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Research Article

Development of a Restructured Protein Based on Low Cost Legumes

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Abstract: The aim of this research was to elaborate a vegetable restructured protein with low cost leguminous plants, using cowpea (*Vigna unguiculata* L.), pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.). Child malnutrition has it's origins on the scarcity of economic resources, the lack of various foodstuffs in one region and also due to the government's promotion and prevention policies that do not meet the full coverage of the most vulnerable regions. The raw and restructured materials were bromatologically characterized on three formulations: F1 (25% cowpea, 25% pea and 25% lentil), F2 (50% cowpea and 25% pea) and F3 (50% cowpea and 25% lentil). A sensory acceptance test was carried out with 50 potential tasters using a 9-point hedonic scale which evaluated attributes of appearance, color, aroma, texture and flavor. We used a randomized block design with three replicates, data were subjected to variance analysis and Tukey's test ($p \leq 0.05$). The lentil, pea and cowpea protein content was 30.94±0.30%, 28.96±0.16% and 26.21±0.40%, respectively. On the acceptance test with the hedonic scale, the tasters did not find significant differences ($p \geq 0.05$) between the attributes of color, appearance, aroma, texture and flavor. The Acceptability Index presented significant differences ($p \leq 0.05$) between formulations three and one and formulation two differed statistically ($p \geq 0.05$) from formulations one and three. The Only flavor had a significant difference ($p \leq 0.05$), in which formulation three was highlighted.

Keywords: Bromatological characterization, formulation, hedonic scale, sensory acceptance, taster

INTRODUCTION

In recent years the eating habits improvement has been a trend in society, therefore, institutions in charge of public policy in the nutrition field, have promoted the use of foods with high nutritional value and low cost such as legumes. A healthy diet should contain a variety of foods to meet all nutritional needs and maintain an optimal state of health. Increasing the consumption of dried legumes such as cowpea, lentil and pea, has been one of the main options for this purpose (Prada *et al*., 2005).

According to the ENSIN (2010), the department of Córdoba's global malnutrition is 4.3% and chronic malnutrition is 16.4%; children under 5 years of age present chronic malnutrition, a higher amount than the one found in the departments of Sucre (18%) and Choco (15.7%). In regards to global malnutrition on this age group, the results reach 12%, surpassing the Atlantic Coast data of (10%) and Colombia's malnutrition (7%) Heredia *et al*. (2007); so it is inferred that many low-income families do not consume the adequate intake of nutrients, because they do not have enough resources in terms of money to satisfy their basic food needs.

A restructured vegetable food is a mixture of many ingredients, on which the main contribution are vegetables, that have been cooked and grounded, resulting in a dough which must have functional characteristics that allow it's preforming, cutting and freezing. In general, the preparation of a restructured vegetable products, begins with a knowledge of the raw material, followed by it's disintegration, additive and condiments addition followed by a subsequent molding and freezing, with or without pre-frying process (De Paula, 2009).

Precisely, one of the major advantages of restructured products is the possibility to modify the final product's composition, by reformulating the original product previously cutted or chopped and in this sense, one could speak of the elimination of some constituents or the incorporation of other new ingredients or additives (Sánchez *et al*., 2004). In earlier studies Walter Jr. *et al*. (2002) developed restructured "Jewel" sweet potatoes for subsequent frying using a calcium-alginate gel system. The final product presented a consistent texture that was positively accepted by the consumer, this indicates that these additives are suitable for restructured foods manufacturing.

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De Paula (2009) developed a restructured product based on malanga, finding that the industrial processing of malanga is viable and can be an alternative that adds value to this raw material. Salinas (2007) elaborated restructured meat concluding that there was no difference in smoothness, juiciness and general acceptance between original steak and the restructured meat, but there was a difference between flavor and appearance.

The municipalities of San Pelayo, Lorica and Cotorra are recognized as significant bean producers, reaching 150, 392 and 320 tons respectively, which makes these three localities very important on the region's bean production (Heredia *et al*., 2007). This data was taken into account, in order to develop a new product with a good nutritional content, produced with legumes that are grown in the country so this can help mitigate the malnutrition crisis that is present in the department of Córdoba.

This study was made in the aim to develop a healthy, low cost restructured vegetable protein that is available to the consumer, made with cowpea legumes (*Vign aunguiculata* L.), peas (*Pisum sativum* L.) and lentils (*Lens culinaris* Medik).

