Research Article

Received: 9 April 2012 Revised: 4 July 2012 Accepted: 13 July 2012 Published online in Wiley Online Library: 30 August 2012

(wileyonlinelibrary.com) DOI 10.1002/jsfa.5852

Composition of two Spanish common dry beans (*Phaseolus vulgaris*), 'Almonga' and 'Curruquilla', and their postprandial effect in type 2 diabetics

Begoña Olmedilla-Alonso,[a*] Mercedes Martín Pedrosa,[b] Carmen Cuadrado,[b] Miguel Brito,[c] Carmen Asensio-S-Manzanera[d] and Carmen Asensio-Vegas[e]

Abstract

BACKGROUND: Legume consumption has been associated with a lower risk of developing type 2 diabetes. However, the type of legume is a modifier of its effect. Two Spanish dry bean varieties – white ('Almonga') and cream ('Curruquilla') – were analyzed and used in a postprandial study in type 2 diabetics to assess glucose, insulin and triacylglycerol in blood.

RESULTS: 'Curruquilla' variety had higher total galactoside (stachyose, mainly), trypsin inhibitors and lectin content than 'Almonga'. The canning liquid was discarded prior to the analysis and the bean consumption by the subjects. The canning process reduced the total α-galactoside content (>50%), practically eliminated trypsin inhibitors, and no lectin content was found. After bean consumption, maximum glucose was obtained at 60 min and was three times lower than that in bread. After bean intake, maximum insulin was produced 60 min with 'Almonga' and occurred later (90 min) with 'Curruquilla' and bread. After 'Almonga' intake, the area under the curve response of triglycerides was 14% lower compared to bread ($P = 0.013$).

CONCLUSIONS: 'Almonga' and 'Curruquilla' are similar in the content of the nutritional but not in that of the antinutritional components. Both beans showed similar effects on blood glucose and insulin in type 2 diabetics and marked differences compared to those of bread in terms of magnitude and time course, but only 'Almonga' rendered a significant reduction in the triglyceridemic response.

Keywords: beans; *Phaseolus vulgaris* L.; postprandial effect; type 2 diabetes; trypsin inhibitors; lectin; oligosaccharides

INTRODUCTION

Legumes – the seeds of different species from the Papilionaceae family that are dried for consumption – are one of the classic foods of the Mediterranean diet that have been consumed less and less over the past two decades. In Spain, an overall decrease in the consumption of legumes, including beans,[1] has been observed over the past 15 years. This has been attributed to a poorer adherence to the Mediterranean diet[2] and to lower agricultural incentives for profitable crops, in spite of the production and registration of new improved agricultural varieties of legumes of high quality.[3]

Major components of pulses are fiber, mainly as soluble fiber, and resistant starch, which show beneficial effects on blood cholesterol, glucose and insulin levels, in addition to improving insulin resistance and increasing satiety.[4,5] Although legumes are of great interest in human and animal nutrition,[5] their use is often limited by the presence of a series of compounds, generally known as antinutritional factors, that impede the absorption of some of their most interesting components, while in some cases they are simply toxic (alkaloids) or cause undesirable physiological side effects (e.g. flatulence).[6] On the other hand, recent research has shown potential beneficial effects of some of these compounds[7] through several complementary and overlapping mechanisms of action.[8,9]

* Correspondence to: Begoña Olmedilla-Alonso, Departamento Metabolismo y Nutrición, Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN)-CSIC, C/José Antonio Novais 10, 28040 Madrid, Spain. E-mail: Bolmedilla@ictan.csic.es

a Departamento Metabolismo y Nutrición, Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN)-CSIC, 28040 Madrid, Spain

b Subdirección General de Investigación y Tecnología (SGIT), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), 28040 Madrid, Spain

c Servicio de Endocrinología y Nutrición, Hospital Universitario Puerta de Hierro-Majadahonda, Madrid, Spain

d Instituto Tecnológico Agrario de Castilla y León, 47071 Valladolid, Spain

e Estación Tecnológica de la Leche, Instituto Tecnológico Agrario de Castilla y León, Palencia, Spain

Legume consumption has been associated with a lower risk of developing various chronic diseases, mainly cardiovascular diseases, but also obesity and type 2 diabetes.[4,10] The World Health Organization has issued several recommendations for reducing overweight, obesity and cardiovascular disease that also are likely to reduce the risk of diet-related diseases, such as type 2 diabetes and obesity. These recommendations include the achievement of adequate intakes of non-starch polysaccharides through regular consumption of wholegrain cereals, legumes, fruits and vegetables.[11] Of the leguminous plants consumed by humans, one of those of greatest importance on a worldwide level is the common bean (*Phaseolus vulgaris* L).

A Spanish research center, the Instituto Tecnológico Agrario de Castilla y León (ITACYL), is developing a bean breeding program to improve the common bean landraces of major interest in Castile and León, a central region in Spain. To date, it has obtained 23 dry bean varieties[12] and we selected two genetically homogeneous varieties of marked commercial interest (high sensorial quality and good agronomic behavior)[3] – the 'Almonga' and 'Curruquilla' varieties, to study the components that have been associated with beneficial and adverse effects, namely protease inhibitors, lectin, oligosaccharides, protein and dietary fiber in raw and canned products, and to assess the effect of their consumption on metabolic aspects involving blood glucose, insulin and triacylglycerol in type 2 diabetics.

