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New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas

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Abstract The effect of different conditions of germination at a semi-pilot scale on the content of available soluble sugars, alpha-galactosides, vitamins B₁ and B₂, and inositol phosphates of beans, lentils and peas have been studied. Results obtained indicated that germination modified the nutritional composition of legumes depending on the type of legume and germination conditions. The storage compounds present in dry seeds (alpha-galactosides and higher forms of inositol phosphates) decreased because they were hydrolysed to glucose, fructose, IP₄ and IP₃, compounds that can serve as a source of energy for the new plant. Vitamin B₂ suffered an important increase after germination whereas vitamin B₁ did not change significantly. To achieve legume flours with enhanced nutritive value, 6 days of germination in the presence of light for beans and lentils, and in darkness for peas can be suggested.

Keywords Legumes · Germination · Available soluble sugars · Alpha-galactosides · Vitamin B₁ · Vitamin B₂ · Inositol phosphates · Functional foods

Introduction

Legumes are high-protein crops that have contributed to the human diet for centuries. They also provide a large amount of available carbohydrates, dietary fibre, water-soluble vitamins and minerals. However, pulses contain antinutritional factors such as trypsin inhibitors, α -galactosides and phytic acid, which can negatively affect their nutritional value, and that is the reason why legume seeds have to be processed prior to their consumption [1].

Germination has been suggested as an inexpensive and effective technology for improving the quality of legumes by enhancing their nutritional value, and germinated soybean constitutes an important proportion of the total consumption of food legumes in China, India, Burma and Indonesia [2], and nowadays are becoming very popular in the western countries.

Germination is generally preceded by soaking the legume seeds in water. Some of the reserve materials of the seeds are degraded and used partly for respiration and partly for synthesis of new cell constituents of the developing embryo during germination, therefore, this process causes important changes in the biochemical, nutritional and sensory characteristics of legumes. Fats and carbohydrates, which often are at surplus levels in the western diets are broken down and starch digestibility increases [3, 4, 5]. Vitamins and secondary compounds, many of which are considered beneficial as antioxidants, often change dramatically during germination [6, 7, 8]. Phytic acid and dietary fibre both affect the uptake of micro-nutrients in the digestive tract and these compounds change differently during the germination process [5, 9]. α -Galactosides content, oligosaccharides that produce flatulence, and trypsin and chymotrypsin inhibitors, which affect the digestion of proteins, can be reduced during germination [5, 10, 11, 12]. The *in vitro* digestibility of proteins increases during germination [13] and the emulsifying capacity of legume proteins increases [14].

Several reports were found in the literature about the effect of germination on the nutritive composition of legumes including soybeans, mung beans or lentils. Most studies have been conducted at lab scale using a single set of germination conditions and no comparisons within legumes germinated in similar conditions have been reported. In this paper we present a systematic study carried out in a semi-pilot scale using different germination conditions in order to know the changes in the content of available soluble sugars, vitamins B₁ and B₂ and inositol phosphates of beans, lentils and peas.

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Material and methods

Legumes

Beans (*Phaseolus vulgaris* L, var. La Granja), lentils (*Lens culinaris* L, var. Castellana) and peas (*Pisum sativum* L, var. Esla) were purchased and used for the germination experiments.

Germination

For every tray in the germinator, 500 g of legume seeds were soaked in 2500 ml of water containing 0.07% sodium hypochlorite solution for 30 min at room temperature. Seeds were then drained off, watered to neutral pH, and soaked in distilled water for 5 h and 30 min. Finally, hydrated seeds were located in six trays and germinated at a pilot scale by layering them over a moist filter paper continuously watered by capillary in a seed germinator (G-120 Snijders, Holland) for 2, 4 and 6 days with light (named 2DL, 4DL and 6DL, respectively) and without light (named 2DNL, 4DNL, 6 DNL, respectively) at 20 °C, 99% relative humidity. Sprouted seeds were freeze-dried and ground to pass through a 0.18-mm sieve for their analysis.

Determination of available soluble sugars and α -galactosides

Analysis of glucose, fructose, sucrose and α -galactosides (raffinose, ciceritol, stachyose and verbascose) were carried out following the method described by Frías et al. [15].

Determination of vitamins B₁ and B₂

A single extraction procedure for vitamins B₁ and B₂ was carried out according to Vidal-Valverde et al. [16]. These vitamins were quantified by HPLC as described in previous papers [16, 17].

Inositol phosphates determination

Inositol hexaphosphate (IP₆), pentaphosphate (IP₅), tetraphosphate (IP₄) and triphosphate (IP₃) were determined by HPLC according to Kozłowska et al. [18].

