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Enrich pasta with faba bean do not impact glycemic nor insulin response but can enhance satiety feeling and digestive comfort when dried at very high temperature.

Short title: Nutritional properties of legume enriched pasta

Author's names

Valérie, Greffeuille[a]; Agnès, Marsset-Baglieri[b]; Nicolas, Molinari[c,d]; Denis, Cassan[a]; Thibault, Sutra[c]; Antoine, Avignon[c,d] and Valérie, Micard[a*].

Authors address

[a] SupAgro-INRA-UM2-CIRAD ; JRU 1208 Agropolymers Engineering and Emerging Technologies, 2 Place Pierre Viala, F- 34060 Montpellier, France

[b] INRA-AgroParisTech-CRNH-IdF; JRU 914 Nutrition Physiology and Ingestive Behavior, F-75005 Paris, France

[c] Centre Hospitalier Régional Universitaire de Montpellier, Montpellier, France.

[d] INSERM U1046, Physiology & Experimental medecine, Montpellier, France; Université Montpellier I, Université Montpellier II, Montpellier, France

*To whom correspondence should be addressed. E-mail : valerie.micard@supagro.fr; JRU IATE, 2 Place Pierre Viala, 34060 Montpellier cedex 01, France. Tel : +33499612889.

Abstract

Enrichment of durum wheat pasta with legume flour enhances their protein and essential amino acid content, especially lysine content. However, despite its nutritional potential, the addition of a legume alters the rheological properties of pasta. High temperature drying of pasta reduces this negative effect by strengthening its protein network. The aim of our study was to determine if these changes in pasta structure alter its *in vitro* carbohydrate digestibility, *in vivo* glycemic, insulin and satiety responses. We also investigated if high temperature drying of pasta can reduce the well-known digestive discomfort associated with the consumption of legume grains. Fifteen healthy volunteers consumed three test meals: durum wheat pasta dried at a low temperature (control), and pasta enriched with 35% faba bean dried at a low and at a very high temperature. When enriched with 35% legume flour, pasta kept its nutritionally valuable low glycemic and insulin index, despite its weaker protein network. Drying 35% faba bean pasta at a high temperature strengthened its protein network, decreased its *in vitro* carbohydrate digestion with no further decrease in its *in vivo* glycemic or insulin index. Drying pasta at a very high temperature reduced digestive discomfort and enhanced self-reported satiety, and was not associated with a modification of energy intake in the following meal.

Key words: legume pasta, drying temperature, GI, II, satiety, digestive comfort

Highlights

High temperature drying strengthened the textural properties of 35% legume pasta, reduced appetite, improved the digestive comfort and does not affect pasta’s low glycemic and insulin indices.

Introduction

Pasta is a very popular staple food that is easy to cook. This source of proteins (12-15% db) and carbohydrates (74-76% db) is traditionally manufactured from durum wheat semolina. An important healthy aspect of pasta is its low glycemic index (GI). The low GI of pasta is generally ascribed to its specific structure, which results from the successive structural changes that occur at macroscopic, microscopic and molecular scale throughout the pasta making process.[1] Hence, the low GI of pasta is generally explained by structural parameters such as compactness[2] and/or by the presence of a strong protein network entrapping starch granules.[3,4]

Despite its advantageously low GI, durum wheat pasta is deficient in two essential amino acids, lysine and threonine, due to a deficiency in durum wheat itself. To improve its amino acid profile, pasta have recently been formulated by mixing durum wheat with different legume flours with a complementary amino acid profile.[5-8] Up to 35% of faba bean flour has been successfully incorporated into pasta, thereby greatly improving its protein content[9] and its theoretical amino acid profile. Although interesting from a nutritional point of view, legume enrichment affects the structure of the pasta by modifying the nature and proportion of pasta components[9], thereby reducing pasta quality attributes (cooking losses, texture and taste).[6] This decrease in pasta quality can be counteracted by drying pasta at high temperatures, which strengthens its protein network.[6,10] Such changes in the structure of the pasta protein network can also affect starch digestibility. This has been demonstrated *in vitro* on pasta enriched with 35% faba bean flour.[11] Increasing the drying temperature of 35% faba bean pasta from 55 °C to 90 °C had a marked impact on its rapidly available glucose content (from 59.6% to 47.2% of total available carbohydrates).[11] Up to now, the impact of incorporating a legume in pasta on its glycemic index (GI) has been sporadically described for chickpea[5] and yellow pea flour[12] but never for faba bean enriched pasta. Although some authors described the glucose, insulin or satiety responses of bread or pretzels enriched with lupin, chickpea or soy flour,[13-15] the insulin index and the satiating properties of legume enriched pasta remain unknown. Concerning the impact of high temperature drying of pasta although demonstrated *in vitro* to slow down the starch digestibility, it has never been demonstrated *in vivo*.

The aim of the present work was thus to determine if, by changing the composition and structure of the pasta, pasta enrichment with a legume and/or high temperature drying affect its carbohydrate digestion profile (glycemia, insulinemia) and the satiety effect, in healthy volunteers. We also investigated if legumes, which are known to cause digestive discomfort when eaten as cooked grains, still have this property when incorporated in pasta.

