JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Germination on the Carbohydrate Composition of the Dietary Fiber of Peas (*Pisum sativum* L.)

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The effect of different conditions of pea germination on dietary fiber (DF) composition was studied. Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were subjected to acid hydrolysis, and the resultant neutral sugars, uronic acids, and Klason lignin were quantified. Germinated peas exhibited significantly higher contents of total dietary fiber (TDF) than the raw sample, due to the increases of both DF fractions. Under darkness conditions, germination exhibited the highest contents of IDF and SDF. Decreasing IDF/SDF ratios showed that the carbohydrate changes did not take place to the same extent during germination, the SDF fraction being the most affected. The detailed chemical composition of fiber fractions reveals increases of cellulose in the IDF of germinated samples, whereas SDF exhibits a decrease of pectic polysaccharides and also increases of polysaccharides rich in glucose and mannose. The DF results were corroborated by a comparative examination of the cell wall carbohydrate composition.

KEYWORDS: Peas (*Pisum sativum* L.); dietary fiber; carbohydrates; germination conditions

INTRODUCTION

Legumes are a good source of protein and calories and they are widely consumed all over the world (*1*). Legumes are often advocated in Western diets because of their beneficial nutritional effects (*2*); they are considered to be good sources of structural carbohydrate mainly due to their higher soluble dietary fiber (SDF) content when compared to other fiber-rich plant foods such as cereals and tubers (*3*, *4*). Increased amounts of legumes are nowadays recommended for inclusion in the diets of diabetics because of their apparent beneficial effect in reducing postprandial glycemia (*5*). However, in some countries legumes are not utilized sufficiently in the human diet because of their antinutritional factors such as lectins and enzyme inhibitors. In the search for possibilities of better utilization and increased consumption of legume seeds, various studies have been done to improve the attractiveness and nutritional value of foods prepared from the seeds (*6*−*8*).

Germination has been identified as an inexpensive and effective technology for improving the quality of legumes, by enhancing their digestibility (*9*, *10*), increasing the content of amino acids (*11*), and reducing the levels of antinutrients commonly found in legumes (*12*, *13*). 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 about the effect of such treatments on the carbohydrate and dietary fiber (*14*, *15*). The study of the above components is relevant in elucidating germination-related metabolic changes associated with the release of storage components and development of new tissues. Germination of legumes involves complex processes that involve the breakdown of macromolecules such as starch and proteins into smaller units (*16*). This results in more easily digestible foodstuffs, making germination a useful process in the development of weaning foods with improved digestibility.

Among the legumes, dry peas provide a good source of protein, vitamins, and a balanced range of minerals. They are also an excellent source of complex carbohydrates and dietary fiber (*17*). In Western countries peas find a place in a variety of day-to-day food dishes.

The aim of this work was to evaluate the effect of the germination process in peas on the content of dietary fiber and its fractions in order to obtain pea flours with high nutritive value, which may be utilized for human consumption as part of the diet or in the fortification of foods.

MATERIALS AND METHODS

Germination. *Pisum sativum* var. Arvense cv. Esla from Valladolid (Spain) were used for the germination experiments, which were performed in duplicate. The germination procedure for pea seeds was as follows: 500 g of seeds was washed with 0.7% sodium hypochlorite, soaked in 2.5 L of distilled water at room temperature for 6 h, and shaken every 30 min. The water was then drained off, and the seeds

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10.1021/jf0207631 CCC: $25.00
Published on Web 01/28/2003

were transferred to a separating funnel, where two different germination conditions were applied: samples were germinated during 2, 4, and 6 days at 20 °C, under darkness (referred to as NL2D, NL4D, and NL6D) and daylight (referred to as L2D, L4D, and L6D) conditions.

A total of 90−100% of the seeds germinated, and the sprouts and the seeds were ground and freeze-dried for analysis.

Total Nitrogen Determination. Total nitrogen content was determined in triplicate from each sample by using the Kjeldahl procedure (*18*).

Dietary Fiber (DF) Determination. Mes-Tris AOAC method 991.43 was used for DF determination (*18*). 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. DF fractions were obtained as indigestible residues after enzymatic digestion of nondietary fiber components; the insoluble residues were isolated by filtration, and soluble fiber was precipitated with ethanol. Dried residues correspond to insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), respectively. Determination of residual ashes and protein (as Kjeldahl N × 6.25) was carried out in the residues for corresponding corrections. Total dietary fiber (TDF) was calculated as the sum of IDF and SDF. Kjeldahl nitrogen and ash contents were assayed according to standard procedures (*18*).

