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Resistance to degradation of reserve proteins in beans after hydration and cooking

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ABSTRACT: Hydration and cooking of beans leads to significant losses in their nutritional quality, but some genotypes may have a greater resistance to protein degradation. Therefore, this study aimed to evaluate the effect of the genotype on both quality and quantity of proteins available in the beans before and after hydration and cooking. The grains of six landrace genotypes from the Active Bean Germplasm Bank of the UDESC (BAFs: 03, 07, 57, 75, 97 and 102) were used "in natura" (control) and subjected to the following treatments: hydration for 8 hours and hydration followed by cooking. The content and electrophoretic profile of the soluble proteins were evaluated. Initially, the protein content ranged from 39.4 (BAF 57) to 51.1 mg g^{-1} FW (BAF 75). Hydration provoked a decrease in the protein content for all genotypes, having it been accentuated with the cooking. BAFs 07 and 75 presented a higher protein content after cooking, 29.1 and 31.4 mg g^{-1} FW, respectively, and concomitantly, higher intensity and presence of protein bands. In conclusion, BAFs 07 and 75 demonstrate a higher resistance to protein degradation and their use may be recommended in crosses aimed at improving the nutritional and technological grain quality.

Key words: *Phaseolus vulgaris* L.; protein profile; SDS-PAGE

Resistência à degradação de proteínas de reserva em grãos de feijão após hidratação e cocção

RESUMO: A hidratação e o cozimento dos grãos de feijão conduzem a perdas significativas na qualidade nutricional, mas alguns genótipos podem apresentar maior resistência à degradação de proteínas. Diante disso, o objetivo deste trabalho foi avaliar o efeito do genótipo na qualidade e quantidade de proteínas disponíveis antes e após a hidratação e cocção dos grãos. Os grãos de seis genótipos crioulos obtidos do Banco Ativo de Germoplasma de Feijão da UDESC (BAFs: 03, 07, 57, 75, 97 e 102) foram utilizados "in natura" (controle) e submetidos aos tratamentos: hidratação por 8 horas e hidratação seguida de cocção. Avaliou-se o teor e o perfil eletroforético de proteínas solúveis. Inicialmente, o teor proteico variou de 39,4 (BAF 57) a 51,1 mg g^{-1} MF (BAF 75). A hidratação provocou redução no teor proteico para todos os genótipos, mas esta foi acentuada com a cocção. Os BAFs 07 e 75 apresentaram maior teor proteico após a cocção, de 29,1 e 31,4 mg g^{-1} MF, respectivamente, e concomitantemente, maiores intensidade e presença de bandas proteicas. Conclui-se que os BAFs 07 e 75 possuem maior resistência à degradação de proteínas e podem ser recomendados para a utilização em cruzamentos direcionados à melhoria da qualidade nutricional e tecnológica de grãos.

Palavras-chave: *Phaseolus vulgaris* L.; perfil proteico; SDS-PAGE

Introduction

The consumption of beans (*Phaseolus vulgaris* L.) stands as a basic protein source for a large portion of the Brazilians, which is why it has a socioeconomic importance in all regions of the country. The consumption of this legume represents one of the pillars of the Brazilian diet, considered as fundamental for food and nutritional security, especially for the poor (Barbosa & Gonzaga, 2012). Brazil remains one of the largest consumers of beans in the world; however, data from CONAB (2020) indicate a drop of roughly 400 thousand tons between the 2009/10 and 2019/20 crops, with this reduction attributed to the high prices practiced in the market due to the reduction of the planted area and the occurrence of adverse climatic conditions. When evaluating only the household consumption, data from the IBGE (2020) indicate a 52% reduction in the acquired grains between the 2002/03 and 2017/18 crops.

In spite of the changes in the consumer market, Brazil excels as a bean producer. Adding to the three crops of 2018, the country produced a total of 3,374,079 tons of beans, about 82,767 tons more than the production in 2017 (IBGE, 2018). It is cultivated throughout the Brazilian territory, under different edaphoclimatic conditions, technological and management levels (Tavares et al., 2017; Pedó et al., 2018). However, in the South region, the cultivated area and production in tons have been considerably decreasing.