Material and methods

Pasta processing and characterization

Manufacturing and cooking

Three types of pasta (spaghetti) were used in this study: (i) durum wheat pasta dried at low temperature (hereafter DW-LT) used as control, (ii) pasta enriched with 35% faba bean flour dried at a low temperature (hereafter F-LT) and (iii) pasta enriched with 35% faba bean flour dried at a very high temperature (hereafter F-VHT). The durum wheat pasta (DW-LT) was processed as described by Petitot et al.[16] and then dried at a low temperature (55 °C) in a pilot-scale drier (AFREM, Lyon, France) in order to reach 12% moisture. Faba bean enriched pasta were processed by replacing 35% of the durum wheat semolina by faba bean flour, as previously described by Petitot et al.[6]. Two drying profiles were then applied to the fresh faba bean pasta: a low temperature of 55 °C (F-LT) like for DW-LT pasta, and a very high temperature (90 °C for 4 h) applied at the end of the LT drying cycle (F-VHT). The diameter of all the dry spaghetti was 1.56 ± 0.02 mm. The dry spaghetti was cooked in Evian water (2 L/100 g) containing 0.7% (w/v) of sodium chloride. The optimal cooking time (OCT) of each pasta was determined according to the official method 66-50 AACC 2000. All analyses of cooked pasta were made using pasta cooked at OCT+1 min.

Composition of pasta.

Table 1 lists the nutritional and biochemical composition of the DW-LT, F-LT and F-VHT pasta. Total proteins (NF V18-120, 1997)[17] and lipids (JORF 08/09/77, 1977)[18] were determined by the Scientific Institute of Hygiene and Analysis (Longjumeau, France). Total carbohydrates were calculated by difference. Non-starch polysaccharide (NSP) analysis was performed by Englyst Carbohydrates LTD (Southampton, U.K.). NSPs were measured using the Englyst procedure including enzymatic starch hydrolysis, precipitation of NSP in ethanol, acid hydrolysis of NSP, and measurement of constituent sugars by HPLC[19]. Bioavailable lysine was calculated as the difference between the total lysine content determined after acid hydrolysis and unavailable lysine content determined by spectrophotometry following 1 fluoro, 2,4 dinitrobenzene assay (NF V18-103, 1985)[20]. All analyses were performed in duplicates and means are given in the Table 1.

Biochemical and rheological characterization of pasta

Protein size distribution of the dried pasta was performed according to the method described in Petitot et al.[16] This two-step extraction method, followed by the separation of proteins on size calibrated size-exclusion high-performance liquid chromatography (SE-HPLC), makes it possible to quantify proteins linked by non-covalent bonds, disulfide, and other covalent bonds. Results are expressed as the percent of total protein in the equivalent raw matter.

The rheological properties of cooked (OCT + 1 min) spaghetti were evaluated using a TA-XTplus (Stable Micro Systems, Scarsdale, USA) texture profile analyzer equipped with a Windows version of Texture Expert software package applying tensile tests. Tensile tests were performed as described previously in Petitot et al.[6] The initial distance between the two tensile grips was 15 mm and the test was performed at a constant deformation rate of (3 mm/s). Breaking stress (MPa) was measured on the stress-strain curve. It corresponds to the maximum extension force that can be applied to the pasta before it breaks and, from a sensory point of view, could represent a product's resistance to deformation.

In vitro starch digestibility

An *in vitro* starch digestion method that mimics human digestion was performed by Englyst Carbohydrates Ltd.[21] on pasta cooked at OCT+1 min. This method is based on HPLC measurement of the glucose released from a test food during timed incubation with digestive enzymes under standardized conditions. The glucose released from starch within 20 min incubation corresponds to rapidly available glucose (RAG). RAG has been found to be a predictor of post-prandial glycemia.[21] The glucose released from starch between 20 and 120 min incubation corresponds to slowly available glucose (SAG). The starch not digested after 120 min corresponds to resistant starch (RS).

In vivo study

Setting

This study was conducted in accordance with guidelines laid down in the declaration of Helsinki. All procedures involving human subjects were approved by the central Ethics Committee Sud Méditerranée III (Nimes, France), according to French law, under trial identification number 2010-A00671-38.

The clinical procedure was carried out during 2.5 months (February-April) in the Endocrinology-Diabetology-Nutrition Department by the Diabetology-Nutrition team of CHRU Lapeyronie Hospital, Montpellier. Biological analyses were performed in the Biochemical and Nuclear Medicine laboratories at the same hospital.

Subjects

Fifteen healthy subjects (8 males and 7 females) participated in the study. Exclusion criteria included subjects with pancreatic, liver or kidney diseases, diabetes, the use of medication likely to affect glycemia or appetite, cigarette smoking, excessive consumption of alcohol, allergy or intolerance to any food ingredients used in the study, dislike of pasta or other food provided for the test meals. We also excluded subjects who were not used to eating three regular meals per day, especially breakfast. Pregnant or breast-feeding women, athletes in training, people with a score >10 on the restraint scale of the Three-factor Eating Questionnaire[22] were also excluded from the study. All volunteers gave their written informed consent to take part in the study and received financial compensation for their participation. The true nature of the study was revealed to them during a debriefing session after the study was completed. The subjects' mean age was 24 +/- 2.9 yrs with a mean BMI of 22.4 +/- 1.8 kg/m^2.

Preloads

Four preloads were used: a glucose solution (50 g in 250 mL of water) as a reference for the determination of glycemia and insulinemia, and the three types of pasta DW-LT, F-LT, and F-VHT. Before serving, the pasta was cooked at their own optimal cooking time + 1 min: 10 min for DW-LT, 10.5 min for F-LT and 13.5 min for F-VHT. In these conditions, the starch in all the pasta tested was gelatinized (i.e. the white core disappearance; approved method 66-50 AACC, 2000). The serving sizes of pasta (180 g, 195 g and 206 g for DW-LT, F-LT and F-VHT of cooked pasta), respectively were chosen to supply 50 g of available carbohydrate whatever the pasta used. The available carbohydrate content of the pasta was determined by the sum of RAG + SAG + fructose calculated from the *in vitro* starch digestibility analysis using the Englyst method.[21]

Menu and foods

All the foods, with the exception of glucose and pasta preloads, were widely available industrial products. The *ad libitum* lunch, served in the laboratory, comprised *Basquaise* chicken (a typical French dish made of small pieces of chicken, rice and sauce mixed together), sweetened fresh cheese, and water to drink. All the food was served separately in generous portions to be sure that each volunteer reached satiety by the end of their meal. The portions were weighed before being served and then reweighed after the subjects had eaten, to obtain the net amount of each food consumed.