Statistical methods

Data were subjected to multifactor analysis of variance using Statgraphics Statistical Graphics 5.0 System Software (Statistical Graphics Corporation, Rockville, MDS) with an IBM Personal Computer.

Table 1 Available soluble sugar content in raw and germinated legumes

	Fructose (% d.m.)	Glucose (% d.m.)	Sucrose (% d.m.)	Total available soluble sugars (% d.m.)
BEANS				
Raw beans	ND	ND	1.02±0.05 ^a	1.02±0.05 ^a
Germination with light				
2 days	0.02±0.00 ^a	ND	0.82±0.02	0.85±0.01
4 days	0.02±0.01 ^a	ND	1.48±0.02	1.15±0.02
6 days	0.04±0.00	ND	1.59±0.06	1.63±0.06
Germination without light				
2 days	0.02±0.00 ^a	ND	0.92±0.02	0.94±0.03
4 days	0.03±0.01 ^a	ND	1.00±0.08 ^a	1.03±0.08 ^a
6 days	0.06±0.01	ND	1.32±0.01	1.38±0.11
LENTILS				
Raw lentils	0.02±0.01	ND	0.63±0.07	0.65±0.07
Germination with light				
2 days	0.25±0.02	0.10±0.02 ^a	0.73±0.06 ^a	1.08±0.06
4 days	0.38±0.05	0.15±0.02	0.77±0.04 ^a	1.30±0.08
6 days	0.94±0.04	0.31±0.04	1.18±0.01	2.42±0.05
Germination without light				
2 days	0.46±0.01	0.10±0.03 ^a	0.96±0.02 ^b	1.53±0.03
4 days	0.64±0.06	0.54±0.07	0.95±0.06 ^b	2.13±0.05
6 days	1.12±0.05	0.87±0.05	1.32±0.01	3.31±0.01
PEAS				
Raw peas	ND	ND	1.73±0.14	1.73±0.14
Germination with light				
2 days	0.01±0.00 ^a	0.02±0.00 ^a	1.38±0.02 ^a	1.41±0.01 ^a
4 days	0.08±0.01	0.10±0.00 ^b	2.74±0.01	2.92±0.01
6 days	0.17±0.01 ^b	0.31±0.01	3.25±0.01	3.75±0.01
Germination without light				
2 days	0.01±0.00 ^a	0.03±0.01 ^a	1.39±0.00 ^a	1.43±0.01 ^a
4 days	0.06±0.00	0.11±0.01 ^b	2.87±0.01	3.04±0.01
6 days	0.16±0.01 ^b	0.38±0.01	3.93±0.01	4.47±0.01

Mean values of three determinations. The same superscript in the same column for the same legume means not significant difference ($P \leq 0.05$).

Table 2 α -Galactoside content in raw and germinated legumes

	Raffinose (% d.m.)	Ciceritol (% d.m.)	Stachyose (% d.m.)	Verbascose (% d.m.)	Total alpha- galactosides (% d.m.)
BEANS					
Raw beans	0.11±0.01	ND	0.50±0.01	ND	0.61±0.01
Germination with light					
2 days	0.07±0.00	ND	0.18±0.01 ^a	ND	0.25±0.01 ^a
4 days	0.05±0.00 ^a	ND	ND	ND	0.05±0.00 ^b
6 days	0.01±0.00	ND	ND	ND	0.01±0.00
Germination without light					
2 days	0.09±0.00	ND	0.18±0.01 ^a	ND	0.27±0.01 ^a
4 days	0.05±0.00 ^a	ND	ND	ND	0.05±0.00 ^b
6 days	ND	ND	ND	ND	ND
LENTILS					
Raw lentils	0.38±0.01	0.62±0.04	1.77±0.08	ND	2.80±0.07
Germination with light					
2 days	0.27±0.01 ^a	ND	ND	ND	0.27±0.01 ^a
4 days	0.18±0.01	ND	ND	ND	0.18±0.01
6 days	ND	ND	ND	ND	ND
Germination without light					
2 days	0.27±0.01 ^a	ND	ND	ND	0.27±0.01 ^a
4 days	0.13±0.01	ND	ND	ND	0.13±0.01
6 days	ND	ND	ND	ND	ND
PEAS					
Raw peas	0.56±0.03	ND	2.24±0.06	2.39±0.01	5.19±0.03
Germination with light					
2 days	0.26±0.01 ^a	ND	0.05±0.00	ND	0.31±0.01 ^{ab}
4 days	0.26±0.01 ^a	ND	ND	ND	0.26±0.01
6 days	0.10±0.01	ND	ND	ND	0.10±0.01
Germination without light					
2 days	0.25±0.00 ^a	ND	0.08±0.01	ND	0.33±0.01 ^a
4 days	0.30±0.01	ND	ND	ND	0.30±0.01 ^b
6 days	0.12±0.00	ND	ND	ND	0.12±0.00

Mean values of three determinations. The same superscript in the same column for the same legume and vitamin means not significant difference ($P \leq 0.05$).

