

EFFECT OF GERMINATION AND COOKING ON IRON CONTENT, PHYTIC ACID AND LECTINS OF FOUR VARIETIES OF CHILEAN BEANS (*Phaseolus Vulgaris*)

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ABSTRACT

The family of *Fabaceae* has been fundamental into the traditional nutrition in South America, including Chile, however in this country has reduce consumption of legumes between children in school age. This investigation pursued establish the impact over nutritional and no nutritional composition of chilean beans (*Phaseolus vulgaris*) exposed to cook and germination. The results obtained has shown the cooked samples has decreased the contain of iron and also diminished in total minerals from ashes analysis, instead the germinated samples increased both. Total proteins determined by Kjeldahl method exhibit no change for cooked and germinated beans in relation to control without treatment, while phytic acid increased and lectins decreased with the action of cook or germination of *P.vulgaris*. All the analysis was supported by statistical assessment and suggest the beans germination is a good alternative to improve the variety of recipes available to prepare beans in other attractive presentations on the subject of promote the children intake.

Keywords: Beans, Lectins, Phytic acid, Germinated, Hemagglutinating.

1. INTRODUCTION

The Chilean bean corresponds to an annual herbaceous plant, belonging to the *Fabaceae* family and the genus *Phaseolus*, which includes five domesticated species, including *P. vulgaris* or common bean. This plant has its primary origin in Mesoamerica, being also domesticated in the Southern Andes region for at least 7000 years [1], [2]. In Chile, the predominating genetic variety is Los Andes del Sur[3] and the cultivation of legumes is located in the south-central part of the country, the BioBío region (formerly eighth region of Chile) being the one with the largest number of hectares planted, of which 40% corresponds to beans [4]. Alas, local economic constraints do not allow competing with countries such as Canada that is one of the main exporters of legumes worldwide. Globally, beans represent 15% of the arable land in the world [5], and are considered by FAO as the most important legume for direct consumption on the planet. Its high protein and dietary fibre content, as well as the presence of phenolic compounds and tocopherols with antioxidant activity, peptides, amino acids and minerals has been well described in the literature.[5]–[7]. However, it is also interesting to consider its benefits in agriculture, with its high nitrogen fixing activity, contribution of organic matter and reduction of pests in soils, as well as its low water consumption whilst obtaining calories, proteins and fats compared to other crops or animal products [4].

In addition to nutritional components, beans also have non-nutritional components. Toxic substances that reduce nutrient utilization and can be classified into two large groups: thermo-stable and thermo-labile. Phytic acid stands out in the former while lectins do so in the latter[8]. Phytic acid forms especially stable complexes with minerals such as zinc, calcium and iron, preventing its absorption in the intestinal tract, resulting in gastrointestinal symptoms such as nausea and diarrhoea. Lectins, also called phytohematoagglutinins, are glycoproteins that have high selectivity towards carbohydrates and can agglutinate red blood cells. Its adverse effects described are related to a reduction in growth and weight loss in laboratory animals, associated with the reduction in the absorption of digestion products in the small intestine[6], [9], [10]. On the other hand, these non-nutritional components have shown anti-tumour and anti-proliferative effects in colorectal cancer cell lines[11]. It has also been observed that the consumption of these anti-nutrients reduces blood sugar levels, which is highly desirable for diseases such as diabetes and hyperlipidemia[12]. Despite this background, the consumption of legumes in Chile has decreased by around 28% in the past decade[13]. Multiple factors influence this decrease in consumption, mostly socio-cultural issues, associating this food with the working class, as well as the time taken for their preparations[14], [15].

Considering this background, our interest focuses on determining the content of these components and assessing the effect of germination and cooking on this composition for four varieties of Chilean beans (*P. vulgaris*). In addition, to contribute to the diversification of preparations for this food in a recipe book created by the Inacap Center for Gastronomic Innovation, encouraging the consumption of beans.