Design

All 15 volunteers participated in five identical sessions, separated by an interval of one week. A list of allowed ingredients for the evening meal prior to the test days was given to all the subjects and they were asked to abstain from alcohol consumption and intense physical effort. Subjects arrived at the laboratory at 8 am after a 10-12 h overnight fast and were installed comfortably in the test room. The 1st and 5th sessions were dedicated to glucose solutions, according to Brouns et al. [23] During the 2nd, 3rd and 4th sessions, subjects received one of the three types of pasta (DW-LT, F-LT or F-VHT) and 250 mL of water, once in random order. Subjects were blinded to which pasta they were receiving. The different preloads were served as breakfast between 8:30 and 9:30 am and had to be entirely consumed within 15 min. No other drinks or foods were allowed until the test meal served as lunch, 200 min after preload ingestion. After lunch, the volunteers left the laboratory.

Visual Analogue Scale (VAS)

In the morning, the volunteers had to fill out a VAS about their appetite sensations before and after preload ingestion, then every 30 minutes thereafter until 180 min, and before and after lunch. The subjects were asked to indicate, on a VAS scale of 0 to 100 mm, how they felt at the moment they completed the following questions: How hungry do you feel now? How full do you feel now? How strong is your desire to eat now? How much food do you think you could eat now? An appetite score was calculated to compare the satiety power of the preloads tested. It is the average between hunger, the desire to eat, prospective food consumption and a 100-fullness score. [24] The palatability of meals (preloads and lunch) was also measured using VAS.

Blood sampling for the determination of glycemia and insulinemia

At the beginning of the experiment, a catheter was inserted into a vein of the forearm to enable frequent blood sampling to measure insulin. Venous samples were collected 5 min before preload ingestion, and then at 0, 30, 60, 90, 120 and 180 min after consuming the preload. Serum insulin was measured by Electrochemiluminescence immunoassay (ECLIA).

Capillary blood was obtained by finger prick and glycemia was measured with a glucometer (Accu-Chek® Performa, Roche Diagnostics) when fasting (5 min before t=0 min), at t=0 min (beginning of the preload consumption) and then at 5, 10, 15, 30, 45, 60, 90, 120 and 180 min.

Digestive comfort

Subjects were asked to rate the global perception of abdominal comfort they felt from 0 to 12h, then from 12 to 24h following the ingestion of the different preloads, using a VAS of 100 mm. The questions were about the feeling of abdominal pain, abdominal discomfort, feeling bloated, feeling gurgling, having flatulence, feeling nauseous and having a headache.

Each term used was specifically defined on the form. An indicator of abdominal discomfort was then calculated as the mean of the rating for each question excluding headache.

Data analysis

The incremental area under the blood glucose response curve (iAUC) during the 120 min following preload ingestion, ignoring the area under the baseline (baseline corresponding to fasting glycemia), was calculated geometrically using the trapezoid method as previously described in Brouns et al. [23] The glycemic index (GI) of each type of pasta was calculated for each individual subject as the iAUC for the pasta preload, expressed as a percentage of the average incremental area under its two blood glucose response curves obtained with the two glucose preloads (GI of glucose solution=100). The GI values of each pasta corresponded to the mean of individual ratios. The insulin index (II) was calculated following the same principle as for the GI.

In addition, glycemic profiles (GP) and a glycemic profile index (GPI) were calculated to characterize the intensity of the glucose response to the ingested pasta preload. The GP of pasta was obtained for each subject by dividing the time (min) during which the blood glucose was above fasting concentration by the incremental peak value of blood glucose (mM) using Graph Pad Prism (Graph Pad Software, San Diego, USA) as described in Rosen et al.[25-27] Insulin profiles (IP) were calculated using the same method. GPI corresponds to the GP of the pasta preload expressed as the percentage of the GP of the glucose solution (GP of glucose solution=100). The insulin profile index (IPI) was calculated using the same method.

GI, II, GP, GPI, IP and IPI were calculated for each subject and the values for each pasta preload are the mean of individual values. Data are expressed as means ± SEM.

Statistical analysis

Statistical analyses were performed with R software (version 3.1.0 (2014-04-10)), the R Foundation for Statistical Computing)**.**

According to a previous internal study, we assumed an effect size of 34 points absolute difference in GI between the different types of pasta, with a similar within-subject deviation (34 points), we calculated that 12 volunteers would be required with a type I error of 0.025 (for multiple test penalization) and a power of 0.8.

Concerning the results of the palatability of the preloads, energy intake at lunch, appetite sensations and digestive discomfort, we compared only the effect of the three types of pasta together by excluding the two glucose tests.

Continuous variables are presented as means ±SD. The Shapiro-Wilk test was used to assess normal distribution. Comparison of continuous variables between two types of pasta was conducted with the paired Student's t-test for variables with a normal distribution and with the Wilcoxon test for paired data for variables with a non-normal distribution. ANOVA or a Kruskal-Wallis test was used to compare multiple groups (more than two foods).

Because each volunteer was evaluated several times (repeated measures) linear mixed-effects models for repeated measures were performed, allowing repeated measures to be taken into account as random variables. Box and Cox transformation was used to normalize the distribution. False discovery rate (FDR) control was used to correct for multiple comparisons.

A two sided P value of less than 0.05 was considered to be statistically significant.

Results and discussion

Impact of legume addition and high temperature drying on the nutritional composition, the protein network structure and the rheological properties of pasta.

