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Research article

DEVELOPMENT OF VEGETABLE SAUSAGE USING NATIVE INGREDIENTS: EFFECT ON PHYSICOCHEMICAL, NUTRITIONAL AND TEXTURAL PROPERTIES

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ABSTRACT

In this work, the changes in the physicochemical, nutritional and textural properties of vegetable sausages prepared from the combination of lentil, rice, oat and carob flours were evaluated. As food hydrocolloids, brea gum and carrageenan (Genuvisco MB 11F) were used. The proximal composition, energy intake, total polyphenols, total dietary fiber, aqueous activity, pH, water retention capacity and textural parameters of each of the samples were determined. The total polyphenol content as well as the total dietary fiber content increased substantially with the addition of carob flour. The amino acid profile was determined for the vegetable sausage made with four types of flour; the composition of essential amino acids meets the amino acid requirements of adults and adolescents. There was significant variation ($p < 0.05$) concerning color among treatments, and the the samples containing carob flour was the darkest colored. The hardness was not different ($p > 0.05$) among the products when carrageenan was replaced by brea gum at the same concentration. However, in the range studied, the products are slightly stickier. The formulation of vegetable sausage incorporating regional ingredients was feasible, generating a product with appropriate textural and nutritional characteristics

1.Introduction

The plant-based diet is a type of diet that promotes care for the environment and reduces the possibility of getting sick from certain diseases.

In a recent work, (Bryant, 2022), 43 studies on the health and environmental sustainability of products made from vegetable proteins compared to similar products of animal origin were reviewed. In terms of environmental sustainability, the former is more sustainable when greenhouse gas emissions, water use, and land use are analysed. In terms of health, they present a series of benefits, including generally favourable nutritional profiles, which aid weight loss and muscle synthesis, and attention to specific health conditions.

A significant number of current consumers choose food products based on vegetable proteins either by adhering to veganism, vegetarianism or by convictions that attend to the care of the environment and health. New food developments include formulation with alternative proteins such as legumes and cereals. Within the offer of vegetarian foods are meat substitute products. In the development of this type of food, an attempt is made to imitate the flavour, texture and even the size of products made with meat (Ahmad *et al.,* 2022; Bakhsh *et al*., 2022; Corrêa *et al.*, 2023; Kamani *et al.*, 2019; Lyu *et al.*, 2023). Thus, for example, in the production of meatless sausages, hamburgers or nuggets, the food industry uses soy as the main ingredient due to its high protein content, high hydration capacity, amino acid composition and other properties. Soybean products, when used in meat products, show positive effects in terms of improving sensory properties, while ensuring stable production, as well as benefits for large-scale production (Munialo *et al.*, 2022). In addition to soybeans, legumes are another important source of vegetable protein. Legumes consumption has been shown to reduce the risk of cardiovascular disease and diabetes (Papandreu *et al.*, 2019). Other likely sources of plant protein studied include mung beans, peanuts, and peas (Samard and Ryu, 2019). Lentil (*Lens culinaris*) is another legume grain with a high concentration of nutrients. Its proteins are incomplete because they lack methionine, an essential amino acid, but if it is combined with other sources of protein such as rice, which is rich in this amino acid, it forms a protein of high biological value.

Another legume of interest in the development of healthy, which is an autochthonous food in the region of the province of San Luis, is the carob tree. Carob trees belong to the *Prosopis* gender, which in Argentina has 27 species, being 13 are of them endemic. Carob pods are characterised by containing highly amount of soluble sugar (40–50%), proteins (3–4%), and lipids (0.4–0.8%). Moreover, carob flour contains substantial amounts of polyphenols, especially condensed tannins. Polyphenols exhibit a wide range of biological properties, and among these, antioxidant activity is the most known (Mom *et al*., 2020). In addition to the high content of phenolic compounds, carob flour is considered a product that contains a high level of dietary fiber, minerals (Fe, Ca, Na, K, P and S) and vitamins (E, D, C, niacin, B6 and folic acid). In view of its high nutritional value, carob flour has been used as an ingredient in functional or healthy foods (Alvarez Sala J, 2017; Babiker *et al*., 2020; Berk *et al*., 2017; Mom *et al*., 2020; Rodriguez *et al*., 2019; Sciammaro *et al*., 2018).