2. MATERIAL AND METHODS

2.1. Plant materials and Reagents

The plant material used corresponds to 4 varieties of Chilean bean seeds (*Phaseolus vulgaris*): found, black, young and white. These were bought in the Central Market of Santiago. Samples are preserved for later analysis.

Lectin (from *Phaseolus vulgaris*), phytic acid sodium salt hydrate, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulphonic acid sodium salt (ferrozine) ≥ 97.0% and Iron standard solution 1 mg/mL Fe were purchased from Sigma-Aldrich and used without prior purification. Defibrinated rabbit blood was purchased at the Public Health Institute of Chile, ISP. Kjeldahl tablets, sulfuric acid 95-97% p.a, boric acid p.a, hydrochloric acid 0.1 and 1 M Titrisol®, 2 M Titripur®, L-ascorbic acid p.a, ammonium acetate p.a, sodium chloride p.a, iron (III) chloride hexahydrate p.a, 5-sulfosalicylic acid dihydrate for synthesis were purchased from Merck and used without prior purification.

The spectrophotometric measurements were performed on a Rayleigh UV-Vis spectrophotometer, China. Nitrogen determination was performed on a SpeedDigester K-425 Buchi digester and distillation was performed on an automatic K-350 Buchi distiller. Hemagglutination readings were performed on Optimus optical microscopes.

2.2. Treatment

The four varieties of beans were subjected to two treatments: germination and cooking. For the germination healthy specimens were selected, washed with 70% ethanol solution and rinsed with distilled water, then subjected to germination in Petri dishes with wet paper in an incubator at 22 °C for 5 days. Once germinated, the beans were dried at 105 °C for 2 hours, crushed to fine powder and stored under vacuum at -20 °C until analysis.

Healthy specimens were selected for cooking and a mass:solvent ratio of 1:10 with deionized water was used. 25 grams of each variety were deposited in a beaker to which 250 mL of deionized water was added.

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This system took two autoclave cycles of 15 minutes each. After this the content was filtered and the beans were dried at 105 °C for two hours, crushed to fine powder and stored under vacuum at -20 °C until analysis.

As raw control, raw beans were used, which were dried at 105 °C for two hours, crushed to fine powder and stored under vacuum at -20 °C until analysis.

2.3. Gravimetric Analysis

The moisture content was determined according to the drying method in an oven with air circulation[16], with few modifications. Approximately 2 grams of a sample are weighed in a porcelain capsule and placed in an oven with air circulation for 2 hours at 105 °C. They are cooled and kept in a desiccator until their final mass is registered. The moisture content (% w/w) is calculated according to the following formula and is reported as the average of three measurements.

$$\% \text{ Moisture} = \frac{\left(\frac{w \text{ of wet sample}}{+ \text{ pan}} \right) - \left(\frac{w \text{ of dried sample}}{+ \text{ pan}} \right)}{\left(\frac{w \text{ of wet sample}}{+ \text{ pan}} \right) - \left(\frac{w \text{ of pan}}{+ \text{ pan}} \right)} \times 100$$

The ash content is determined according to the muffle heating method[16], with few modifications. Approximately 2 grams of sample are weighed in a porcelain crucible and they are placed in a muffle oven for 24 hours at 550 °C. They are cooled and preserved in desiccator until their final mass is registered. The percentage of ashes (% w/w) is calculated according to the following formula and is reported as the average of three measurements.

$$\% \text{ Ash} = \frac{(w \text{ of ash})}{(w \text{ of wet sample})} \times 100$$