Substituting faba bean flour for 35% of durum wheat semolina during processing of the pasta modified its macronutrient composition notably by increasing its protein and fiber (NSP) contents and reducing its total carbohydrate contents (Table 1). The use of faba bean also increased the amount of available lysine in the legume enriched pasta. At the opposite, the use of high temperature for pasta drying reduced the availability of lysine. This lysine loss could be associated to the formation of Amadori compounds during the Maillard reaction[28] as demonstrated by the change in color parameters in pasta dried at high temperature (increase of pasta redness, decrease in pasta yellowness and darkening).[6] However, even if lysine content of faba bean pasta decrease with VHT drying, F-VHT pasta still contained more than twice the concentration of available lysine than traditional pasta.

The study of protein size distribution in DW-LT, F-LT and F-VHT pasta highlighted changes in their protein network structure. Respectively 71±1.4, 81±0.2 and 24±0.3% of DW-LT, F-LT and F-VHT pasta proteins were non-covalently linked. 26±0.2, 16±0.8 and 55±0.4 % were linked by disulfide bonds, and respectively 3±1.2, 3±0.5 and 21±0.1% were linked by other covalent bonds. Including faba bean flour in pasta therefore weakened the protein network in the dried pasta compared to durum wheat pasta. Conversely, drying at a very high temperature strengthened the protein network with 76% of the proteins linked with covalent bonds in F-VHT. According to our previous studies on faba bean pasta,[9,11] the differences in protein structure between DW-LT and F-LT and between F-LT and F-VHT remained significant, even though less accentuated, after cooking.

The addition of faba bean also significantly ($p<0.05$) increased the resistance of the pasta to extension (F-LT breaking stress was 0.141 MPa versus 0.077 MPa for DW-LT). Resistance also increased significantly when the faba bean pasta was dried at high temperature (F-VHT breaking stress= 0.221 MPa). Similar changes in textural properties were previously observed by Petitot et al.[6] in pasta enriched with 35% legume flour [6] or with 15% pea fiber.[29]

***In vitro* carbohydrate digestibility of pasta**

The amount of total available carbohydrates was significantly lower in faba bean enriched pasta than in durum wheat pasta (table 2), in agreement with our previous work on faba bean pasta.[9] The faba bean pasta also contained significantly ($p<0.05$) higher amounts of resistant starch. This finding is in agreement with previous studies reporting a low rate of starch hydrolysis in legumes.[30,31] However in our case, this did not lead to a significant difference in the percentages of RAG and SAG between DW-LT and F-LT. Conversely, the very high drying temperature applied to the faba bean pasta significantly ($p<0.05$) reduced the proportion of its RAG content (F-VHT versus F-LT or DW-LT pasta). The ratio of SAG in F-VHT was therefore significantly ($p<0.05$) higher than in DW-LT and F-LT. These results are in accordance with those of our previous study on faba bean pasta.[9,11]

The use of high temperature drying created a strongly aggregated protein network in F-VHT pasta since 76% of the proteins were linked via covalent bonds. This highly aggregated protein network has been shown to reduce *in vitro* starch digestibility in durum wheat [32,16] as well as in faba bean enriched pasta.[11] With regard to the RAG and SAG ratio obtained in our study, F-VHT pasta contains lower amount of Rapidly Available Glucose when compared to F-LT or DW-FT pasta. According to the literature, RAG is a strong predictor of postprandial glycemia.[33] The difference in the rate of *in vitro* carbohydrate digestion thus indicated that F-VHT pasta would produce a lower glycemic response *in vivo* than F-LT or DW-LT.

***In vivo* glucose response and glycemic index**

The profile of the glycemic response to the glucose solution was classical, with a high peak at 30 min and a drop under the baseline after 120 minutes (Figure 1). No significant difference was observed between the three pasta profiles. The blood glucose concentrations caused by all three types of pasta were significantly lower ($p<0.05$) than that caused by the glucose solution from 10 to 90 min after the ingestion of the preload. No drop under baseline was observed for any pasta after 120 or even 180 minutes. When subjects ingested DW-LT or F-LT pasta, a small second "peak" of glycemia was observed at 120 min.

The incremental area under the curve (iAUC) and the maximum incremental peak (iPeak max) in glycemia obtained with glucose solution were significantly higher than the values obtained for pasta, whereas peak time did not significantly differ. Thus, the glycemic profile was significantly lower for glucose solution compared to pasta. The GP value calculated at 180 min (data not shown ; 44.2 ± 8.7 min/mM) was closed to the values previously reported at 180 min for white wheat bread [25-27] or white wheat porridge.[25] There was no difference in glucose incremental area under the curve (iAUC), in the maximum peak in blood glucose concentrations (iPeak max), or in peak time values among the three types of pasta (table 3). The GI calculated at 120 min did not differ significantly among the three types of pasta and corresponded to low-GI foods;[34] nor was there a significant difference in the GP and GPI among the pasta. All the indexes calculated at 180 min gave similar results.