Chemical Analysis of DF Components. The composition of DF was determined after acid hydrolysis of fiber residues obtained according to the AOAC modified method 991.43 (*18*), where soluble fraction was obtained by dialysis for 48 h at 4°C against distilled water.

IDF residues (100.0 ± 0.1 mg) were subjected to 12 M H_2SO_4 treatment for 3 h at room temperature, followed by dilution to 0.6 M H_2SO_4 hydrolysis at 100 °C for 3 h. In addition, the insoluble fiber residues were also hydrolyzed by 0.6 M H_2SO_4 at 100 °C for 3 h. SDF residues (100.0 ± 0.1 mg) were only hydrolyzed with 0.6 M H_2SO_4 at 100 °C for 3 h (*19*). The acid hydrolysis released the different fiber components, neutral sugars, and uronic acids. The insoluble residue after 12 + 0.6 M H_2SO_4 hydrolysis was recovered quantitatively over a glass filter (Pyrex no. 4), washed thoroughly with pure water, and dried for 18 h at 105 °C corresponding to Klason lignin residue.

The hydrolysates were neutralized using AG4-X4 resin (Bio-Rad Laboratories, Richmond, CA). The neutral sugar composition of the hydrolysates was determined by HPLC using a microguard column (Aminex Carbo-P, Bio-Rad, Richmond, CA) in series with a carbohydrate analysis column (Aminex HPX-87P heavy metal, 300 mm × 7.8 mm, Bio-Rad Laboratories, Richmond, CA) operated at a flow rate of 0.5 mL/min using a refractive index detector. Galactose and rhamnose coelute from this column. The amounts of sugars present were computed using System Gold 7.0 version software after calibration with standard sugars (Sigma, St. Louis, MO). Erythritol (Sigma), added just before neutralization, was used as the internal standard. Recoveries from the hydrolytic procedure were determined by subjecting standard sugars to the total analytical procedure (*20*).

Uronic acids were determined colorimetrically by adapting the 3-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen (*21*) with D-galacturonic acid (Sigma) (*19*).

Preparation of Alcohol-Insoluble Residue (AIR). Pea flours were homogenized in a Waring blender (Fisher Scientific Instrument, Loughborogh, U.K.) with cold ethanol (85% v/v final concentration). The cold homogenate was transferred to a stainless steel beaker and homogenized with an Ystral homogenizer (Scientific Instruments Ltd., Manchester, U.K.) for 1 min. The homogenate was filtered through 100 μm nylon mesh (John Stannier and Co., Manchester, U.K.). The residue was further homogenized twice in 85% (v/v) ethanol before being extracted with phenol buffer with Tris (250 mL, pH 7; Sigma) for 45 min (*22*). The buffered phenol was prepared by the addition of 100 mL of 500 mM Tris, pH 7.5, to 200 g of phenol. The suspension was stirred and allowed to stand for 8 h. The residue was recovered by centrifugation and washed three times with ethanol (85% v/v). The residue was then resuspended in 90% DMSO and left to stir overnight. A number of extractions in DMSO were performed to completely remove the starch. The sample was checked as starch free by staining with 3% I_2/IK. The sample was washed with acetone and dried overnight in a fume cupboard.

Table 1. Content of Insoluble, Soluble, and Total Dietary Fiber and Its Distribution in Germinated Peas (Percent DM)[a]

sample	IDF	SDF	TDF	IDF/SDF
raw	9.7 ± 0.7a	5.6 ± 0.4a	15.3	1.73
L2D	10.0 ± 0.8a	9.5 ± 0.8b	19.5	1.05
L4D	10.6 ± 0.7a	9.1 ± 0.8b	19.7	1.16
L6D	11.5 ± 0.8b	9.2 ± 0.9b	20.7	1.25
NL2D	8.6 ± 0.6a	6.9 ± 0.4c	15.5	1.24
NL4D	11.7 ± 0.6b	12.7 ± 0.9d	24.4	0.93
NL6D	13.2 ± 0.7c	13.8 ± 1.0d	27.0	0.96

[a] Mean ± SD ($n = 6$). Mean values of each column followed by a different letter significantly differ when subjected to DMRT ($P < 0.05$).

