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Article

Lentil flour as an alternative source of protein

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Abstract

Lentils have a high nutritional value and are sustainably grown, due to their protein content, they are an alternative source of protein. Their transformation into flour would facilitate the development of foods, benefiting their nutritional profile and contributing to the diversification of protein sources. The objective was to evaluate the protein quality of green and red lentil flour. The research was conducted at the Faculty of Chemistry-Pharmacobiology, Morelia, Michoacán in 2023. Lentil flours were obtained by grinding the seeds and by traditional cooking (94 °C). A proximate chemical analysis was performed and protein fractions were determined (Osborne method). Protein quality was determined by Score and PDCAAS. Green lentils are high in protein and fiber, red lentils in carbohydrates and ashes. The protein fractions are the majority and were globulins and albumins, and they decreased after cooking. Red lentil flour presented a better digestibility-corrected amino acid profile, with high Score and PDCAAS. In conclusion, lentil flour is an alternative protein source to develop nutritious foods. Future research could optimize its functional and sensory properties through technologies such as extrusion, expanding its use in caloric-protein diets.

Keywords:

Lens culinaris M., legumes, protein fractions, protein quality.

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Introduction

Legumes are important in human nutrition and a remarkable source of protein (22 to 32%); this percentage depends mainly on the type of seed and variety (Sáenz-Reyes *et al*., 2022). Lentils contain essential aminoacids (leucine, lysine, threonine, and phenylalanine) and are deficient in sulfur aa: methionine, cysteine, and tryptophan (Mefleh *et al*., 2021).

Lentils are essential for food security due to their protein content, helping to reduce malnutrition and other diseases (Bessada *et al*., 2019). They are a sovereign and sustainable resource, with a long shelf life, stable crops, adaptable to the climate, and contribute to nitrogen fixation, benefiting other crops and reducing the use of fertilizers (Prada *et al*., 2024).

Lentil varieties are distinguished by their color: yellow, orange, red, green, brown, and black, with differences in texture, composition, and appearance (Chandler *et al*., 2022). Le Puy green lentils are the most produced and consumed in Mexico, they are small in size and yellowish-green in color (Meenal *et al*., 2023).

Red lentils, produced mainly in Canada, have reddish-orange hues and are devoid of skin, which reduces their cooking time, facilitates digestion, and gives them a less vegetal flavor. However, their fiber content is lower (Kaale *et al*., 2022).

The main parts of lentils are cotyledon (89%), containing proteins and carbohydrates, testa (10%), with polyphenols, and embryonic axis (1%) (Samaranayaka *et al*., 2024). In Mexico, Michoacán concentrates the cultivation of green lentils (90-95%), the area of which has increased (Sáenz-Reyes *et al*., 2022). Red lentils, although valued, are imported and little used in Mexican cuisine.

Lentils are traditionally consumed in stews, purees, side dishes, appetizers, or desserts; as they are a rich source of proteins with essential aminoacids, they can be complemented with the essential aminoacids of cereals, providing quality proteins. At the same time, they provide carbohydrates, micronutrients and fiber (Chandler *et al*., 2022).

The consumer profile has changed; in this sense, the development of lentil-based products: flour, sprouts, protein extrudates and isolates, among others, are alternatives to diversify their consumption, taking advantage of their contribution in proteins, minerals, carbohydrates, among others (Soto *et al*., 2023).

The protein content in lentils is 20-25%, most of these proteins are storage proteins (80%): globulins (65-70%) and albumins (10-15%) (Gilani, 2021). Their amino acid composition is characterized by a low concentration of methionine and cysteine, and they are rich in arginine, aspartic acid, glutamic, and lysine (Kumar *et al*., 2022).

The proteins in lentils are classified as structural, biologically active, and reserve (Boye *et al*., 2009). To separate the protein fractions (PFs) from the reserve proteins, the Osborne method is used, which classifies them according to their solubility in different solvents and their isoelectric pH, obtaining four types of fractions (Kumar *et al*., 2022).

