Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 107 (2008) 1045–1052
Food Chemistry
www.elsevier.com/locate/foodchem

Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes

María A. Martín-Cabrejas [a,*], María Felicia Díaz [b], Yolanda Aguilera [a], Vanesa Benítez [a], Esperanza Mollá [a], Rosa M. Esteban [a]

[a] Departamento de Química Agrícola, Facultad de Ciencias, Universidad Autónoma de Madrid (UAM), 28049 Madrid, Spain
[b] Instituto de Ciencia Animal, Carretera Central, Km 47, 1/2, San José de las Lajas, La Habana, Cuba

Received 18 April 2007; received in revised form 20 July 2007; accepted 10 September 2007

Abstract

Influence of light exposure during germination on structural and soluble carbohydrates including total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble (SDF) dietary fibre fractions and also raffinose family oligosaccharides (RFOs) was studied in non-conventional legumes *Vigna unguiculata* (cowpea), *Canavalia ensiformis* (jack bean), *Stizolobium niveum* (mucuna), and *Lablab purpureus* (dolichos), and compared to a well known and used *Glycine max* (soybean). Non-conventional legumes were rich in DF, mainly IDF, which represented 93–97% of TDF. It was relevant the proportion of protein that remained associated to the insoluble DF matrix. Non-conventional legumes exhibited important levels of RFOs but their profile was different depending on the tropical seed. The germination of seeds produced changes in the carbohydrate fraction, mainly an increase of TDF in most instances, except for soybean, and a drastic reduction of RFOs, from 98% to 63%, with the corresponding increase in the amounts of total soluble sugars.

Keywords: Germination; Non-conventional legumes; Dietary fibre; Soluble carbohydrates; α-Galactosides

1. Introduction

The role of seed legumes in the diets of animal and man in developed countries is well documented (Waterlow, 1994). There is a lack of sufficient animal protein, hence it is necessary to search for alternative sources of protein from lesser-known legumes in lieu of expensive and scarce animal protein. Recent research efforts are being directed to this area to identify and evaluate under-exploited legume food sources as alternative protein crops for the future. This development has stimulated research on the utilisation of some under-utilised legumes such as *Vigna unguiculata* (cowpea), *Canavalia ensiformis* (jack bean), *Stizolobium niveum* (mucuna), *Lablab purpureus* (dolichos), which are potential sources of plant protein for many developing countries (Alabi & Alausa, 2006; Janardhanan, Vadivel, & Pugalenthi, 2003). The protein quality of these wild pulses seems to be similar to that of most edible legumes and they provide a large amount of structural carbohydrates mainly due to their higher DF content when compared to other fibre rich plant foods such as cereals and tubers (Guillon & Champ, 2002). They are beneficial for health, with low glycemic index and the potential to decrease serum cholesterol levels.

For efficient utilisation and consumer acceptance of legume seed flours, it is desirable to study the content of antinutritional factors commonly found in the seeds such as enzyme inhibitors, lectins, phenolics, phytates, cyanoglycosides, α-galactosides..., which may have adverse effects for human and animal nutrition (Messina, 1999). Indeed that is the reason why the seeds have to be processed before being consumed (Liener, 1989). However, some antinutrients, such as α-galactosides are heat-stable and are not eliminated by the heating process (Onyenekwe, Njoku, & Ameh, 2000; Egounlety & Aworh, 2003).

* Corresponding author. Tel.: +34 91 4978678; fax: +34 91 4973830.
E-mail address: maria.martin@uam.es (M.A. Martín-Cabrejas).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.09.020

α-Galactosides are common in pulses and the major producers of flatus when consumed in large quantities, although α-galactosides may also have a beneficial effect by increasing the bifidobacteria population in the colon (Bouhnik et al., 2004; Tuohy, Rouzaud, Brück, & Gibson, 2005). These oligosaccharides called also raffinose family oligosaccharides (RFOs) include raffinose, stachyose and verbascose and have important functions in many plant seeds (Dey, 1985). They cannot be hydrolysed and absorbed in monogastric animals due to the lack of α-galactosidase (EC.3.2.1.22) activity in the small intestine. Microorganisms in the large intestine utilize these sugars, leading to gases and short-chain fatty acids (SCFAs). The SCFAs play specific physiological roles, which are beneficial, whereas the gases are responsible for the digestive discomfort in humans and diarrhoea in animals. For the above reasons, it would be desirable for most of the population to remove α-galactosides from pulses by technological or genetic means.

In this regard, germination has been identified as an inexpensive and effective technology for improving the quality of legumes, by enhancing their digestibility increasing the content of amino acids (Chang & Harrold, 1988) and reducing the levels of antinutrients (Vidal-Valverde et al., 2002). Numerous investigations into the effects of germination on protein, starch and antinutritional factors have been carried out in legumes. However, there is a paucity of literature on the effect of such treatments on the oligosaccharide content and dietary fibre fraction of the under-exploited legumes (Alabi & Alausa, 2006; Janardhanan et al., 2003).

Thus, the present study was carried out with the aim to evaluate the influence of the germination process on the dietary fibre fractions and the profiles of oligosaccharides in four non-conventional legumes as compared to a well known and used legume (*Glycine max*) in order to obtain legume flours with high nutritive value.

2. Materials and methods

2.1. Samples

Seeds of *V. unguiculata* (L.) Walp, *C. ensiformis* (L.) DC, *S. niveum* (L.) DC, *L. purpureus* (L.) Sweet and *Glycine max* (L.) Merrill were grown and supplied by the Instituto de Ciencia Animal (La Habana, Cuba).