MATERIALS AND METHODS

This research was carried out at the University of Córdoba, Berástegui, municipality of Ciénaga de Oro, located geographically at 120 masl between 8° 52' 52.95" North Latitude and 75° 42' 8.77" West Longitude with respect to the Greenwich meridian, this location has an average temperature of 29°C and a relative humidity of 80%. For the execution of this project the facilities of the Vegetable Pilot Plant were used and the Food Analysis and Sensory Laboratories, that belong to the Food Engineering Department.

Bromatological characterization of raw materials: The main raw materials used for the elaboration of the restructured vegetable protein were bromatologically characterized: cowpea, peas and lentils, which were obtained in a local market of the city of Montería. The bromatological characterization was performed through the analysis of moisture (AOAC 925.10), ethereal extract (AOAC 920.85), raw protein (AOAC 920.87), ashes (AOAC 923.03) and crude fiber (AOAC 920.86), according to AOAC methodology (2012). The total carbohydrate percentage was calculated according to the following formula:

$$\% \text{ Carbohydrates} = 100 - (\% \text{ moisture} + \% \text{ ethereal extract} + \% \text{ raw protein} + \% \text{ ash} + \% \text{ crude fiber})$$

The samples were studied in triplicate for each of one the raw materials used.

Table 1: Ingredients used in the elaboration of protein restructuration (g/100 g of product)

Ingredients	F1	F2	F3
Cowpea bean	25	50	50
Vetch	25	25	
Lentil	25		25
Bean	4	4	4
Carrot	4	4	4
Head onion	2	2	2
Chives	2	2	2
Coriander	2	2	2
Pepper	2	2	2
Garlic	2	2	2
Chili pepper	2	2	2
Salt	2	2	2
Egg	1	1	1
Wheat flour	2	2	2

Formulation development for the elaboration of restructured protein from legumes: Three different formulations were developed (Table 1), the universe consisted of 2 kg for each one of the different formulations.

At the initial stage of the restructured vegetable protein process, legumes (lentil, cowpea and pea) were soaked for a period of 2 h, on which the water was regularly changed to avoid the presence of soluble sugars (triose and tetrose) responsible for the fermentation process (Montoya, 2012). Subsequently, a separate pre-cooking for each of the leguminous was carried out, on which a pressure cooker was used during a 5 min period, in order to facilitate it's manipulation. Once the legumes were cooked (lentils, cowpeas and peas), a milling process was carried out with a Javar mill, model M22 series 05 of 2 Hp, with the addition of carrot, string beans, onion, chives, chili and cilantro, which were added in controlled quantities depending on the required amount for each one of the formulations (Table 1). After this, a mixture was made by adding the rest of the ingredients.

In order to give it an appropriate shape and texture the mixture was embedded, using an "ALIFAN" sheath (polyethylene/polyamide blend of 5 cm diameter), then put through a blanching process at a temperature of 75 to 80°C (on the thermal center) for 30 min, then it was allowed to cool to room temperature.

Once the cooking process ended, the product was frozen at -24°C and then sliced, making slices of 0.5 cm each, finally it was fried in palm oil at 170°C for 4 min, using a discontinuous electric fryer which has a capacity of 5 L.

Bromatological characterization of restructured vegetable protein: The three formulations of restructured vegetable protein were bromatologically evaluated according to the methodology used for the raw material characterization, this was made in the aims of knowing the main present nutrients in each formulation and their influences on each one of the treatments. Samples were analyzed in triplicate for each one of the formulations.

Acceptance determination of the selected restructured vegetable protein: The restructured protein acceptance was evaluated using a hedonic scale of 9 points, where 1 is "It is extremely disgusting" and 9 "I extremely like it ", in order to evaluate color, appearance, aroma, texture and flavor attributes.

The samples were served on white plastic plates, coded with randomized three-digit numbers, presented simultaneously to the tasters in random order, this study was carried out with the participation of 50 potential product consumers that were verbally recruited.

The Acceptability Index (AI) of the product was also calculated according to Peuckert *et al*. (2010). For it's calculation, the following expression was adopted:

$$AI (\%) = A \times 100/B$$

where,
A = Average grade obtained for the product
B = Maximum score given to the product

The AI with good repercussion has been considered ≥70%.