On the other hand, the desirable organoleptic characteristics in a sausage made without meat require the addition of gums or food hydrocolloids. Studies carried out on this subject, (Majzoobi *et al*., 2017), showed that the protein and fat contents of meatless sausages (12.8% protein and 13.3% fat) were like those of common meat sausages (12–14% protein and 13–15% fat). However, certain problems associated with texture, low water retention capacity and dark colouration were found. The incorporation of carrageenan and mannan gum managed to correct some of these deficiencies. These studies support the need to study a new hydrocolloid for food use to improve the textural aspects of this type of product. Brea gum is a natural hydrocolloid obtained from a forest species (*Cercidium praecox*) belonging to the legume family Brea gum is a viscous and uncrystallisable substance, which differs from resins by being soluble in water. It has various properties such as thickener, gelling agent, emulsifier and stabilizer. Physically, it is very similar to gum arabic, so it could be a good substitute (Bertuzzi *et al*. 2012). Its incorporation into the Argentine Food Code was carried out in 2013 in chapter XVIII, article 1398, section 72.1 as a thickener, stabilizer and emulsifier. Studies on its application in the preparation of edible films, bakery production and microencapsulation of corn oil can be found in the bibliography (Spotti *et al*., 2016; Castel *et al*., 2017; López and Jimenez, 2016; Slavutsky *et al*., 2018).

Based on the above, the hypothesis is raised that the incorporation of carob flour will improve the nutritional properties of the product and the use of brea gum as a new hydrocolloid will allow it to replace an existing additive on the market.

In this work, it is proposed to develop a vegetable sausage, using as sources of vegetable proteins: lentil, rice, oat, and carob flour. The similarities and differences of two natural hydrocolloids: brea gum and carrageenan in the textural properties of the products are also analysed.

2. Materials and methods

2.1. Materials

The raw materials and additives used in the development of the product included: lentil, rice, oat, and carob flour as sources of vegetable protein and fiber; brea gum and carrageenan (CP Kelco, Copenhagen, Denmark) as food hydrocolloids; sunflower oil; spices mix; salt and synthetic casing. The two regional ingredients: *Prosopis* flour and brea gum were provided by the Cooperativa Raíces del Bosque Nativo in the province of San Luis (Argentina). The rest of the mentioned ingredients were purchased in the local trade.

2.2. Experimental design

In the development of the proposed vegetable sausage, an experimental mix design was applied. The response variable will depend on the proportions of the components. The response variables were the protein content of the product and the textural parameters. The controllable factors corresponded to the compositions (percentage wt.) of the vegetable protein sources. Table 1 shows the combinations obtained.

The experiences were repeated using:

- Stage 1: 0.8 %wt. carrageenan (C)
- Stage 2: 0.8 %wt. brea gum (BG)
- Stage 3: 1.5 %wt. (C+BG)

The limits of each of them were established from experience and previous tests.

Analysis of variance (ANOVA) was performed using the free R software with a confidence interval of 95%. The results of the evaluated variables were presented as mean ± standard deviation of three determinations.

Table 1. Experimental mix design

Experience	Lentil flour	Rice flour	Oat flour	Carob flour
1	0.9	0.1	0.0	0.0
2	0.1	0.9	0.0	0.0
3	0.1	0.1	0.8	0.0
4	0.6	0.1	0.0	0.3
5	0.1	0.6	0.0	0.3
6	0.1	0.1	0.5	0.3

2.3. Preparation of Sausages

Sausages were formulated based on 0.8% spices and 0.6% salt, 14% sunflower oil, 42.3% water, a percentage of hydrocolloid and the rest with the mixture of vegetable proteins according to the mix design of Table 1. The ingredients were mixed in a food processor operating at 1500 rpm for 20 minutes. The sausages were filled and later the samples were pasteurized in a water bath at 80 °C for 20 min. Then they were rapidly cooled in cold water (8 °C) until they reached room temperature. The samples were stored at 4 °C for 24 h before their characterisation.

2.4. Chemical characterisation of sausage formulations

Protein was determined with the AOAC 2001.11 method (6.25 to convert nitrogen content into protein content), fats using AOAC 991.36 method, moisture with the AOAC 945.15 method and ash with AOAC 923.03 method. Carbohydrate content was calculated by difference.

The analyses were carried out in triplicate. The energy value was calculated based on the following calorie contents: 9 $kcal \cdot g^{-1}$ for fats and 4 $kcal \cdot g^{-1}$ for proteins and carbohydrates (FAO, 2003).

The determination of total dietary fiber was carried out according to the AOAC 991.43 standard.

