31.013 (AOAC 1984).

Total carbohydrates were determined as the difference between 100 and the sum of the other components (proteins, lipids, ashes and moisture).

Crude fiber (i.e., cellulose, lignin and part of the total hemicellulose) was determined by a single step method (CAA 1999), employing defatted samples. Samples were submitted to digestion in sulfuric acid (0.255 N) under refluxing (30 min) and then NaOH (3.52 g/100 mL) was added to perform the alkaline digestion for 30 min. After acidifying up to pH = 5, fiber (including minerals content) was determined by filtering through an ashless filter paper and weighing the solids after drying at 105C. Ash content must be discounted, so the samples were then calcinated at 550C. Weight difference was considered the crude fiber content.

Iron and calcium content were determined by flame atomic absorption with a Shimadzu AA6650 equipment (Kyoto, Japan) using a hollow cathode lamp and air acetylene flame. An exactly weighed quantity of ash was dissolved in nitric acid of 0.1 N. Measurements were performed at 248.3 and 422.7 nm for iron and calcium, respectively. In all cases, calibration curves were obtained with certified standards. Results were expressed in mg/100 g product.

All the assays were performed in duplicate.

Physical Properties

Water activity (A_w) was determined at room temperature in an AquaLab QuickStart series 3TE (Water Activity Meter, Decagon, Pullman, WA). Assays were performed in triplicate.

Color measurements were performed with a Minolta Chroma Meter CR-300 colorimeter (Tokyo, Japan). Samples were cut into small pieces (diameter = 2 cm, height = 1 cm). Color was recorded using the CIE-L*a*b* scale. L^* is related to luminosity, a^* is red-green chromaticity value and b^* is yellow-blue chromaticity value.

Texture Profile Analysis

Product texture parameters were evaluated using a TA.XT2i Texture Analyzer (Stable Micro Systems, Surrey, U.K.) with a software Texture Expert for Windows, version 1.2. Cylindrical samples (diameter = 2 cm, height = 1 cm) were obtained from cooked product. Each sample was submitted to two cycles of compression up to 30% of the original height with a cylindrical probe (diameter = 7.5 cm). Force–time curves were obtained at a crosshead speed of 0.5 mm/s. Product hardness, adhesiveness, elasticity and cohesiveness were determined in six replicates. Hardness was defined as the maximum force registered during the first compression cycle. Adhesiveness is the negative area obtained during the first cycle. Cohesiveness was determined as the ratio between the positive area of the second cycle and the positive area of the first cycle. Elasticity was calculated as the distance between the beginning and the maximum force of the second compression cycle.

Microbiological Assays

Twenty-gram samples of raw paste or cooked product were homogenized for 1 min with 180 mL of 0.1% peptone water in stomacher (Stomacher 400, Seward Medical, London, England). The following procedures were applied:

Pour Plate Procedure. This was used for total microbial count. Plate count agar (Merck, Whitehouse Station, NJ) was inoculated with 1 mL of each dilution in triplicate (37C for 48 h). A colony counter (Inolex, Philadelphia, PA) was used and results were expressed as colony forming units per gram (cfu/g).

Most Probable Number (MPN) Method for Coliform (AOAC 1984, Method 46016). Three tubes were seeded into lauryl sulfate tryptose broth with 1 mL of inocula of 1:10, 1:100 and 1:1,000 dilutions in triplicate (48 h, 35C). Positive tubes were transferred to (1) brilliant green lactose bile (48 h, 35C) and (2) Escherichia coli broth (48 h, 45.5C). Results were informed as coliforms and fecal coliforms bacteria/g, respectively. Positives tubes of EC broth were transferred on Levine's eosin methylene blue agar (EMB; 24 h, 35C). Typical colonies were transferred to nutritive agar to perform the biochemical test IMViC (Indol, Methyl Red, Voges Proskauer and Citrate) for classification as *Escherichia coli.*

Microbiological Challenge Testing. In order to evaluate the effectiveness of the cooking process, a fresh culture of *E. coli* was selected for the microbiological challenge testing according to Vestergaard (2001). Usually, a generic strain of *E. coli* can be used instead of *E. coli* O157:H7. *E. coli* was chosen because it is an usual pathogen in meat products and particularly in cooked sausage-type products like morcilla (Oteiza *et al.* 2003). *E. coli* isolated from meat products was provided by the National Reference Laboratory ANLIS, "Dr. Carlos Malbrán," Buenos Aires, Argentina. The raw product was inoculated at a level of 10^7 cfu/g and two different times of cooking were performed at 100C: for 90 min or for 120 min. The number of *E. coli*/g in the cooked products was evaluated in EMB agar (Merck) with incubation at 37C for 24 h. The results were expressed as cfu/g.