Table 2. Content of Protein in Germinated Peas and in Their Fractions of Insoluble, Soluble, and Total Dietary Fiber (Percent DM)[a]

sample	initial protein	protein IDF	protein SDF	protein TDF
raw	25.5 ± 0.1a	1.8 ± 0.1a	7.7 ± 0.1a	9.6
L2D	24.3 ± 0.2a	0.9 ± 0.1b	8.6 ± 0.1b	9.5
L4D	25.1 ± 0.2a	1.1 ± 0.1c	8.3 ± 0.1c	9.4
L6D	24.9 ± 0.2a	1.2 ± 0.3c	6.6 ± 0.1d	7.8
NL2D	24.6 ± 0.3a	0.9 ± 0.1b	10.0 ± 0.2e	10.9
NL4D	24.0 ± 0.6a	0.7 ± 0.1c	8.4 ± 0.1bd	9.1
NL6D	25.8 ± 0.3a	1.3 ± 0.1c	5.7 ± 0.1f	7.0

[a] Mean ± SD ($n = 6$). Mean values of each column followed by a different letter significantly differ when subjected to DMRT ($P < 0.05$).

Sequential Extraction of AIR. AIR (1 g) was suspended in cyclohexane-*trans*-1,2-diamine-*N*,*N*,*N*′,*N*′-tetraacetate (CDTA, sodium salt, 0.05 M, 100 mL, pH 7; Aldrich, Poole, U.K.) twice and stirred for 2 h at 1 °C; the residue was then extracted in Na_2CO_3 (0.05 M, 100 mL; containing 0.02 M $NaBH_4$) twice, at 1 °C and then at 20 °C and KOH (0.5 M, 100 mL; containing 0.02 M $NaBH_4$) as described by Waldron and Selvendran (*23*). The supernatants were filtered, neutralized when required, and dialyzed exhaustively with distilled water prior to concentration and freeze-drying. Cell-wall neutral sugars and uronic acids were analyzed as described previously.

Statistical Analysis. Results were analyzed using Duncan's multiple-range test (DMRT) (*24*). Differences were considered to be significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Of nutritional interest is the study of dietary fiber fractions due to their important physiological properties. The levels of IDF and SDF were important in this pea variety (**Table 1**), which are higher than those found in cereal seeds (*25*) and slightly lower compared to other legumes (*26*, *27*). Thirty-six percent was in the SDF form, a percentage significantly higher than in the DF from many other plant foods (*25*).

The germination process had a significant impact on these values (**Table 1**), showing the largest changes in peas germinated in darkness conditions. The highest IDF levels were found at 6 days of germination in darkness, which resulted in a 36% increase in IDF content. In contrast, the presence of light resulted in an increase of only 18% in the IDF level. The increase of IDF values is due to higher gravimetric residues found in germinated peas, accompanied by a lower protein content associated with the fiber matrix. A 63% solubilization of this component (**Table 2**), which can be more easily hydrolyzed by the endogenous proteases activated by this process, was observed.

SDF also increased in all germination conditions. In the presence of light, the levels of SDF increased by 65% after 2

Table 3. Composition of Insoluble Dietary Fiber in Germinated Peas

	carbohydrates[a] (%)						total sugars[b]	Klason lignin[c]	total	
sample	Glc	Xyl	Gal/Rha	Ara	Man	UA	(g kg^{-1} of DM)	(g kg^{-1} of DM)	(g kg^{-1} of DM)	UA/NS
raw	44	6	4	20	1	25	88.0 ± 0.7a	7.3 ± 0.8a	95.3	1.00
L2D	67	4	tr	5	6	18	90.4 ± 3.7a	4.2 ± 0.6b	94.6	3.60
L4D	76	5	tr	5	2	12	118.1 ± 2.5d	2.3 ± 0.7c	120.4	2.40
L6D	77	4	tr	8	1	10	116.0 ± 4.0d	2.2 ± 0.5c	118.2	1.25
NL2D	58	3	tr	12	7	20	97.6 ± 1.0b	4.1 ± 0.7b	101.7	1.67
NL4D	81	4	tr	3	3	9	136.3 ± 2.5c	6.2 ± 0.8a	142.5	3.00
NL6D	71	4	tr	8	5	12	136.8 ± 5.1c	9.3 ± 1.0d	146.1	1.50

[a] Data expressed as percentage of total sugars. [b] Mean ± SD ($n = 3$). Mean values of each column followed by a different letter significantly differ when subjected to DMRT ($P < 0.05$). [c] Mean ± SD ($n = 6$). Mean values of each column followed by a different letter significantly differ when subjected to DMRT ($P < 0.05$).

days postgermination, and this was maintained after 4 and 6 days. In contrast, germination and growth in darkness resulted in a continuing increase in SDF over the 6 day period, which was 2.5 times higher than the control at 6 days. This is in agreement with observations in germinated seeds (lupin, soybean, and black bean) that showed higher contents of both SDF and IDF components (*28*).