2.2. Germination

The germination procedure for seeds was as follows: four portions of 150 g of seeds were washed with 0.7% sodium hypochlorite, soaked in 450 ml of distilled water at room temperature for 6 h, and shaken every 30 min. The water was then drained off, and the seeds were transferred to a separating funnel, where different germination conditions were applied: samples were germinated under darkness (0 h-L), 12 h of light daily (12 h-L) and daylight (24 h-L) conditions (Díaz, Torres, González, & Node, 2004). In all cases, the germination was carried out at 25 °C for 96 h and seeds were sprayed daily with distilled water in order to maintain an adequate hydration level. The sprouts and the seeds were ground and freeze-dried for analysis. The germination experiments were performed in duplicate and the physical characteristics of germinated legumes are presented in Table 1.

2.3. Determination of soluble carbohydrates

The extraction method of soluble carbohydrates was carried out in legume flour according to a procedure described previously by Sánchez-Mata, Peñuela-Teruel, Cámara-Hurtado, Díez-Marqués, and Torija-Isasa (1998). The sample extract was vacuum evaporated at 30 °C to dryness, the concentrated sugars were redissolved in deionised water and sonicated for 5 min, finally, filtered using Whatman 41 paper and made up to 10 ml with MilliQ water. Samples were passed through a Sep pak® C_{18} cartridge (Waters, Midford, MA, USA). An aliquot of 2 ml of filtrate was mixed with 8 ml of acetonitrile and filtered through a 0.54 μm Millex membrane prior to injection. The soluble carbohydrates were determined by HPLC using an amino bonded column (3.9 × 300 mm column, Waters), isocratic pump and refractive index detector. The mobile phase was acetonitrile: water (65:35 v/v), at a flow rate of 1 ml min^{-1} and room temperature.

Quantification of peaks was performed using the external standard method. An estimate of the amount of ciceritol (with no commercial standard available) was made, using the calibration curve of the previous peak (raffinose), corrected by molecular weight. Standard sugars were obtained from Merck (Darmstadt, Germany).

2.4. Dietary fibre determination

Mes-Tris AOAC method 991.43 was used for DF determination (AOAC, 1995). Two replicates of each sample were taken to complete the six-sample analysis method. The principle of the method was based on the use of three enzymes (heat-stable α-amylase, protease and amyloglucosidase) under different incubation conditions in order to remove starch and protein components. Dietary fibre fractions were obtained as indigestible residues after enzymatic digestion of nondietary fibre components; the insoluble residues were isolated by filtration and soluble fibre was precipitated with ethanol. Dried residues correspond to insoluble dietary fibre (IDF) and soluble dietary fibre (SDF), respectively. Determination of residual ashes and proteins (as Kjeldahl N × 6.25) was carried out in the residues for corresponding corrections. Total dietary fibre (TDF) was calculated as sum of IDF and SDF. Kjeldahl nitrogen and ash contents were assayed according to standard procedures (AOAC, 1995).

Table 1
Changes in seed/seedings' biomass and percent of germination at different light conditions of germination

Legume	Light conditions of germination	% Increase in fresh weight of seeds/seedings	Development of radicle (cm)	% Germination
Cowpea	Control	–	–	–
	0 h-L	192	4.9	98
	12 h-L	240	7.3	96
	24 h-L	164	4.4	98
Jack bean	Control	–	–	–
	0 h-L	147	4.7	100
	12 h-L	142	4.5	99
	24 h-L	118	4.6	100
Dolichos	Control	–	–	–
	0 h-L	134	5.9	84
	12 h-L	105	5.2	83
	24 h-L	112	4.4	81
Mucuna	Control	–	–	–
	0 h-L	72	2.3	88
	12 h-L	101	2.1	63
	24 h-L	77	1.8	54
Soybean	Control	–	–	–
	0 h-L	109	4.6	96
	12 h-L	110	4.4	96
	24 h-L	99	4.0	96

2.5. Statistical analysis

Means comparison was performed using Duncan's multiple range test (DMRT) (Bender, Douglass, & Kramer, 1989). Significance was defined as $P \leqslant 0.05$.

3. Results and discussion

The study of the effect of germination may provide useful information for optimization of use of these legume seeds as food products, since germination has proved beneficial for the nutritional quality of common seeds. Table 1 indicates the changes in biomass and percent of germination under different light conditions. Fresh weight of seedlings increased from 72% to 240%. Cowpea showed the highest increase in fresh weight and in water content, while mucuna and soybean seeds exhibited lower increases. The results obtained are similar to those found for soybean (Kumar, Rani, Pandey, & Chauhan, 2006) and few data were found in literature for the non-conventional legumes (Maass, 2006). The success of this processing on the non-conventional legumes was high, jack bean (100%), cowpea (98%) and dolichos (84%), were similar to common seeds, while mucuna only reached 54% in daylight conditions. Regarding to development of radicles, light conditions of germination (24 h L) seemed to show the lowest length of radicles in all studied legumes. Mucuna was the non-conventional legume with the least size of radicle, while cowpea and dolichos exhibited the greatest lengths.

