Chickpea flour ingredient slows glycemic response to pasta in healthy volunteers

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Abstract

Diets with a low glycaemic index have been shown to improve glucose tolerance in both healthy and diabetic subjects. However, there is a need for a more diversified range of foods with a low glycaemic response. The object of this work was to make a spaghetti, containing chickpea flour, as a food having a low postprandial glycaemic response. Twelve healthy volunteers consumed three test meals containing 50 g of carbohydrates: white bread, wheat spaghetti and spaghetti, in which the wheat was partially substituted by chickpea flour. Blood samples were collected over 2 h after consumption of meals to evaluate the glycaemic response and to determine the glycaemic index. Nutritional composition and in vitro starch hydrolysis of pasta were determined. The incorporation of chickpea flour increased the mineral, fat and indigestible compound content of the pasta, but total starch content was not affected. Starch hydrolysis was lower in both types of pasta than in white bread, but the difference was greater in the case of pasta made with chickpea flour. The glycaemic indices (GI) of both types of pasta were in the normal range for lente carbohydrates but were significantly lower in the pasta containing chickpea flour ($GI_{wheat\ spaghetti}$: 73 ± 5; $GI_{wheat-chickpea\ spaghetti}$: 58 ± 6). The inclusion of chickpea flour, as an ingredient in pasta products, evidently provide a food with a low glycaemic response and could help in achieving a wider range of low-GI foods for the consumer.
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1. Introduction

A recent joint FAO/WHO expert consultation recommended increased consumption of low-glycaemic index (GI) foods (FAO/WHO, 1998). There is increasing evidence that a low-glycaemic-index diet can be beneficial in that it improves metabolic control of hyperlipidaemia in diabetic patients as well as in healthy subjects (Frost et al., 1999; Jenkins et al., 1994).

GI is a ranking of foods based on the postprandial blood glucose response compared with a reference food (Jenkins et al., 1981). The main factor that influences GI is the rate of digestion or absorption of the carbohydrates present in a food. The rate and extent of starch digestion instigate a number of physiological functions that have different effects on health, including reduction of the glycaemic and insulinaemic responses to a food

(Björck, Lilberg, & Östman, 2000), hypocholesterolaemic effects (De Deckere, Koots, & Van Amelsvoort, 1995) and protective effects against colorectal cancer (Cassidy, Bingham, & Cummnings, 1994).

Dried legume seeds generally promote slow and moderate postprandial blood glucose increase (García-Alonso, Goñi, & Saura-Calixto, 1998; Phillips, 1993). Several factors can contribute, such as the rigidity of cotyledonary cell walls, the intrinsically low enzyme susceptibility of legume starches, and the presence of polyphenols and other α-amylase inhibitors (Granfeldt & Björck, 1991; Tovar, 1992). Moreover, a high proportion of non-digestible carbohydrates, such as resistant starch, non-starch polysaccharides and oligosaccharides, contribute to a low glycaemic response (Björck et al., 2000; Ou, Kwok, Li, & Fu, 2001; Van Loo et al., 1999). Legumes are also a source of high-quality protein (Rincón, Martínez, & Ibañez, 1998). Despite their nutritional value, consumption of legumes has been decreasing in recent years (MAPA, 2002).

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Pasta, on the other hand, is a popular food with a high rate of acceptability in many population groups (fitness enthusiasts, children, adolescents, the elderly). Moreover, new ingredients can be readily incorporated in industrial pasta-making processes.

Both traditional pasta and legumes are considered as low-GI foods (Björck et al., 2000).

The number of low-GI foods is very limited, and so a much wider range of low-GI products will be required to make a well-balanced low-GI diet practicable (Björck et al., 2000).

The object of this work was to make a common type of pasta (spaghetti) using chickpea flour as an ingredient, to achieve a nutritious, slowly-digestible food, rich in dietary fibre and starch, in order to diversify the range of available foods with a low glycaemic response.