These increases are likely to be of interest because of their physiological and nutritional implications. SDF increases the viscosity of stomach contents, thereby reducing the rate of mixing and absorption of nutrients, whereas IDF reduces intestinal transit time and increases the bulk of the food mass (*3*).

In accordance with these results, germinated peas exhibited significantly higher contents of TDF than the corresponding raw sample (**Table 1**), because of the increases of both DF fractions. Insoluble/soluble fiber ratios are important from both dietary and functional perspectives. To be acceptable, a dietary fiber ingredient must perform in a satisfactory manner as a food ingredient (*29*). The changes promoted by germination conditions are also reflected in the IDF/SDF ratio. Interestingly, germination in darkness results in the SDF becoming the major fiber fraction. Therefore, the different germination treatments appears to alter the dietary and functional characteristics of the fiber. Thus, germinated peas, similarly to other germinated seeds, are a good source of DF, and their soluble and insoluble component levels are substantially higher than those found in nongerminated seeds (*30*, *31*).

The effect of germination treatments on the protein content is summarized in **Table 2**. In the first stage of the process a marked decrease of this component was observed in IDF. This is consistent with germination-related hydrolysis of protein to lower molecular weight components (small peptides, amino acids, etc.), which would be not retained in the insoluble matrix.

The profile of the sugar composition of IDF (**Table 3**) indicated similarities of the IDF constituents of the Esla pea with that of other legume seeds (*26*). The bulk of the IDF of raw pea mainly comprised carbohydrates, glucose (44%), uronic acid (25%), and arabinose (20%) being the main sugar constituents, followed by xylose, galactose, and mannose, which appeared in minor amounts. The glucose component was mainly cellulosic in origin; ~10% was released by 0.6 M H_2SO_4 hydrolysis (*29*). This result confirms the removal of starch during DF preparation. From these results, cellulose and polyuronides were inferred to be the main polysaccharides of IDF. The residues obtained after acid hydrolysis were referred to as Klason lignin; they seemed to be higher than expected, possibly due to the presence of cell wall or coprecipitated intracellular protein residues (*32*). Martín-Cabrejas et al. (*33*) confirmed the presence of coprecipitated protein in Klason lignin of beans (~35%).

Table 4. Composition of Soluble Dietary Fiber in Germinated Peas

	carbohydrates[a] (%)						total sugars[b]	
sample	Glc	Xyl	Gal/Rha	Ara	Man	UA	(g kg^{-1} of DM)	UA/NS
raw	29	2	7	5	3	54	21.8 ± 2.1a	4.50
L2D	40	4	6	21	7	22	26.7 ± 2.2b	0.82
L4D	24	3	6	28	10	29	38.2 ± 2.7c	0.85
L6D	21	3	5	21	9	41	41.1 ± 1.7c	1.58
NL2D	27	2	3	24	18	26	39.4 ± 3.2c	0.96
NL4D	30	3	4	21	22	21	47.3 ± 4.6c	0.84
NL6D	19	2	5	28	28	18	51.8 ± 2.5d	0.55

[a] Data expressed as percentage of total sugars. [b] Mean ± SD ($n = 3$). Mean values of each column followed by a different letter significantly differ when subjected to DMRT ($P < 0.05$).

In the germination processes the changes observed were considered to be consequences of the metabolic reaction undergone in the seeds during germination.The enzymatic−chemical DF procedure (**Table 3**) exhibited the same trend as the enzymatic−gravimetric DF method (**Table 1**); the IDF yield was 55% higher in samples germinated under darkness conditions compared to 34% under light. In both dark and light conditions, germination resulted in a relative increase in the proportion of cellulosic glucose and a concomitant reduction in the percentage of pectic polysaccharides. An increase of cellulose has been previously reported in legume seeds (*28*).

Furthermore, chemical analysis of the IDF fraction of raw peas showed that the levels of neutral sugars and uronic acids determined after 12 + 0.6 M acid hydrolysis were higher than those obtained by 0.6 M H_2SO_4 in all samples (data not shown). The carbohydrate composition of the mild hydrolysis showed the presence of pectic polysaccharides, as inferred from the percentage of uronic acids (58% of the total), which probably will build up the middle lamella (homogalacturonans). Thus, these results point out that cellulose microfibrills were closely connected to hemicelluloses (xyloglucans) and pectic polysaccharides such as homogalacturonans and also arabinans. Numerous studies show that cellulose microfibrills were strongly associated with xyloglucans in plant food cell walls (*16*).