2.4. Protein Nitrogen Determination

The nitrogen content of the beans studied was determined by the Kjeldahl method. Approximately 1 g of dried beans are weighed and transferred to a digestion tube. A catalyst tablet and 10.0 mL of concentrated sulfuric acid are added. Blanks are prepared by adding a catalyst tablet and 10 mL of concentrated sulfuric acid to the digestion tube. The tubes are mounted in the digester rack and this is taken to the digestion block, connecting the vapor extraction system. Digestion is maintained for two hours, confirming that these must be clear and do not have carbonized material. The tubes are cooled and diluted with 50 mL of deionized water, stirring until completely dissolved. Each tube is then taken to the distillation system and a flask with boric acid (4% w/v) and two drops of the red methyl indicator and one drop of methylene blue are placed to collect the distillate. Distillation is carried out for 10 minutes for each tube. Finally, the distillate titration is performed using standardized 0.1 M HCl until the indicator changes colour from green to pink. The consumed volume of standard HCl is recorded and the calculations are performed according to the following formula.

$$\%N = \frac{(mL \text{ HCl std} - mL \text{ blank}) \times M \text{ HCl std} \times 1,4007}{g \text{ sample}}$$

$$\% \text{ Protein} = \%N \times \text{Protein Factor}$$

The protein factor used is 6.25. The measurements were made in triplicate.

2.5 Iron Determination

The iron content was determined from the ashes of the four varieties of beans in its two treatments and in the control by the method of Ferrozine[16], with slight modifications. Calibration standards were prepared containing 0, 2, 4, 6, 8 and 10 µg Fe/mL from a standard solution of 10 µg Fe/mL. Samples were prepared by dissolving approximately 0.5 g of ashes in a small portion of 1 M HCl and then 0.1 M HCl was added to 50 mL in a volumetric flask. For measurements, 0.500 mL of standards or samples are taken in 10 mL test tubes. 1.250 mL of ascorbic acid (0.02%, freshly prepared in 2 M HCl) is added and placed on a vortex mixer and then allowed to stand for 10 minutes. 2.000 mL of ammonium acetate (30% w/v) is added and stirred on a vortex mixer. 1.250 mL of Ferrozine (1 mM in water) are added, stirred on a vortex mixer and allowed to stand in the dark for 15 minutes. Finally, the absorbance at 562 nm is read against a blank containing 0.500 mL of deionized water instead the sample. The results are listed as µg Fe/g dry bean and reporting the average of three determinations.

2.6 Phytic acid determination

The phytic acid (PA) content was determined by the colorimetric method with Wade reagent[17], with minor modifications. The extraction was carried out on 0.5 g of beans that were mixed with 10 mL of 2.4% HCl in a centrifuge tube that was subjected to orbital agitation at 220 rpm for 16 hours and centrifuged at 1000 g for 20 minutes at 10 °C. Crude extracts are collected for PA analysis. The crude extracts are transferred to test tubes containing 1 g of NaCl, stirred orbitally at 350 rpm for 20 minutes and allowed to stand at -20 °C for 20 minutes. This mixture is centrifuged at 1000 g for 20 minutes at 10 °C and 1.000 mL of the supernatant is diluted to 10 mL with deionized water. For quantification, 3.000 mL of the supernatant or standards are mixed with 1.000 mL of the Wade reagent (0.03% FeCl₃·6H₂O and 0.3% Sulfosalicylic acid), mixed vigorously in a vortex stirrer and centrifuged at 1000 g for 10 minutes at 10 °C. The absorbance at 500 nm is recorded on a spectrophotometer and the results are expressed as mg PA/g bean. All trials were done in sextuplicate.

2.7 Lectin determination

The Lectin determination was performed by the red blood cell agglutination method[18]. First, hematoagglutinins are extracted on 1 gram of dried beans using 10 mL of 0.9% NaCl in a centrifuge tube. The mixture is stirred for one minute in a vortex mixer and then left in a shaker for 1 hour. The suspension is allowed to stand in the refrigerator at 4 °C overnight and then centrifuged at 14190 g for 10 minutes at 4 °C. After filtering, the supernatant is successively diluted in 1.5 mL Eppendorf tubes. A dilution range of 0 in tube 1 to 1: 8192 (extract: saline, v/v) in tube 14 and a final volume of 0.5 mL was used. 0.05 mL of 2% rabbit red blood cell solution was added to each tube, stirred and allowed to stand for 2 hours. Hemagglutinin activity (HA) was determined microscopically by taking a drop of each tube, transferring it to an object holder and covering it with a coverslip. At least five cells added together are considered positive evidence of agglutination. A blank was prepared containing 0.05 mL of saline and 0.05 mL of red blood cell solution. A positive control was also prepared by mixing 0.05 mL of lectin solution (1:1, m/v in phosphate buffered saline, pH = 6.8). The lowest dilution of the extracts produced by a positive agglutination is defined as that which contains a Hemagglutinin Unit (HU). The specific activity of each bean (HU/mg bean) is calculated according to the following formula.