Of nutritional interest is the study of dietary fibre fractions due to their relevant physiological properties. The levels of TDF were remarkable in these raw non-conventional legumes (Table 2), and higher than those found in cereal seeds (Asp, 1996) and in other legumes such as peas (Martín-Cabrejas et al., 2003), beans (Martín-Cabrejas et al., 2004; Rehiman, Rashid, & Shah, 2004) and chickpeas (Martín-Cabrejas et al., 2006). Comparing the studied non-conventional legumes, it could be seen that mucuna and dolichos showed the highest levels of TDF, however, they were lower than those of soybean. The contents of IDF represented 93–97% of the TDF for the studied legumes. Thus, the SDF fraction constituted a small portion (3–7%) of the dietary fibre of these legumes. These results are in accordance with those found in the literature for cowpea (Veena, Urjo, & Puttaraj, 1995), soybean (Chang, Sang, Eun, & Young, 2006), jack bean (Akpapunam & Sefa-Dedeh, 1998; Betancur-Ancona, Peraza-Mercado, Miguel-Ordóñez, & Fuertes-Blanco, 2004) and mucuna (Siddhuraju, Becker, & Makkar, 2000). The studied non-conventional legumes showed high contents of IDF, from 303 to 399 mg kg^{-1} DM, although they were lower than that found in soybean. Regarding to SDF, the contents of this fraction showed remarkable differences depending on the legume species. Cowpea was the legume with the lowest amount of SDF, whereas mucuna and soybean showed the highest contents of this fibre fraction. This fact could exert an influence on the physiological effects and also the physico-chemical properties of DF. IDF/SDF fibre ratios are a variant related to structural and also sensorial properties (Jenkins, Kendall, & Ransom, 1998). The resultant IDF/SDF ratios of the studied legumes were very high. Thus, the incorporation of these legume fibrous residues could be used to aid in treatment of constipation, by reducing intestinal transit time through an increase in peristaltic movements.

Different studies have shown that the germination process has a significant impact on DF fractions (Chang et al., 2006; Mahadevamma & Tharanathan, 2004; Martín-Cabrejas et al., 2003), but those changes are dependent on the studied legume and germination conditions. In the present study, a general increase in the level of TDF of legumes germinated in darkness was observed, except for soybean. This last legume showed a decrease of TDF (20%), regardless the germination conditions, as a consequence of the marked decrease of IDF (from 521 g kg^{-1} DM to 407 g kg^{-1} DM). These results agreed with those found for MACS-13 (Madhurima & Mangala, 2003), a recently developed soybean variety which exhibited 69% reduction of DF, however germination of small black soybean significantly increased IDF and SDF contents (Chang et al., 2006). In contrast, the under-utilized legumes exhibited different behaviour depending on light hours during germination. Cowpea showed a general increase of TDF and IDF fraction under all germination conditions, showing the highest levels in darkness (increases of 14% and 12% in TDF and IDF, respectively). Likewise SDF

Table 2
Content of insoluble, soluble, and total dietary fibre and its distribution in raw and processed legume flours (g kg^{-1} DM)

Samples	Light conditions of germination	IDF	SDF	TDF	IDF/SDF
Cowpea	Control	303.2 ± 16.7^{a}	9.0 ± 2.0^{a}	312.2	33.7
	0 h-L	$338.8 \pm 27.2^{a.b.c}$	17.1 ± 1.4^{b}	355.9	19.8
	12 h-L	327.4 ± 2.5^{b}	11.9 ± 2.5^{a}	339.3	27.6
	24 h-L	332.1 ± 17.3^{c}	6.7 ± 3.5^{a}	338.8	49.6
Jack bean	Control	317.4 ± 4.4^{b}	15.1 ± 3.2^{a}	332.5	21.1
	0 h-L	355.5 ± 4.3^{c}	7.0 ± 3.6^{a}	362.5	50.7
	12 h-L	$317.1 \pm 6.6^{a.b}$	9.4 ± 7.0^{a}	326.5	33.6
	24 h-L	310.1 ± 2.0^{a}	29.8 ± 2.4^{b}	339.9	10.4
Dolichos	Control	399.0 ± 16.9^{b}	21.2 ± 5.2^{b}	420.2	18.8
	0 h-L	422.9 ± 2.0^{c}	38.1 ± 4.1^{c}	461.0	11.1
	12 h-L	387.4 ± 9.7^{b}	9.1 ± 0.1^{a}	396.6	42.4
	24 h-L	364.7 ± 8.2^{a}	14.3 ± 1.7^{b}	379.0	25.5
Mucuna	Control	397.6 ± 9.6^{a}	29.0 ± 5.7^{a}	426.6	13.7
	0 h-L	415.2 ± 5.8^{b}	37.2 ± 5.3^{a}	452.4	11.2
	12 h-L	417.9 ± 14.8^{b}	31.4 ± 4.4^{a}	449.3	13.3
	24 h-L	388.8 ± 4.8^{a}	28.4 ± 13.8^{a}	417.2	13.7
Soybean	Control	520.6 ± 5.3^{d}	27.1 ± 0.8^{b}	547.6	19.2
	0 h-L	473.2 ± 17.3^{c}	22.3 ± 0.1^{a}	495.5	21.2
	12 h-L	441.9 ± 5.9^{b}	33.0 ± 1.2^{c}	474.8	13.4
	24 h-L	407.2 ± 14.9^{a}	28.9 ± 0.2^{b}	436.1	14.1

Mean values of each column followed by different superscript letter significantly differ when subjected to Duncan's multiple range test ($p < 0.05$). (Mean ± SD ($n = 4$)).

fractions increased in germinated cowpea (97% increase in darkness conditions) with the exception of seeds germinated under 24 h light processing. For the rest of legumes the germination in darkness conditions involved lower increases of TDF than in cowpea (10% in dolichos, 9% in jack bean, and 6% in mucuna), and in case of light conditions, the influence of germination on TDF content was not clear except for dolichos which exhibited a slight decrease of TDF (10% and 6% in 12 h-L and 24 h-L, respectively). This loss of total dietary fibre was observed in both fibre fractions, with decreases of SDF of around 57% and 32% in 12 h-L and 24 h-L conditions, respectively. The IDF/SDF ratios did not show any clear tendency thus, the carbohydrate changes did not take place to the same extent during the germination process. Therefore, the effect of germination was dependent on both the type of legume and the light conditions of this processing (Chang et al., 2006; Mahadevamma & Tharanathan, 2004; Martín-Cabrejas et al., 2003).