The carbohydrate composition of SDF of raw peas contained uronic acid as the main sugar component (**Table 4**) followed by glucose. The pectic polysaccharides thus comprised 66% of total sugars. Interestingly, the level of arabinose found in this fraction was very low (1.09 g kg^{-1} of DM) unlike in other legumes (*26*). The germination process produced a marked increase of the sugar content; the highest increases occurred when germination was carried out in darkness conditions. The SDF level was up to 2.4 times higher than the control, arabinose being the main sugar responsible for these increases. The marked decrease in UA/NS ratio observed in germinated samples is consistent with the depolymerization of pectic polysaccharides

Table 5. Composition of Total Dietary Fiber in Germinated Peas

	carbohydrates[a] (%)						total sugars	total sugars + Klason lignin	
sample	Glc	Xyl	Gal/Rha	Ara	Man	UA	(g kg^{-1} of DM)	(g kg^{-1} of DM)	UA/NS
raw	41	5	4	18	1	31	109.8	117.8	1.41
L2D	61	4	1	9	6	19	117.1	121.3	1.90
L4D	62	4	1	9	4	20	159.2	161.5	2.00
L6D	64	4	1	13	4	14	154.2	156.4	1.00
NL2D	49	3	1	16	10	21	137.0	141.1	1.23
NL4D	68	3	1	7	8	13	183.6	189.8	1.62
NL6D	58	3	1	13	11	14	188.6	197.9	1.00

[a] Data expressed as percentage of total sugars.

Table 6. Composition of Alcohol-Insoluble Residue in Germinated Peas

	carbohydrates[a] (%)						total sugars	
sample	Glc	Xyl	Gal/Rha	Ara	Man	UA	(g kg^{-1} of DM)	UA/NS
raw	40	7	5	23		24	98.7 ± 4.1a	0.86
L6D	44	6	6	21		23	116.0 ± 7.9b	0.85
NL6D	52	6	5	17	1	19	166.2 ± 10.8c	0.86

[a] Data expressed as percentage of total sugars.

from IDF, mainly arabinans. Soluble uronic acids were lost by the action of endo-polygalacturonase, as demonstrated in other plant foods during this process (*16*). Furthermore, it was observed that the soluble fraction exhibited higher amounts of mannose than the insoluble fraction in all samples; these results, besides the increases of glucose, suggest the biosynthesis of polysaccharides rich in mannose and glucose such as mannans and glucomannans during the germination process.

Significant differences were detected in the carbohydrate composition of TDF from different germination conditions (**Table 5**). The levels of glucose and mannose in any germinated pea samples were higher compared to the control. This suggests the synthesis of polysaccharides besides the synthesis of new proteins and RNA reported (*34*, *35*) during this process. In keeping with the IDF and SDF trends, TDF exhibited a clear loss of pectic polysaccharides as cell wall soluble components. The UA/NS ratio of TDF in raw sample was lower than that observed in SDF, thus indicating the higher solubility of less branched pectic polysaccharides. However, in most of the germinated peas the UA/NS ratios in SDF were lower than those of TDF, which showed the significant solubilization effect of germination on branched pectic polysaccharides.

In the present study, it was observed that the processing treatment of germination, particularly under darkness conditions, significantly increased the IDF and SDF contents compared to raw sample. DF has important therapeutic implications for certain conditions such as diabetes and hyperlipidemia and may have preventive implications for others. The main effect of DF appears to be that it prolongs gastric emptying time, which is dependent on the physical form of the fiber and viscosity (*36*). The physical properties and physiological action of DF is also dependent on the plant cell wall integrity. An important function of the plant cell wall is to provide an insoluble matrix that may trap nutrients. The cell wall polysaccharides are mobilized during seedling germination; they provide soluble carbohydrates for the young seedling, which can be used for the formation of other cell components and for respiration to provide energy (*16*). As part of this study, we have investigated the carbohydrate composition of cell wall polymers of peas from different conditions of germination (L6D and NL6D samples) to confirm the DF results.

Table 7. Carbohydrate Composition of Germinated Pea AIR Extracted and Insoluble Residue