2. Materials and methods

2.1. Materials

Two types of pasta (spaghetti) were provided by Rio Dulce SA, (Guadalajara, Spain). Pasta 1 was processed with durum wheat flour (wheat spaghetti) and pasta 2 was elaborated from a mixture (75/25) of durum wheat flour and chickpea flour (wheat–chickpea spaghetti). Both types of spaghetti were boiled in water for 10 min.

A commercial white bread (BIMBO, S.A. Spain) was purchased from the local supermarket. The crust was separated, and the fresh crumb was used as reference food in both in vitro and in vivo studies.

2.2. Analytical methods

Fresh bread crumb and boiled spaghetti were dried and milled ($\theta = 0.5$ mm) prior analytical determinations. Determinations were done in quadruplicate.

2.2.1. Total starch (TS)

The procedure followed for the determination of TS has been reported previously (Goñi, García-Alonso, & Saura-Calixto, 1997). Samples (50 mg) were dispersed in 2 M KOH to hydrolyze the starch and samples were incubated (60 °C, 45 min, pH: 4.75) with amyloglucosidase (102 857, Roche Diagnostics, S.L. Barcelona, Spain). Starch was measured as glucose with Peridochrom Glucose GOD-PAP (676 543, Roche Diagnostics, S.L. Barcelona, Spain). The glucose content was determined and the starch was calculated as glucose (mg)×0.9.

2.2.2. Resistant starch (RS)

Resistant starch (RS) was measured by the procedure of Goñi et al. (1997). Briefly, protein and digestible starch were removed after treating with pepsin (Merck

7190, Darmstadt, Germany; 40 °C, 1 h, pH 1.5), and -amylase (Sigma A-3176, Madrid, Spain; 37 °C, 16 h, pH 6.9), respectively. After centrifugation, residues were dispersed with 2 M KOH to dissolve RS, incubated with amyloglucosidase and glucose was quantified spectrophotometrically using the GOD-PAP reagent (676543, Roche Diagnostics, Barcelona, Spain). RS was calculated as glucose×0.9.

2.2.3. Total, soluble and insoluble dietary fiber

Dietary fibre was analyzed by the AOAC method (Prosky, Asp, Schweizer, De Vries, & Furda, 1992), modified in our laboratory (Mañas & Saura-Calixto, 1993). Samples were treated with heat stable α-amylase (Sigma, A-3306), protease (Sigma, P-3910, Madrid, Spain) and amyloglucosidase (Sigma, A-9913, Madrid, Spain), followed by centrifugation (15 min, 3000 g) instead of filtration to separate the soluble and insoluble fractions. Ethanol precipitation of soluble dietary fibre (SDF) was substituted by dialysis against water. Supernatants were transferred into dialysis bags (12,000–14,000 Da MWCO, Medicell International, London) and exhaustively dialyzed against water (48 h, 25 °C, water flow: 7 l/h).

Dialysater were hydrolyzed with 1 M sulphuric acid at 100 °C for 90 min. Neutral sugars in the soluble dietary fibre were quantified by gas liquid chromatography (GLC) (Englyst & Cummings, 1988) and uronic acids by the Scott method (Scott, 1979). A Shimadzu GC-14 A chomatograph (Shimadzu Co., Kyoto Japan), fitted with a flame ionization detector and connected to a C-R4A Chomatopac computer system was used. A SP-2330 capillary column (30 m×0.32 i.d. Cat. No. 2-4073, Supelco, PA) was used. Analytical conditions were as follows: column temperature 240 °C (isothermal), injector temperature 270 °C, detector temperature 270 °C, carrier gas nitrogen.

2.2.4. Total, soluble and insoluble portions of indigestible fraction (IF)

There were measured according to the procedure previously reported (Saura-Calixto, García-Alonso, Goñi, & Bravo, 2000). Spaghetti, recently boiled, was successively incubated with pepsin and α-amylase to eliminate digestible components. Samples were centrifuged (3000 g, 15 min) and supernatants removed. Residues were dried overnight at 105 °C and quantified gravimetrically as the insoluble IF. Supernatants were dialyzed against water for 48 h at 25 °C. Dialysates were then hydrolyzed and neutral sugars and uronic acids were determined as described above.