$$\frac{HU}{mg} = \frac{D_a \times D_b \times S}{V}$$

Where,

D_a = Dilution factor in tube 1 (1 unless the solution is diluted)

D_b = Dilution factor in the tube showing a HU

S = mL original extract / mg of bean

V = mL of the extract in tube 1

2.8 Statistical Analysis

All determinations were made at least in triplicate and the results are presented as average ± standard deviation. Two-factor ANOVA was performed and Tukey test considering significant differences for p values less than 0.05. All analyses were done with Microsoft Excel 2017 (Microsoft Office 2017) and GraphPad Prism version 6.01 2012 (GraphPad Software Inc 2012).

3. RESULTS AND DISCUSSION

3.1. Gravimetric analysis

The results of moisture and ash content are shown in Table 1. The Black bean has the highest percentage of moisture with 9.19%, while the Miss bean shows the lowest of the raw varieties with 6.58%. Although the Black bean shows the highest percentage of moisture in the germination treatment, it is the Miss variety that holds the highest amount of water in the process, increasing its content almost 8 times. Our results are correlated with those obtained for common bean flour, with values that vary between 6.4 and 8.9% humidity[19]. Figure 1 shows the effect of the treatment on the moisture and ash content. It is observed that cooking increases the water content at least 7 times, again showing that Miss bean has the biggest increase. The statistical analysis shows an extremely significant influence of the treatment, accounting for 99.81% of the total variance.

Table 1. Effect of the treatment on the % of moisture and ashes for the four varieties of raw, sprouted and cooked beans. The results correspond to the average of three replicates and their standard deviation.

Type	% Moisture			% Ash		
	Raw	Germinated	Cooked	Raw	Germinated	Cooked
Black	9.19 ± 0.06	54.26 ± 0.35	70.40 ± 0.04	4.31 ± 0.08	4.45 ± 0.06	2.47 ± 0.04
Found	8.72 ± 0.12	50.39 ± 0.74	70.41 ± 0.65	3.90 ± 0.16	4.39 ± 0.04	2.30 ± 0.15
White	8.33 ± 0.09	51.57 ± 0.82	71.03 ± 1.23	4.14 ± 0.06	4.60 ± 0.14	2.50 ± 0.05
Miss	6.58 ± 0.06	51.71 ± 0.24	71.24 ± 1.88	3.95 ± 0.34	4.25 ± 0.06	2.73 ± 0.34

The ash content is an important parameter in food because it represents the content of available minerals. Our results show that the Black bean is the variety with the highest amount of ashes (4.31%) for raw beans, while the White bean is the variety that generates the highest amount of ashes germinated with 4.60% and finally the cooked Miss bean with 2.73%. A review on the chemical composition of common beans shows ash values for raw beans that are similar in magnitude and error as those obtained in our study[6]. Although the differences observed between varieties are not significant in the ash content, the treatment shows significant influence of the germination on the raw bean, increasing in ranges that go from 3 to 11%, being the Found bean the one that shows the bigger increase. These results are shown in Figure 1.