	carbohydrates[a] (%)						total	
sample	Glc	Xyl	Gal/Rha	Ara	Man	UA	(g kg^{-1} of DM)	UA/NS
CDTA-1								
raw	18	2	3	5	2	70	8.2	8.75
L6D	3	3	5	5		84	6.4	8.40
NL6D	3	1	3	4		89	8.9	12.71
CDTA-2								
raw	20		10	10		60	1.3	1.5
L6D			6	6		88	2.8	8.0
NL6D			8	15		77	1.3	3.3
Na_2CO_3-1								
raw		6		18		76	2.3	3.2
L6D	6	6		18		70	2.7	2.4
NL6D		4	7	11		78	2.8	3.7
Na_2CO_3-2								
raw	8	8	8	25		51	1.6	1.0
L6D				31		69	2.4	2.2
NL6D			8	25		67	1.2	2.0
0.5 M KOH								
raw	43	29		28			1.1	
L6D	47			27		26	2.3	0.36
NL6D	29			29		42	1.4	0.56
1 M KOH								
raw		100					1.0	
L6D	26	13	35			26	3.4	0.35
NL6D	33	8	41	10	8		3.9	
residue								
raw	57	6	3	18		16	75.6	0.20
L6D	60	6	3	17	1	13	89.5	0.15
NL6D	61	5	3	20	1	10	132.3	0.11

[a] Data expressed as percentage of total sugars.

The bulk of AIR in control pea comprised carbohydrate, mainly cellulose and pectic polysaccharides, as inferred from the levels of glucose, uronic acids, arabinose with minor quantities of xylose and galactose (**Table 6**). Thus, the carbohydrate composition was broadly similar to that of TDF previously reported. The absence of starch from AIR was indicated by a lack of staining with I/I_2 and by the release of only 10% of the glucose after hydrolysis with 0.6 M sulfuric acid (*37*).

The total carbohydrate and carbohydrate compositions of AIRs from both germination conditions confirmed the previous DF results: an increase of glucose and a decrease of uronic acids and arabinose during the process. Germination under darkness conditions induced the most relevant changes. The AIRs were subjected to a detailed fractionation based on the method of Redgwell and Selvendran (*38*). The procedure is designed to minimize β-eliminative degradation of pectic polymers (*23*).

The polysaccharides released by the first sequential extractions were predominantly pectic in nature (**Table 7**). The CDTA treatments solubilized most of the extractable pectic polymers.

The UA/NS (uronic acid/arabinose + galactose) ratio was highest in CDTA-1 polysaccharides, indicating that these were less branched than the polysaccharides extracted by Na_2CO_3 or KOH. The KOH-insoluble residues showed the highest total carbohydrate levels (>81%). They were rich in glucose (>57%), the remaining carbohydrate consisting mainly of pectic components. The ratios of UA/NS of residues were lower than the AIRs and reflect the insolubility of more highly branched pectic polysaccharides in the residues, as was observed in IDF fractions (**Table 3**).

Germination of pea seeds released small quantities of carbohydrates into CDTA-2, Na_2CO_3-1, Na_2CO_3-2, 0.5 M KOH, and 1 M KOH, approximately 5, 1, 2, 2, and 3% of the total, respectively. These consisted of mainly uronic acid, arabinose, and, in a lesser quantity, galactose. Glucose appeared only in KOH fractions. Germination had no major effect on the extractability of the cell wall polysaccharides in CDTA, Na_2CO_3, or KOH, but a relatively large increase in the level of total polysaccharides was found in the residues. NL6D exhibited the highest content of carbohydrates, although the sugar composition was similar to that of L6D. Thus, the results corroborated that previous finding that germination under darkness conditions exhibited a higher activity in the carbohydrate metabolism for the development of new tissues (*16*).

In conclusion, the above work has demonstrated that the germination process, in particular under darkness conditions, tends to modify the structure of cell wall polysaccharides of pea seeds possibly by affecting the intactness of tissue histology and disrupting the protein−carbohydrate integration. This will involve extensive cell wall biosynthesis and therefore the production of new dietary fiber. Therefore, the modifications in the germinated pea cell walls will involve changes in their physicochemical properties and, also, physiological effects when ingested. Germination of peas appears to be an effective and promising process for increasing the functionality of this legume and, therefore, enhancing the quality of the product.

ABBREVIATIONS USED

IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber; UA/NS, uronic acids: neutral sugars; AIR, alcohol-insoluble residue; CDTA, cyclohexane-*trans*-1,2-diamine-*N*,*N*,*N*′,*N*′-tetraacetate sodium; DM, dry matter.

LITERATURE CITED

(1) Sgarbieri, V. C. Composition and nutritive value of beans (*Phaseolus vulgaris* L.). In *Nutritional Value of Cereal Products, Beans and Starches*; Bourne, G. H., Ed.; World Review Nutrition Diet: Basel, Switzerland, 1989; Vol. 60, pp 132−198.

(2) Doughty, J.; Walker, A. *Etude FAO: Alimentation et Nutrition*; FAO: Rome, Italy, 1982.

(3) Olson, A.; Gray, G. M.; Mei-Chen, C. Chemistry and analysis of soluble dietary fiber. *Food Technol*. **1987**, *41* (2), 71−80.

