

## Prolonged storage affects the nutritional quality and cooking time of ayocote beans

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### Abstract

The consumption of ayocote beans is recommended because of their high protein content, but rural producers prefer to consume common beans and store ayocote seeds for occasional consumption or until their demand in the market makes their commercialization more profitable. The effect of the storage period on the cooking time and nutritional quality of ayocote bean seeds was studied. During 2021, the content of carbohydrates, ash, fiber, fat, and protein was determined, as well as the cooking times of ayocote seeds of a variety with black grain produced in different years (2017, 2018, 2019, and 2020) and localities. One variety of ayocote beans with white seed and one of common black beans (CPV-F52) produced in 2020 were used as controls. Data were analyzed under a completely randomized design with three repetitions; the comparison of means was made with Tukey's test ( $p < 0.05$ ). The ayocote seeds of 2017 and 2018 presented similar percentages of protein (22.49% and 22.77%) and cooking times (253 and 226 min), while those produced in 2020 had 25.43% and 108 min, respectively; the CPV-F52 variety presented a protein content of 27.61% and a cooking time of 105 min. The protein content of ayocote bean seeds decreases as the storage period increases, while the cooking time increases proportionally to the duration of storage.

### Keywords:

*Phaseolus coccineus*, protein content, seed cooking.

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Common beans (*Phaseolus vulgaris* L.) are a legume grown in different parts of the world, in very diverse regions, environments, and climates. In Mexico, they are one of the most important crops since they are part of the daily source of proteins, calories, B vitamins, and minerals in the diet of Mexicans; in addition, they are a source of economic income for producers who grow this species (Cid *et al.*, 2014).

Their protein content is 14-33%, and they are characterized because they are deficient in sulfur amino acids and tryptophan but contain large amounts of lysine. They have 1.5-6.2% lipids with different fatty acids, especially monounsaturated and polyunsaturated, such as oleic, linoleic, and linolenic (Bolaños-Silvestre, 2011). Ayocote beans (*Phaseolus coccineus* L.), also known as patol, frijolón, pak and cimarrón, are used as human food in an immature and dry state. They are a species adapted to varied environments, show high genetic diversity due to their high percentage of natural crossing (14.7%), and therefore require an agronomic management different from that of common beans (Muruaga *et al.*, 1992), which hinders the purity of native varieties.

The species is a productive option in different regions of Mexico. This type of bean is a source of nutrients such as protein, dietary fiber, and carbohydrates for its starch content. However, like common bean seeds, ayocote bean seeds may exhibit antinutritional factors, including enzyme inhibitors, hemagglutinins, flatulence factors, tannins, phenols, and phytic acid.

In common bean seeds, cooking completely removes hemagglutinins, but the trypsin they contain is more resistant to heat (Antunes and Sgarbieri, 1980). Proximate chemical analyses of the ayocote beans show that the concentration of protein in their seeds is higher in the black ones (23.8%), which showed the highest concentration compared to the purple beans (21.93%) (Teniente-Martínez *et al.*, 2016).

Prolonged storage, for months or years, or short periods under inadequate conditions causes the deterioration of the quality of the grain, decreasing its commercial value (Jacinto-Hernández, 2017). The main change observed is the darkening of the testa and the decrease in the culinary quality of the grain (Martín *et al.*, 1997). In this sense, the quality of the bean seed is not only determined by the physical appearance, uniform size, similar color, and without damage or defects but also by a timely inspection of its production and field harvest, appropriate processing, and storage (Cid *et al.*, 2014).

Additionally, the quality of the bean grain is determined by the variety, agronomic management, crop conditions, and later storage of the grain. The main factors that influence the deterioration of grain in storage are the high moisture content in the stored grain, the high temperature of the environment or warehouse, and the presence of fungi, bacteria, and rodents (Cid *et al.*, 2014).

During storage, the seed can absorb moisture from the environment or release moisture into it until it reaches an equilibrium with the relative humidity of the environment; if the relative humidity is high, the seed will deteriorate more quickly, so the high moisture content in the seed and the environment causes deterioration, as the rate of respiration, loss of vigor and germination of the seed increases (FAO, 2012).

Storage under conditions that damage the seed, such as high temperature and relative humidity, causes the phenomenon of hardness for cooking domesticated beans but not for wild beans (Peña-Valdivia *et al.*, 1999). The most frequently observed post-harvest changes are the darkening, also called 'oxidation', of the grains of white testa and hardening, which in turn causes an increase in their cooking time.

Both oxidation and hardening are mainly caused by prolonged storage time or inadequate storage conditions (Liu, 1995), although hardness can also be of genetic origin. Bean cooking time, a characteristic that determines consumer preference, is considered important in genetic improvement programs, especially in Mexico, where 96% of the beans produced are consumed through homemade preparations (Jacinto-Hernández, 2002).