Table 3 indicates the content of protein in raw and germinated legumes and protein associated to IDF and SDF residues. The raw non-conventional legumes showed important levels of protein ranging from 293 to 321 g kg^{-1} DM, similar or even higher to those found in literature, for mucuna (Adebowale, Adeyemi, & Oshodi, 2005; Siddhuraju et al., 2000), jack bean (Betancur-Ancona et al., 2004), cowpea (Giami, Okusa, & Emelike, 2001), dolichos (Alabi & Alausa, 2006) and common legumes (Messina, 1999). Nevertheless, raw soybean exhibited the highest amount of protein (434 g kg^{-1} DM). With respect to germinated legumes, in some cases, germination process at 0 h-L and 12 h-L conditions promoted an increase of the level of protein, except for mucuna and soybean, while germination under light for 24 h caused no changes or even decreases (jack bean and mucuna) of protein content.

The nutritive value of these legumes depends on the availability of the seed proteins for digestion and absorption in the digestive tract. Thus, due to the physico-chemical characteristics of dietary fibre, a proportion of protein remained associated to the fibre matrix being less nutritionally available. The distribution of protein between fibre fractions showed that significant percentages of protein still remained insoluble in DF which represented between 23–43% of the total protein and was mainly associated to IDF fraction (≅90%). In soybean and mucuna, the percentages of proteins associated to DF were lower (27 and 23%, respectively) than those found in the other legumes. The results brought about that a portion of protein was linked to the insoluble matrix (cellulose, hemicelluloses, lignin, polyphenols, ...) and was not hydrolyzed to lower molecular weight components (small peptides, amino acids, etc.), being retained in this fibre fraction (Bravo, 1998).

However, no significant changes on the protein associated to the DF fractions were observed as consequence of germination, except for mucuna (0 h-L and 12 h-L) and cowpea (12 h-L) which exhibited decreases (9–27% of the IDF protein). Thus, the variations of IDF values were mainly due to the gravimetric residues found in germinated seeds, but not accompanied by a lower protein content associated with the fibre matrix as was observed in germinated peas (Martín-Cabrejas et al., 2003).

Table 3
Content of total protein and protein associated to insoluble, soluble, and total dietary fibre of raw and processed legume flours (g kg^{-1} DM)

Samples	Light conditions of germination	Total protein	IDF protein	SDF protein	TDF protein
Cowpea	Control	305.6 ± 23.8^{a}	113.1 ± 6.1^{b}	11.9 ± 0.6^{c}	125.0
	0 h-L	372.8 ± 17.5^{b}	113.1 ± 6.0^{b}	9.0 ± 0.5^{a}	122.1
	12 h-L	430.3 ± 19.3^{c}	86.7 ± 4.8^{a}	11.9 ± 1.0^{c}	98.6
	24 h-L	333.4 ± 16.3^{a}	105.6 ± 6.3^{b}	10.1 ± 0.5^{b}	115.7
Jack bean	Control	321.2 ± 13.7^{b}	102.1 ± 4.9^{a}	9.7 ± 0.6^{b}	111.8
	0 h-L	313.5 ± 14.0^{b}	110.8 ± 5.4^{a}	15.1 ± 0.8^{c}	125.9
	12 h-L	364.9 ± 17.0^{c}	104.8 ± 4.8^{a}	6.6 ± 0.5^{a}	111.4
	24 h-L	274.3 ± 12.7^{a}	109.3 ± 4.7^{a}	13.7 ± 0.9^{c}	123.0
Dolichos	Control	292.8 ± 16.7^{a}	113.7 ± 6.1^{a}	10.8 ± 0.9^{b}	124.5
	0 h-L	327.0 ± 15.9^{b}	92.0 ± 4.9^{a}	20.8 ± 1.2^{d}	112.8
	12 h-L	354.1 ± 18.1^{b}	101.3 ± 6.3^{a}	14.1 ± 1.0^{c}	115.4
	24 h-L	284.9 ± 14.3^{a}	91.8 ± 5.4^{a}	2.0 ± 0.1^{a}	93.8
Mucuna	Control	309.3 ± 15.1^{b}	71.7 ± 4.7^{c}	2.0 ± 0.1^{a}	71.7
	0 h-L	283.4 ± 14.7^{b}	52.4 ± 3.9^{a}	4.6 ± 0.3^{c}	57.0
	12 h-L	306.6 ± 15.1^{b}	65.1 ± 4.3^{b}	2.9 ± 0.1^{b}	68.0
	24 h-L	246.8 ± 12.4^{a}	75.5 ± 5.0^{c}	4.3 ± 0.4^{c}	79.0
Soybean	Control	433.8 ± 20.9^{a}	115.8 ± 6.7^{a}	7.8 ± 0.3^{d}	123.6
	0 h-L	455.9 ± 22.7^{a}	97.5 ± 6.0^{a}	2.7 ± 0.2^{b}	100.2
	12 h-L	449.4 ± 23.5^{a}	114.6 ± 6.2^{a}	2.2 ± 0.2^{a}	116.8
	24 h-L	442.7 ± 25.1^{a}	117.8 ± 5.9^{a}	3.3 ± 0.2^{c}	121.1

Mean values of each column followed by different superscript letter significantly differ when subjected to Duncan's multiple range test ($p < 0.05$). (Mean ± SD ($n = 4$)).