(4) Marlett, J. A.; Vollendorf, N. W. Dietary fiber content and composition of vegetables determined by two methods of analysis. *J. Agric. Food Chem*. **1993**, *41*, 1608−1612.

(5) Champ, M.; Gourgue, N.; Faisant, N.; Leclerc, C. Glycemic response to starchy food: Importance of dietary fiber per se versus fiber in relation to food structure. In *Physicochemical Properties of Dietary Fibre and Effect of Processing on Micronutrients Availability*; Amado, R., Barry, J.-L., Frolïch, W., Eds.; Commission of the European Communities: Belgium, 1994; pp 95−103.

(6) Ghazy, M. A. Effect of gamma-irradiation on some antinutritional factors in kidney bean (*Phaseolus vulgaris* L.) seeds. *J. Agric. Res. Dev*. **1990**, *30*, 734−739.

(7) Frías, J.; Vidal-Valverde, C.; Kozlowska, H.; Tabera, J.; Honke, J.; Hedley, C. Natural fermentation of lentils. Influence of time, flour concentration, and temperature on the kinetics of monosaccharides, disaccharide, and α-galactosides. *J. Agric. Food Chem*. **1996**, *44*, 579−584.

(8) Khalil, M. N. Effect of soaking, germination, autoclaving on chemical and biological value of guar compared with faba bean. *Nahrung—Food* **2001**, *45* (4), 246−250.

(9) Reddy, N. R.; Sathe, S. K.; Salunkhe, D. K. Carbohydrates. In *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization*; Salunkhe, D. K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. 1, pp 65−131.

(10) Schulze, H.; Savelkoul, F. H.; Verstegen, M. W.; van der Poel, A. F.; Tamminga, S.; Groot Nibbelin, S. Nutritional evaluation of biologically treated white kidney beans (*Phaseolus vulgaris* L.) in pigs: ileal and amino acid digestibility. *J. Anim. Sci.* **1997**, *75*, 3187−3194.

(11) Chang, K. C.; Harrold, R. L. Changes in selected biochemical components *in vitro* protein digestibility and amino acids in two bean cultivars during germination. *J. Food Sci.* **1988**, *53*, 783−787.

(12) Frías, J.; Díaz-Pollan, C.; Hedley, C. L.; Vidal-Valverde, C. Evolution of trypsin inhibitor activity during germination of lentils. *J. Agric. Food Chem*. **1995**, *43*, 2231−2234.

(13) Vidal-Valverde, C.; Frías, J.; Estrella, I.; Gorospe, M. J.; Ruiz, R.; Bacon, J. Effect of processing on some antinutritional factors of lentils. *J. Agric. Food Chem*. **1994**, *42*, 2291−2295.

(14) Vidal-Valverde, C.; Frías, J. Changes in carbohydrates during germination of lentils. *Z. Lebensm. Unters. Forsch*. **1992**, *194*, 1−4.

(15) Urbano, G.; López-Jurado, M.; Hernández, J.; Fernández, M.; Moreu, M. C.; Frías, J.; Díaz-Pollán, C.; Prodanov, M.; Vidal-Valverde, C. Nutritional assessment of raw, heated and germinated lentils. *J. Agric. Food Chem*. **1995**, *43*, 1871−1877.

(16) Brett, C.; Waldron, K. W. Cell wall degradation and biotechnological applications. In *Physiology and Biochemistry of Plant Cell Walls*; Black, M., Charlwood, B., Eds.; Chapman and Hall: London, U.K., 1996; pp 206−221.

(17) Adsule, R. N.; Lawande, K. M.; Kadam, S. S. Pea. In *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization*; Salunkhe, D. K., Kadam, S. S., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. 1, pp 215−251.

(18) AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Washington, DC, 1995.

(19) Martín-Cabrejas, M. A.; Jaime, L.; Karanja, C.; Downie, A. J.; Parker, M. L.; López-Andréu, F.-J.; Maina, G.; Esteban, R. M.; Smith, A. C.; Waldron, K. W. Modifications to physicochemical and nutritional properties of hard-to-cook beans (*Phaseoulus vulgaris* L.) by extrusion cooking. *J. Agric. Food Chem*. **1999**, *47*, 1174−1182.

(20) Jaime, L.; Mollá, E.; Fernández, A.; Martín-Cabrejas, M. A.; López-Andréu, F. J.; Esteban, R. M. Structural carbohydrate differences and potential source of dietary fiber of onion (*Allium cepa* L.) tissues. *J. Agric. Food Chem*. **2002**, *50*, 122−128.