In accordance with the results of *in vitro* carbohydrate digestion, which revealed no significant differences between the RAG ratios of the DW-LT and F-LT pasta when faba bean flour was used to replace a high proportion of durum wheat, the GI of the pasta did not change. Several studies have shown that the incorporation of a legume (10% to 30% substitution) in cereal-based products such as bread,[13,14,35,36] cake,[37] biscotti,[12] pretzel,[15] chapattis[38] or pasta,[5] significantly reduced[5,12,14,35-38] or tended to reduce[14] the glycemic response. Concerning pasta, only two studies reported the effect of incorporating a legume on its glycemic response.[5,12] The first study reported that the incorporation of 25% chickpea flour into spaghetti resulted in a significantly lower GI (58.9) than the GI of traditional spaghetti

(72.8).[5] However, in the second study, Marinangeli et al.[12] showed that the addition of 30% whole yellow-pea flour to pasta failed to reduce and even slightly increased the GI (93.3) compared to a whole wheat flour pasta (83.6). In our study, the GI of the pasta enriched with 35% faba bean (41.9 for F-LT and 49.4 for F-VHT) did not differ significantly from the GI of traditional durum wheat pasta (52.3). The effect of adding a legume to pasta therefore appears to differ depending on the nature and ratio of the legume used for enrichment, and also probably on the structure of the initial pasta matrix used as a reference. The GI values of our enriched pasta (F-LT or F-VHT) are of interest as they classify our legume pasta in the category of low GI foods according to the International Standards Organisation classification (ref number à ajouter). They had an even lower GI than the 25% chickpea or 30% whole yellow pea flour pasta analyzed by Goni et al.[5] and Marinangeli et al.[12] which could explain why no additional reduction of GI was obtained in our study with VHT drying. A low GI of pasta is generally explained by structural parameters such as its compactness[2] and/or the presence of a strong protein network that entraps the starch granules.[3] In our study, the protein network structure of pasta was weakened by incorporating faba bean (81% versus 71% of non-covalent linked proteins in the F-LT pasta compared to the DW-LT pasta). Thus, if the pasta maintained a low GI when 35% faba bean was incorporated (F-LT Pasta), this cannot be explained by a strengthening of protein network at supramolecular scale. The higher breaking stress of F-LT (0.141 MPa) *vs* DW-LT (0.077 MPa) traduced however a modification of the structure of the pasta due to faba bean addition. This modification could have occurred at higher organizational scales, e.g. macro or microscopic, and could be responsible for the low F-LT GI.

The use of the high drying temperature for the legume-enriched pasta strengthened the structure of the protein network (76% of covalently linked proteins) and also significantly altered the texture of the F-VHT pasta, which was more resistant to deformation than the DW-

LT or F-LT (F-VHT breaking stress: 0.221MPa). However, even though these modifications in the structure of the pasta at supramolecular and macromolecular scales had a significant impact on carbohydrate digestion *in vitro*, no effect was identified *in vivo*. The *in vitro* digestion rates, and particularly the RAG of food, have been reported to be good indicators of GI.[21] However, in our study, the *in vitro* results of carbohydrate digestion failed to match *in vivo* results, contrary to the results of the study by Goñi et al.,[5] in which *in vivo* and *in vitro* results of carbohydrate digestion of chickpea enriched pasta showed similar tendencies.[5] It is possible that the great variability of human response to the ingestion of food has lowered the highlighted effect observed *in vitro*, in controlled conditions. The heterogeneous and complex conditions occurring during the *in vivo* digestion of starch are not reproduced by current *in vitro* digestion protocols. Hasjim et al. [39] have therefore demonstrated that the RAG and SAG contents analyzed using *in vivo* (pigs) and *in vitro* digestion could be different. The preparation step used prior to *in vitro* starch digestion procedures has also been recently demonstrated to affect the predictive glycemic response and hence increase differences attributed to food composition or structure. [40] At the end, slowly and rapidly digested starchy foods were also demonstrated to be able to elicit a similar glycemic response in healthy men due to a differential glucose metabolism. [41]

Insulin response and insulinemic index

Only a few studies have reported the glycemic index of legume enriched pasta,[5,12] and, to our knowledge, no study has previously determined insulin response to this mixed food.

The insulin profile reflects the glucose profile commented above, with a high peak at 30 min and a drop under baseline over 120 minutes (Figure 2). The three types of pasta produced similar insulinemia curves with a significantly ($p<0.05$) lower concentration of insulin than the glucose solution until 120 min and no drop under baseline even after 180 min. When subjects ingested F-LT pasta, a small second “peak” of insulinemia was observed at 120 min.

The addition of the legume to pasta associated with high temperature drying significantly increased the intensity of the insulin peak for F-VHT compared to DW-LT and to F-LT (table 3). Since the peak duration (180 min) was identical for the three types of pasta, this difference in peak intensity led to a significant difference ($p<0.05$) in the Insulin Profile (IP) and in the Insulin Profile Index (IPI) of the two pasta that included faba bean compared to the durum wheat pasta. The addition of the legume to pasta also tended to accelerate (peak time) insulin response, as emphasized by the thermal treatment of F-VHT pasta ($p<0.05$).

Although GI was not modified by the faba bean enrichment in pasta, the insulin response was more intense and more rapid in the legume-enriched pasta than in the DW-LT pasta. This effect on insulin secretion is probably due to the higher protein content and particularly to insulinogenic amino acid in the faba bean pasta, as previously suggested by other authors in studies on bread made with chickpea flour consumed as part of breakfast,[14] cakes made from whole soy powder consumed alone or with paddy rice,[37] in a pre-meal protein drink containing soy protein isolate and other amino acids [42] and in several foods enriched with cocoa powder.[43]

Palatability of preloads and lunch, energy intake at lunch and subjective satiety

The palatability of the F-VHT pasta (39.6 ± 5.4 mm) was significantly lower than the two others pasta (56.5 ± 4.8 and 58.2 ± 5.6 mm for F-LT and DW-LT respectively). There was no difference of palatability between F-LT and DW-LT.

The palatability of lunch did not significantly differ as a function of the pasta ingested as preloads, with a score of 54.4 ± 3.8, 49.9 ± 4.4 and 48.6 ± 3.9 mm on the VAS for lunch after F-LT, F-VHT and DW-LT preload ingestion, respectively.

Concerning energy intake at lunch, there was no significant pasta preload effect (3517.9 ± 351.2, 3160.5 ± 254.6 and 3412.6 ± 318.9 kJ for lunch after ingestion of F-LT, F-VHT and DW-LT preload, respectively).