Experimental design: For the bromatological characterization of the raw materials and for the restructured one, a random block design was used in the experiment assembly, with three repetitions for each treatment, the results were subjected the Analysis of Variance (ANOVA) and the Tukey test ($p \leq 0.05$) in order to compare the means

For the analysis of the obtained data from the acceptance test (9-point hedonic scale), a randomized block design, the Analysis of Variance (ANOVA) and Tukey's test ($p \leq 0.05$) were used. All results were analyzed using SAS software free version for universities.

RESULTS AND DISCUSSION

Bromatological characterization of the raw material: The obtained results from the proximal analysis of legumes showed significant differences ($p<0.05$) between moisture, ash, fat, protein, fiber and total carbohydrates values that were evaluated, as can be seen in Table 2.

The studied legumes types were mainly selected because of their high carbohydrate content and their protein content, which present maximum values of 51.92 and 30.94%, respectively. On the other hand, the moisture and ashes values in the raw materials were moderately low as is normal in these products, however, it was observed that the lentil presented higher moisture value (2.39%) compared to cowpea (1.11%) and pea (1.54%) (Table 2). The moisture values found in the lentil are similar to those of Álvarez (2014), which reported a content of 2.88%, unlike Polo (2012), which found a content of 12.22%.

Umaña *et al*. (2013) reported values of 10.41% for moisture content in cowpea beans and Cerón *et al*. (2016) of 12.91% for pea, values higher than those found in this study (Table 2). However, it could be said that the results found in this study, are within the accepted limit of dry legumes that must have a maximum of 13% humidity, to reduce the risks of contamination and the proliferation of bacteria that can affect the freshness and health of the grain (Sarmento, 2012).

In regards to the results of ash content, the obtained measure on lentils (3.06%) is similar to that reported by Maldonado *et al*. (2002) which is (2.95%). In the case of cowpea beans a value of 2.29% was found, lower than that reported by Guzmán (2012) (4.12%), peas reported a lower value of (2.74%), similar to that found by Cerón *et al*. (2016) (2.67%).

It was found that lentils presented the highest percentage of protein, with 30.94%, followed by 28.96% for peas and 26.21% for the cowpea beans (Table 2). Umaña *et al*. (2013) compared different legumes, including lentils and cowpea beans, finding lower values than those in this study, with a protein percentage of 23.99% for lentils and 22.56% for beans, respectively.

Similarly, Cerón *et al*. (2016) reported a lower value for peas (20.18%). In contrast, Mune Mune *et al*. (2008) states that cowpea beans have many major protein contents, which can be corroborated by Onwuliri and Obu (2002) who obtained data close to 40.0%. Sarmento (2012) obtained 38.6% for lentils, a higher value than the one found in this study. Therefore considering that the average percentage of protein in legumes is between 20-25%, we observe that the values obtained in this research are higher than those reported in the literature, however the literature supports wide differences between data in protein, which may be due to changes in biochemical composition induced by genetic improvement (Castellón *et al*., 2003) and environmental factors such as the nitrogen and sulfur presence in the soil, which strongly influences the accumulation on the seed (Tabe *et al*., 2002).

The fat content of the studied legumes presented relatively high values, being the cowpea beans the one that showed the highest value (3.18%), as reported by Vargas and Villamil (2012) and contrary to Umaña *et al*. (2013) who reported a fat content of 0.39%. Followed by lentils which presented a value of 2.86%, a higher result than the one reported by Umaña *et al*. (2013) (0.95%) and peas had the lowest value (2.10%), but higher than the one reported by Cerón *et al*. (2016) (1.27%).

Table 2: Bromatological composition of the legumes used

Composition (%)	Cowpea beans	Lentil	Vetch
Moisture	1.11±0.02	2.39±0.05	1.54±0.02
Ash	2.29±0.08	3.06±0.08	2.74±0.02
Protein	26.21±0.40	30.94±0.30	28.96±0.16
Grease	3.18±0.00	2.86±0.02	2.10±0.07
Fiber	15.26±0.05	16.61±0.29	13.16±0.11
CHO	51.92±0.41	44.13±0.63	51.48±0.14

*: Average of three replicates±standard deviation

Table 3: Bromatological characterization of three restructured proteins from legumes