In the Santa Catarina state, bean cultivation is usually by small producers, accounting for 4.3% of the total produced in the country in 2018 (IBGE, 2018). The state average yield reached up to 1,979 kg ha^{-1} in the 2017/18 crop (CONAB, 2018), but only 76 seed production fields were registered in that crop with the Ministry of Agriculture, Livestock and Food Supply – MAPA, totaling 2,246 ha (Brasil, 2018). Santa Catarina has a wide diversity of bean genotypes (Pereira et al., 2009), including the landrace genotypes with breeding potential when aiming at high yield, resistance to diseases and pests, genetic and physiological quality for producing grains and high nutritional content. Resurging into use these genotypes can favor the breeding programs by incorporating traits of interest in commercial cultivars (Coelho et al., 2010b). Furthermore, these materials are important sources of food and income for the family farming in the state (Pereira et al., 2011), an activity that favors conserving the genetic resources of landrace beans through the sustainable use.

Studies aiming the characterization of landrace bean genotypes from the Bean Active Germplasm Bank (BAF), part of the Santa Catarina State University – UDESC, have been conducted for over a decade (Coelho et al., 2007a, 2010b, 2010c; Pereira et al., 2009, 2011; Michels et al., 2014; Ehrhardt-Brocardo & Coelho, 2016; Gindri et al., 2017). Some of these demonstrated the potential of the landrace genotypes in obtaining a higher protein content when compared to commercial genotypes (Pereira et al., 2011), as well as a shorter cooking time (Coelho et al., 2007b). This aspect is related to the water penetration capacity in the grains due to the impermeability of the integument (Morais et al., 2010) or cotyledons, resulted from chemical modifications occurring during storage (Castellanos et al., 1995) that increase the cooking time.

In addition to these characteristics, hydration tests have been performed prior to the cooking for introducing or disposing genotypes, aiming mainly at the genetic improvement (Bordin et al., 2010). Hydration can reduce the cooking time, but when prolonged it can lead to significant losses in the protein content of the grains. However, some genotypes may be more resistant to protein degradation and loss of nutritional quality.

Striving to complement the characterization studies and indicate genotypes for commercialization or for crossbreeding in breeding programs for improving the nutritional and technological quality of grains, this study had as its objective to evaluate different landrace bean genotypes for protein availability, before and after grain hydration and cooking, seeking to recommend them for consumption.

Materials and Methods

The landrace bean genotypes, produced in Santa Catarina and from the UDESC Bean Active Germplasm Bank, comprising BAF 03, BAF 07, BAF 57, BAF 75, BAF 97 and BAF 102, were multiplied by cultivation in a commercial crop located at 27°47'33.5" south latitude and 50°18'23.0" west longitude, in the municipality of Lages, Santa Catarina, Brazil. The submitted samples of at least 1,000 g were homogenized by the mechanical method in a sample divider, by successive divisions and subsequent combination of the parts. After homogenized for at least 3 times, the samples were successively divided in order to obtain the working samples (Brasil, 2009). The grains were previously standardized to 12% humidity in an air-circulation oven. Table 1 illustrates the common name and source of used genotypes.

For protein extraction, untreated or "*in natura*" (control) grains were used and subjected to the following treatments: hydration and hydration followed by cooking.

a. Hydration: grains were immersed in distilled water, in the proportion of 6.25 mL for each 1 g of beans, with temperature controlled at 25 °C, for 8 hours;

b. Hydration followed by cooking: after soaking, the beans were cooked in boiling distilled water in a Mattson-type cooker, which consists of 25 vertical stilettos with a 1-mm diameter tip and a mass of 90 g each, which are supported on

Table 1. Common name and source of landrace bean genotypes (BAFs).

Genotype	Common name	Source
03	Manchinha	Palmitos (SC)
07	Preto Lages	Lages (SC)
57	Preto	Cunha Porã (SC)
75	Serrano	Formigueiro (RS)
97	Charque	Iraí (RS)
102	México 309	Goiânia (GO)

the beans during the cooking. The sample was considered as cooked when the stilettos punctured 13 grains.