The levels of monosaccharides, disaccharides and raffinose family oligosaccharides (RFOs) are presented in Table 4. The presence of ciceritol was also considered, although it could not be confirmed, due to the lack of a commercial standard available. The raw legumes analyzed differed from each other in the different amounts of total soluble sugars, ranging from 38.4 to 63.7 g kg^{-1} of DM. Mucuna showed the highest levels of total soluble sugars, sucrose and maltose being the main components (≅44% and 22% of the total sugar content, respectively). The RFOs content (21.7 g kg^{-1} DM) was similar to that of dolichos and accounted for 34% of the total sugar content, lower than in the other legumes, jack bean (70%), cowpea (66%) and soybean (57%). The main α-galactosides, raffinose and stachyose, which were also detected in earlier reports (Oboh et al., 2000; Siddhuraju et al., 2000), appeared in mucuna at similar levels. Soybean exhibited also high level of total soluble sugars, similar to those of mucuna; however stachyose was the predominant sugar (45%) followed by sucrose (36%). The level of RFOs in soybean in the present study (34.7 g kg^{-1} DM) was low when compared to the values reported earlier (Egounlety & Aworh, 2003), which could be due to differences in the environmental conditions, as well as genotypes studied and ripeness of seeds.

The rest of the legumes (cowpea, jack bean and dolichos) showed lower contents of total soluble sugars (55.0, 41.9 and 38.4 g kg^{-1} DM, respectively) although their RFOs contents were different depending on the legume. Cowpea showed RFOs levels similar to that of soybean, while jack bean and specially dolichos had lower levels. However, in every case RFOs content accounted for more than 50% of total soluble sugars. The profile of oligosaccharides is different depending on the tropical legume. Stachyose is found to be the main flatulent oligosaccharide in cowpea and dolichos, accounting for 59% and 46% of total sugars, respectively. The contents of RFOs of cowpea and dolichos investigated in this study were comparable to those found in the literature (Egounlety & Aworh, 2003; Onyenekwe et al., 2000). However, the results obtained in raw jack bean showed that raffinose was the main oligosaccharide (29%), along with another galactoside corresponding to a peak after raffinose and before stachyose; it was probably ciceritol and other digalactocyclitols, and comprised 27%. Stachyose is also found as the minor galactoside (14%), similar to that found by other authors (Pugalenthi, Siddhurajue, & Vadivel, 2006). Therefore, the galactoside fraction found in these legume seeds was mainly the raffinose family typical of common legume seeds (Vidal-Valverde et al., 1998, 2002).

Regarding the monosaccharide contents, these tropical legumes showed small amounts of fructose, glucose and galactose, which represented around 4% of the total sugar content. These results are in agreement with the earlier reports in common legumes (Frías et al., 1996; Martín-Cabrejas et al., 2006; Vidal-Valverde et al., 2002).

Germination process produces a general increase in the total soluble sugars (from 20 to 161%), accompanied by a drastic decrease in the α-galactoside content (from 98 to 63%). This behaviour is reported by earlier studies with conventional legumes (Frías et al., 1996; Vidal-Valverde et al., 1998, 2002). The highest increases of total soluble

Table 4
Content of soluble carbohydrate in raw and processed legume flours (g kg^{-1} DM)