Sensory Assessment

Thirty nontrained panelists evaluated the following attributes: appearance, consistency, flavor and global acceptability using a linear hedonic scale (10 cm). One end corresponded to the qualification "dislike extremely," the center to "neither like nor dislike" and the opposite end to "like extremely."

Statistical Analysis

Systat software (version 5.02, Systat Inc., San Jose, CA) was used to perform the analysis of variance in sensory evaluation tests. Statistica for Windows (version 5.0, StatSoft Inc.) was employed for mixture experimental design and response surface analysis.

RESULTS AND DISCUSSION

Composition and Nutritional Evaluation

Protein contents of the ingredients (liver: 19.9%, tripe: 19.2% and soybean seed: 41.4%) were similar to those reported by USDA-NDB (2005a,b,c).

Table 2 shows the values for moisture, ash, proteins, lipids (ether extract) and crude fiber for the different formulations assayed. Moisture content ranged from 66.62 to 72.35 g/100 g and protein contents were similar or even higher than those of available commercial products (meat, sausages). Even formulation 2 (67.0-g tripe/100 g, 16.5-g soybean/100 g, 16.5-g liver/100 g) having the lowest protein content, showed a value close to those of beef meat (18.3- to 20.0-g protein/100 g; INTA 2006). On the other hand, the lipid content of all the formulations was similar to that of slim beef meat (García *et al.* 1994). Crude fiber ranged between 1.5 and 4.5% by weight, and was proportional to soy content.

A_w varied between 0.977 and 0.990 for all the formulations. These results indicate that all products are highly susceptible to microbial spoilage.

Formulation 5 (41.6-g tripe/100 g, 16.8-g soybean/100 g soybean and 41.6-g liver/100 g) exhibited the highest calcium and iron contents (34.01 and 2.98 mg/100 g, respectively). Sodium content was mainly determined by salt addition during product preparation, so final quantity could be adjusted.

The iron content ranged between 1.39 and 2.98 mg/100 g product (wet basis). Formulations 1, 4, 5 and 6 exhibited the highest iron contents. Flour and egg, used as binding agents, contributed to a minor part of the found total iron. The iron bioavailability depends on the form in which iron is present in the diet. This mineral can be associated with a heme group (known as heme iron) or as a divalent or trivalent iron (nonheme iron). Heme iron is found in meat, chicken and fish. Nonheme iron is found in some other foods (soy for example). The bioavailability of iron is 15 to 35% for heme iron, but only 2 to 20% for the nonheme iron. It depends on the iron status of the body and in the case of the nonheme iron, on the composition of the meal (Hambraeus 1999). With respect to the bioavailability of iron in these products, even though the presence of fitates from soy may interfere in the absorption of nonheme iron, it has been reported that the extra iron contribution from soy would compensate this inhibiting effect on bioavailability (Hallberg and Rossander 1982).

Caloric contributions of each formulation (Kcal/100 g) are shown in Table 2.

Texture Attributes

Mean textural parameters for the different formulations are shown in Table 3. The best adjustment of experimental data was obtained when second-order interactions were considered, so second-order polynomials were applied. Table 4 shows the coefficients of the obtained polynomials and statistic parameters related to model adjustment. However, the differences between predicted values and real values using a confirmatory test revealed that the surfaces obtained could be used only as tendency indicators and not with a predictive

TABLE 2.
COMPOSITION OF THE SEVEN FORMULATIONS (F)

F	Moisture (g/100 g)	Proteins (g/100 g)	Lipids (g/100 g)	Fiber (g/100 g)	Ashes (g/100 g)	Carbohydrates (*) (g/100 g)	Ca (mg/100 g)	Fe (mg/100 g)	Na (mg/100 g)	Caloric value (Kcal/100 g)
1	67.69	23.56	3.63	2.00	2.37	2.75	29.08	2.38	159.08	130
	(0.11)	(0.28)	(0.16)	(0.07)	(0.01)	(0.56)	(0.02)	(0.03)	(0.03)	
2	72.35	17.32	3.79	1.50	1.88	4.66	15.07	1.39	198.67	116
	(0.02)	(0.01)	(0.03)	(0.07)	(0.02)	(0.08)	(0.02)	(0.04)	(0.02)	
3	66.62	20.42	3.87	3.10	2.72	6.37	25.95	1.48	125.86	130
	(0.40)	(0.40)	(0.06)	(0.20)	(0.14)	(1.00)	(0.03)	(0.04)	(0.02)	
4	67.64	20.24	3.22	4.50	2.61	6.29	25.82	2.60	146.54	117
	(0.30)	(0.47)	(0.12)	(0.35)	(0.09)	(0.96)	(0.01)	(0.02)	(0.04)	
5	71.42	19.16	3.44	2.41	2.14	3.84	34.01	2.98	131.72	113
	(0.56)	(0.06)	(0.12)	(0.02)	(0.06)	(0.80)	(0.04)	(0.01)	(0.01)	
6	72.13	17.97	3.22	2.5	1.44	5.24	32.78	2.38	130.43	112
	(0.36)	(0.37)	(0.03)	(0.14)	(0.27)	(1.03)	(0.04)	(0.01)	(0.01)	
7	71.58	22.23	3.26	3.1	1.81	1.12	21.57	1.80	117.38	118
	(0.93)	(0.65)	(0.14)	(0.14)	(0.02)	(1.74)	(0.03)	(0.04)	(0.03)	