Results and discussion

Tables 1, 2, 3 and 4 collect the content of available soluble sugars, α -galactosides, vitamins B₁ and B₂, and inositol phosphates of raw and germinated bean, lentil and pea seeds in different conditions.

The observed content of total available soluble sugars in beans, lentils and peas was 1.02%, 0.65% and 1.73%, respectively (Table 1). Sucrose was the soluble sugar found at the highest level in the raw legumes studied (1.02, 0.63 and 1.73% in beans, lentils and peas, respectively). Fructose and glucose were not detected in beans and peas. In lentils, only very low level of fructose was found (0.02%) (Table 1).

The content of total available soluble sugars increased during germination, except in beans and peas at the beginning of the process (2 days of germination), where a decrease was observed. When the time of germination increased the available soluble sugars rose gradually, and the content of available soluble sugars in lentils and peas

was larger when germination was performed in darkness. The content of sucrose underwent a significant ($P \leq 0.05$) increase during germination in the legumes under study, which was more pronounced after 6 days of germination. However, in beans and peas sucrose undergoes first a decrease during the first 2 days of germination and then an increment after 4 and 6 days. The presence of light during germination led to higher sucrose levels in germinated beans while the opposite effect was observed for lentils and peas. Fructose appeared in beans and peas, and glucose appeared in lentils and peas after germination, and their amounts were higher when the germination was carried out without light.

Table 2 shows the changes in α -galactosides of beans, lentils and peas during germination. Peas presented the highest level of total α -galactosides (5.19%). Ciceritol was only detected in raw lentils (0.62%), whilst verbascose was only detected in raw peas (2.39%). Raffinose and stachyose levels were higher in peas (0.56 and 2.24%, respectively) and lower in beans (0.11 and

Table 3 Vitamin content in raw and germinated legumes

	Vitamin B ₁ (mg/100 g d.m.)	Vitamin B ₂ (mg/100 g d.m.)
BEANS		
Raw beans	0.75±0.02 ^a	0.28±0.01 ^a
Germination with light		
2 days	0.73±0.03 ^{ab}	0.31±0.03 ^a
4 days	0.71±0.02 ^{abc}	0.43±0.02 ^b
6 days	0.69±0.02 ^c	0.51±0.02 ^c
Germination without light		
2 days	0.73±0.02 ^{abc}	0.31±0.02 ^a
4 days	0.72±0.04 ^{abc}	0.43±0.01 ^b
6 days	0.70±0.01 ^{bc}	0.52±0.02 ^c
LENTILS		
Raw lentils	0.52±0.03 ^a	0.20±0.01
Germination with light		
2 days	0.51±0.02 ^a	0.24±0.00 ^a
4 days	0.50±0.01 ^a	0.34±0.01 ^b
6 days	0.49±0.01 ^a	0.47±0.01 ^c
Germination without light		
2 days	0.51±0.02 ^a	0.24±0.01 ^a
4 days	0.50±0.02 ^a	0.34±0.01 ^b
6 days	0.50±0.02 ^a	0.47±0.02 ^c
PEAS		
Raw peas	0.74±0.04 ^a	0.15±0.01
Germination with light		
2 days	0.75±0.02 ^a	0.24±0.01 ^a
4 days	0.77±0.01 ^a	0.39±0.01 ^b
6 days	0.75±0.02 ^a	0.35±0.01
Germination without light		
2 days	0.71±0.03 ^b	0.24±0.01 ^a
4 days	0.74±0.03 ^{ab}	0.30±0.02
6 days	0.71±0.01 ^b	0.40±0.00 ^b

Mean values of three determinations. The same superscript in the same column for the same legume means not significant difference ($P \leq 0.05$).

0.50%, respectively). The complete elimination of ciceritol and stachyose in lentils, and verbascose in peas was observed after 2 days of germination. In beans and peas, stachyose was eliminated after 4 days of germination. Raffinose was completely eliminated in lentils after 6 days of germination, and was also eliminated in beans when the process was carried out for 6 days in absence of light. In peas, however, raffinose was still present after 6 days of germination, but only in minor amounts (0.10 and 0.12%) (Table 2).