MATERIAL AND METHODS

Plant material

We employed two improved and registered Spanish dry bean varieties,[13] developed by the ITACYL in cooperation with the Spanish Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA): 'Almonga')[3] and 'Curruquilla'. 'Almonga' (NRVP 20 064 637 and # TOV 002 319) is a large (67 g per 100 seeds) white dry bean in the *planchada* market class and 'Curruquilla' (NRVP 20 064 639 and # TOV 002 323) is a large (54 g per 100 seeds) cream dry bean in the *canela* market class. Both are of good culinary quality, according to a trained sensory panel, with very soft integument and highly buttery albumen.

These bean varieties have a short vegetative cycle with an acceptable yield and were multiplied in the field for one year at one location (Valladolid, Spain), in an isolated test plot with varietal purity control, and harvested at plant maturity. Standard cultivation practices were employed. A random sample (500 g) was taken after harvest of the mature beans for analysis of the nutritional profile, quality and bioactive compounds. The rest of the seeds (about 30 kg per variety) were used for canning. Based on experience in previous work with these two varieties,[12] the harvested seeds were canned by cooking at 115 °C for 40 min at a pressure of 1800 kg, and subsequent cooling to 45 °C for 25 min.

Canned seeds were drained, rinsed with distilled water and frozen. Then, raw and cooked beans were freeze-dried, milled into flour and passed through a 1 mm sieve (Tecator, Cyclotec 1093, Höganäs, Sweden) prior to chemical analysis. Two replicates of each sample were taken to complete each of the following analyses.

Protein, dietary fiber, starch, amylase/amylopectin and soluble sugars

For the determination of nitrogen, the Kjeldahl method (AOAC 979.09) was followed.[14] A conversion factor of 5.40 was utilized to calculate protein from grams of nitrogen.[15]

A Mes-Tris method (AOAC 991.43) was used for dietary fiber determination.[14] Dietary fiber was obtained as an indigestible residue after enzymatic digestion of non-dietary fiber components.

Starch was determined polarimetrically by the official analytical method (34a) of the Spanish Agricultural Ministry.[16] The content of amylose and amylopectin was analyzed using the kit purchased from Megazyme (Wicklow, Ireland), based on a concanavalin A precipitation procedure.[17]

The concentration of soluble sugars in the seed flours was determined by high-performance liquid chromatography (HPLC).[18] Individual sugars were quantified by comparison with external standards of pure standards. A linear response was evident in the 0–5 mg mL^{-1} range, with a correlation coefficient of 0.99.

Trypsin inhibitor (TI) activity and lectins

Seed flours (25 mg) were extracted by stirring with 1 mL of 50 mmol L^{-1} HCl for 1 h at +1 °C and centrifuged (14 500 × *g* for 10 min). The resultant clear supernatants were stored at −20 °C.

Quantitative TI measurements were performed and trypsin inhibitor units (TIU) were defined according to the assay described by Welham and Domoney.[19] TI was determined using α-*N*-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA) as the trypsin substrate. TIU (mg per flour) were calculated from the absorbance read at 410. One unit of TIU was defined as that which gave a reduction in A_{410nm} of 0.01, relative to trypsin control reactions, using a 10 mL assay volume.[20]

Bean flour (100 mg) was extracted with 0.1 mol L^{-1} phosphate-buffered saline (PBS) (pH 7.4).[21] Hemagglutinating activity in the pH 7.0 buffer extracts was estimated by a serial dilution procedure using rat blood cells.[22] The amount of material (g) that caused agglutination of 50% of the erythrocytes was defined as that containing 1 hemagglutinating unit (HU).

The assays were reproducible to ±1 dilution; values expressed as the mean of four separate measurements and Processor (kidney bean) was included in each assay as a control. In addition to the hemagglutination assay, a competitive indirect enzyme-linked immunosorbent assay (ELISA) for quantification of PHA (*Phaseolus vulgaris* lectin) in bean samples was performed according to Hajós *et al.*[23] with some modifications. Plates were coated overnight at 4 °C with 0.1 µg mL^{-1} PHA in 0.01 mol L^{-1} PBS. Standard PHA diluted in PBST (0.01 mol L^{-1} PBS containing 0.1% Tween 20, pH 7.4) or bean samples with unknown content of lectin were added, followed by rabbit anti-PHA IgG antibody, diluted 1 : 32 000 with PBST. After incubation for 1 h, goat anti-rabbit IgG biotin conjugate diluted with PBST (1 : 2000, v/v) was added. After washing, Extravidin-peroxidase diluted 1 : 500 was added. After incubation for 1 h, a solution of *o*-phenylene-diamine (OPD) – H_2O_2 was added and the reaction was stopped by adding 50 µL of 3 mol L^{-1} H_2SO_4 and the optical density measured at 492 nm.

Subjects and study design

Type 2 diabetics (n = 12, five women), treated with diet and metformin as monotherapy, were recruited among patients in the Endocrinology and Nutrition Department at Hospital Universitario Puerta de Hierro-Majadahonda (HUPH, Madrid, Spain). Inclusion criteria: age range 50–76 years old, body mass index (BMI) range 25–35 kg m^{-2}, metformin as the only drug used to treat diabetes, and glycohemoglobin (HbA1c), as measure of long-term control of diabetes, within or close to the upper limit of normal (i.e. <8.5%; normal range: 4.3–6.3%). This postprandial crossover study was approved by the Clinical Research Ethics Committee of HUPH (Acta no. 223). Subjects were informed about the study and gave their written consent.