Based on the above, this research has as its main purpose to generate information about the effect of the storage period on the cooking time of the seeds and their nutritional quality. This

research was carried out in the Laboratory of Physicochemical, Biochemical, and Biological Analysis of the Puebla Campus of the College of Postgraduates.

Ayocote seeds of a black variety identified as Population 89 were studied, which were harvested in the following years and localities: 2017 in San Buenaventura Tecalzingo, Municipality of San Martín Texmelucan, 2018 in Calpan, Municipality of San Andrés Calpan, 2019 in Moyotzingo, Municipality of San Martín Texmelucan and 2020 in Santa María Zacatepec, Municipality of Juan C. Bonilla. The seed of Population 84, which is an ayocote with white grain, produced in 2020 in the municipality of Huejotzingo, Puebla, and the registered variety of common black beans CPV-F52, obtained in the same year in San Andrés Calpan, were included as controls.

A completely randomized experimental design was used, with three repetitions of each variable evaluated. The data obtained were subjected to an analysis of variance and mean test (Tukey  $p < 0.05$ ). Statistical analyses were performed using the GLM procedure of the Statistical Analysis System (SAS) statistical package (SAS, 2020).

Fifty seeds of each of the varieties used in the study were taken, placed in the industrial mill (Micro-Mill Grinder), and ground for 4 min until a fine meal was obtained. The meal obtained from the milling was used for the different bromatological analyses, where the nutritional content (protein, fat, carbohydrates, fiber, ash) was determined by proximate analyses. To obtain the amount of crude protein, the Kjeldahl method of the AOAC (1990) manual of official chemical techniques was used.

To quantify the crude fiber content, the digestion apparatus (Ankom 200/220 fiber analyzer) and Ankom F57 filter bags were used. To calculate the fat content, the Soxhlet method was followed through an ethereal extract; using the Soxhlet equipment and hexane as solvent. The total carbohydrate (TC) content was determined by calculating the difference between the original weight of the sample and the sum of the weight of water, fat, protein, crude fiber, and ash. To calculate the percentage of total carbohydrates, the following formula was used: % TC = 100 - (% moisture + % ash + % protein + % fat + % crude fiber). To determine the ash content, the method based on the incineration of the sample was used (Nollet, 1996).

To specify the cooking times of the seeds, the soaking method was used, which consists of the seeds to be analyzed being deposited in a container to which a volume of 100 ml of drinking water at room temperature was added for a period of 11 h. After soaking, the 25 bean seeds per repetition of each variety were cooked in CINSA aluminum Teflon-coated pots with a glass lid and pressure exhaust valve of 16 cm in diameter and a capacity of 1.4 L, for which a Mabe gas stove with four grills was used. The cooking time of the 75 seeds was determined by a sensory test between the thumb and index finger to perceive the softness of the seed (NMXF-090-S-1978).

Table 1 shows the mean squares of the chemical variables of the ayocote bean and common bean populations. There were statistical differences at  $p \leq 0.001$  and  $p \leq 0.05$ . The protein content presented a statistically significant difference, which indicates that one of the treatments is different from the others in the percentage of protein, which means that the type of seed that presents the highest protein content will have a higher nutritional quality. The same happened for the variables of moisture, fat, and carbohydrates; in the case of ash and fiber contents, no statistically significant differences were observed.



**Table 1. Mean squares of chemical variables in samples of ayocote and common beans (CPV-F52).**

Source of variation	df	Moi (%)	Prot (%)	Ash (%)	Fat (%)	Carb (%)	Fiber (%)	CT (min)
Origin	5	42.39 <sup>***</sup>	32.99 <sup>**</sup>	5.99 ns	1.78 <sup>*</sup>	197.29 <sup>***</sup>	2.67 ns	10777.7 <sup>***</sup>
Error	12	3.94	5.44	6.6	0.57	9.16	1.2	219.5
CV		19.62	10.14	44.91	23.16	6.33	15.75	9.57
Mean		10.11	22.99	5.72	3.25	47.81	6.97	154.83

df= degrees of freedom; Moi= moisture; Prot= protein; Carb= carbohydrates; CT= cooking times; CV= coefficient of variation; ns= non-significant difference, \*, \*\* and \*\*\*= statistical significance at  $p \leq 0.05$ ,  $p \leq 0.01$  and  $p \leq 0.001$ , respectively.

Regarding the results of cooking times, there was a statistically significant difference ( $p \leq 0.001$ ) for varieties with different storage times because the period in which the seeds remain in storage affects them negatively, causing a deterioration of the grain, which generates a longer cooking time compared to new seeds.