Concerning the cumulative energy intake for preloads and lunch (energy of pasta + energy of *ad libitum* lunch), there was no significant effect of the pasta preload (4635.2 ± 351.2, 4355.2 ± 254.6 and 4508.8 ± 318.9 kJ for F-LT, F-VHT and DW-LT preloads, respectively).

Hunger, the desire to eat, and prospective consumption decreased just after consumption of the preload and increased progressively until lunch. The opposite pattern was observed for sensations of fullness. Compared with DW-LT and F-LT, F-VHT given at breakfast resulted in significantly higher self-reported satiety as expressed by the appetite score (Figure 3). Between DW-LT and F-VHT, significance values for time-by-treatment interactions in the models for hunger, fullness, desire to eat and appetite score were P=0.005, P=0.008, P=0.02 and P=0.006 respectively. In the same way, significance values for time-by-treatment interactions in the models for prospective food consumption, desire to eat, fullness and appetite score were P=0.02, P=0.02, P=0.02 and P=0.01, between F-LT and F-VHT, respectively. No difference was observed between F-LT and DW-LT for all the appetite sensations reported.

Few studies have reported the impact of legume protein enrichment of a food on energy intake and satiety.[13-15, 44] Our results are in accordance with those reported by Hall et al.[13] (bread including 10% Australian sweet lupin as part of breakfast) and Johnson et al.[14] (bread with 24.3% of chickpea or extruded chickpea flour as part of breakfast), who reported that legume enrichment of bread did not affect satiety or food intake. In contrast, Lee et al. [44] observed a decrease in food intake 3 h after the ingestion of a portion of lupin enriched bread compared to white wheat bread, which could be attributed to the fact that the two breads used in that study had large difference in their protein content.

Consumption of F-VHT pasta led to a significant reduction in the appetite score between F-VHT and the two other types of pasta. This reduction could also be due to the different texture of the F-VHT pasta compared to the two other types of pasta, as described by their rheological properties. Indeed numerous studies [45-48] have examined the relations between the oral processing characteristics of a food, satiation, and appetite sensations. It appears that softer textures result in less chewing activity, lower oro-sensory exposure times, lower expected satiation and lower appetite sensations. Conversely, harder textures increase the total oral processing time, satiation and appetite sensations. Because of their increased hardness, F-VHT pasta could have extended chewing time compared to the two other types of pasta, which could have reduced their appetite score.

Digestive comfort

No difference was observed between the three types of pasta in feeling gurgling or flatulence. F-VHT significantly reduced abdominal discomfort (p=0.01) and feeling bloated (p=0.02) compared to F-LT with a score of 3.2±4.7 and 6.1±9.5 versus 11.3±20.3 and 14.8±23.7 mm on the VAS, respectively. As a result, the indicator of abdominal discomfort was significantly lower (p=0.007) for F-VHT (5.6±5.8 mm) than for F-LT (12.0±16.3 mm). No difference in this indicator was observed between F-LT and DW-LT (7.5 ± 10.4 mm), nor between F-VHT and DW-LT. Legumes are often considered to cause abdominal upset. This effect is generally primarily attributed to the presence of alpha-galactosides found in appreciable concentrations in legume grains.[49] The reduction in abdominal discomfort observed with the F-VHT pasta could be explained by the additional high temperature drying treatment applied to this pasta in comparison to the F-LT pasta. Several processes have been reported to reduce the alpha-galactoside content of legumes including soaking, cooking, germinating, fermentation and adding enzymes.[49] More specifically concerning faba bean, Vidal-Valverde et al.[50] reported that dry heating (120°C, 1 atm for 15 min) led to a 56% reduction in alpha-galactoside content. The high temperature drying applied to the faba bean pasta could therefore have reduced their alpha-galactoside content, thereby improving digestive comfort, as observed for the F-VHT pasta in our study. Beside abdominal discomfort, these compounds can also contribute by their fermentation to positive change in the human microbiote composition [49], asking the question of the health benefits of removing them from food.

Conclusion

This is the first study of the glycemic and insulin responses to faba bean enriched pasta as well as it evaluated satiety of subjects after ingestion of such the composite pasta. Our results emphasize to what extent modifying the structure of the food matrix by changing manufacturing conditions can affect the nutritional characteristics of pasta. High rates of incorporation (up to 35%) of faba bean changed the structure of the pasta at different scales. This was demonstrated by changes in the rheological properties and by a weakening of the protein network of F-LT. These changes in the structure of F-LT pasta were not reflected in its GI and II indices *in vivo*. The pasta enriched with 35% faba beans thus remained a nutritionally valuable food, with a low glycemic index along with an increase in nutritional values (higher protein, lysine and fiber content) compared to its 100% durum wheat homolog. Preliminary results, obtained *in vitro*, lead us to think that the GI of the faba bean pasta could be further reduced by using high temperature drying treatment. But even if structural changes, such as a strengthened protein network and higher resistance to deformation, were obtained in the F-VHT pasta, this did not lead to a reduction in the GI. However, the use of high temperature drying for the pasta enriched with faba bean improved its global digestive comfort and led to a decrease in appetite after eating. The impact of processing conditions on food structure and their relation to the appetite sensation remains largely unknown and could be an innovative way to modulate the feeling of satiety after food consumption.