Light conditions germination	Ribose	Arabinose	Fructose	Glucose	Galactose	Sucrose	Maltose	Mannotriose	Raffinose	Ciceritol	Stachyose	RFOs	Total sugars
Cowpea control	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.1 ± 0.1^{a}	n.d.	2.0 ± 0.1^{a}	13.9 ± 0.8^{a}	0.6 ± 0.1^{a}	0.7 ± 0.1^{a}	4.0 ± 0.3^{b}	0.4 ± 0.1^{a}	32.2 ± 2.0^{b}	36.6	55.0
0 h-L	0.4 ± 0.1^{a}	0.6 ± 0.1^{b}	0.1 ± 0.1^{a}	1.8 ± 0.1^{c}	4.8 ± 0.2^{b}	46.9 ± 1.8^{b}	10.1 ± 0.8^{b}	9.2 ± 0.7^{b}	0.6 ± 0.3^{a}	1.8 ± 0.1^{b}	1.6 ± 0.1^{a}	4.0	77.9
12 h-L	0.4 ± 0.1^{a}	0.7 ± 0.2^{b}	0.1 ± 0.1^{a}	0.7 ± 0.2^{a}	5.7 ± 0.2^{c}	45.4 ± 2.1^{b}	9.8 ± 0.5^{b}	8.5 ± 0.7^{b}	0.5 ± 0.2^{a}	2.0 ± 0.2^{b}	n.d.	2.5	73.8
24 h-L	0.4 ± 0.1^{a}	0.6 ± 0.2^{b}	0.1 ± 0.1^{a}	1.2 ± 0.2^{b}	6.2 ± 0.3^{c}	48.7 ± 2.5^{b}	10.6 ± 0.7^{b}	8.9 ± 0.8^{b}	0.5 ± 0.1^{a}	1.7 ± 0.1^{b}	n.d.	2.2	78.9
Jack bean control	n.d.	n.d.	n.d.	1.6 ± 0.2^{a}	n.d.	9.4 ± 0.7^{a}	1.2 ± 0.6^{a}	n.d.	11.8 ± 0.9^{c}	11.1 ± 0.5^{b}	5.9 ± 0.1^{c}	28.8	41.0
0 h-L	n.d.	n.d.	n.d.	8.6 ± 0.1^{c}	2.2 ± 0.2^{a}	51.4 ± 4.1^{c}	3.3 ± 0.2^{b}	17.9 ± 1.3^{b}	0.8 ± 0.2^{a}	n.d.	1.3 ± 0.1^{a}	2.1	85.5
12 h-L	n.d.	n.d.	n.d.	9.6 ± 0.3^{d}	2.5 ± 0.1^{a}	59.2 ± 4.3^{c}	3.6 ± 0.3^{b}	30.2 ± 2.2^{c}	0.6 ± 0.1^{a}	n.d.	n.d.	0.6	105.7
24 h-L	0.6 ± 0.1^{a}	n.d.	n.d.	6.8 ± 0.2^{b}	1.9 ± 0.1^{a}	38.6 ± 2.8^{b}	6.8 ± 0.5^{c}	12.0 ± 0.9^{a}	2.5 ± 0.2^{b}	1.3 ± 0.1^{a}	2.2 ± 0.1^{b}	6.0	74.1
Dolichos control	n.d.	n.d.	n.d.	n.d.	1.3 ± 0.1^{a}	14.9 ± 0.9^{a}	n.d.	n.d.	4.3 ± 0.4^{c}	0.4 ± 0.1^{a}	17.5 ± 0.7^{c}	22.2	38.4
0 h-L	0.2 ± 0.1^{a}	n.d.	1.0 ± 0.2^{b}	0.5 ± 0.1^{a}	2.0 ± 0.2^{b}	67.8 ± 5.8^{c}	6.5 ± 0.5^{b}	7.3 ± 0.6^{a}	1.2 ± 0.2^{a}	0.8 ± 0.1^{b}	1.6 ± 0.1^{a}	3.6	88.9
12 h-L	0.3 ± 0.1^{a}	n.d.	1.9 ± 0.2^{c}	0.5 ± 0.1^{a}	3.9 ± 0.2^{c}	71.9 ± 5.9^{c}	8.6 ± 0.4^{c}	7.7 ± 0.6^{a}	2.9 ± 0.3^{b}	1.1 ± 0.1^{c}	1.9 ± 0.1^{b}	5.9	100.7
24 h-L	0.4 ± 0.1^{a}	n.d.	0.6 ± 0.1^{a}	0.5 ± 0.1^{a}	9.8 ± 0.3^{d}	39.8 ± 2.8^{b}	4.7 ± 0.3^{a}	11.1 ± 1.0^{b}	n.d.	1.2 ± 0.1^{c}	1.6 ± 0.1^{a}	2.8	69.7
Mucuna control	n.d.	n.d.	0.1 ± 0.1^{a}	n.d.	0.4 ± 0.1^{a}	27.8 ± 1.9^{a}	13.7 ± 1.1^{a}	n.d.	10.9 ± 0.5^{c}	n.d.	10.8 ± 0.7^{c}	21.7	63.7
0 h-L	n.d.	n.d.	0.5 ± 0.1^{b}	0.2 ± 0.1^{a}	0.5 ± 0.1^{a}	67.6 ± 4.9^{b}	20.5 ± 1.5^{b}	0.4 ± 0.1^{a}	n.d.	0.6 ± 0.1^{a}	2.9 ± 0.1^{b}	3.5	93.2
12 h-L	n.d.	n.d.	0.5 ± 0.1^{b}	0.2 ± 0.1^{a}	0.6 ± 0.1^{a}	64.7 ± 4.5^{b}	21.9 ± 1.6^{b}	0.5 ± 0.1^{a}	0.2 ± 0.1^{a}	0.8 ± 0.1^{a}	2.5 ± 0.1^{a}	3.5	91.9
24 h-L	n.d.	n.d.	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}	0.6 ± 0.2^{a}	95.7 ± 7.9^{c}	37.3 ± 2.9^{c}	0.6 ± 0.2^{a}	4.8 ± 0.2^{b}	0.7 ± 0.1^{a}	2.6 ± 0.1^{a}	8.1	143.2
Soybean control	n.d.	n.d.	n.d.	2.4 ± 0.6^{a}	n.d.	22.1 ± 1.8^{a}	2.0 ± 0.3^{a}	0.2 ± 0.1^{a}	6.7 ± 0.2^{d}	0.5 ± 0.1^{a}	27.5 ± 1.3^{b}	34.7	61.4
0 h-L	1.0 ± 0.3^{b}	n.d.	0.2 ± 0.1^{a}	4.9 ± 0.3^{b}	0.6 ± 0.1^{a}	39.7 ± 2.2^{c}	8.5 ± 0.7^{c}	16.9 ± 0.9^{c}	3.7 ± 0.1^{c}	1.3 ± 0.1^{b}	n.d.	5.0	76.8
12 h-L	0.2 ± 0.1^{a}	n.d.	0.5 ± 0.1^{b}	5.0 ± 0.4^{b}	1.2 ± 0.1^{b}	47.3 ± 2.5^{d}	8.8 ± 0.7^{c}	6.7 ± 0.7^{b}	2.7 ± 0.1^{b}	1.0 ± 0.1^{b}	n.d.	3.7	73.4
24 h-L	1.2 ± 0.1^{a}	n.d.	1.4 ± 0.4^{c}	3.2 ± 0.4^{a}	1.8 ± 0.1^{c}	26.1 ± 1.8^{b}	5.5 ± 0.4^{b}	5.9 ± 0.5^{b}	2.1 ± 0.1^{a}	1.8 ± 0.3^{c}	2.1 ± 0.1^{a}	6.0	51.1

Mean values of each column followed by different superscript letter significantly differ when subjected to Duncan's multiple range test ($p < 0.05$). (Mean ± SD ($n = 4$)). n.d. non-detected. RFOs: raffinose family oligosaccharides (raffinose + stachyose + ciceritol).

sugars were exhibited by dolichos, jack bean and mucuna. The effect of germination on RFOs content was more accentuated in jack bean and cowpea which experienced the largest reduction of α-galactosides, reaching 98% and 94%, respectively, followed by dolichos (87%), soybean (86%) and mucuna (84%). These results are greater than the corresponding RFOs reductions obtained by soaking, cooking and comparable to fermentation processing or addition of α-galactosidase (Egounlety & Aworh, 2003; Pugalenthi et al., 2006). Thus, germination was an efficient process to reduce the levels of α-galactosides in these under-utilized legumes although its influence was different depending on the type of legume. The appreciable losses of oligosaccharides caused during germination are due to the increased activity of the enzyme α-galactosidase which hydrolyses the α-1-6-galatosidic linkages thereby causing an increase in the total soluble sugar content.