2.2.5. Protein

Two types of spaghetti were analyzed for total nitrogen in a FP-2000 Dumas nitrogen/protein determinator (Lek, FP-2000, LEKO Corporation, St Joseph, MI,

USA). Samples were combusted in the pure oxygen environment of the furnace. After passing through a thermo-electric cooler to remove water, an aliquot from the combustion gases was taken. Gases were passed through and all nitogen-containing materials reduced to nitrogen and detected by a thermal-conductivity cell. An air blank was carried out and the instrument calibrated with EDTA. Protein was calculated as nitrogen $\times 6.25$.

2.2.6. Lipids

Lipids were extracted in a Soxhlet extractor (Soxtec System HT 1043 Tecator, Sweden) with petroleum ether at 40–60 °C (AOAC, 1995). Lipid content was determined gravimetrically after drying at 60 °C to constant weight.

2.2.7. Ash

Samples were digested in 50% HNO_3 (suprapure) at 550 °C to total calcination and gravimetrically quantified.

2.3. In vitro starch hydrolysis

An in vitro method previously reported was used (Goñi et al., 1997). A portion of each type of spaghetti containing 50 mg of total starch (TS) was boiled in water for 10 min. Sample preparation and analysis were carry out in the same tube. Boiled spaghetti and a portion of white bread (50 mg of TS) were successively incubated with pepsin (Sigma P-7000) and porcine pancreatic α-amylase (Sigma A-3176) at 37 °C with continuous shaking. Samples (1 ml) were withdrawn every 30 min for 3 h and were inactivated at 100 °C in shaking water bath. They were hydrolyzed to glucose with amyloglucosidase and glucose released was measured with Peridochrom Glucose GOD-PAP reagent (676543, Roche Diagnostic, Barcelona, Spain) and converted into starch by multiplying by 0.9. The rate of hydrolysis was expressed as the percentage of total starch hydrolyzed at different times.

2.4. In vivo study

Twelve healthy female volunteer aged 23.25 ± 2.42 years, with normal body mass indices (22.46 ± 1.79 kg/m^2) took part in this study. None of the subjects were under medication. The Ethic Committee of the Nutrition Department, Complutense University, Madrid, Spain approved the study protocol, and all volunteers gave their written informed consent.

Each subject consumed all three test foods (white bread, wheat spaghetti and wheat–chickpea spaghetti) after an overnight fast. Each volunteer took part in the experiment on three non-successively days. On the first experimental day, subjects consumed white bread (50 g starch). The second day, they ingested boiled wheat spaghetti (50 g starch), and the last day they ingested boiled wheat-chickpea spaghetti (50 g starch). Foods were eaten within 10–12 min and 250 ml of water was drunk with the meals.

Capillary blood samples were taken using a Softclix (Accu-chek, Roche Diagnostics, Barcelona, Spain) in the fasting state and 15, 30, 45, 60, 90, and 120 min after each meal. Blood samples were taken on test strips and analysed, with Accutrend-Sensor, for glucose (Roche Diagnostics, Barcelona, Spain). The glycemic indices were calculated following the procedure of Wolever, Jenkins, Jenkins, and Josse (1991) from the incremental blood glucose area in relation to the corresponding area obtained after white wheat bread used as reference food.

2.5. Statistical analysis

Results are expressed as mean values and standard deviation of their mean. Data were analyzed using an one-way analysis of variance (ANOVA) to determine the significance of mean differences between groups, by using the Stat Graphics computer programme (SAS/STAT version 6, SAS Institute, Cary, NC). Significant level was $P < 0.05$.