Albumins (25.1-31%) are soluble in water and have functional properties such as enzymes and lectins. They participate in protein degradation during germination. They also include defense proteins: trypsin inhibitors and lectins. In legumes, albumins are a source of lysine and methionine (Patto *et al*., 2019).

Globulins are reserve proteins soluble in saline solutions, they constitute between 26.2-34.6.5% in legumes and are composed of legumin and vicilin (Martín-Cabrejas, 2019). To a lesser extent are prolamins, soluble in alcohol-water (50-80%), main PF in cereals (corn and wheat), rich in proline and glutamine. Finally, glutelins are soluble in acidic or alkaline media (Boye *et al*., 2009).

Protein quality refers to the amount of essential aminoacids and their digestibility. In legumes, it is lower than in animal proteins due to their low methionine and cysteine content, the resistance of some proteins to digestion, and the presence of anti-nutritional compounds that affect their digestibility (Khazaei *et al*., 2019).

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A biologically complete protein contains all essential aminoacids in amounts equal to or greater than that established for each aminoacids in a reference protein. Proteins that have one or more limiting aminoacids are incomplete as they limit protein synthesis and cannot be fully used (Chandler *et al*., 2022).

The amino acid computation or protein Score compares the amount of limiting aminoacids in a protein with a reference protein. To determine the Score, values of the essential aminoacids, lysine, tryptophan, threonine, methionine, and cysteine, are used since they are aminoacids that are usually limited in some foods (Avilés-Gaxiola *et al*., 2017).

Protein digestibility corresponds to the proportion of nitrogen ingested and absorbed. The human body excretes between 10 and 25% of ingested nitrogen, one part comes from unabsorbed dietary nitrogen and the other part from protein (Suleiman *et al*., 2019).

The digestibility-corrected aminoacids (PDCAAS) calculation method assesses the nutritional quality of protein sources. The PDCAAS of a protein is determined by the calculation of aminoacids (between 0 and 1), multiplied by the digestibility value of the protein (0.8) (Boye *et al*., 2009; Avilés-Gaxiola *et al*., 2017).

Lentils are an alternative in the search for sustainable proteins due to their protein content, low production cost, and sustainable cultivation. Lentil flour has the potential to develop high-protein foods, so it is important to evaluate its quality and the effect of cooking on its PF. Therefore, the objective was to analyze the protein quality of green and red lentil flours to determine their potential as an alternative source of protein.

Materials and methods

Study material

Lentil (*Lens culinaris* M.) seeds of green and red varieties. The green lentils of the Verde Valle® brand and the red lentils of the Lima Limón® brand were acquired in a local market in Morelia, Michoacán, Mexico. They were transferred to the MC Víctor Manuel Rodríguez Alcocer Biotechnology Laboratory of the Faculty of Chemistry-Pharmacobiology of the UMSNH.

Raw lentil flour and cooked lentil flour

The raw lentil seed (125 g) was dry-crushed in a Golden Wall® electric mill to a particle size of 0.25 mm, and the flour was vacuum sealed for analysis. Another batch of lentils (125 g) was traditionally cooked in 400 ml of water at 94 °C: 15 min (green lentil) and 7 min (red lentil). The cooked seeds were dehydrated in a Hamilton Beach® dehydrator (12 h at 50 °C). Finally, they were crushed in the electric mill and vacuum sealed for analysis.

Proximate chemical analysis

Of the chemical composition of green and red lentils, the following was determined: moisture, ash, crude protein, oil, total fiber and carbohydrates (AOAC, 2005). Nitrogen was determined by the Kjeldahl method. The protein content was calculated as nitrogen, using the factor 6.25 to convert nitrogen to crude protein. The oil was obtained by the Soxhlet method by hexane extraction for 4 h.

The ashes were calculated from the remaining weight after the incineration of the sample at 550 °C for 2 h. Moisture was determined based on the weight loss of the sample after drying at 110 °C for 4 h. Carbohydrates were obtained by difference of the total from what was analyzed.