Table 2 presents the proximate chemical analysis for protein and fiber contents in meals of the different varieties of ayocote beans and the common bean variety. It is observed that the concentration of protein varies according to their storage time, with the common black beans (CPV-F52) (27.61%) being the ones that showed the highest concentration compared to the beans of the POB-89, black, and of origin 2017 (22.49%).

**Table 2. Chemical composition of meals of ayocote and common beans.**

Variety	Year of harvest	Protein (%)	Carb (%)	Fat (%)	Fiber (%)
CPV-F52	2020	27.61 a	44.09 c	3.03 ab	5.67 a
POB-89	2020	25.43 a	49.77 abc	4.64 a	6.41 a
POB-89	2018	22.77 ab	53.52 ab	3.11 ab	7.06 a
POB-89	2017	22.49 ab	48.09b c	2.87 ab	7.34 a
POB-89	2019	21.72 ab	57.19 a	3.47 ab	6.86 a
POB-84	2020	17.91 b	34.08 d	2.37 b	8.48 a
LSD		6.39	8.29	2.06	3.01

Carb= carbohydrates. Values in the same column with the same letter are not statistically different (Tukey,  $p \leq 0.05$ ).

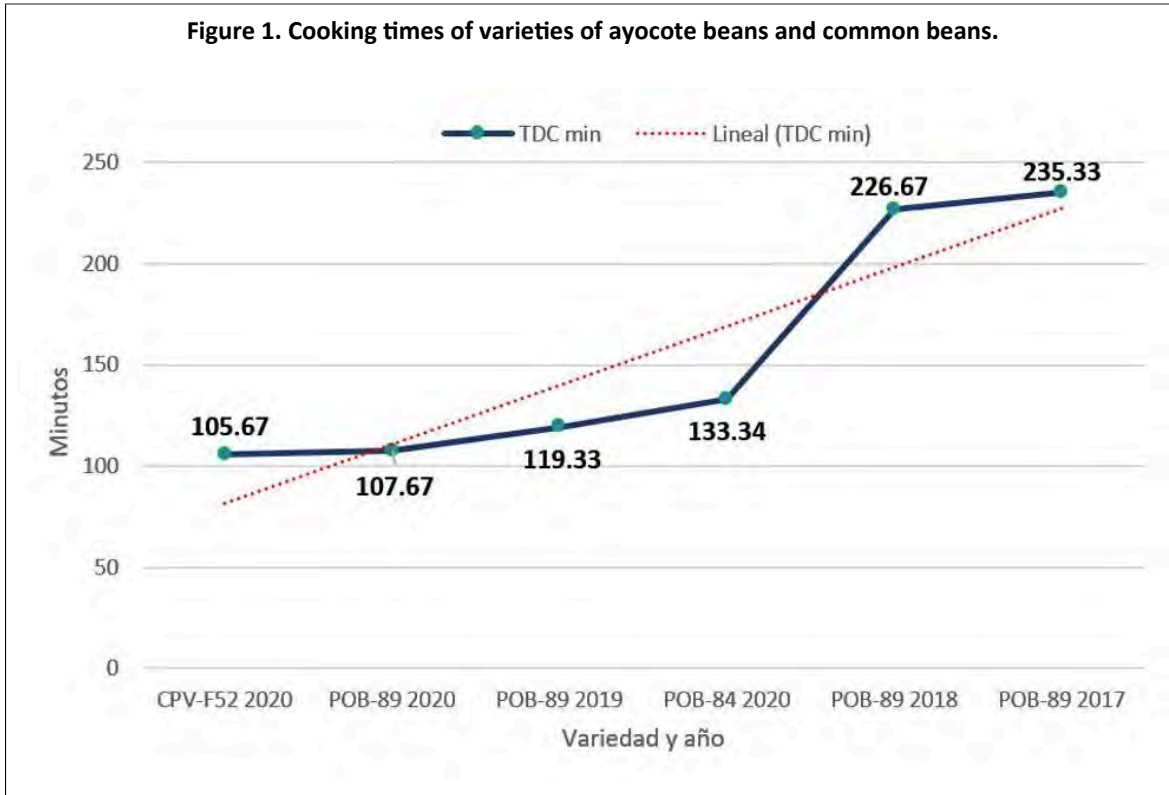
The results obtained through the analysis for common black beans were similar to those reported by Bolaños-Silvestre (2011) for saltillo pinto beans, while the results obtained for ayocote beans were lower compared to the black ayocote (23.8%) reported by Teniente-Martínez *et al.* (2016), except for the case of black POB-89 with origin in 2020. This shows that seeds with a shorter storage period will have better nutritional quality, and consequently, seeds with a prolonged storage period will be of lower nutritional value, mainly in protein content.