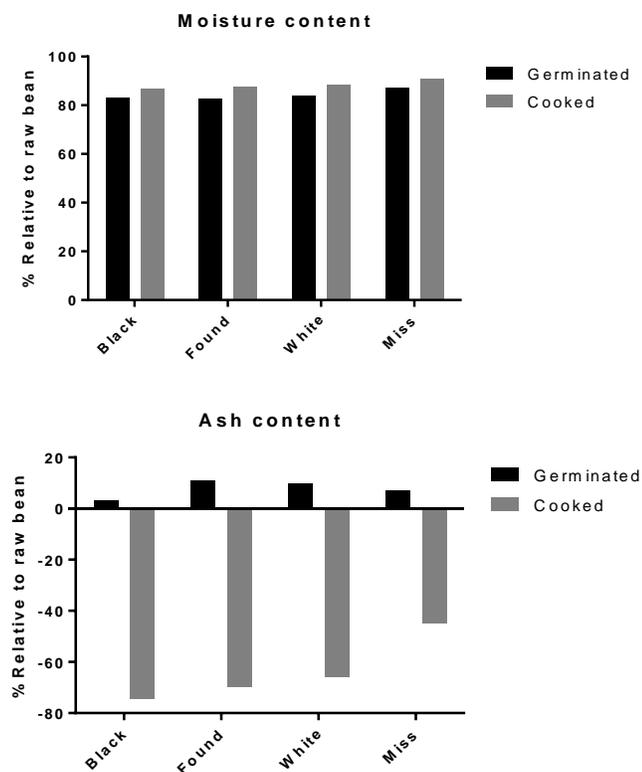


Figure 1. Percentage of gain or loss in the moisture and ash content of the different treatments. The values were calculated from the content of the raw beans.

Interestingly, the cooking treatment significantly decreases the amount of ashes, which would potentially reduce the availability of minerals for the food intake. The greatest effect is observed in the Black bean, with a decrease of 74% while the Miss bean presents the smallest variation with a decrease of 44%.

3.2 Nitrogen content

The nitrogen content results are shown in Table 2. The % N varies significantly between the raw bean varieties, from 3.29% in the White bean to 3.79% in the

Black bean. A similar behaviour is observed for cooked bean varieties, with values ranging from 3.36% in beans found to 4.39% in black beans. For germinated beans, there are no significant variations between them, the Black bean being the variety that contributes more nitrogen with 3.92%. All results are in agreement with those reported for common bean [5], [6], [19], [20]. Although it is not possible to establish a significant effect of the treatment on the nitrogen content, germination causes increases with respect to the raw bean, which vary between 1.8 and 7.2%, finding the greatest variation in the bean found, as can be seen in Figure 2. The cooking shows a more variable behaviour in terms of the change in the nitrogen content, with increases ranging from 0.3% and 13.7%, where the Black bean shows the greatest increase, however the Miss bean results in a 1.6% decrease, which could be explained by the dissolution of soluble proteins [19].

Table 2. Effect of the treatment on the nitrogen content for the four varieties studied. The results correspond to the analysis of triplicate samples and are presented as the average and its standard deviation.

Type	% N		
	Raw	Germinated	Cooked
Black	3.79 ± 0.06	3.92 ± 0.16	4.39 ± 0.28
Found	3.35 ± 0.01	3.61 ± 0.21	3.36 ± 0.01
White	3.29 ± 0.04	3.42 ± 0.08	3.45 ± 0.17
Miss	3.75 ± 0.13	3.82 ± 0.21	3.69 ± 0.00