Determination of protein fractions (PFs) of lentil flour

Osborne’s (1924) method was used. The solvents for the extraction of the PFs were: distilled water, 5% sodium chloride. One gram of lentil flour (raw and cooked) was placed in a test tube with 14 ml of solvent, with a dissolution time of 12 h. It was then centrifuged at 4 000 rpm for 20 min in a Dauz®

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electric centrifuge, and the supernatant was adjusted to pH 4.5 with 1N HCL and centrifuged at 4 000 rpm for 10 min. The precipitated PFs were washed with distilled water and dried at 40 °C for 12 h. Three trials were performed to calculate the percentage of protein in each fraction: % protein = (protein extracted in the fraction (g)) / (amount of flour (g)) x 100.

Determination of the protein quality of lentil flour of green and red varieties

The protein quality of lentil flour was determined by calculating the digestibility-corrected aninoacids score (PDCAAS). Tables of chemical composition of foods were consulted on the amount of protein in lentils, the content of essential aminoacids, and the protein digestibility of the seed. The aminoacids standard for children and adults specified in the WHO/FAO/UNU (2007) was used as the standard protein.

The protein score was calculated using the equation:

$$\text{Score}=\frac{\text{mg amino acids in the study protein}}{\text{mg amino acids in the standard protein}} \text{x } 100$$

Finally, based on the digestibility for lentil flour reported in the literature, the PDCAAS was calculated using the equation:

$$\text{PDCAAS}=\frac{\text{SCORE x protein digestibility}}{100}$$

Statistical analysis

The statistical analysis of the data was performed with the JMP statistical package, version 11 (SAS institute, 2011). An Anova and a *pos-hoc* Tukey mean comparison test were performed, with a significance level (α= 0.05) to establish the differences between the means.

Results and discussion

Proximate chemical composition of green and red lentils

In the analysis of the chemical parameters of green and red lentils (Table 1), it was observed that green lentils presented, with statistically significant differences ($p\leq$ 0.05), the highest moisture content (8.16%) compared to the red variety (7.3%).

Table 1. Proximate chemical composition of lentils (*Lens culinaris* M.), green and red varieties (%).

Sample (%)	Lentil variety	
	Green	Red
Moisture	8.16 ±0.27[a]	7.3 ±0.15[b]
*Protein	27.07 ±0.14[a]	25.83 ±0.02[b]
Oil	1.56 ±0.39[a]	1.03 ±0.16[a]
Ashes	2.72 ±0.03[b]	2.92 ±0.03[a]
Total dietary fiber	26.93 ±0.15[a]	23.2 ±0.02[a]
Total carbohydrates	33.56 ±0.33[b]	39.72 ±0.28[a]

The values are means on a dry basis ± the standard deviation (n= 3). *N x 6.25, total carbohydrate by difference. Means within each row with equal letters do not show significant differences ($p\leq$ 0.05)

A moisture of more than 15% in legumes favors the generation of fungi, contamination, and decomposition, affecting the structure of the seed. It can induce germination due to the activation of enzymes, compromising the integrity of the seed and its nutritional quality (NOM-247-SSA1-2008).

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The green lentils had a significantly higher concentration ($p \leq 0.05$) of oil (1.56%) than the red lentils (1.03%), with a difference of 0.53%. Wyss *et al*. (2020) found that different varieties of lentils have between 1-3% oil, except for soybeans and peanuts (23.31% and 50.46%, respectively).

Legumes contain between 2 and 5% ash (potassium, magnesium, iron and manganese) (Bessada *et al*., 2019). These, like other components, vary according to the crop, environment, and genotype (Chandler *et al*., 2022). The green lentils have 2.72% ash and the red lentils (2.92%) (Table 1). Values similar (2.83%) to those reported by Gallegos-Tintoré *et al*. (2004).

Red lentils have more carbohydrates (39.72%) than green lentils (33.56%). They include starch, oligosaccharides and fiber (Kaale *et al*., 2022); these generate a prolonged feeling of satiety, their slow digestion and low glycemic index are associated with a reduction in obesity and cardiovascular diseases (Ros-Berruzco *et al*., 2021).