The patients received a standardized breakfast of beans (275 g) or white bread (114 g) (control), with olive oil (20 mL) to increase their palatability. Assays were carried out on three different days, at one-week intervals. Beans supplied 374 kcal ('Almonga') and 369 kcal ('Curruquilla'), and bread 320 kcal. The amounts of carbohydrates supplied in the study were: 57.75 g by 'Almonga', 52.25 g by 'Curruquilla' and 59.3 g by bread. Total carbohydrate content in beans was calculated by difference (total solids minus protein, fat, ash and dietary fiber) and that of bread (white wheat bread) from food composition tables.[24] Bread was used as a control since, in the Spanish diet, it is a basic food item that accompanies the main meals (breakfast, lunch and dinner).

Subjects arrived at the lab after an overnight fast (at least 10 h after consumption of their usual dinner, as had been requested) and, after collection of baseline blood samples, were given the assigned treatment and instructed to finish it within 10–15 min. Water consumption, up to 200 mL, was allowed during the study. Blood samples were collected over a 6 h period, at baseline and 30, 60, 90, 120, 180, 240 and 360 min after breakfast. Glucose and insulin were analyzed in all the samples; cholesterol (total) was analyzed every 2 h; and triglycerides, at baseline, after 4 h and at the end of the study.

Blood glucose was analyzed by the hexokinase method, and triglycerides and cholesterol by Advia® 2400 Analyzer (Siemens Medical Solutions Diagnostics Ltd, Sudbury, UK). Insulin was analyzed by chemiluminescent immunometric assay (Immulite 2000; Siemens Medical Solutions Diagnostics Ltd). Hemoglobin A1c was analyzed by HPLC (Adams HA-8160, Menarini, Naples, Italy).

Statistical methods

The baseline characteristics of the subjects are expressed as the mean plus or minus standard deviation. The normal distribution of the data was assessed (Kolmogorov–Smirnov test) and the differences between subjects at the beginning of the study by means of Student's *t*-test. There were no statistically significant differences at baseline.

A sample size of 11 subjects was considered on the basis of a mean baseline blood glucose value of 120 mg dL^{-1} (SD = 17) to obtain a 15% difference in blood glucose (18 mg) with 90% power and an alpha error of 0.05.

The postprandial responses of glucose, insulin and triglycerides were assessed by one-way ANOVA with a Tukey post hoc test and by a generalized estimating equation (GEE) model using 6 h area under the curve (AUC) data and adjusted for volunteer and type of food. All reported *P*-values are based on a two-sided test and compared to a significance level of 5%. SPSS v.19 (SPSS Inc., Chicago, IL, USA) software was used for all statistical calculations.

RESULTS

Bean composition

Table 1 shows the soluble sugar contents, TI and PHA contents and the effect of canning on the nutritional components of 'Almonga' and 'Curruquilla' beans. Both seeds had a similar protein content. Raw 'Curruquilla' had higher fiber content than raw 'Almonga'. Raw 'Almonga' and 'Curruquilla' had insoluble fiber contents of 25% and 30%, respectively, and the soluble fiber content was about 6% in both (data not shown). Amylopectin was the main component of the starch fraction. No significant change was observed in any nutritional component after canning.

Stachyose was the major α-galactoside in raw and canned samples (>80%) and it was reduced by 40% and 51.4% in 'Almonga' and 'Curruquilla', respectively. The greatest sugar reduction (66.5%) corresponded to raffinose in the 'Almonga' variety. The higher total galactoside content was shown in the 'Curruquilla' variety and it was reduced by canning (about 53% in 'Almonga' and 50% in 'Curruquilla'). Ciceritol was not detected in the canned samples.

The 'Curruquilla' variety had higher TI content than 'Almonga'. The canning process (soaking and cooking) reduced these amounts further, to negligible concentrations in both varieties. An initial evaluation of the lectin content was carried out using the hemagglutination assay. The results indicate that the raw samples differed significantly in their PHA content; the highest

Table 1. Concentration of protein, dietary fiber, starch, amylose, amylopectin, soluble sugars, trypsin inhibitors and PHA, and influence of processing on their content (dry matter basis)

Sample	Raw 'Almonga'	Canned 'Almonga'	Raw 'Curruquilla'	Canned 'Curruquilla'
Protein (mg g^{-1})	229.0 ± 5.0	225.0 ± 7.0	239.0 ± 9.0	234.0 ± 18.0
Dietary fiber (mg g^{-1})	294.0 ± 24.0	288.0 ± 17.0	325.0 ± 29.0	294.0 ± 19.0
Starch (mg g^{-1})	427.0 ± 42.0	409.0 ± 18.0	386.0 ± 25.0	412.0 ± 18.0
Amylose (%)	33.6 ± 2.7	36.2 ± 2.9	34.5 ± 1.5	30.3 ± 2.0
Amylopectin (%)	66.4 ± 3.1	63.8 ± 2.6	65.5 ± 3.2	69.7 ± 2.3
Sucrose (mg g^{-1})	23.4 ± 0.43^a	22.3 ± 0.30^b	22.6 ± 0.08^b	18.8 ± 0.27^c
Maltose (mg g^{-1})	0.6 ± 0.02^a	1.7 ± 0.13^b	0.7 ± 0.06^c	0.5 ± 0.03^a
Melibiose (mg g^{-1})	4.9 ± 0.58^a	2.1 ± 0.05^b	5.2 ± 0.08^a	1.0 ± 0.09^c
Raffinose (mg g^{-1})	4.0 ± 0.08^a	1.4 ± 0.04^b	3.3 ± 0.08^c	2.5 ± 0.12^d
Ciceritol (mg g^{-1})	1.6 ± 0.07^a	n.d.	1.0 ± 0.08^b	n.d.
Stachyose (mg g^{-1})	22.9 ± 0.64^a	13.7 ± 0.15^b	27.5 ± 0.25^c	13.4 ± 0.09^b
Total α-galactosides (mg g^{-1})	28.5 ± 0.62^a	15.0 ± 0.19^b	31.8 ± 0.51^c	15.9 ± 0.18^d
Trypsin inhibitors (TIU mg^{-1})	27.7 ± 0.84^a	0.6 ± 0.03^b	38.3 ± 0.99^c	0.4 ± 0.02^d
PHA (mg g^{-1})	0.7 ± 0.004^a	n.d.	12.9 ± 0.79^b	n.d.