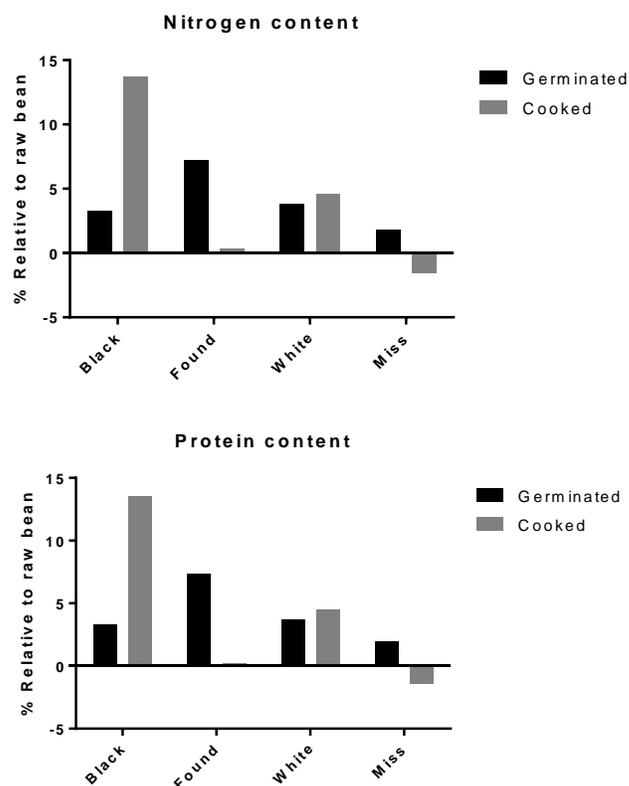


Figure 2. Percentage of gain or loss in the nitrogen and protein content of the different treatments. The values were calculated from the content of the raw beans.

Protein content is an important parameter from the point of view of food. A characteristic of beans is its high protein intake. The results of the analysed samples can be seen in Table 3. A similar trend to nitrogen content is observed for protein content, the bean type being the most influential factor in the variations found. The Black bean is the variety that makes the greatest contribution of nitrogen with 23.7%, 24.51% and 26.96% for the raw, germinated and cooked treatment, respectively. The data obtained are comparable to those

reported for *P. vulgaris* [6], [7], [19] and show the high protein contribution of the Chilean beans analysed.

Table 3. Effect of the treatment on the protein content for the four varieties studied. The results correspond to the analysis of triplicate samples and are presented as the average and its standard deviation.

Tipo	% Protein		
	Raw	Germinated	Cooked
Black	23.70 ± 0.38	24.51 ± 0.95	27.39 ± 1.75
Found	20.93 ± 0.04	22.58 ± 1.35	20.98 ± 0.04
White	20.59 ± 0.25	21.39 ± 0.53	21.56 ± 1.05
Miss	23.40 ± 0.85	23.86 ± 1.32	23.08 ± 0.01

3.3. Iron content

Iron is an important micronutrient for human metabolism and its presence in the bean makes this food an excellent mineral intake in the diet. The effect of the treatments on the iron content can be seen in Table 4. The results show a significant increase of two to five times for the iron content in the four germinated bean varieties compared to the raw bean, which can be due to the hydrolysis of iron compounds due to high water retention in germination.

Table 4. Effect of treatment on iron content for the four varieties of beans analysed. The results correspond to the average and its standard deviation for three replicas. ND = not detectable.

Type	Iron content ($\mu\text{g Fe/g bean}$)		
	Raw	Germinated	Cooked
Black	17.7 ± 0.5	30.0 ± 4.4	ND
Found	12.8 ± 0.7	41.1 ± 1.4	ND
White	15.0 ± 0.40	74.2 ± 2.9	ND
Miss	16.8 ± 1.3	47.1 ± 0.5	ND

The Black bean shows the highest iron value in raw beans with 16.8 $\mu\text{g Fe/g}$ bean while the White bean shows the highest value for germinated beans with 74.2 $\mu\text{g Fe/g}$ bean. These values are similar to those reported for Chilean beans in their iron content [21]. In addition, the White bean shows the highest increase (79.8%) in iron content, while the Black bean the lowest (41.0%) for germination. Interestingly cooked beans in all its varieties have iron contents below the limits of detection of the method, suggesting the different forms of iron are indeed present in the cooking water, thus making it gastronomically relevant.

The statistical analysis shows an extremely significant influence of both factors, type of bean and treatment, as well as their interaction, so it is possible to establish that the germinated bean significantly increases its iron content, while the cooked bean loses this mineral by dissolution, which is in agreement with the results obtained for moisture and ash content of the beans analysed in this study.