Composition (%)	F1	F2	F3
Moisture	2.30±0.09[ab]	2.68±0.24[a]	2.06±0.06[b]
Ash	2.50±0.01[c]	3.51±0.15[a]	3.02±0.01[b]
Protein	15.89±0.09[a]	15.99±0.06[a]	14.89±0.07[b]
Grease	17.70±0.48[NS]	18.28±0.17[NS]	17.38±0.43[NS]
Fiber	25.44±0.30[a]	21.42±0.26[b]	18.50±0.43[c]
CHO	56.36±0.08[c]	57.61±0.38[b]	83.03±0.73[a]

*: Average of three replicates±standard deviation; **: Different letters in the same column denote significant statistical differences ($p \le 0.05$)

As for the fiber content, the lentils presented the highest percentage with 16.61%, followed by cowpea beans (15.27%) and peas (13.16%), these values are much higher than those reported by different authors for the same legumes. Polo (2012) reported values of 5.17 and 6.32% for lentils and peas respectively, while Guzman (2012) found 4.24% for cowpea beans.

Regarding the carbohydrate content, we observed that cowpea beans showed the highest percentage with (51.92%), followed by peas and lentils with 51.48 and 44.13%, respectively (Table 2). Vargas and Villamil (2012) reported values of 68.51% for cowpea beans, 56.40% for lentils and 52.50% for peas, obtaining higher values than those found in this study.

Bromatological characterization of the restructured vegetable protein: The established formulations to obtain the restructured vegetable protein from legumes, generated statistically significant differences ($p \le 0.05$) on the bromatological characterization for the variables of moisture, ash, carbohydrates, fiber and protein, however, the fat content was not statistically significant ($p \ge 0.05$) (Table 3).

When evaluating each one of the physicochemical variables of the restructured product, we observed that the three formulations presented a high content of carbohydrates and fiber (Table 3), compared to the other variables, which is common in legumes since these contain a large amount of starches (made up of 30% amylase and 70% amylopectin), which is attributed to health benefits, contributing to a glucose release reduction and inducing a drop in the glycemic index, while fiber is involved in gastrointestinal health (León and Rosell, 2007). In relation to the protein percentage, F1 and F2 did not present significant statistical differences among themselves ($p \ge 0.05$), yielding values of 15.89 and 15.99% (Table 3) respectively, but are statistically different ($p \le 0.05$) in relation to F3 with 14.89%, which shows that the bean/pea mixture provides less protein content than the other mixtures. Vigo (2014) reported protein values of 20.06% for restructured alpaca with pecan inclusion, as well as Boari (2014) for restructured chicken (21.7%), obtaining values higher than those found in this study. Therefore, results obtained for restructured with legumes mixture, suggest that their consumption may be of great importance when replacing animal protein.

The moisture content between the three formulations is higher in F2 with 2.68% being is statistically significant ($p \le 0.05$) in relation to formulation F3 with 2.06% (Table 3), indicating that the moisture percentage is higher when cowpea beans and lentils are mixed compared to the three legumes mixture. An explanation for this result could be that lentils present a higher moisture volume than cowpea beans and peas (Table 2). F1 has no significant differences ($p \ge 0.05$) to F2 and F3. These results differ from those reported by Boari (2014) for their restructured made from chicken, reporting humidity contents up to 63.03% and those from Vigo (2014) that obtained higher values for their restructured alpaca including pecan by publishing a humidity content of 65.55%.

As for the ash content, F2 stood out with a proportion of 3.51%, being statistically significant ($p \le 0.05$) in relation to F2 with 2.50% and F3 with 3.02% (Table 3). These data are higher than those reported by Vigo (2014) (1.48%) and similar to that of Serrano (2006) who elaborated a restructured meat protein with the addition of walnut (3.51%).

Regarding the fat percentage, the fat absorption of the different formulations was not statistically significant ($p \ge 0.05$), it is observed that the formulation containing more water is F2, which is the one that presents a higher oil absorption, however, this fact not statistically representative to show significant differences between them, even though F2 presented a higher value in the fat content.