3. Results and discussion

The two types of spaghetti had similar high proportions of protein, but the association of wheat and chickpeas improves the level of quality protein in the mixture. Lysine is a limiting amino acid in cereals but not in legumes, and methionine is a limiting amino acid in legumes but not in cereals. When cereals and legumes are combined, the quality score of the combined proteins may be much higher than each of the individual values (Hegarty, 1995).

The incorporation of chickpea flour also increased the mineral and fat contents of pasta, contributing to elevate the nutritional value of the food. Composition values are shown in Table 1.

Table 1
Proximal composition of two types of spaghetti[a]

	Wheat spaghetti (100% wheat flour)	Wheat–chickpea spaghetti (75% wheat flour and 25% chickpea flour)
Protein[b]	16.2 ± 0.02	17.0 ± 0.02
Fat	1.81 ± 0.01a	2.60 ± 0.02b
Total starch[b]	74.1 ± 2.24	72.9 ± 1.99
Resistant starch	2.92 ± 0.64a	3.78 ± 0.12b
Digestible starch[b]	71.1 ± 1.65	69.9 ± 0.91
Ash	0.77 ± 0.10a	1.71 ± 0.18b

[a] Values are means $\pm$ standard deviation ($n = 4$). Different letters in a row indicate significant differences ($P < 0.05$).
[b] Values not statistically different.

The pasta had a high percentage of total starch (74% of dry matter). A high proportion of the total starch was rendered digestible by cooking, and only a small amount of resistant starch (RS) remained in the samples (Table 1). The amount of potentially available starch was similar in both samples. Digestible starch was digested significantly more slowly than in the case of the reference white bread; however, the degree of starch hydrolysis was lowest at all points on the curve when chickpea flour was used in the pasta (Fig. 1). This could be a consequence of other factors such as the presence of non digestible constituents of chickpea, such as RS, oligosaccharides, polyphenols and lectins (Rincón et al.,
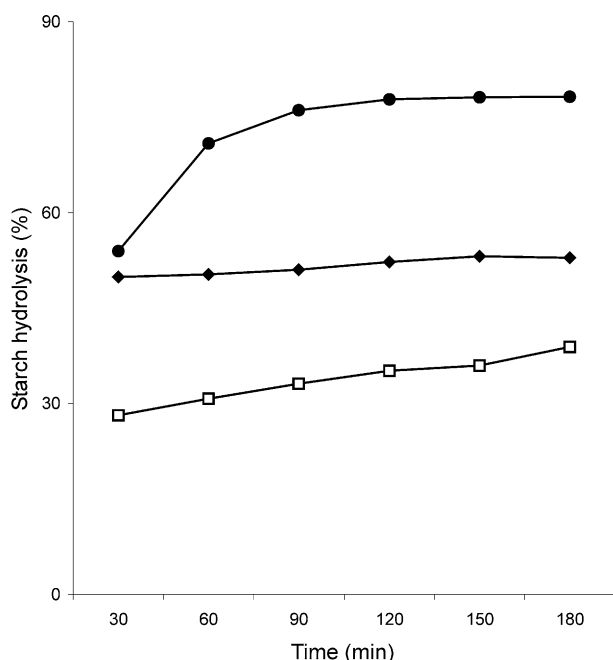
1998; Saura-Calixto et al., 2000), and of the cooking process.