Values are means ± SE ($n = 4$).
Mean values in the same row followed by different superscript letters are significantly different ($P < 0.05$).
PHA, *Phaseolus* lectin; n.d., not detected.

level corresponded to 'Curruquilla' (40 g kg^{-1}) and it was very low in the case of 'Almonga' (0.32 g kg^{-1}). Indirect competitive ELISA was also applied. The result also showed that 'Curruquilla' variety had a higher lectin content than 'Almonga' and no lectin content was found in processed beans assessed by ELISA.

Postprandial effect of bean intake in type 2 diabetics

The postprandial effect of two varieties of Spanish beans was assessed in a postprandial crossover study in type 2 diabetics with a short history of diabetes (range: 5–54 months), using white bread as control and evaluating the response of the glucose, insulin and triglyceride levels in blood. Baseline characteristics of the diabetics were: 66.4 ± 6.2 years of age, 30.1 ± 3.6 kg m^{-2} BMI, 118.5 ± 17.4 mg glucose dL^{-1}, 13.4 ± 9.6 µU insulin mL^{-1}, 167.2 ± 26.7 mg total cholesterol dL^{-1}, 160.8 ± 68.1 mg triglycerides dL^{-1}, 5.8 ± 0.6 HbA1c (%) and 2.6 ± 1.4 years of duration of diabetes. There were no significant differences in the glucose, insulin and triglyceride concentrations in blood at the start of each of the three days of assays.

Concentrations of glucose, insulin and triglycerides in serum increased over the first 6 h after bean or bread intake. Beans elicited lower glycemic responses than white wheat bread during the first 2 h and there was a slow decline in the blood glucose after bean intake. Although glucose concentrations dropped below fasting levels after 6 h; this decrease was less pronounced than with bread (Fig. 1). Insulin responses showed trends similar to that of the blood glucose responses, although the lower responses in insulin after bean intake compared to bread intake occurred at 90 and 120 min – a shorter period of time than for glucose (30–120 min). The maximum insulin concentrations over baseline were 19.2 ± 34.9 µIU mL^{-1} at 60 min with Almonga and 21.3 ± 11.8 µIU mL^{-1} at 90 min after Curruquilla. The mean differences in the glycemic and insulinemic responses of beans *versus* bread are shown in Table 2 and mean responses to the two beans were similar. Means in the glycemic (mg dL^{-1}) responses during the 6 h postprandial study are shown in figure 1.

The maximum increments in glucose and insulin concentrations over baseline and the time at which they were reached are shown in Fig. 1. The maximum glucose level was obtained at 60 min and was similar with the two varieties of beans ('Almonga': 149.8 ± 28.8 mg dL^{-1}; 'Curruquilla': 145.3 ± 22.4 mg dL^{-1}), and nearly three times lower than that obtained with bread (Fig. 1). The maximum insulin concentration was produced 60 min after the intake of 'Almonga' (33.2 ± 34.7 µIU mL^{-1}), but was not detected until minute 90 in the case of 'Curruquilla' (32.7 ± 15.7 µIU mL^{-1}) and bread (66.2 ± 34.7 µIU mL^{-1}). There were no differences between the two varieties of beans in terms of the maximum glucose and insulin concentrations.

The correlation between glucose and insulin responses were weak as the highest determination coefficients (R^2) were 0.264 (bread) and 0.250 ('Almonga') and the lowest was 0.119 ('Curruquilla').

The glucose, insulin and triglyceride responses to bean and to bread intake, expressed as AUC at 6 h, are shown in Table 3. The AUC responses to bread differed from those found with beans in terms of magnitude and time course, especially those corresponding to the glucose and insulin concentrations. The glucose response following consumption of the two varieties of beans did not differ significantly. The insulin responses to bean intake were similar ($P = 0.686$) and more than twofold lower than that observed with bread ($P = 0.000$). On comparing the AUC responses of triglycerides after intake of beans and of bread, 'Almonga' showed a significant difference ($P = 0.013$), whereas 'Curruquilla' did not.

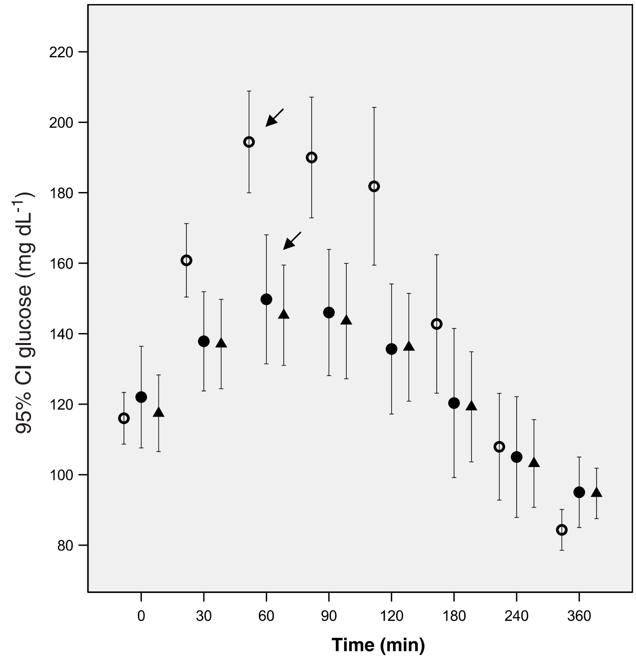

Figure 1. Postprandial glycemic response to bread and bean intake. ○, Bread; ●, 'Almonga'; ▲, 'Curruquilla'; CI, confidence interval. Arrows indicate the maximum blood glucose levels for both beans and bread.