The total phenol content was determined by the Folin-Ciocalteu colorimetric method from the methanolic extract. 50 µL of sample were incubated with 200 µL of the Folin-Ciocalteu reagent. After 1 hour of incubation at room temperature in the dark, sodium carbonate solution (7.5 %w/v) was added and mechanically stirred for 1 hour in the same conditions. The absorbance at 760 nm was measured using an SP Spectrum 2100 UV/SP spectrophotometer. A calibration curve was made with gallic acid (0-100 $mg \cdot l^{-1}$). The measurement was carried out in triplicate and the total phenolic content was expressed in mg of gallic acid equivalents per 100 g of dry sample.

Amino acid composition was analysed based on ISO 20976–1:2019 (AOAC Method 994.12). Laboratorio Rapela (Buenos Aires, Argentina) performed protein hydrolysis, amino acid analysis and quantification. Individual amino acids were measured and quantified by High Pressure Liquid Chromatography (HPLC).

2.5. Determination of pH and water activity

The pH was determined on ten grams of each sample, grounded in small sizes, mixed with 100 ml of distilled water, homogenizing for 2 min. Then, the pH was measured using a digital pH meter calibrated at room temperature.

The water activity (aw) was measured in triplicate in sausages (at 25°C), using Aqualab equipment. The procedure included filling a chopped sausage sample into a measuring capsule (2/3 of its height), placing it in the measuring part of the apparatus and lasted until the equilibrium was established

2.6. Colour determination

The determination of the measure of the colour of the vegetarian sausage was carried out by recording the L*a*b* coordinates in a ColourTec PCM digital colorimeter. The values of L*(light/dark), a*(red/green) and b*(yellow/blue) were obtained. The measurements were performed at room temperature immediately after the sausages were cut

2.7. Determination of water release

A thin slice of each sample (1 g) was cut and placed between two filter papers (Whatman No. 1) of known weight. It was pressed with 1 kg of weight for 20 min at room temperature. The weight gain of the filter papers was determined gravimetrically using an analytical balance. The weight gain of the filter papers divided by the initial weight of the sausage (1 g) multiplied by 100 is reported as percentage of water release from the samples, which has a negative correlation with the water retention capacity.

2.8. Texture profile analysis (TPA)

The texture properties of the samples were studied using a texture analyser (Texture Analyser, TA Plus, stable Microsystems. Surrey, England). The test was carried out with a blade probe. A test speed of 1 $mm \cdot s^{-1}$ and a cutting distance of 25 mm were used.

3.Results and discussions

3.1. Chemical composition of sausage formulations

Table 2 shows the experimental data of the proximal composition of the tested formulations. The results for humidity, protein, fat and ashes show that there are significant differences ($p < 0.05$). Protein contents of vegetable sausage ranged between 6.2 and 9.37%. The highest value in protein content responds to the formulation with the highest amount of lentil flour. Other authors, (Corrêa *et al.,* 2023; Marti-Quijal *et al.,* 2019; Priya *et al.,* 2022), have found total protein values that vary between 2.33% to 23% depending on whether they use legume and cereal flours or textured vegetable proteins.The fat medium used was sunflower oil, which has a low saturated fat content.

Table 2. Proximal composition of vegetable sausages

	M1	M2	M3	M4	M5	M6
			Carrageenan (0.8 %)			
Humidity ($g\cdot kg^{-1}$)	479±1^{a}	476±12^{a}	486±6.2^{a}	470±26^{b}	473±20^{b}	481±25^{c}
Proteins ($g\cdot kg^{-1}$)	93±0.2^{a}	77±2.8^{b}	62±0.1^{b}	64±0.1^{b}	63±5.0^{a,b}	66±4.9^{b}
Fat ($g\cdot kg^{-1}$)	117±22^{a}	111±33^{b}	113±2^{a,b}	114±2^{a,b}	115±2^{a,b}	118±20^{a}
Ashes ($g\cdot kg^{-1}$)	13±2.4^{a}	11±1.4^{b}	12±1.6^{a,b}	14±5.0^{a}	12±1.8^{a,b}	13±4.7^{a}
Carbohydrates ($g\cdot kg^{-1}$)	297±59	323±68	326±11	337±8.2	335±75	320±76
Energy supply ($Kcal\cdot kg^{-1}$)	2618	2604	2575	2638	2636	2615
Polyphenols ($mg\cdot kg^{-1}$)	82.6	--	--	1344.3	--	--
Total dietary fiber ($g\cdot kg^{-1}$)	63.0±1	--	--	102.0±2	--	--

Mi, where i is the experience number of the experimental design in Table 1. Means within files with different letter (a, b or c) are significantly different ($p < 0.05$).