The content of vitamins B₁ and B₂ in raw beans, lentils and peas was 0.75, 0.52 and 0.74 mg/100 g d.m., and 0.28, 0.20 and 0.15 mg/100 g d.m., respectively (Table 3). Germination did not produce any significant ($P \leq 0.05$) variation in the content of vitamin B₁ of lentils and peas. In germinated beans no significant ($P \leq 0.05$) changes were observed during the first 4 days, and a little but significant decrease ($P \leq 0.05$) was observed after 6 days (9 and 7%, respectively). Light during germination

did not affect vitamin B₁ content of legumes. Regarding vitamin B₂ content, in general, it increased progressively during the germination of legumes. In beans and lentils, no significant differences ($P \leq 0.05$) were found between experiments carried out with and without light, while in peas the highest content was observed after 4 days in presence of light (0.39 mg/100 g d.m.), and after 6 days in absence of light (0.40 mg/100 g d.m.) (Table 3).

Table 4 shows the evolution in the content of inositol phosphates during germination of beans, lentils and peas. The levels found for IP₆ and IP₅ in raw seeds were 4.78 and 1.65 g/kg d.m. in beans, 6.17 and 0.39 g/kg d.m. in lentils, and 3.48 and 0.51 g/kg d.m. in peas, respectively, while IP₄ and IP₃ were not detected in any of the investigated legumes. IP₆ and IP₅ decreased significantly as a result of the germination process, the reduction being, in general, more important in legumes germinated for 6 days. In beans and peas, there were not detected significant differences ($P \leq 0.05$) between experiments carried out in presence or absence of light, whereas in lentils the IP₆ reduction was higher in experiments carried out in absence of light. IP₄ and IP₃ appeared in germinated legumes and in general, the highest levels were found after 6 days of germination.

There are little systematic studies in the literature regarding the effect of the different germination conditions on the nutritional value of legumes. In most cases, existing published data are not comparable, mainly because of the different germination conditions. Published results are referred mostly to soybean, mung bean and lentils germinated at lab scale: in a layer on sponges [19, 20] placed on a cheesecloth bag [21, 22, 23, 24, 25], in petri dishes [9, 26, 27, 28, 29], between paper [30, 31, 32, 33] or in separation funnels [5, 8, 11, 34, 35, 36]. This large amount of data makes results difficult to compare, and growth conditions during germination have a key role in the composition of the final product. Most of the authors agree that germination leads to a gradual decrease of the α -galactosides and that total removal can be obtained after 6–8 days [5, 27, 28, 30, 34, 37] and this is highly in accordance with our results. Puwastien and King [30] and Lowell and Kuo [38] reported that during germination of winged beans the α -galactosidase activity increased, which is accompanied by the removal of α -galactosides and an increment of sucrose.

Light and the humidity of the seed during germination affect the composition of sprouts. Abdullah and Baldwin [23] observed a sharp increase of thiamin, riboflavin, niacin and ascorbic acid after the germination of soybean and mung bean for 3 days on damp cheesecloth and rinsed three times daily. Prodanov et al. [35] reported that the content of thiamine, riboflavin and total and available niacin of lentils and faba beans depended on the frequency of rinsing, presence of light and time of germination when seeds were processed in separation funnel at 20 °C. Sierra and Vidal-Valverde [8] observed that vitamin B₁ decreased by 83% and vitamin B₂ increased by two times after germination of peas for 6 days