Authors information

Corresponding author

E-mail: valerie.micard@supagro.fr; JRU IATE, 2 Place Pierre Viala, 34060 Montpellier cedex 01, France. Tel: +33499612889. Fax: 04 99 61 30 76

Note

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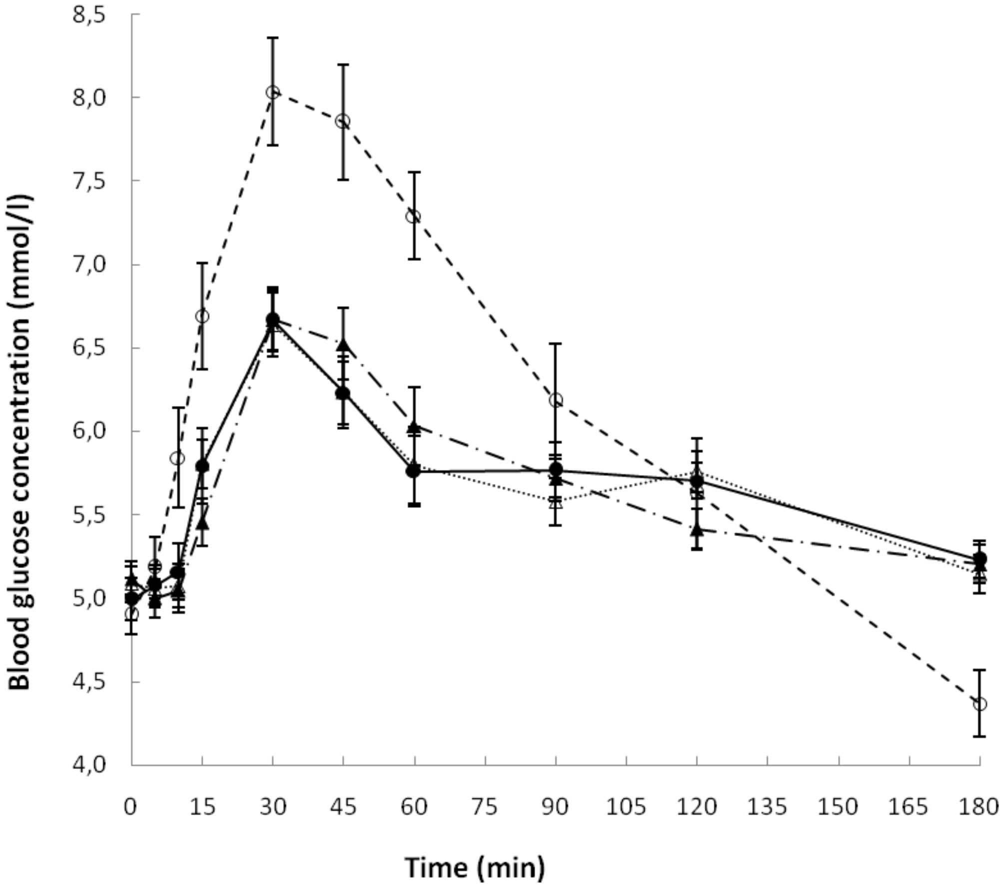
Figures captions

Figure1: Fasting and postprandial blood glucose response to the ingestion of glucose solution or pasta. Values at different time point correspond to the mean of 15 subjects except for DW-LT (n=14). Glucose solutions were tested two times by each 15 subjects. Values are means, with standard errors of the means represented as vertical bars. Glucose solution; DW-LT; F-LT; F-VHT.

Figure 2: Serum insulin concentration (nmol/L) of subjects in response to the ingestion of glucose solution or pasta. Values at different time point correspond to the mean of 15 subjects except for DW-LT (n=14). Glucose solutions were tested two times by each subject. Values are means, with standard errors of the means represented as vertical bars. Glucose solution; DW-LT; F-LT; F-VHT.

Figure 3: Hunger, fullness, desire to eat, prospective food consumption and appetite scores rated by visual analogue scales (VAS) throughout the test (n=15). Values are means ±SEM. Glucose DW-LT; F-LT; F -VHT. Time-by-treatment interactions in the models were for hunger, fullness, desire to eat and appetite score between DW-LT and F-VHT. Time-by-treatment interactions in the models were significant for prospective food consumption, desire to eat, fullness and appetite score between F-LT and F-VHT.

Figure 1



Blood glucose concentration (mmol/l)
8,5
8,0
7,5
7,0
6,5
6,0
5,5
5,0
4,5
4,0
0
15
30
45
60
75
90
105
120
135
150
165
180
Time (min)