3.4. Phytic acid content

The phytic acid content varies significantly between the four varieties studied, both in raw, sprouted and cooked beans, as can be seen in Table 5. For raw beans, values vary between 2.03 and 3.06 mg/g, the white bean being the one with the highest contribution of phytic acid. For sprouted beans, the values are distributed between 2.96 and 4.86 mg/g with the Black bean being the variety that provides the most phytic acid. Finally, for the cooking treatment, values between 3.04 and 4.51 mg/g are observed, the Black bean again being the one with the highest phytic acid content. These values are comparable to those obtained in a study with 50 varieties of common bean, with an average value of 3.102 mg/g of phytic acid [10].

The analysis of variance shows that the type of beans, the treatment and their interaction have significant effects on the phytic acid content. All varieties show an increase in the content of this component with the germination treatment, with the White bean being the one that shows the smallest increase. However, although a similar trend is observed in cooked beans, the White variety shows a slight decrease due to treatment. Figure 3 shows that for the Black bean, although there is an increase of 53.1 and 49.5% in the PA content for the germinated and cooked bean respectively, these values do not differ statistically, therefore a discrepancy between germination and cooking is not observed. A similar behaviour is shown in the Miss bean, allowing to establish that the treatment affects the content of PA, but in the same way. A study with common beans (*P. vulgaris*) from Canada [18] shows phytic acid contents that are almost four times greater than those obtained for our varieties, which can be explained by genetic, geographical, soil, climatic and other conditions well described in the literature [3], [10], [18], [22]–[27].

Table 5. Phytic Acid Content in the four varieties studied by two treatments. The results correspond to the analysis of six-fold samples and are presented as the average and its standard deviation.

Type	Phytic acid content (mg PA/g bean)		
	Raw	Germinated	Cooked
Black	2.28 ± 0.2	4.86 ± 0.05	4.51 ± 0.12
Found	2.03 ± 0.14	3.58 ± 0.10	4.42 ± 0.27
White	3.06 ± 0.22	3.60 ± 0.29	3.04 ± 0.18
Miss	2.11 ± 0.08	2.96 ± 0.28	3.40 ± 0.29

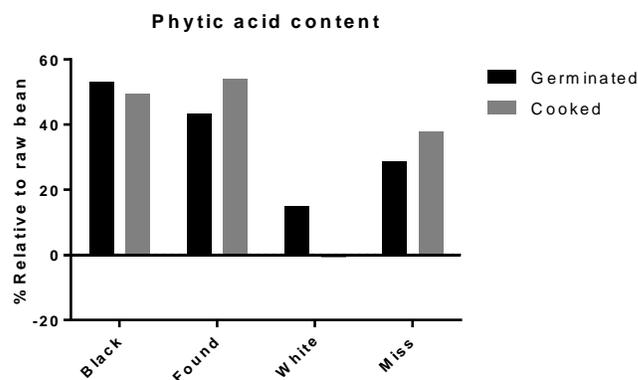


Figure 3. Percentage of gain or loss in the phytic acid content of the different treatments. The values were calculated from the content of the raw beans

3.5. Lectin content

The results of the hemagglutinating activity analysis of the four varieties of raw, sprouted and cooked beans are shown in Table 6 and in Figure 4. Among the raw beans, the black variety shows the highest hemagglutinating activity, with 6.29 HU/mg while the white bean shows the lowest activity with 6.20 HU/mg. A similar behaviour is observed among the germinated beans, the Black and White beans being the ones that show the highest and lowest lectin content, respectively. Statistical analysis shows that the treatment, the bean variety and its interaction significantly affect the content of lectins, reducing to undetectable amounts of lectins in cooking. These results are consistent with those shown by other studies where cooking and germination treatment significantly reduce the amount of lectins [18], [24], [28]. However, our results show values that are fourteen-fold lower compared to those of Canadian beans, a trend similar to that observed with phytic acid. The low levels of these components in the varieties analysed could even have beneficial effects depending on the extensive biological activity reported against microorganisms, antitumor, immunomodulatory, blood lipid reduction, among others [11], [24], [29]–[31].