Table 3. Amino acids profile of vegetable sausage (M6) and the amino acid requirements in different age groups according to FAO/WHO (1985). mgAA*(g protein)$^{-1}$

Amino acids (AA)	(M6)	Infants	Children (2 to 5 years)	Children (10 to 12 years)	Adults
Essential-amino acids					
Leucine	72.89	93	66	44	19
Isoleucine	27.33	46	28	28	13
Lysine	55.81	66	58	44	16
Methionine	9.11	----	----	----	----
Methionine+Cystine	29.61(*)	42	25	22	17
Phenil alanine	46.70	----	----	----	----
Phenil alanine + Tyrosine	82.01	72	63	22	19
Threonine	36.45	43	34	28	9
Valine	36.45	55	35	25	13
Cystine+Cysteine (as Cysteic acid)	20.50	----	----	----	----
Tyrosine	35.31	----	----	----	----
Tryptophan	10.25	17	11	9	5
Total essential amino acids	350.80				
Non essential-amino acids					
Alanine	50.11	----	----	----	----

Arginin	77.45	----	----	----	----
Aspartic acid + Asparagine	112.76	----	----	----	----
Glutamic acid + Glutamine	206.15	----	----	----	----
Glycine	56.95	----	----	----	----
Histidine	27.33	26	19	19	16
Proline	59.23	----	----	----	----
Hydroxiproline	7.97	----	----	----	----
Ornithine	1.14	----	----	----	----
Taurine	1.14	----	----	----	----
Total non essential amino acids	600.23				
Total determined aminoacid	951.03				

(*)Methionine+ Cystine+Cysteine

Recent studies (Domingo *et al.*, 2023), which analyzed the effect of replacing animal fat with vegetable fat (corn, palm oil and margarine) in meat sausages obtained the best results from a sensory and nutritional point of view when using corn oil. As can be seen (Table 2), both the polyphenol content and the total dietary fiber content substantially increase with the addition of carob flour. The fiber content found exceeds values obtained with other high protein formulations based on textured vegetable proteins (Bakhsh *et al.*, 2022).The nutritional value of vegetable sausage is determined not only by its protein content, but also by its amino acid profile. In terms of amino acid composition, legumes protein varies from cereal grain protein because it contains much more lysine and threonine while being deficient in tryptophan and sulphur containing amino acids such as methionine and cysteine. Table 3 shows the amino acid profile of the vegetable sausage made with the four types of flour under study (M6). The same table shows the amino acid requirements in different age groups according to FAO, 2003. From the data, it is observed that the composition of essential amino acids meets the amino acid requirements of adults and adolescents. These age ranges correspond to the potential consumers of this type of products. The combination of cereals and legumes in this formula made it possible to compensate for the deficiencies of each type of flour.

3.2. Determination of pH, water activity (aw), colour and water release

Table 4. Physicochemical properties (aw, pH and L, a, b parameters) and weight losses of sausage samples with 0.8% of carrageenan

Variable	M1	M2	M3	M4	M5	M6
L	52.92±5.57^{a}	61.89±3.32^{b}	65.71±3.48^{c}	19.82±1.92^{d}	18.01±2.94^{d}	43.31±3.75^{e}
a	3.25±1.98^{a,b}	8.28±2.43^{c}	4.24±3.05^{b}	0.45±0.05^{a}	0.56±0.05^{a}	3.82±1.05^{b}
b	16.80±4.02^{a}	13.51±4.82^{a,b}	16.38±2.53^{a,b}	7.90±1.90^{b,c}	6.93±1.91^{c}	15.34±2.92^{a,b}
aw (25 °C)	0.97±0.01	0.97±0.01	0.97±0.01	0.96±0.01	0.97±0.00	0.96±0.01
pH	5.85±0.05^{a}	5.73±0.30^{a}	5.85±0.16^{a}	5.60±0.39^{a,b}	5.33±0.57^{c}	5.43±0.60^{b,c}
			Weight losses (%)			
Brea gum (BG)	6.43±0.60	4.25±0.60	9.07±0.70	5.40±0.60	5.59±0.10	11.05±0.10
Carrageenan (C)	5.01±0.11	4.01±0.13	8.45±0.11	5.05±0.13	5.04±0.60	10.21±0.20
(BG+C)	6.45±0.20	3.45±0.20	8.01±0.30	5.10±0.20	7.30±0.60	10.35±0.60

Means within files with different letter (a, b, c, d or e) are significantly different ($p < 0.05$).