Comparing individual α-galactosides, stachyose was the main oligosaccharide that showed the largest reduction when these legumes were submitted to the different germination conditions, followed by raffinose and ciceritol. This is because α-1-6-galatosidase, during germination first attacks stachyose followed by raffinose (Dey, 1985).

Mono- and oligosaccharides other than α-galactosides were also affected by processing; an important increase of sucrose, maltose and mannotriose was shown. The levels of sucrose were significantly higher in germinated jack bean than in raw seed, followed by dolichos, cowpea, mucuna and soybean. The contents of maltose also increased but to a lesser extent. Glucose, galactose and fructose not even present in most of studied legumes suffered a large increase as a consequence of starch and galactosides hydrolysis and increase of α-amylase activity (Martín-Cabrejas et al., 2003; Vidal-Valverde et al., 2002). The predominance of galactose throughout the germination period suggests that the α-D-galactosidase acts on the oligosaccharides at the galactose moety (Egounlety & Aworh, 2003).

The results obtained in the present work indicate that the changes depend on the germination conditions and also the type of legume. In cowpea, a general increase of total soluble sugars in germinated seeds was shown (40%), but no relevant differences were detected in the total soluble sugar contents due to different light conditions during germination. However, mucuna, jack bean and dolichos exhibited a different behaviour, and the presence of light during germination led to higher levels of total soluble sugars.

In conclusion, the legume seeds selected for this study showed particular characteristics. The germination of these non-conventional seeds under different light conditions produces changes in the carbohydrate fraction, including an increase of TDF in most instances accompanied by an overall decrease in the content of α-galactosides and a corresponding increase on the amounts of total soluble sugars. Thus, these changes contribute to the increase of the nutritional value of these non-conventional seeds for human nutrition. This fact results in foodstuffs more digestible, making germination a useful process in the development of weaning foods with improved digestibility.

Acknowledgements

The authors wish to thank the University Autónoma of Madrid (UAM-SCH) for their financial support to the cooperation with Latin America (Project 2005–2006).

References

Adebowale, Y. A., Adeyemi, A., & Oshodi, A. A. (2005). Variability in the physicochemical, nutritional and antinutritional attributes of six *Mucuna* species. *Food Chemistry, 89*, 37–48.

Akpapunam, M. A., & Sefa-Dedeh, S. (1998). Some physicochemical properties and anti-nutritional factors of raw, cooked and germinated jack bean (*Canavalia ensiformis*). *Food Chemistry, 59*, 121–125.

Alabi, D. A., & Alausa, A. A. (2006). Evaluation of the mineral nutrients and organic food content of the seeds of *Lablab purpureus*, *Leucaena Leucocaphala* and *Mucuna utilis* for domestic consumption and industrial utilization. *World Journal of Agricultural Sciences, 2*, 115–118.

AOAC (1995). *Official methods of analysis* (16th ed.). Washington: Association of Official Analytical Chemists.

Asp, N. G. (1996). Dietary carbohydrates: Classification by chemistry and physiology. *Food Chemistry, 57*, 9–14.

Bender, F. E., Douglass, L. W., & Kramer, A. (1989). *Statistical methods for food and agriculture*. New York: Food Products Press.

Betancur-Ancona, D., Peraza-Mercado, G., Miguel-Ordóñez, Y., & Fuertes-Blanco, S. (2004). Physicochemical characterization of lima bean (*Phaseolus lunatus*) and jack bean (*Canavalia ensiformis*) fibrous residues. *Food Chemistry, 84*, 287–295.

Bouhnik, Y., Raskine, L., Simoneau, G., Vicaut, E., Neut, C., Flourié, B., et al. (2004). The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. *American Journal of Clinical Nutrition, 80*, 1658–1664.

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews, 56*, 317–333.

Chang, H.-L., Sang, H.-O., Eun, J.-Y., & Young, S.-K. (2006). Effects of raw, cooked and germinated small black soybean powders on dietary fibre content and gastrointestinal functions. *Food Science and Biotechnology, 15*, 635–638.

Chang, K. C., & Harrold, R. L. (1988). Changes in selected biochemical components *in vitro* protein digestibility and amino acids in two bean cultivars during germination. *Journal of Food Science, 53*, 783–787.

Dey, P. M. (1985). D-Galactose containing oligosaccharides. In P. M. Dey (Ed.), *Biochemistry of storage carbohydrates in green plants* (pp. 5–129). London: Academic Press.

Díaz, M. F., Torres, V., González, A., & Node, A. (2004). Biotransformaciones en el germinado de *Vigna unguiculata*. *Revista Cubana de Ciencia Agrícola, 38*, 91–95.

Egounlety, M., & Aworh, O. C. (2003). Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms). *Journal of Food Engineering, 56*, 249–254.

Frías, J., Vidal-Valverde, C., Kozlowska, H., Tabera, J., Honke, J., & Hedley, C. L. (1996). Natural fermentation of lentils. Influence of time, flour concentration, and temperature on the kinetics of monosaccharides, disaccharides and α-galactosides. *Journal of Agricultural and Food Chemistry, 44*, 579–584.