In regards to the fiber content, F1 showed the highest fiber percentage with 5.44% presenting significant differences ($p \le 0.05$) in relation to F2 with 21.42 and 18.50% with F3 (Table 3), this can be

Table 4: Acceptance tests and Acceptance Index (AI) results

Attributes	Average score	Acceptability index (%)
Formulation F1		
Color	6.26±1.44^a	69.6±2.03^a
Appearance	6.28±1.01^a	69.8±4.01^a
Aroma	6.84±1.07^a	76.0±1.10^a
Texture	6.54±1.19^a	72.7±0.96^a
Flavor	6.98±1.11^a	77.6±0.98^b
Formulation F2		
Color	6.40±1.39^a	71.1±1.61^a
Appearance	6.50±1.11^a	72.2±1.72^a
Aroma	6.90±0.88^a	76.7±1.84^a
Texture	6.68±0.89^a	74.2±0.53^a
Flavor	7.22±0.95^a	80.2±1.21^{ab}
Formulation F3		
Color	6.38±1.30^a	70.9±1.45^a
Appearance	6.34±1.00^a	70.4±2.33^a
Aroma	6.88±0.96^a	76.4±1.51^a
Texture	6.66±1.08^a	74.0±2.14^a
Flavor	7.34±1.02^a	81.6±1.72^a

Values in the columns for each attribute followed by the same letter do not differ from each other by the Tukey test ($p \leq 0.05$)

considered as normal since it gathers the contributed amounts by the three used legumes.

Sensory acceptance of the restructured vegetable protein: The level of acceptance (using the hedonic scale of 9 points) for the different formulations of the restructured vegetable protein was made with 50 tasters that evaluated the attributes of color, appearance, aroma, texture and flavor, yielding the results established in Table 4.

The tasters revealed that there were no significant differences ($p \geq 0.05$) between the formulations in terms of the evaluated attributes, indicating that the means between the samples are similar to the experimental error (Watts *et al*., 1989). The formulations were located between the hedonic terms "I lightly like it" and "I moderately like it" for the attributes of color, appearance, aroma and texture; while for the flavor attribute among they were located between the hedonic terms "I mildly like it" to "I like it very much" (Table 4).

According to Chen and Trout (1991) the product's color plays a very important role because it attracts the consumer and helps in the product's acceptance, otherwise discoloration can be a problem because it diminishes the acceptability of the product. Likewise appearance is one of the most important properties for restructured products (Jena and Bhattacharya, 2003) and it often is the only attribute on which we base the decision to buy or consume a product (Vilije, 2010).

According to Vigo (2014), the texture in these products changes according to the variations that are carried out during the restructuring process. According to Pagador (2003) products that have lost less water will be softer and those that lose more water, will be less soft and more pleasing to the consumer's taste, thus conferring a greater importance to this attribute as one of the quality determinants.

According to Acebrón and Dopico (2000) within the organoleptic properties, flavor always stands out, since it identifies the product and it directly influences it's acceptance.

The results for the Acceptability Index (AI) (Table 4) show that only the flavor attribute presented significant differences ($p \leq 0.05$), F3 stands out with the highest value (81.6%) which is statistically equal to F2 and this is statistically equal ($p \leq 0.05$) than F1.

According to Peuckert *et al*. (2010) for a food to be accepted by the public consumer it must have an AI greater than or equal to 70%. In this way it can be said that the formulations obtained a good acceptance in general, with exception to F1 on the color and appearance attributes. Therefore F3 stands out on the acceptance test, being the selected sample, which contains a mixture of 50% cowpea beans and 25% lentils.

CONCLUSION

The raw materials (cowpea, lentil and pea) used in the elaboration of the restructured vegetable protein presented values of humidity (1.11-2.39 g/100 g), ashes (2.29-3.06 g/100 g), crude protein (26.21-28.96 g/100 g), crude fat (2.10-3.18 g/100 g), crude fiber (13.16-16.61 g/100 g) and carbohydrates (44.13-51.92 g/100 g).

The formulations of the restructured proteins were characterized by their high carbohydrate and fiber content, in comparison to other variables. The protein percentage in formulations 1 and 2 did not present statistically significant differences ($p \geq 0.05$), yielding values of 15.89 and 15.99% respectively, but in turn, they are statistically significant ($p \leq 0.05$), in relation too formulation 3 (14.89%), showing that the cowpea beans/peas mixture provides less protein content than the other mixtures.

In the acceptance test with hedonic scale, the tasters didn't find significant differences ($p \geq 0.05$) among attributes of color, appearance, aroma, texture and flavor. For the Acceptability Index (AI) there is a difference ($p \leq 0.05$) between formulations 3 and 1 and formulation 2 differs statistically ($p \geq 0.05$) between formulations 1 and 3; as all ratings were above 70%. For most of the attributes the tasters located their hedonic terms on the pleasing scale. In general there was a good acceptance of the restructured vegetable protein being F3 the one that stands out, which contains the mixture of 50% cowpea beans and 25% lentils.

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