Table 4 Inositol phosphates content (g/kg d.m.) in raw and germinated legumes

	IP6 (g/kg d.m.)	IP5 (g/kg d.m.)	IP4 (g/kg d.m.)	IP3 (g/kg d.m.)	Total inositol phosphates (g/kg d.m.)
BEANS					
Raw beans	4.78±0.29	1.65±0.12	ND	ND	6.43±0.34
Germination with light					
2 days	2.59±0.06 ^a	0.62±0.02 ^a	0.60±0.02 ^{abc}	0.26±0.01	4.06±0.05 ^a
4 days	2.40±0.11 ^b	0.57±0.02 ^b	0.59±0.04 ^{bc}	0.29±0.02 ^a	3.84±0.09 ^b
6 days	2.42±0.07 ^b	0.43±0.04 ^c	0.58±0.04 ^c	0.31±0.01 ^b	3.74±0.07 ^c
Germination without light					
2 days	2.55±0.08 ^{ab}	0.60±0.01 ^a	0.62±0.0 ^a	0.24±0.01	4.00±0.07 ^a
4 days	2.44±0.12 ^{ab}	0.55±0.02 ^b	0.59±0.01 ^{abc}	0.28±0.00 ^a	3.96±0.11 ^b
6 days	2.41±0.04 ^b	0.41±0.02 ^c	0.61±0.03 ^{ab}	0.31±0.02 ^b	3.74±0.06 ^c
LENTILS					
Raw lentils	6.17±0.10	0.39±0.04	ND	ND	6.57±0.07
Germination with light					
2 days	4.58±0.06	0.22±0.02 ^a	0.08±0.01 ^a	0.11±0.01 ^{ab}	4.99±0.04
4 days	3.73±0.07	0.15±0.02 ^b	0.10±0.01 ^{bc}	0.10±0.01 ^b	4.08±0.07
6 days	2.37±0.04	0.11±0.01 ^c	0.11±0.01 ^c	0.11±0.01 ^{ab}	2.71±0.06
Germination without light					
2 days	4.30±0.04	0.22±0.03 ^a	0.08±0.01 ^a	0.12±0.01 ^a	4.72±0.07
4 days	3.33±0.03	0.17±0.01 ^b	0.09±0.02 ^{ab}	0.11±0.01 ^{ab}	3.70±0.04
6 days	1.86±0.06	0.11±0.01 ^c	0.11±0.01 ^c	0.12±0.01 ^a	2.21±0.07
PEAS					
Raw peas	3.48±0.03	0.51±0.03	ND	ND	3.99±0.03
Germination with light					
2 days	1.71±0.04	0.44±0.02 ^a	0.04±0.01 ^a	0.05±0.00 ^a	2.24±0.05
4 days	1.16±0.04 ^a	0.26±0.02 ^b	0.06±0.01 ^b	0.07±0.01 ^b	1.55±0.05 ^a
6 days	0.88±0.04 ^b	0.12±0.01 ^c	0.08±0.01 ^c	0.12±0.01 ^c	1.20±0.04 ^b
Germination without light					
2 days	1.59±0.02	0.40±0.02 ^a	0.05±0.01 ^{ab}	0.05±0.01 ^a	2.09±0.02
4 days	1.23±0.07 ^a	0.25±0.02 ^b	0.06±0.01 ^b	0.07±0.01 ^b	1.61±0.07 ^a
6 days	0.90±0.04 ^b	0.14±0.01 ^c	0.08±0.01 ^c	0.12±0.01 ^c	1.24±0.05 ^b

Mean values of three determinations. The same superscript in the same column for the same legume means not significant difference ($P \leq 0.05$).

in the dark. In our work carried out at a semi-pilot scale, no changes in vitamin B₁ were observed, but the rise in vitamin B₂ was between 2.3 and 2.6 times after 6 days of germination in the dark.

Some authors have reported that the content of phytic acid (inositol hexaphosphate, IP₆) is reduced as a consequence of the germination of different legumes [16, 37, 38, 39, 40, 41]. El-Mahdy et al. [42] reported that germination of lentils led to a marked decrease in phytic acid and a considerable increase in inorganic phosphorus as well as non-phytate and organic phosphorus. Honke et al. [43] found a large degradation of IP₆ during germination of pea, faba bean and lupin for 8 days and the degradation rate depended on the type of legume. Hydrolysis of IP₆ to lower inositol phosphates and free phosphorus during germination is catalysed by phytase enzyme, which is activated even during soaking process [44]. The activity of this enzyme depends on the species and variety of the seed and Reddy et al. [45] have reported increases during germination. Beal and Metha [46] obtained a 75% reduction of IP₆ content and a 12-fold of

phytase activity after 10 days of pea germination. Honke et al. [43] found the appearance and subsequent increment of IP₃ and IP₄ in pea and faba bean during 8 days of germination while IP₃ was not detected in lupins.

Results obtained in the present work indicate that, as a consequence of germination, the storage compounds present in dry seeds (such as α -galactosides and higher forms of inositol phosphates) are hydrolysed to metabolites and can serve as a source of energy for the new plant (glucose, fructose, IP₄ and IP₃). Secondary metabolites such as vitamins were modified differently and, while thiamin did not change during germination, riboflavin increased significantly.

In conclusion, it has been shown that germination is an inexpensive treatment that modify markedly the nutritional composition of beans, lentils and peas and that the nutritive quality of the germinated product depend on the germination conditions. In our work, germination led to an important increase in the content of vitamin B₂, the removal of α -galactosides responsible of the appearance of glucose and fructose, and a noticeable reduction on

IP₆ and IP₅. To achieve legume flour with enhanced nutritive value it is suggested that the germination of beans and lentils takes place in the presence of light and peas in darkness during 6 days.

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