Table 2. Mean differences in the glycemic (mmol L^{-1}) and insulinemic (pmol L^{-1}) responses during the 6 h postprandial study

	'Almonga' vs. bread	'Curruquilla' vs. bread
Glucose (30 min)	−1.28 ± 0.44	−1.32 ± 0.44
(95% CI)	(−2.37, −0.18)	(−2.46, −0.23)
P	0.019	0.015
Glucose (60 min)	−2.48 ± 0.56	−2.73 ± 0.56
(95% CI)	(−3.86, −1.10)	(−4.11, −1.34)
P	<0.0003	<0.0000
Glucose (90 min)	−2.44 ± 0.61	−2.58 ± 0.61
(95% CI)	(−3.95, −0.94)	(−4.08, −1.08)
P	0.001	0.001
Glucose (120 min)	−2.56 ± 0.68	−2.54 ± 0.68
(95% CI)	(−4.24, −0.90)	(−4.19, −0.88)
P	0.002	0.002
Insulin (90 min)	−240.30 ± 73.62	−232.66 ± 73.62
(95% CI)	(−421.56, −59.73)	(−413.92, −52.09)
P	0.007	0.009
Insulin (120 min)	−208.35 ± 67.37	−189.60 ± 67.37
(95% CI)	(−372.95, −43.06)	(−354.20, −24.31)
P	0.011	0.022

CI, confidence interval.

DISCUSSION

Legumes improve markers of longer-term glycemic control in humans[4,25] and their consumption is recommended as a means of optimizing diabetes control.[26–28] However, the glycemic benefits

Table 3. Glucose (mmol L^{-1} h^{-1}), insulin (pmol L^{-1} h^{-1}) and triglyceride (mmol L^{-1} h^{-1}) responses expressed as mean AUC ± SE (95% CI) and *P*-value for pairwise comparisons between each bean and bread

	Glucose	Insulin	Triglycerides
Almonga*	388.5 ± 20.5 (348.4, 428.6) $P = 0.014$	8419.3 ± 1940.1 (4616.8, 12221.7) $P = 0.000$	126.0 ± 90.1 (96.6, 155.5) $P = 0.013$
Curruquilla*	384.5 ± 15.3 (354.6, 414.4) $P = 0.000$	8844.6 ± 1463.6 (5976.2, 11713.0) $P = 0.000$	134.6 ± 23.1 (89.4, 179.9) $P = 0.264$
Bread	434.5 ± 13.2 (408.6, 460.4)	14109.5 ± 1937.5 (10311.9, 17907.0)	145.8 ± 21.0 (104.6, 187.0)

* *P*-value obtained in the pairwise comparisons between beans: glucose ($P = 0.654$), insulin ($P = 0.686$), triglycerides ($P = 0.396$).
CI, confidence interval.

appear to be modified by pulse type, and the interpretation of results regarding benefits from *Phaseolus vulgaris* (black, white, pinto, red and white kidney beans) is complicated by the marked interspecific and intravarietal heterogeneity.[4] In this context, the present study focused on two Spanish varieties of *Phaseolus vulgaris*, white ('Almonga') and cream ('Curruquilla') beans, from the seeds to the biological response in type 2 diabetics, including the analysis of components associated with beneficial and adverse effects.

'Curruquilla' and 'Almonga' varieties correspond to commercially available types of great interest in the European market,[28] showing excellent agricultural features, highlighting their resistance to the major diseases of the Spanish cultivars, such as halo blight[29] and viral diseases[30] and a high sensorial quality and a nutritional profile similar to that of other varieties studied.[31]

In this study, the major nutritional and antinutritional components of beans were analyzed in two forms (raw and boiled–canned), as the physical form and processing are important factors affecting their composition and metabolic responses.[32] The nutritive compound content was within the range reported for other beans.[33,34] Although the chemical composition of pulses is affected by processing,[33] the cooking/canning process had no significant effect ($P > 0.05$) on protein, dietary fiber or starch content. Different authors show that heat treatment of beans increases the content of insoluble dietary fiber and this is attributed to the presence of different amounts (2–7%) of fiber-associated resistant starch.[33,35,36] However, the slight decrease observed in the total fiber content of cooked 'Almonga' and 'Curruquilla' beans is not statistically significant. According to some authors, there are no significant changes in the total amount of dietary fiber after cooking.[34,37] Kutoš *et al.*[36] reported a decrease in the total dietary fiber and resistant starch contents in canned beans. Martín-Cabrejas *et al.*[38] observed slight decreases in total dietary fiber contents in fermented and autoclaved Carilla beans. The slight increase in the starch content in 'Curruquilla' may be attributed to the loss of soluble solids during the canning process, which would increase the concentration of starch in cooked seeds,[33] or may be caused by an increase in resistant starch mainly due to amylase retrogradation.[34] 'Almonga' and 'Curruquilla' have an amylose content of over 30%, like all legume starches.[39] Similar amounts of total and insoluble dietary fiber were found in kidney and pinto beans.[33,36,37,40]

The soluble sugar content reported in beans, chickpeas, lupin and soybean[36,38,41] is similar to that of raw 'Almonga' and 'Curruquilla' beans. The reduction observed after the canning process was mainly due to lixiviation of the sugars during the processing of the beans, both into the soaking medium and into the canning liquid.[36,42] Both liquids were discarded before dehydrating the cooked seeds for the chemical analysis. The canning liquid was also drained prior to bean consumption by the subjects. Although α-galactosides are responsible for digestive discomfort in humans (flatus), the presence of a small amount in the diet may have a beneficial effect by increasing the bifidobacterial population in the colon.[43]

The concentration of TI detected in the raw samples is similar to that of other beans[44] and its reduction after the cooking process (>98%) is in agreement with other studies,[44,45] indicating that these substances do not interfere with protein digestion.