The two types of spaghetti contained similar amounts of RS and DF. However, the indigestible fraction (IF) was significantly higher in pasta with chickpeas (Table 2). IF covers a broader spectrum than DF. IF comprises non-starch polysaccharides, lignin and other compounds resistant to the digestive enzymes that can affect starch digestion, such as RS, resistant protein and oligosaccharides; these reach the large intestine, where interaction with colonic microflora can modify the behaviour conditions of the intestinal ecosystem and may be beneficial to the health of the host (Goñi & Martín, 2001). Moreover, chickpea flour contains non-digestible oligosaccharides, mainly stachyose, (1.5% dry matter) (Rupérez, 1998). These were not quantified in either DF or IF, but they represented only a small percentage of the sample; they are considered as prebiotic ingredients and could have beneficial effects for the host (Van Loo et al., 1999).
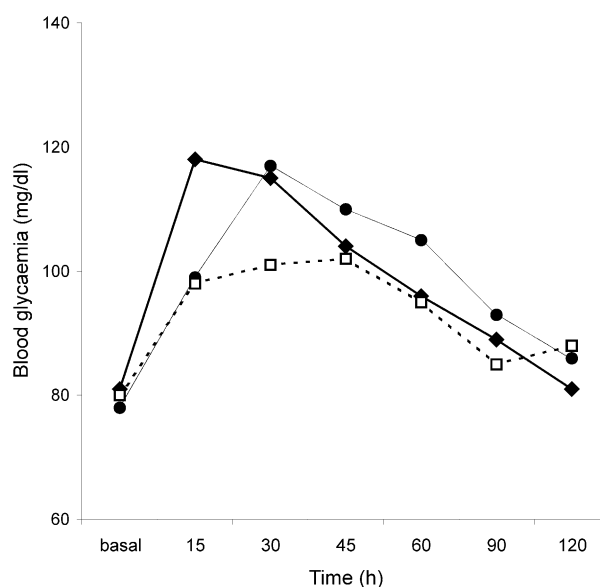

Fig. 1. In vitro starch hydrolyis (%) in white bread -●-, boiled wheat spaghetti -◆- and boiled wheat-chickpeas spaghetti (75:25) -□-.

Fig. 2. Mean blood glucose concentrations in healthy subjects after intake of white bread -●-; boiled wheat spaghetti -◆- and boiled wheat–chickpea spaghetti (75:25) -□-.

Table 2
Indigestible fraction and total dietary fibre content of two types of spaghetti[a]

	Wheat spaghetti (100% wheat flour)	Wheat–chickpea spaghetti (75% wheat flour and 25% chickpea flour)
Dietary fibre		
Insoluble[b]	6.36±0.12	6.50±0.16
Soluble[b]	3.03±0.07	2.91±0.08
Total[b]	9.39±0.12	9.41±0.16
Indigestible fraction		
Insoluble	11.9±0.79a	13.9±0.65b
Soluble	2.10±0.42a	3.10±0.44b
Total	14.0±0.82a	17.0±0.64b

[a] Mean±standard deviation ($n=4$). Different superscript letters in a row indicate significant differences ($P < 0.05$).
[b] Values not statistically different.

Table 3
Clinical data upon admission to the study, experimental fasting glucose levels and glycemic index (GI)

Number of subjects	12
Sex	Females
Age (y)	23.25±2.42
Body mass index (kg/m²)	22.46±1.79
Fasting glucose (mg/dl)	80.0±7.26
GI$_{wheat\ spaghetti}$	72.8±4.64a
GI$_{wheat–chickpea\ spaghetti}$	58.9±6.38b

Different letters denote statistically significant differences ($P \leqslant 0.05$).

Clinical data of volunteers are shown in Table 3. In vivo (Fig. 2) and in vitro (Fig. 1) results showed similar tendencies. The postprandial rises in blood glucose concentrations were smaller in subjects given spaghetti than those given white bread (Fig. 2). Chickpea flour reduced the hyperglycaemia peak and the total hyperglycaemia phase (area under curve). Maximum plasma glucose was reached during the first 30 min, but values were significantly lower for 45 min after wheat–chickpea spaghetti consumption. This helped to keep glucose levels moderate for up to 120 min.

The glycaemic index for two types of pasta was in the normal range for lente carbohydrate, but the index was significantly lower when the pasta contained chickpea flour ($GI_{wheat\ spaghetti}$: 73 ± 5; $GI_{wheat–chickpea\ spaghetti}$: 58 ± 6). Pasta products containing chickpea flour, presenting a low glycaemic response, could help broaden the range of low-GI foods available to the consumer. Further research is needed into the effect of non-digestible dietary components on the glycaemic response.

Acknowledgements

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