The soluble proteins were extracted as according to the methodology of Brown et al. (1981). 100 mg of fresh weight (FW) of the cotyledons were used, macerated with the aid of liquid nitrogen, and 1 mL of the 0.5 mol L^{-1} NaCl solution, pH 2.4 was then added to it. The sample was vortexed for 1 minute and subsequently kept under agitation for 30 minutes. The homogenate was centrifuged for 20 minutes at 10,000 *g*, at room temperature (20 °C). The protein content was determined in a spectrophotometer at 595 nm according to the method of Bradford (1976), using bovine serum albumin (BSA) as standard.

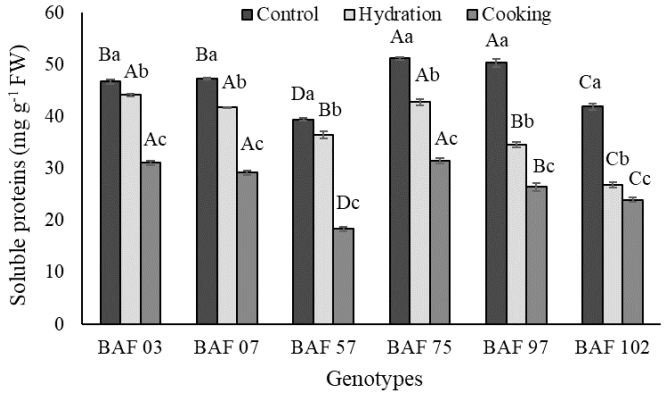
Aliquots of soluble protein (20 µg for cooked samples and 15 µg for the others) were mixed in an equal volume of sample buffer containing 0.5 mol L^{-1} of Tris buffer solution (pH 6.7), 20% glycerol, SDS 2%, 2-mercaptoethanol 5% and bromophenol blue. The protein separation was through a vertical electrophoresis system, by using the SDS-PAGE polyacrylamide gel composed of separation gel at 12% and concentration gel at 3% (Laemmli, 1970), under a constant voltage of 80 V per gel, for approximately 16 hours. The electrophoretic separations of the proteins were performed in triplicate. A standard of molecular weight markers from 6.5 to 97.4 kDa was used. The gels were stained with the coomassie blue at 0.1% (Alfenas, 1998).

The experiment was conducted in a completely randomized design, where the protein extractions were in three replicates. The analysis of variance and Tukey test at 5% probability were performed in order to separate the means. The gels were analyzed qualitatively by observing the presence and intensity of the bands within them, aided by the Gel Analyzer 19.1 program. Additionally, multivariate analyzes of clustering and of principal components (PCA) were performed. The variables were standardized through standardization and the Euclidean distance between the genotypes was calculated with the UPGMA agglomeration algorithm. All analyzes were performed by using the R program (R Core Team, 2018).

Results and Discussion

The initial soluble protein content of the genotypes ranged from 39.4 mg g^{-1} FW (BAF 57) to 51.1 mg g^{-1} FW (BAF 75), but there were no significant differences ($p \leq 0.05$) between the genotypes BAF 75 and BAF 97, which had the highest protein levels (Figure 1). Other authors have found a mean of 87 g kg^{-1} of soluble proteins in seeds of 34 bean genotypes, using similar methods of extraction and quantification (Pereira et al., 2011).

The hydration of the grains caused a reduction in protein content for all genotypes, which was accentuated by cooking them (Figure 1). The highest protein degradation after hydration occurred for the genotype BAF 97 (reduction of 15.8 mg g^{-1} FW in comparison to the control) and for the BAF 57, after cooking (reduction of 18.0 mg g^{-1} FW in comparison to hydrated grains). Comparing the control to the cooked grains,



Uppercase letters compare the genotypes while lowercase letters compare the control, hydration and cooking treatments for each genotype by the Tukey test ($p \leq 0.05$).

Figure 1. Total soluble protein content in grains of bean landrace genotypes. The values stand for the mean (n=10) of three replicates for each treatment and the vertical bars indicate the standard error of the mean (ANOVA).

BAF 97 had the largest reduction in its protein content, which was of 23.9 mg g^{-1} FW, while BAF 03 had the lowest protein degradation, 15.7 mg g^{-1} FW. After the cooking, BAFs 75, 03 and 07 had the highest total protein content, with the values of 31.4, 31.0, and 29.1 mg g^{-1} FW, respectively.