The protein content in the green variety was 27.07%, higher ($p \leq 0.05$) than the red variety (25.83%). Suleiman *et al*. (2019) found 20-28% in green and red lentils. Reyes-Bautista *et al*. (2023); Khazaei *et al*. (2019) reported lower values (22.4% and 25.16%). In comparison, chickpeas (*Cicer arietinum*) contain (23.56%-21.34%), black beans (*Phaseolus vulgaris* L.) (18.50%), broad beans (*Vicia faba*) (27.17%), and peas (*Pisum sativum*) (22.64%) (Chandler *et al*., 2022).

Protein fractions (PFs) in lentil flour of green and red varieties

The major PFs in green and red lentil flours, raw and cooked, are shown in Table 2. The major PFs for both varieties were globulins (19.19%-18.35%) and albumins (17.1%-16.76%), significantly higher in the green variety. These results are comparable to those by Reyes-Bautista *et al*. (2023) in lentil flour, who reported: albumins (46.41%) and globulins (26.5%).

Table 2. Concentration of means of the total protein fractions in raw and traditionally cooked lentil flour of green and red varieties.

Protein fraction	Protein extracted (%)	
	Green lentils	Red lentils
Globulins	12.72 ±0.04^{aA}	18.35 ±0.02^{aB}
Albumins	18.35 ±0.05^{bA}	12.95 ±0.03^{aB}
Glutelins	4.32 ±0.02^{cA}	5.25 ±0.05^{bA}
Prolamins	2.177 ±0.01^{cA}	1.177 ±0.01^{cA}

The values are means on a dry basis ± the standard deviation (n= 3) of the protein fractions extracted (%). Different lowercase letters indicate that means within the same variety of lentils are significantly different from each other. Different capital letters indicate that the means between the green and red lentil varieties are also significantly different ($p \leq 0.05$).

The PFs of albumins and globulins in raw and cooked green lentil flour (Figure 1), raw lentil flour presented the highest percentage (albumins 18.35-12.95% and globulins 12.72-18.35%); however, they are not statistically different. When lentils are cooked, albumins (9.83-9.33%) and globulins (10.58-10.33%) decrease.

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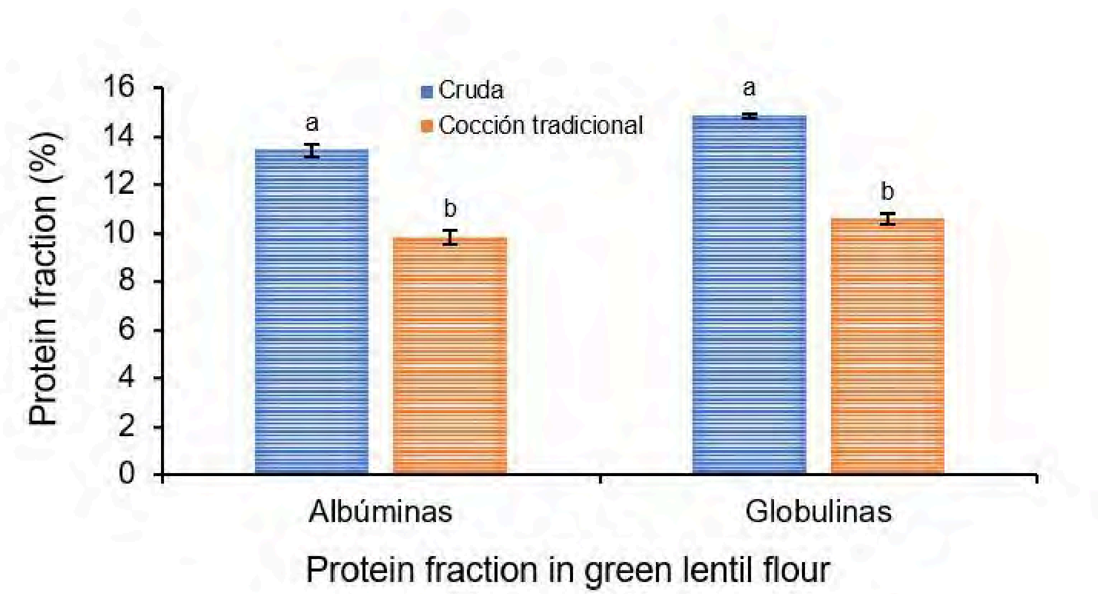
Figure 1. Albumin and globulin fractions for raw and cooked green lentil flour. Each value represents the mean ± standard deviation of three replications of the same sample. Values with different letters are significantly different with a *p*≤ 0.05.