(21) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem*. **1973**, *54*, 484−489.

(22) Huber, D. J. Acidified phenol alters tomato cell wall pectin solubility and calcium content. *Phytochemistry* **1991**, *30*, 2523−2527.

(23) Waldron, K. W.; Selvendran, R. R. Cell wall changes in immature asparagus stem tissue after excision. *Phytochemistry* **1992**, *31*, 1931−1940.

(24) Bender, F. E.; Douglass, L.; Kramer, A. *Statistical Methods for Food and Agriculture*; Bender, F. E., Douglass, L., Kramer, A., Eds.; Food Products Press: New York, 1989.

(25) Anderson, J. W.; Bridges, S. R. Dietary fibre content of select foods. *Am. J. Clin. Nutr.* **1988**, *47*, 440−447.

(26) Gooneratne, J.; Majsak-Newman, G.; Robertson, J. A.; Selvendran, R. R. Investigation of factors that affect the solubility of dietary fiber, as nonstarch polysaccharides, in seed tissues of mung bean (*Vigna radiata*) and black gram (*Vigna mungo*). *J. Agric. Food Chem.* **1994**, *42*, 605−611.

(27) Veena, A.; Urooj, A.; Puttaraj, S. Effect of processing on the composition of dietary fibre and starch in some legumes. *Nahrung* **1995**, *35* (2), 132−138.

(28) Trugo, L. C.; Donangelo, C. M.; Trugo, N. M. F.; Bach Knudsen, K. E. Effect of heat treatment on nutritional quality of germinated legume seeds. *J. Agric. Food Chem.* **2000**, *48*, 2082−2086.

(29) Ariza, N. Análisis de carbohidratos en guisantes (*Pisum sativum* L.) Esla. *Estudio de Componentes Nutricionales y Antinutricionales en Guisantes: Influencia de la Variedad y del Proceso de Germinación*; Universidad Autónoma de Madrid: Madrid, Spain, 2000; pp 229−287.

(30) Augustin, J.; Klein, B. P. Nutrient composition of raw, cooked, canned, and sprouted legumes. In *Legumes: Chemistry, Technology and Human Nutrition*; Matthews, R. H., Ed.; Dekker: New York, 1989; pp 187−217.

(31) Donangelo, C. M.; Trugo, L. C.; Trugo, N. M. F.; Eggum, B. O. Effect of germination of legume seeds on chemical composition and on protein and energy utilization in rats. *Food Chem.* **1995**, *53*, 23−27.

(32) Martín-Cabrejas, M. A.; Waldron, K. W.; Selvendran, R. R.; Parker, M. L.; Moates, G. K. Ripening-related changes in the cell walls of Spanish pear. *Physiol. Plant.* **1994**, *91*, 671−676.

(33) Martín-Cabrejas, M. A.; Esteban, R. M.; Pérez, P.; Maina, G.; Waldron, K. W. Changes in physicochemical properties of dry beans (*Phaseolus vulgaris* L.) during long-term storage. *J. Agric. Food Chem.* **1997**, *45*, 3223−3227.

(34) Abdel-Rehim, F.; Abdel-Mobdy, E.; Mohamed-Abdel, O.; Erdmann, V. A.; Lippmann, C. The changes of protein patterns during one week of germination of some legume seeds and roots. *Food Chem.* **1995**, *52* (4), 433−437.

(35) Mohamed-Abdel, O.; Erdmann, V. A.; Lippmann, C.; Abdel-Rehim, F.; Abdel-Mobdy, E. Effect of germination on the ribonucleic acids (RNA) of some legume seeds (*Vicia faba*, *Cicer arietinum* and *Lupinus termes*). *Food Chem.* **1995**, *52* (4), 427−432.

(36) Selvendran, R. R. The plant cell wall as a source of dietary fibre: chemistry and structure. *Am. J. Clin. Nutr.* **1984**, *39* (2), 320−337.

(37) Selvendran, R. R.; O'Neil, M. A. Isolation and analysis of cell walls from plant material. *Methods Biochem. Anal.* **1987**, *32*, 25−153.

(38) Redgwell, R. J.; Selvendran, R. R. Structural features of cell-wall polysaccharides of onion (*Allium cepa*). *Carbohydr. Res.* **1986**, *157*, 183−199.

Received for review July 11, 2002. Revised manuscript received November 7, 2002. Accepted November 8, 2002. This study was supported by Comisión Interministerial de Ciencia y Tecnología ALI96-0480 and forms part of the Ph.D. of N.A.

JF0207631