Giami, S. Y., Okusa, M. O., & Emelike, J. N. (2001). Evaluation of selected food attributes of four advanced lines of ungerminated and

germinated Nigerian cowpea (*Vigna unguiculata* (L.) Walp.). *Plant Foods Human for Nutrition, 56*, 61–73.

Guillon, F., & Champ, M. M.-J. (2002). Carbohydrate fractions of legumes: Uses in human nutrition and potential for health. *British Journal of Nutrition, 88*(2), S293–S306.

Janardhanan, K., Vadivel, V., & Pugalenthi, M. (2003). Biodiversity in Indian underexploited/tribal pulses. In P. K. Jaiwal & R. P. Singh (Eds.), *Improvement strategies for leguminosae biotecnology* (pp. 353–405). London: Kluwer Academic Publishers..

Jenkins, D. J. A., Kendall, C. W. C., & Ransom, T. P. P. (1998). Dietary fibre, the evolution of the human diet and coronary heart disease. *Nutrition Research, 18*, 633–652.

Kumar, V., Rani, A., Pandey, V., & Chauhan, G. S. (2006). Changes in lipoxygenase isozymes and trypsin inhibitor activity in soybean during germination at different temperatures. *Food Chemistry, 99*, 563–568.

Liener, I. E. (1989). Control of antinutritional and toxic factors in oilseeds and legumes. In E. W. Lusas, D. R. Erickson, & W. Nip (Eds.), *Food uses of whole oil and protein seeds* (pp. 344–371). Illinois: American Oil Chemistry Society.

Maass, B. L. (2006). Changes in seed morphology, dormancy and germination from wild to cultivated hyacinth bean germplasm (*Lablab purpureus*: Papilionoideae). *Genetic Resources and Crop Evolution, 53*, 1127–1135.

Madhurima, D., & Mangala, G. (2003). Effect of sprouting on nutrients, antinutrients and *in vitro* digestibility of the MACS-13 soybean variety. *Plant Foods Human for Nutrition, 58*, 11–16.

Mahadevamma, S., & Tharanathan, R. N. (2004). Processing of legumes: Resistant starch and dietary fiber contents. *Journal of Food Quality, 27*, 289–303.

Martín-Cabrejas, M. A., Aguilera, Y., Benítez, V., Molla, E., López-Andréu, F. J., & Esteban, R. M. (2006). Effect of industrial dehydration on soluble carbohydrates and dietary fiber fractions in legumes. *Journal of Agricultural and Food Chemistry, 54*, 7652–7657.

Martín-Cabrejas, M. A., Ariza, N., Esteban, R. M., Mollá, E., Waldron, K. W., & López-Andréu, F. J. (2003). Effect of germination on the carbohydrate composition of the dietary fiber of peas (*Pisum sativum* L.). *Journal of Agricultural and Food Chemistry, 51*, 1254–1259.

Martín-Cabrejas, M. A., Sanfiz, B., Vidal, A., Molla, E., Esteban, R. M., & López-Andréu, F. J. (2004). Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry, 52*, 261–266.

Messina, M. J. (1999). Legumes and soybeans: Overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition, 70*, 439–450.

Oboh, H. A., Muzquiz, M., Burbano, C., Cuadrado, C., Pedrosa, M. M., Ayet, G., et al. (2000). Effect of soaking, cooking and germination on the oligosaccharide content of selected Nigerian legume seeds. *Plant Foods Human for Nutrition, 55*, 97–110.

Onyenekwe, P. C., Njoku, G. C., & Ameh, D. A. (2000). Effect of cowpea (*Vigna unguiculata*) processing methods on flatus causing oligosaccharides. *Nutrition Research, 20*, 349–358.

Pugalenthi, M., Siddhurajue, P., & Vadivel, V. (2006). Effect of soaking followed by cooking and the addition of α-galactoside on oligosaccharides levels in different *Canavalia* accessions. *Journal of Food Composition and Analysis, 19*, 512–517.

Rehiman, Z., Rashid, M., & Shah, W. H. (2004). Insoluble dietary fibre components of food legumes as affected by soaking and cooking processes. *Food Chemistry, 85*, 245–249.

Sánchez-Mata, M. C., Peñuela-Teruel, M. J., Cámara-Hurtado, M., Díez-Marqués, C., & Torija-Isasa, E. (1998). Determination of mono-, di-, and oligosaccharides in legumes by high-performance liquid chromatography using an amino-bonded silica column. *Journal of Agricultural and Food Chemistry, 46*, 3648–3652.

Siddhuraju, P., Becker, K., & Makkar, H. P. (2000). Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilized tropical legume, *Mucuna pruriens* var. Utilis. *Journal of Agricultural and Food Chemistry, 48*, 6048–6060.

Tuohy, K. M., Rouzaud, G. C. M., Brück, W. M., & Gibson, G. R. (2005). Modulation of the human gut microflora towards improved health using prebiotics. *Assessment of efficacy. Current Pharmaceutical Design, 11*, 75–90.

Veena, A., Urjo, A., & Puttaraj, S. (1995). Effect of processing on the composition of dietary fibre and starch in some legumes. *Die Nahrung, 39*, 132–138.

Vidal-Valverde, C., Frías, J., Sierra, I., Blázquez, I., Lambein, F., & Kuo, Y.-H. (2002). New functional legume foods by germination: Effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology, 215*, 472–477.

Vidal-Valverde, C., Frías, J., Sotomayor, C., Díaz-Pollán, C., Fernández, M., & Urbano, G. (1998). Nutrients and antinutritional factors in faba beans as affected by processing. *Zeitschrift für Lebensmittel Untersuchung und Forschung, 207*, 140–145.

Waterlow, J. C. (1994). Childhood malnutrition in developing nations: Looking back and looking forward. *Annual Review of Nutrition, 14*, 1–19.