The significant reduction in protein content occurred for all genotypes after hydration, and in a similar fashion, the cooked genotypes had a reduction in protein content in relation to the hydrated grains. Cooking also caused a significant reduction in the content of all essential amino acids in seeds of *P. angularis* and *P. calcaratus* (Chau et al., 1997). In *P. vulgaris*, extrusion cooking reduced the soluble proteins levels and affected the solubility and digestibility of the protein, in addition to reducing the amino acids availability (Alonso et al., 2000).

Differently from the observed regarding the soluble proteins, some authors reported a tendency towards an increase in the crude protein content (insoluble fraction) in *P. vulgaris* grains after cooking (Pujolà et al., 2007; Wang et al., 2010). This occurs due to the loss of soluble solids during cooking, which would increase the concentration of protein in cooked grains (Wang et al., 2010). However, due to the sharp loss of dry weight during the hydration and cooking stages of *P. vulgaris* grains, the recommended is to prepare the grains with no prior hydration, or using the hydration water for cooking, in order to maintain a higher nutritional content (Pujolà et al., 2007).

Protein degradation was also observed through changes in the electrophoretic profile of the grains, characterized by the presence of seven main protein bands with molecular weights between 55 and 17 kDa (Figure 2). Besides having the lowest protein content among the evaluated genotypes (Figure 1), BAF 57 was the only that did not have one of the seven main bands, comprised in the molecular weight of approximately 28 kDa, also having low band intensity of 17 kDa (Figure 2).

One of the most intense bands is in the range of 45 kDa, which has been related to the presence of phaseolins, the most important reserve proteins in grains, which represent from 40

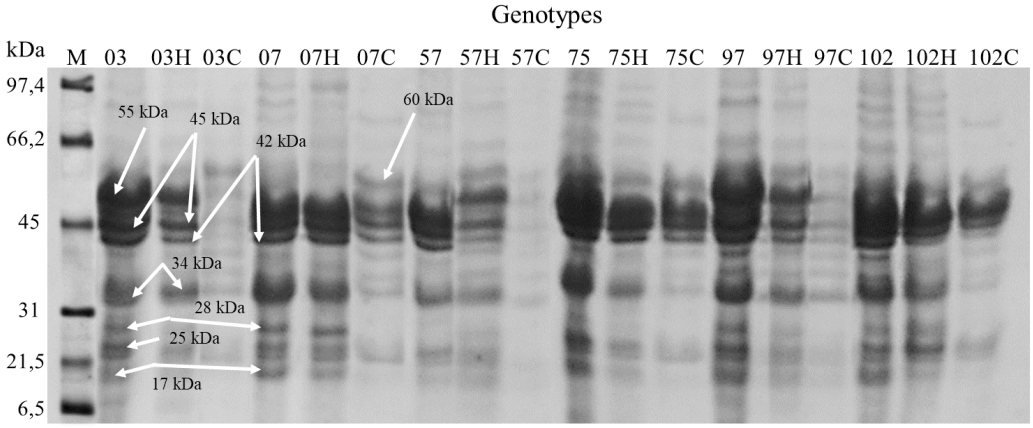


Figure 2. Electrophoretic profile (SDS-PAGE) of soluble proteins in creole bean genotype grains. M – Molecular weight markers; H – After hydration for 8 hours; C – After hydration followed by cooking.

to 50% of the total protein in *P. vulgaris* (Rovalino-Córdova et al., 2019). Phaseolin is considered as one of the main markers in characterizing the diversity and phylogenetic relationship within the species (Jannat et al., 2019). This protein has high resistance to the activity of proteolytic enzymes during hydrolysis and, thus, has limited digestibility, restricting its nutritional value in raw conditions, requiring heat treatment (cooking) for improving its digestibility (Montoya et al., 2008; Rovalino-Córdova et al., 2019).