Temperature can denature proteins and break peptide bonds, altering their structure. Cooking causes Maillard reactions, where the amino groups of proteins interact with sugars, decreasing the availability of essential aminoacids. In addition, changes in pH during cooking can modify the isoelectric point, affecting protein solubility and functionality (Khazaei *et al*., 2019). Regarding albumin PF, its percentage in raw flour (14.48%) is significantly higher than in cooked flour (9.33%). In globulins, it decreased from 15.57% to 10.33% in cooked flour.

Albumins, which are soluble in water, undergo denaturation during cooking, which changes their native structure as the seeds hydrate (Lam *et al*., 2016). At high temperatures, they form complexes with other proteins, hydrolyzing enzymatically (Arango *et al*., 2012).

In raw and cooked red lentil flour, the PFs of albumins and globulins decrease with cooking (Figure 2). The albumin content is higher in raw lentil flour (13.44%) than in cooked lentil flour (9.83%). Similarly, globulins are more abundant in raw flour (14.87%), decreasing significantly in cooking.

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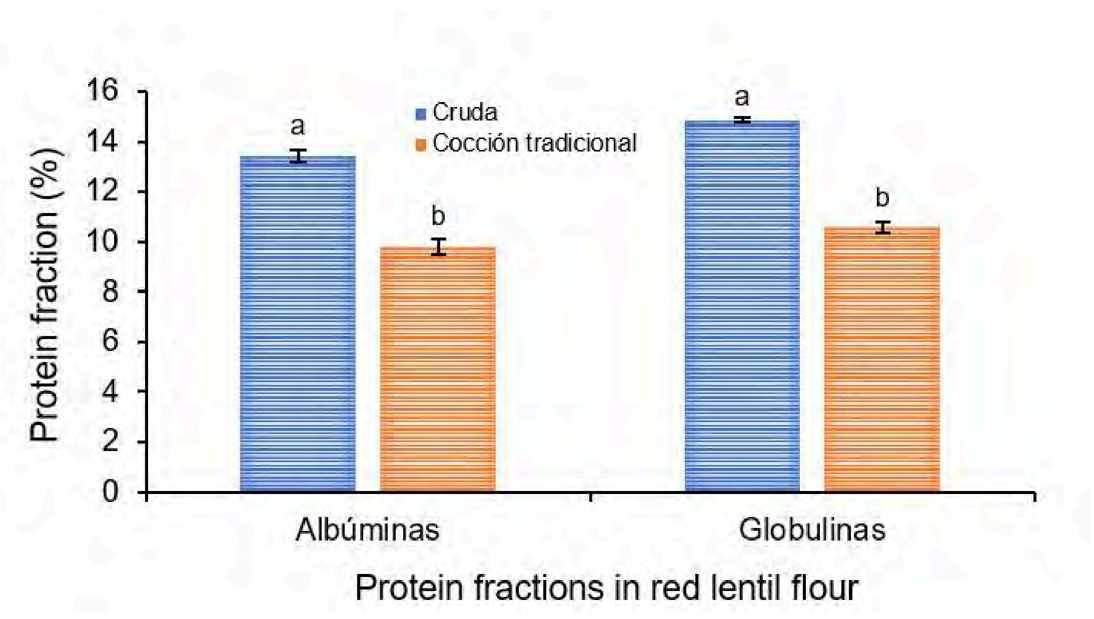
Figure 2. Albumin and globulin fractions for raw and cooked red lentil flour. Each value represents the mean ± standard deviation of three replications of the same sample. Values with different letters are significantly different with a *p*≤ 0.05.