The amount of lectin (PHA), the major toxic antinutritional factor limiting the use of *P. vulgaris* seeds, is in agreement with that described by other authors.[22,46,47] Although, in general, lectins are more resistant to heat denaturation than other plant proteins, the heat processing applied during canning eliminates the PHA content. Boiling improves the nutritional profile of beans as thermolabile toxic components (i.e. lectins and trypsin inhibitors) and oligosaccharides (responsible for flatulence) are reduced, but the protein and fiber contents are maintained.[9]

Glucose and insulin responses to the two canned bean varieties were compared to that of white wheat bread, a basic food item in the Spanish diet that accompanies main meals. Changes in glucose and insulin levels occurred during the first 2 h, but not from 180 min on, in accordance with previous postprandial studies.[33,48,49] The glucose response to the intake of the two varieties of beans and bread is much greater and persists longer (from 30 to 120 min) than the insulin response in blood, which occurs from minute 90 to 120. As the postprandial glycemic spike and degree of fluctuation of blood glucose seem to be more clinically adverse than sustained hyperglycemia,[48] the lower glucose responses obtained throughout the postprandial period (11.5% on average *versus* bread) and its slow decline in blood even after 6 h, along with the short period in which the insulin increment takes place (~39% total average *versus* bread), support the high relevance of bean consumption.

Postprandial glucose is one of the parameters targeted to reach a good metabolic control when treating diabetic patients[50] and it should not exceed 140 mg dL^{-1}. This goal is reached with bean intake (no statistical differences between both beans) but not with bread.

Several attempts have been made to identify the mechanism and compounds by which legumes might improve glycemic control, but some uncertainties remain and seem to be due to the intact structure of the grain or legume more than to a specific component.[25] Although the insulinemic response to transient postprandial hyperglycemia after a meal containing digestible carbohydrates is considered to be dose dependent,[50] the correlations obtained in our study are weak.

The portion size in our study (275 g) represents one-third of the amount of beans recommended on a weekly basis in the US Dietary Guidelines for Americans.[51] As the amount ingested is one of the factors influencing the response,[4,52] it must be taken into account in comparisons between studies, along with the type of beans, study design (postprandial or medium- or long-term intervention), effect of food consumption prior to postprandial glycemic response (known as the second meal effect) and other foods ingested as potential modifiers of the response. In our study, although the second meal effect cannot be ruled out because the subjects did not eat a standardized dinner on the pre-study days, and it seems improbable, as a recent study reported, that the effect did not last over a period of 9.5 h in healthy subjects.[53] The effect of three types of beans (pinto, black and red kidney), as well as rice, on postprandial glycemic response in type 2 diabetics has recently been described[28] in a report that showed differences in the glycemic response, despite the small differences between them in terms of calories, protein and fat composition, a finding that could be explained by the differences in the content of fiber, as pinto and dark red kidney had nearly twice that of black beans. However, in our study, the two varieties, when canned, did not differ with respect to the content of dietary fiber and starch.

The blood lipid profile is improved by legume consumption,[54] and a high postprandial lipemia is a characteristic metabolic abnormality of a number of lifestyle-related conditions.[55] In our study, triglyceridemia was measured at baseline and 2 and 4 h after food intake, and 'Almonga' but not 'Curruquilla' intake rendered a significant reduction (14% *versus* that of bread) in the AUC response. The reduction obtained in postprandial triglyceridemia was within the range described in other short- and medium-term studies, although the comparison is difficult owing to the different designs and amounts consumed. A greater reduction, 25%, was obtained by Jenkins *et al*.[56] using a higher amount of legumes (335 g mixed legumes, including beans) in a long-term study; a less marked reduction (11%) was described by Anderson *et al*.[57] after consumption of 240 g of navy and pinto beans combined; and no effect was reported by Finley *et al*.[58] after 12 weeks of daily consumption of 130 g pinto beans.

In conclusion, the two Spanish bean varieties analyzed – 'Almonga' and 'Curruquilla' – are similar in content of the nutritional but not the antinutritional components. Raw 'Curruquilla' has a higher level of trypsin inhibitors, hemagglutination activity and lectin content, all of which are reduced by cooking. Both bean varieties had similar effects on blood glucose and insulin in type 2 diabetics, which differed from those produced by bread in terms of magnitude and time course, but only 'Almonga' rendered a significant reduction in the triglyceridemic response.

ACKNOWLEDGEMENTS

This study was funded by the INIA (RTA2008-007) and ITACYL (PEP 2006/00891). We thank the Biochemical Analysis Department at HUPH (E Donoso, F Granado, I Blanco), and MA Sanz and A Ibeas-García from ITACYL.