Table 4 shows the results of aqueous activity, colour parameters and pH for the sausage samples with 0.8% of carrageenan. The luminosity and a* parameters were significantly modified for the samples containing carob flour, observing a decrease in luminosity and products with a darker coloration. The b* value show a significant difference ($p < 0.05$). Carob flour, has the lowest L*value with high ash content, (Ammar *et al.,* 2022) indicating a significantly darker flour. The results of the sensory analysis of the product (not shown here) indicated that the samples containing carob flour showed a different colour than meat sausage, a particular flavour associated with "regional products", a homogeneous texture and good acceptability.

Table 5. Attributes of the texture profile of vegetarian sausage with different hydrocolloids (mean± deviation standard; n = 3).

Hydrocolloid	M1	M2	M3	M4	M5	M6
			Hardness (N)			
Carrageenan (C)	1.19±0.07	3.26±0.68	2.38±0.25	2.34±0.06	2.73±0.15	2.51±0.07
Brea gum (BG)	3.00±0.48	4.37±0.28	0.83±0.03	1.97±0.14	3.04±0.41	2.04±0.01
BG + C	2.00±0.60	3.01±0.00	0.70±0.00	1.00±0.01	2.59±0.10	1.05±0.05
			Chewiness (mJ)			
Carrageenan (C)	4.07±0.07	0.18±0.08	0.12±0.05	1.04±0.06	0.05±0.00	1.00±0.01
Brea gum (BG)	5.56±0.02	0.01±0.00	1.96±0.01	2.65±0.01	0.10±0.05	1.38±0.01
BG + C	4.02±0.60	0.10±0.00	1.09±0.00	1.50±0.11	0.08±0.00	0.74±0.05
			Stickness (mJ)			
Carrageenan (C)	1.14±0.05	0.10±0.00	1.00±0.05	1.05±0.01	0.14±0.00	0.10±0.01
Brea gum (BG)	2.66±0.30	0.12±0.07	1.49±0.14	2.49±0.27	0.17±0.00	1.00±0.01
BG + C	1.38±0.50	0.23±0.00	1.30±0.00	3.00±0.31	0.23±0.00	0.08±0.00

The water activity values show that it is a very favourable medium for microbial growth and samples should be stored under refrigeration.

The values of water release (Table 4), are statistically significant differences ($p<0.05$) for the flour combination block, but there is no significant effect of the type of hydrocolloid.

The experimental results show that products made with oat flour, (M3 y M6), are those with the lowest water retention. Vazquez *et al.,* (2017), studied the effect of replacing wheat flour with oat flour in bread making and found that mixtures containing oats had a lower water retention capacity.

3.3. Textural analysis

Table 5 shows the TPA parameters of the sausages for sample with the hydrocolloid combinations studied. Since a P-value is less than 0.05, the flour combination factor has a statistically significant effect on hardness, stickness and chewiness with a 95.0% confidence level.

When the experimental data for brea gum are analysed, the R-squared statistic indicates that the models adjusted for the three parameters explain 88.4, 97.9 and 95.6% of the variability of hardness, chewiness and adhesiveness respectively.

Hardness is maximized as the amount of rice flour increases while chewiness and adhesiveness parameters increase with lentil flour concentration.

The analysis of variance of several factors for the hardness parameter showed that there are no statistically significant differences when the type of hydrocolloid is modified. However, both chewiness and stickiness showed statistically significant differences between carrageenan and brea gum with a 95% confidence level.

Stickiness is a measure of the energy required to overcome the surface attraction of the sausage when chewed. Products made with brea gum are somewhat sticky than when carrageenan is used. Chewyness, which is the product of hardness, cohesiveness and elasticity, represents the work necessary to disintegrate a food until it is ready to be swallowed; this parameter is larger when brea gum is used.

4. Conclusions

The incorporation of two regional raw materials in the formulations of vegetarian sausages produced changes in the physicochemical, textural and nutritional properties. From the nutritional point of view, the presence of carob flour allowed obtaining food with a higher content of polyphenols and fibers. The combination of the four flours studied allowed us to obtain a food that meets the amino acid requirements for adolescents and adults. A statistically significant decrease was observed in the parameters of luminosity and reddish colouration of the samples that contained carob flour. The textural characteristics of the vegetable sausages when brea gum was used as an additive are good. The hardness was not different ($p > 0.05$) among the products when carrageenan was replaced by brea gum at the same concentration. However, in the range studied, the products are slightly stickier.

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