Cooking reduced albumins and globulins by 7.9% in green lentil flour and by 10% in red lentil flour. However, it is necessary for direct consumption or if raw flour will be used for other foods since heat treatment removes anti-nutritional compounds: protease inhibitors, lectins, tannins, and saponins (Prada *et al*., 2024).

It should be noted that albumins comprise enzymes, protease and amylase inhibitors, and lectins. Globulins are classified into legumin (11S) and vicilin (7S), a third protein is convicilin. Convicilin has sulfur aminoacids, absent in vicilin (Lam *et al*., 2016).

Determination of the protein quality of lentil flour. Protein score and PDCAAS

The score determines the percentage of limiting aminoacids in a food matrix, it is the one with the lowest proportion with respect to the same aminoacids in the reference protein (Fernández-Orozco *et al*., 2003). Table 3 summarizes the calculation of the protein score and the protein digestibility-corrected aminoacids calculation, including limiting aminoacids

Table 3. Chemical score and digestibility-corrected amino acid score in green and red lentil flour.

Food	Weight (g)	Protein (g)	N^{bc}	Content of essential amino acids in the protein of foods (mg g^{-1} of N)^{d}			
				Lysine	Methionine + cysteine	Threonine	Tryptophan
Red lentils	30	7.85	1.25	608.75	139	360	80.62
Mg g^{-1} N				487	103.73	288	64.49
Essential amino acid reference standard				320	111.2	170	43

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Food	Weight (g)	Protein (g)	N[bc]	Content of essential amino acids in the protein of foods (mg g^{-1} of N)[d]			
				Lysine	Methionine + cysteine	Threonine	Tryptophan
Amino acid calculation mg g^{-1} of N				1.52	0.93	1.69	1.49
PDCAAS				1.24	0.76	1.38	1.22
Green lentils	30	8.42	1.34	653.25	139	387.12	86.43
Mg g^{-1} of N				487.5	103.73	288	64.5
Essential amino acid reference standard				320	156	170	43
Amino acid calculation mg g^{-1} of N				1.5	0.66	1.69	1.5
PDCAAS				1.2	0.52	1.35	1.2

a= corresponds to the amount of protein in 30 g of lentils; bN= nitrogen; c= g equivalents of nitrogen, it is obtained by dividing the protein content of lentils by 6.25; d= values obtained by multiplying the amount of nitrogen in lentils by the amount of lysine, methionine + cysteine, threonine and tryptophan; e= represents the content of each amino acid in one gram of nitrogen in the sample.

The green lentils had a lower score in methionine and cysteine (0.66%, 0.93%) and PDCAAS (0.52 and 0.76) compared to the red lentils, these being the limiting aminoacids. The red variety presented better values in lysine, threonine, and tryptophan (1.5, 1.69, 1.5) and PDCAAS (1.2, 1.35, 1.2). The maximum PDCAAS for essential aminoacids is 1 (milk, egg, and soybeans) (Ros-Berruzco *et al*., 2021). Even with limiting aminoacids, lentil flour is rich in protein and essential aminoacids, being suitable for protein and sustainable diets.

Conclusions

Lentil flours are nutritious options to be reintroduced into the diet. Both varieties are alternatives to animal protein. Although raw flour contains more albumins and globulins that decrease with cooking, this can improve their digestibility by reducing anti-nutritional factors. Red lentil flour presented higher protein quality, with a better aminoacids profile and digestibility.

Technologies such as extrusion or fermentation can improve physicochemical and sensory properties. Microencapsulation is proposed for the stability of the bioactive compounds of green lentil flour and to study their effect on the reduction of cholesterol and blood pressure.

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