The reduction in the intensity and presence of protein bands were found in all genotypes after cooking, however, the maintenance of bands with approximately 55 and 34 kDa also took place in all genotypes. This reduction was less sharp in the cooked grains from BAFs 07, 75 and 102. BAF 07 was the one that showed the smallest differences in the number and intensity of bands between the protein profile in grains both untreated and after hydration, having a greater total number of bands after the cooking.

As for the BAF 57 cooked grains, which had the lowest protein content, the lowest band intensity was also observed in them, showing the occurrence of protein denaturation due to the high temperature. This genotype was considered promising for incorporation in breeding programs due to its high yield (Coelho et al., 2007a), although it did not demonstrate any using potential when aiming at protein grains for consumption.

In some genotypes, such as BAF 07, the appearance of a band in the range of 60 kDa after cooking and/or hydration occurred, possibly from the hydrolysis of higher molecular weight proteins that are in the control group. This band has been associated with the presence of Heat Shock Proteins (HSPs), synthesized and accumulated in response to the stress, mostly thermal (Araújo et al., 2003; Wang et al., 2014), as the occuring during cooking. A special group of HSPs, the chaperones, has also been found in this molecular weight range and are involved in maintaining protein homeostasis, recovering damaged proteins, preventing, maintaining or renaturing denatured proteins (Dahiya & Buchner, 2019), important functions in cells that have been subjected to thermal stresses (Borges & Ramos, 2005). Other authors related the expression of bands in the 65, 45, 25 and 18 kDa ranges to responses to other stress types, such as the water stress (Coelho et al., 2010a).

The lectin from *P. vulgaris*, called phytohemagglutinin (PHA), is a protein with molecular weight of approximately 28 kDa (Ehrhardt-brocardo, 2017) and 34 kDa (Sell & Costa, 2000). PHA has a potent agglutinating activity on erythrocytes and stimulates the cell mitosis (Sell & Costa, 2000; Kaltner et al., 2018). When consumed, it can exert anti-nutritional effects and cause toxicological manifestations, having a reduction in its concentration due to denaturation in cooked grains of *P. vulgaris* (Sun et al., 2019). On the other hand, the band of approximately 42 kDa can indicate the presence of the mitochondrial enzyme formate dehydrogenase (FDH), which takes part in important biosynthetic reactions and is considered as an universal stress marker protein, involved in responses to various types of abiotic and biotic stresses (McNeilly et al., 2018; Wang et al., 2018).

The analysis of protein content based on clustering genotypes by the Euclidean distance identified the formation of two distinct groups: the first formed by the untreated (control) and hydrated genotypes, and the second by the genotypes after cooking; with such groups formed by considering the cut-off level at the Euclidean distance equal to 3.0 (Figure 3).

The cophenetic correlation coefficient was relatively high (r = 0.80), demonstrating that the dendrogram representation is coherent with the original data and there is classification and structure. The formation of these two groups is related to the greater degradation that proteins went through after the cooking, in relation to hydrated grains and control. Results observed by the decrease in the levels of total soluble proteins (Figure 1) and by the lower band intensity in all BAFs submitted to cooking (Figure 2).

The analysis of the main components pointed out that the first two components represented 89.3% of the total variation. Analyzing component 1, a set was highlighted to the right of the axis, formed by the genotypes after cooking. As for component 2, on the upper part is a predominance of BAFs 07, 75 and 102 after cooking; 03 and 75 hydrated; and 57