In terms of fiber content, the results show that the varieties of the black POB-89 are similar to each other, compared to the seeds of the white POB-84 control, which showed a value numerically higher than the other seeds, while the control CPV-F52 presented a lower value; even so, its fiber content is similar to that of ayocote seeds. These results are similar to those obtained by Bolaños-Silvestre (2011) for saltillo pinto beans.

Regarding the carbohydrate content, no effect of the storage period on this variable was detected in the ayocote bean seeds. In general, it was observed that black ayocote seeds, regardless of their age, had higher contents than the common bean or white ayocote bean seeds. Something

similar has been reported in the cooking of broad bean seeds, as protein contents decreased in boiled broad beans, but carbohydrate contents increased (Maya-Ocaña, 2009).

The same trend was observed for fat content, but in this case, the seeds of Population 89 with a shorter storage period presented the highest values for this variable. Figure 1 shows the cooking times of the seeds of ayocote beans and common beans. A great variation was observed, which had a proportional relationship between the storage time of the grain and the time required for cooking it; that is, in general, the longer the storage time, the longer the seeds will take to cook, and vice versa.



The length of the storage period is directly related to the cooking time of the seeds. Prolonged storage, for months or years, under inadequate conditions causes deterioration of grain quality (Jacinto-Hernández, 2017). Storage under conditions that damage the seed, such as high temperature and humidity, causes the phenomenon of hardness for cooking domesticated beans but not for wild beans (Peña-Valdivia *et al.*, 1999).

In similar works, it is reported that the cooking time of common bean grains is in the range of 92 to 154 min, while for ayocote beans, this can be up to 317 minutes (Martínez-López, 2018); this was evident for the seed of the common beans CPV-F52 and the seed of the population 89 origin 2020, since these seeds had a shorter cooking time. Ayocote seeds with origins in 2017 and 2018 had a longer cooking time, with 235.33 and 226.67 min, respectively; these cooking times were longer than those reported by Muñoz-Velázquez *et al.* (2009) in beans native to the state of Hidalgo.

Cooking gelatinizes the starch, alters the texture, and improves the flavor of the legumes. Moderate heat increases protein availability in most legumes and weakens the intercellular binding material so that pressure from a fork or teeth causes intact starch-filled cells to separate (Charley, 1999). Seeds with a shorter storage period have better ease of water absorption (Peña-Valdivia *et al.*, 1999); this is evident in the case of the seeds of the POB-89 of origins 2017 and 2018, which presented a longer cooking time because they are seeds with a longer storage period, which causes the hardening of the seed and decreases the quality of the grain.

This implies a longer time for the preparation of foods, and that these are of lower nutritional value, there is the disadvantage of incurring an additional expense for the consumption of fuel necessary for the cooking of the seeds, as has been demonstrated with different varieties of corn (Roque-Maciél *et al.*, 2016). Two factors are known to cause slow or poor cooking: the 'hard testa', which describes a physical state in which the seeds are unable to soak enough water due to the partial impermeability of the testa, and the 'hardness to cooking', which refers to the texture of the cotyledon, which induces longer cooking time (Liu, 1995; Reyes-Moreno and Paredes-López, 1993).

Other characteristics intrinsic to the seed, such as the thickness, composition, and microstructure of the testa, can affect the hardness of the seed; in addition, changes during postharvest, such as lipid oxidation, formation of insoluble pectates and modifications of cell wall components, can also irreversibly alter seeds (Morales-Santos *et al.*, 2017). Softness for cooking and protein content are important characteristics for bean improvement. The user desires a fast-hydrating, low-cooking time bean that produces a broth with a good appearance, flavor, and texture (Jacinto-Hernandez, 2002).

## Conclusions

Prolonged storage of seeds decreases their nutritional quality, particularly the protein content, which is the compound with the highest nutritional value. It also proportionally affects cooking times since seeds stored for three or four years took longer to cook because the seeds harden and have a lower ease of water absorption. It is recommended not to store the seeds of ayocote beans for more than two years since this causes their aging, which not only affects cooking times, generating an additional cost and greater energy consumption, but also decreases their nutritional quality.

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Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 April 2023
Date accepted: 01 June 2023
Publication date: 29 September 2023
Publication date: August 2023
Volume: 14
Issue: 29 Suppl Especial
Electronic Location Identifier: e3542
DOI: 10.29312/remexca.v14i29.3542

### Categories

Subject: Investigation note

### Keywords:

**Keywords:**

Phaseolus coccineus  
protein content  
seed cooking

### Counts

Figures: 1  
Tables: 2  
Equations: 0  
References: 22  
Pages: 0