REFERENCES

1 MERCASA, *Alimentación en España 2009: Legumbres*. Madrid, pp. 118–129 (2009).
2 Varela Moreiras G, Ávila Torres JM, Cuadrado Vives C, del Pozo de la Calle S, Ruiz Moreno E and Moreiras Tuny O, Valoración de la dieta española de acuerdo al Panel de Consumo Alimentario del Ministerio de Agricultura Pesca y Alimentación (MAPA). Fundación Española de la Nutrición. MAPA. Madrid (2008).
3 Asensio C, Asensio-S-Manzanera MC and López R, Registration of 'Almonga' planchada dry bean. *Crop Sci* **4**:2125 (2005).
4 Sievenpiper JL, Kendall CWC, Esfahani A, Wong JMW, Carleton AJ, Jiang HY, *et al*, Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. *Diabetologia* **52**:1479–1495 (2009).
5 Leterme P, Recommendations by health organizations for pulse consumption. *Br J Nutr* **3**:S239–S242 (2002).
6 Muzquiz M and Wood JA, Antinutritional factors, in *Chickpea Breeding and Management*, ed. by Yadav SS, Redden R, Chen W and Sharma B. CAB International, Wallingford, UK, pp. 143–166 (2007).
7 Gelencsér E, Novel research strategy for safe use of legume proteins in human nutrition. *Acta Alim* **38**:61–70 (2009).
8 Campos-Vega R, Loarca-Piña G and Oomah BD, Minor components of pulses and their potential impact on human health. *Food Res Int* **43**:461–482 (2010).
9 Schlemmer U, Frolich W, Prieto RM and Grases F, Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Mol Nutr Food Res* **53**:S330–375 (2009).
10 Schröder HJ, Protective mechanism of the Mediterranean diet in obesity and type 2 diabetes. *J Nutr Biochem* **18**:149–160 (2007).
11 *WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases*. WHO Technical Report Series 916, Geneva (2003).
12 Asensio Vegas C, Asensio-S-Manzanera MC, López Pérez R, Fernández Lobato S and Ibeas García A, *Catálogo de variedades de judías-grano del ITACyL* (2nd edn). Instituto Tecnológico Agrario de Castilla y León, Palencia (2006).
13 Boletín Oficial del Estado (BOE) de 9 de marzo de 2007, por la que se conceden títulos de obtención vegetal en el Registro de Variedades Protegidas. BOE **59:** 10273–10274.
14 *AOAC, Official Methods of Analysis* (16th edn). Association of Official Analytical Chemists, Washington, DC (1995).
15 Mossé J, Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds: a reappraisal of its definition and determination. Variation according to species and to seeds protein content. *J Agric Food Chem* **38**:18–24 (1990).
16 *Official Methods of Analysis*. Vol. 1: *Official Methods of Analysis of Feed and their Raw Materials*. 34 (a). Starch (Polarimetric method). Spanish Ministry of Agriculture, Madrid (1993).
17 Haase N, A rapid test procedure for estimating the amylase content of pea starch. *Plant Breed* **111**:325–329 (1993).
18 Muzquiz M, Rey C, Cuadrado C and Fenwick R, Effect of germination on oligosaccharide content of lupin species. *J Chromatogr* **607**:349–352 (1992).
19 Welham T and Domoney C, Temporal and spatial activity of a promoter from a pea enzyme inhibitor gene and its exploitation for seed quality improvement. *Plant Sci* **159**:289–299 (2000).
20 Welham T, O'Neill M, Johnson S, Wang TL and Domoney C, Expression patterns of genes encoding seed trypsin inhibitors in *Pisum sativum*. *Plant Sci* **131**:13–24 (1998).
21 Trugo L, Muzquiz M, Pedrosa MM, Ayet G, Burbano C and Cuadrado C, Influence of malting on the composition of non-nutrient components of different seeds. *Food Chem* **65**:85–90 (1999).
22 Grant G, Legumes, in *Toxic Substances in Crop Plants*, ed. by D'Mello JPF, Duffus CM and Duffus JH. Royal Society of Chemistry, Cambridge, UK, pp. 49–67 (1991).
23 Hajós G, Gelencsér E, Pusztai A, Grant G, Sakhri M and Bardocz S, Biological effects and survival of trypsin inhibitors and the agglutinin from soybean in the small intestine of the rat. *J Agric Food Chem* **43**:165–170 (1995).
24 Moreiras O, Carbajal A, Cabrera L and Cuadrado C, *Tablas de Composición de Alimentos* (14th edn). Ediciones Pirámide, Madrid (2009).
25 Venn BJ and Mann JI, Cereal grains, legumes and diabetes. *Eur J Clin Nutr* **58**:1443–1461 (2004).
26 Mann JI, De Leeuw I, Hermansen K, Karamanos B, Karlström B, Katsilambros N, *et al*, Evidence-based nutritional approaches to the treatment and prevention of diabetes mellitus. *Nutr Metab Cardiovasc Dis* **14**:373–394 (2004).
27 Bantle JP, Wylie-Rosett J, Albright AL, Apovian CM, Clark NG, Franz MJ, *et al*, Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. *Diabetes Care* **31**(Suppl 1):S61–S78 (2008).
28 Thompson SV, Winham DM and Hutchins AM, Bean and rice meals reduce postprandial glycemic response in adults with type 2 diabetes: a cross-over study. *Nutr J* **11**:23 (2012).
29 Santalla M, De Ron AM and Voysset O, European bean market classes, in *Catalogue of Bean Genetic Resources*, ed. by Amurrio M, Santalla M and De Ron AM. MBG-CSIC-PHASELIEU, Fundación Barrié de la Maza, Pontevedra, Spain, pp. 79–94 (2001).
30 Asensio C and Zan M, Cristóbal V, Identification of *Pseudomonas syringae* pv. *phaseolicola* races in the Central Region of Spain. *Annu Rep Bean Improv Coop* **41**:74–75 (1998).