Table 6. Effect of germination and cooking on the haemagglutinating activity in the four varieties of beans analysed. The results are presented as the average and the standard deviation of 6 replicates. ND = Not Detectable.

Type	Lectin content (HU/mg bean)		
	Raw	Germinated	Cooked
Black	6.29 ± 0.10	5.96 ± 0.13	ND
Found	6.21 ± 0.04	3.17 ± 0.06	ND
White	6.20 ± 0.17	3.06 ± 0.21	ND
Miss	6.24 ± 0.24	3.07 ± 0.01	ND

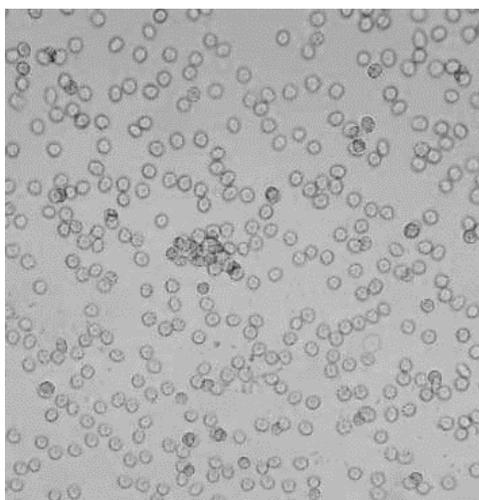
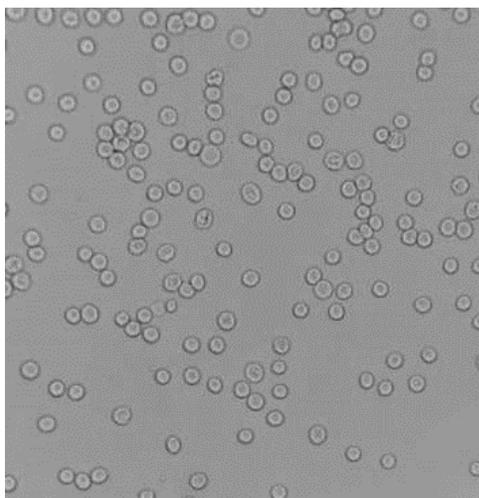


Figure 4. Photographs of the agglutination. Negative control (left) and positive agglutination for Miss Raw (right). The increase is 400x.

4. CONCLUSIONS

Considering the food importance of beans, as well as the systematic reduction of its consumption in Chile, the effect of germination and cooking on the content of phytic acid and lectins in four varieties of Chilean beans was studied for the first time, in order to find new gastronomic applications possibly promoting its consumption in the population.

Our results show that the mineral content available for intake significantly decreases during cooking, while germination causes an increase in these micronutrients, particularly with iron. Likewise, it is possible to establish that the protein content of the bean is not significantly affected by the treatments.

The analysis of non-nutritional components makes it possible to establish that the treatment significantly influences the content of phytic acid, however this is significantly lower than that reported for Canadian beans, highly consumed in Chile. On the other hand, the lectin content of Chilean beans shows a similar trend, being 14 times lower than that of Canadian beans, allowing to establish that the antinutritional content of Chilean beans is significantly lower than that of imported beans.

This background allows us to conclude that the germination of Chilean beans is presented as an important nutritional alternative for flour production, considering the heat treatment to which they are subsequently subjected. Likewise, cooking is presented as a favourable alternative to the reduction of these nutritional and antinutritional components, so cooking water should also be used in gastronomic applications.

Finally, the low content of non-nutritional compounds presents in the varieties studied could have positive impacts on the overall nourishment and health of the Chilean population.

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