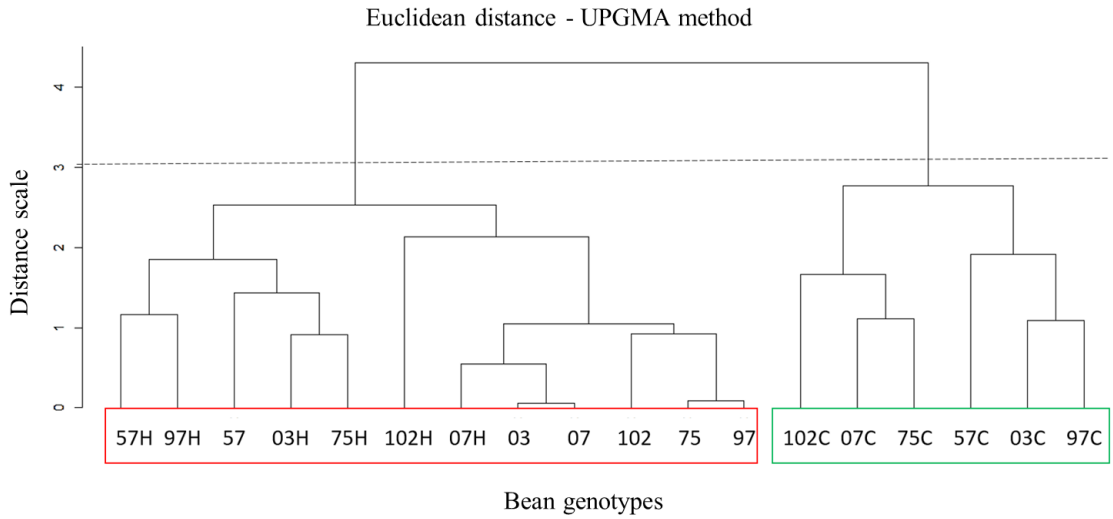


Figure 3 . Dendrogram of the Euclidean distance among the genotypes 03, 07, 57, 75, 97 and 102 with no treatment and subjected to hydration (H) or cooking (C). Cut-off level at the Euclidean distance equal to 3.0 (dashed line) with the formation of two groups, first formed by hydrated and untreated genotypes (red) and second by the genotypes subjected to cooking (green). Cophenetic correlation index r = 0.80.

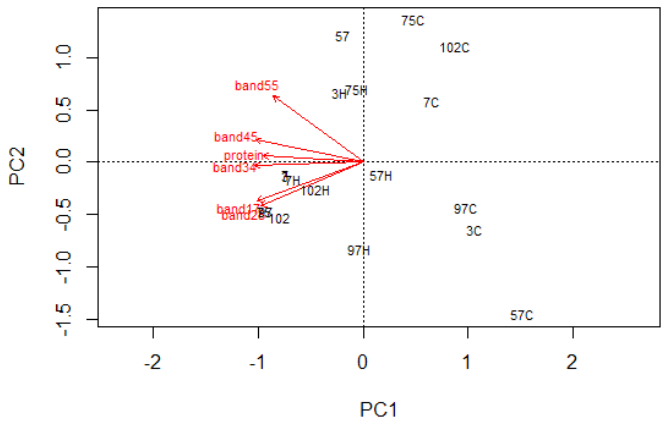


Figure 4. Ordering diagram based on axis 1 and 2 of the Principal Component Analysis, representing 89.3% of the total variation of the evaluated bean genotypes, namely: protein: soluble proteins; and bands 17, 28, 34, 45 and 55; C: cooking; H: hydrated.

with no treatment (control). The other genotypes are at the bottom of the component 2 (Figure 4).

The most important variables for component 1 were the bands 34 and 45 kDa, in which BAFs 07, 75, 97 and 102 with no treatment demonstrated a greater intensity of these bands for the above mentioned variables. While, for component 2, the band of approximately 55 kDa was more relevant among the variables, with all genotypes with no treatment and after hydration demonstrating a high intensity. This separation pointed out that BAFs 07, 75, 97 and 102 suffered less protein degradation, and consequently the grains have a greater availability of this said reserve. Beans are a low cost and high nutritional quality protein source for human consumption (Durigon et al., 2015). In this regard, the consumption of grains with a higher protein content is a beneficial alternative, since data from the IBGE (2020) allow estimating that beans are still the main protein source for the low-income population. Furthermore, bean consumption is highly recommended by the Ministry of Health (Brasil, 2014).

The bean genotypes BAF 07 and BAF 75 had a greater resistance to protein degradation, thus showing their superior nutritional quality. However, these results indicate the importance of new studies evaluating the resistance to hydration and cooking of other nutritional components, enabling the incorporation of genes of interest in genotypes already used for genetic improvement through breeding.

Conclusions

Genotypes BAF 07 and BAF 75 had high protein content and less protein denaturation, as evidenced by the electrophoretic profile, even after hydration and cooking.

These same genotypes can be recommended for consumption and for crossbreeding in programs aimed at improving the nutritional quality of the grains.

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