31 Saiz MC, de Blas G, Carazo J, Fresno J, Romero S and Castro S, Incidence and characterization of bean common mosaic virus isolates in Spanish bean fields. *Plant Dis* **79**:79–81 (1995).
32 Broughton WJ, Hernández G, Blair M, Beebe S, Gepts P and Vanderleyden J, Beans (*Phaseolus* spp.): model food legumes. *Plant Soil* **252**:55–128 (2003).
33 Tovar J, Granfeldt Y and Björck IM, Effect of processing on blood glucose and insulin responses to starch in legumes. *J Agric Food Chem* **40**:1846–1851 (1992).
34 Wang N, Hatcher DW, Tyler RT, Toews R and Gawalko EJ, Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). *Food Res Int* **43**:589–594 (2010).
35 Almeida-Costa GE, Silva K, Pissini SM and Costa A, Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chem* **94**:327–330 (2006).
36 Kutoš T, Golob T, Kac M and Plestenjak A, Dietary fibre content of dry and processed beans. *Food Chem* **80**:231–235 (2003).
37 Tovar J, Bioavailability of carbohydrates in legumes: digestible and indigestible fractions. *Arch Latinoam Nutr* **44**:36S–40S (1994).
38 Martín-Cabrejas MA, Aguilera Y, Benítez V, Mollá E, López-Andréu FJ and Esteban RM, Effect of industrial dehydration on the soluble carbohydrates and dietary fiber fractions in legumes. *J Agric Food Chem* **54**:7652–7657 (2006).
39 Peñalver C, Herrera IM and Tovar J, Indigestible starch associated to dietary fiber residues from cooked legume seeds consumed in Venezuela. *Interciencia* **32**:620–623 (2007).
40 Candela M, Astiasaran I and Bello J, Cooking and warm-holding: effect on general composition and amino acids of kidney beans (*Phaseolus vulgaris*), chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*). *J Agric Food Chem* **45**:4763–4767 (1997).
41 Martín-Cabrejas MA, Sanfiz B, Vidal A, Mollá E, Esteban R and López-Andréu J, Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). *J Agric Food Chem* **52**:261–266 (2004).
42 Ibrahim SS, Habiba RA, Shatta AA and Embaby HE, Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. *Food Nahrung* **46**:92–95 (2002).
43 Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, Flourié B, *et al*, The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose–response relation study. *Am J Clin Nutr* **80**:1658–1664 (2004).
44 Guillamón E, Pedrosa MM, Burbano C, Cuadrado C, Sánchez MC and Muzquiz M, The trypsin inhibitors present in seed of different grain legume species and cultivar. *Food Chem* **107**:68–74 (2008).
45 Rehman Z and Shah WH, Thermal heat processing effect on antinutrients, protein and starch digestibility of food legumes. *Food Chem* **91**:327–331 (2005).
46 Burbano C, Muzquiz M, Ayet G, Cuadrado C and Pedrosa MM, Evaluation of antinutritional factors of selected varieties of *Phaseolus vulgaris*. *J Sci Food Agric* **79**:1468–1472 (1999).
47 Martín-Cabrejas MA, Aguilera Y, Pedrosa MM, Cuadrado C, Hernández T, Díaz S, *et al*, The impact of dehydration process on antinutrients and protein digestibility of some legume flours. *Food Chem* **114**:1063–1068 (2009).
48 Wolever TMS, Katzmanrelle L, Jenkins AL, Vusksan V, Josse RG and Jenkins DJA, Glycemic index of 102 complex carbohydrate foods in patients with diabetes. *Nutr Res* **14**:651–669 (1994).
49 Brand-Miller JC, Stockmann K, Atkinson S, Petocz P and Denyer G, Glycemic index, postprandial glycemia, and the shape of the curve in healthy subjects: analysis of a database of more than 1000 foods. *Am J Clin Nutr* **89**:97–105 (2009).
50 *Guidelines for Management of Postmeal Glucose International Diabetes Federation*. Lesaffre, Brussels (2007).
51 US Dietary Guidelines for Americans. [Online]. US Department of Health and Human Services (2005). Available: www.healthierus.gov/dietaryguidelines [6 June 2011].
52 Winham DM and Hutchins AM, Baked bean consumption reduces serum cholesterol in hypercholesterolemic adults. *Nutr Res* **27**:380–386 (2007).
53 Fletcher JA, Perfield JW II, Thyfault JP and Rector RS, The second meal effect and its influence on glycemia. *J Nutr Disord Ther* **2**:108 (2012).
54 Bazzano LA, Thompson AM, Tees MT, Nguyen CH and Winham DM, Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis* **21**:94–103 (2011).
55 Lairon D, Play B and Jourdheuil-Rahmani D, Digestible and indigestible carbohydrates: interactions with postprandial lipid metabolism. *J Nutr Biochem* **18**:217–227 (2007).
56 Jenkins DJ, Wong GS, Patten R, Bird J, Hall M, Buckley GC, *et al*, Leguminous seeds in the dietary management of hyperlipidemia. *Am J Clin Nutr* **38**:567–573 (1983).
57 Anderson JW, Gustafson NJ, Spencer DB, Tietyen J and Bryant CA, Serum lipid response of hypercholesterolemic men to single and divided doses of canned beans. *Am J Clin Nutr* **51**:1013–1019 (1990).
58 Finely JW, Burell JB and Reeves PG, Pinto bean consumption changes SCFA profiles in fecal Fermentations, bacterial populations of the lower bowel, and lipid profiles in blood of humans. *J Nutr* **137**:2391–2398 (2007).