Values are the mean of duplicates. Standard errors are shown in parentheses. Carbohydrates were determined by difference.

TABLE 3.
TEXTURAL PARAMETERS FOR THE DIFFERENT FORMULATIONS

Formulation	Hardness (N)	Adhesiveness (N s)	Cohesiveness (dimensionless)	Elasticity (mm)
1	30.9 (1.8)	2.11 (0.46)	0.50 (0.01)	3.80 (0.10)
2	25.8 (3.2)	0.059 (0.02)	0.56 (0.01)	7.59 (0.76)
3	33.5 (0.7)	3.98 (0.64)	0.47 (0.09)	2.66 (0.75)
4	26.1 (1.8)	2.45 (0.29)	0.47 (0.01)	3.24 (0.10)
5	22.7 (1.1)	1.18 (0.24)	0.53 (0.00)	3.68 (0.15)
6	36.5 (2.9)	0.56 (0.22)	0.51 (0.01)	3.23 (0.13)
7	26.6 (1.5)	2.04 (0.27)	0.50 (0.01)	3.42 (0.20)

Values are the mean of six replicates. Standard errors are shown in parentheses.
N, Newton; s, second.

TABLE 4.
POLYNOMIAL COEFFICIENTS AND STATISTICAL PARAMETERS

Parameters	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	Adjusted R^2	P
Hardness	25.85	33.63	31.00	26.47	–24.53	–26.46	0.9593	0.139
Adhesiveness	0.15	3.81	2.09	–4.76	–	–	0.8781	0.025
Cohesiveness	0.56	0.47	0.50	–0.021	–	–0.064	0.9995	0.0003
Elasticity	7.55	2.69	3.83	–6.96	–7.41	–	0.9724	0.019

purpose. Contour plots are shown in Fig. 1. Products showed the highest hardness values when the ground soybean content increased (Fig. 1a). Adhesiveness was rather low for all the formulations; this attribute showed the lowest value for the formulation containing a higher proportion of beef tripe and the highest value was obtained for the product rich in soybean (Fig. 1b). With respect to elasticity, the values increased with the proportion of beef tripe (Fig. 1c). This fact can be related to the presence of connective tissue (rich in elastin and collagen) in beef tripe; this tissue is not easily destroyed by cooking, remaining a highly extensible matrix material with a high work of fracture (Lillford 2000). Elastin is a highly insoluble, fibrous protein found in tissues requiring a high degree of elasticity. This protein is regarded as having a rubber-like structure consisting of several peptide chains cross-linked at intervals by stable chemical bonds (Trevor and Bailey 1981). Elastin is characterized by the relevant presence of glycine, randomly distributed throughout the molecule; the high content of glycine can be correlated to the flexibility of the polypeptide chains. So, like other protein elastomers, it is capable of stretching and recoiling (Debelle and Tamburro 1999). Cohesiveness (Fig. 1d) values are similar among the different formulations (0.47 to 0.56). These

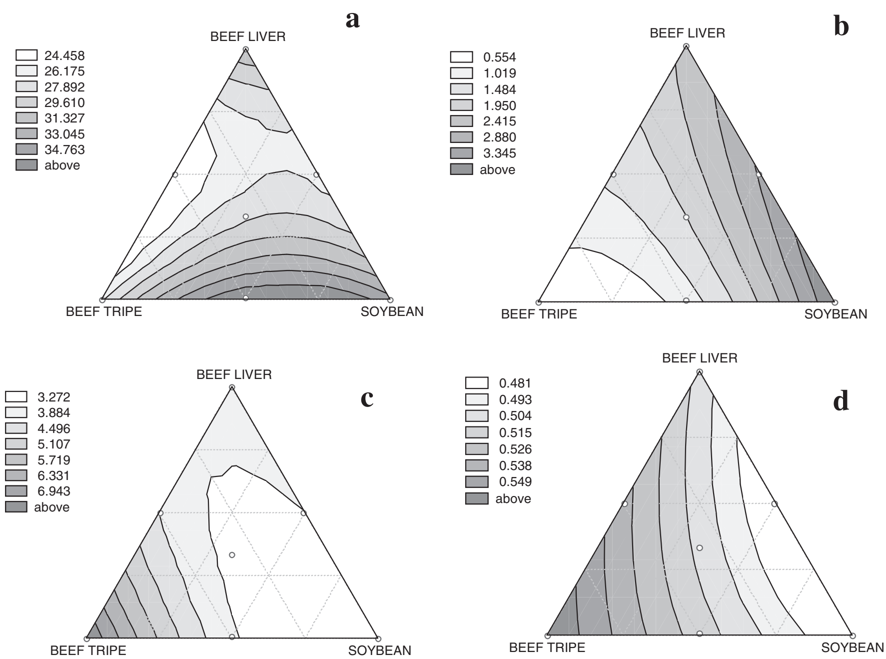

FIG. 1. CONTOUR PLOTS FOR THE DIFFERENT TEXTURAL ATTRIBUTES
(a) Hardness, (b) adhesiveness, (c) elasticity and (d) cohesiveness.

results are according with the good integrity maintained by all these products when they are cut and manipulated. Maximum cohesiveness corresponded to the sample with the maximum proportion of beef tripe, rich in collagen (formulation 2). The arrangement of collagen fibrils played an important role in determining the properties of a tissue. When the tissue is heated, denatured collagen may exude and set to a gel on cooling (Trevor and Bailey 1981); this could be the principal cause for the marked cohesiveness observed in this formulation.

Color Analysis

Chromaticity parameters (a* and b*) did not show a defined tendency. L* (luminosity) ranged between 55.2 (formulation 1) and 63.4 (formulation 3). Formulations with higher contents of soy led to the higher values of L because the presence of soy ground particles made the paste clearer.

Metamioglobine and metahemoglobine formation during thermal treatment is probably the main cause for the final dark color of these products. This fact is usual in products containing liver as an ingredient.

Sensorial Evaluation

Scores for appearance, consistency and global acceptance were not significantly different ($P < 0.05$) for the assayed formulations. With respect to flavor, formulations corresponding to the triangle vertexes in the experimental design (1, 2 and 3) had the lowest scores, probably due to the predominant presence of one ingredient. These samples showed mean scores between 3.88 and 4.72 in a scale ranging from 0 to 10. On the contrary, formulation 7 corresponding to the center of the triangle, was the more accepted (5.64). This formulation had similar proportions of each main ingredient and its flavor seemed to be the more equilibrated one. However, considering that beef liver, soy and beef tripe are marked flavored, this would be a critical point to be improved in order to attain a more acceptable product.

Microbiological Assays

Before thermal treatment, the noninoculated paste showed microbial counts up to 10^5 cfu/g because of the microbial contribution from raw ingredients (mainly beef liver and beef tripe). In spite of the high counts found for total coliforms (440 MPN/g) and fecal coliforms (440 MPN/g), *E. coli* was not detected. As expected, inoculated paste exhibited higher total microbial counts ($1.5–5.5 \times 10^7$ cfu/g) than noninoculated paste and *E. coli* was present.

All thermal treatments were efficient, conducting to total microbial counts lesser than 100 cfu/g and absence of coliforms and particularly of *E. coli*. These results indicate that the thermal treatment of 90 min at 100C is enough for assuring the product safeness even in those samples with a high initial microbial charge. Actually, Argentinean Food Law only prohibits the presence of any pathogenic microorganisms in meat products but no time–temperature standards are established.

CONCLUSIONS

A mixture approach allowed establishing ranges for a more adaptable product preparation by combining the three principal ingredients (beef tripe, liver and soybeans) in proportions ranging between 16.5 and 67%. All the obtained formulations combine animal and vegetal proteins (17.32–25.56 g/100 g) and may represent a significant contribution of fiber (1.5 to 4.5 g/100 g) and minerals (1.44–2.72 g/100 g), particularly iron, to diet. The formulated sausage-type products were different in texture, color and global acceptance depending on composition. Textural attributes varied according to mixture proportions; when beef tripe content increased, elasticity and cohesiveness increased probably due to the contribution of collagen and elastin from tripe.

Hardness and adhesiveness increased with soybean content. Color was mainly determined by the incorporation of liver due to metamioglobine and metahemoglobine formation and the product was clearer when soybean content increased. The most accepted mixture, as determined by sensorial evaluation, was that with equal proportions of each main ingredient; this mixture had 22.2-g protein/100 g, 1.80 mg/100-g iron and 3.1-g fiber/100 g. From a microbiological point of view, in spite of the high A_w values of all the formulations, the thermal treatment proposed for the domestic scale procedure demonstrated to be safe.

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