Figure 2

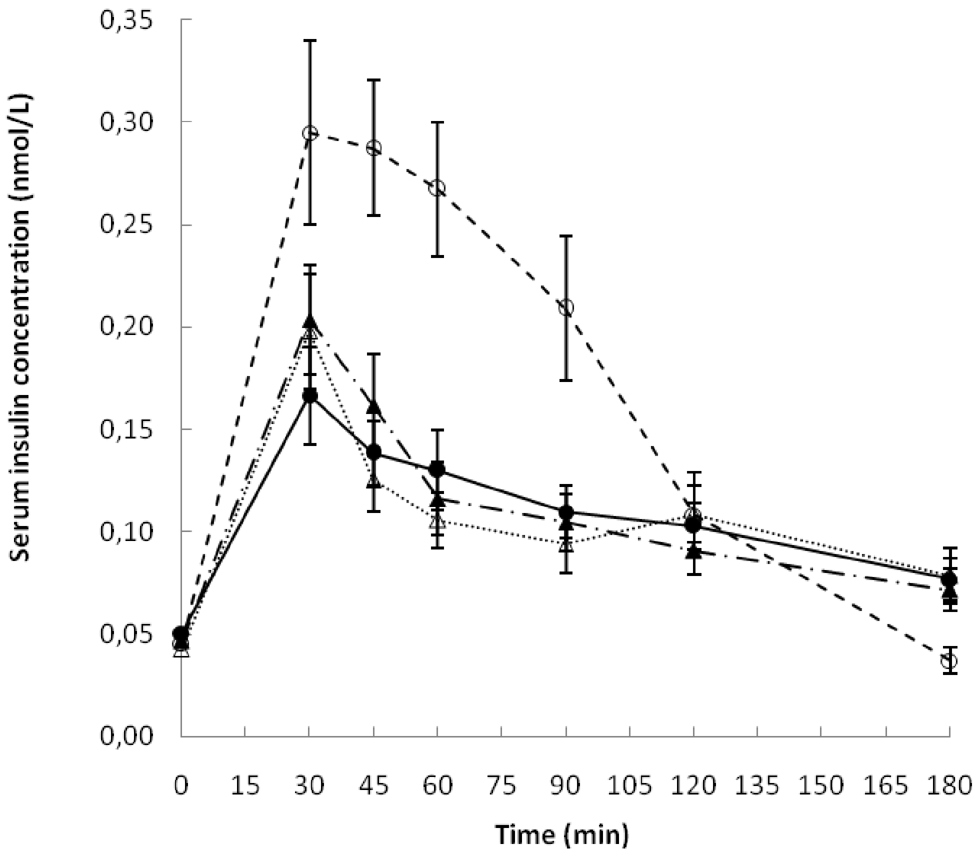
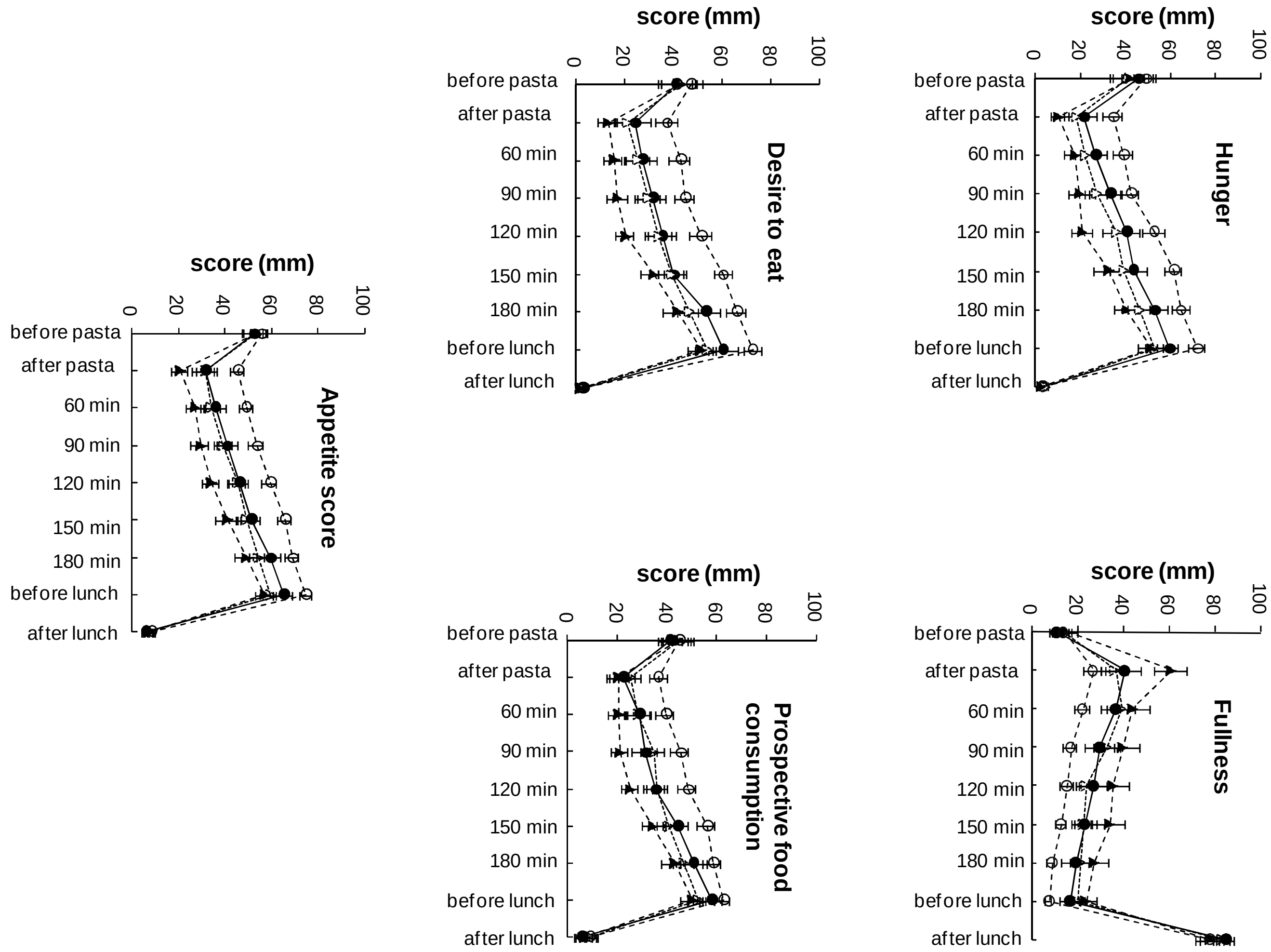

0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
Serum insulin concentration (nmol/L)
0 15 30 45 60 75 90 105 120 135 150 165 180
Time (min)

Figure 3


Hunger
score (mm)
0
20
40
60
80
100
before pasta
after pasta
60 min
90 min
120 min
150 min
180 min
before lunch
after lunch
Fullness
Desire to eat
Prospective food consumption
Appetite score

Tables

Table 1: Nutritional composition of pasta (per 100g cooked pasta)

Pasta	**DW-LT**	**F-LT**	**F-VHT**
Energy (kJ)	609	573	580
Total carbohydrate (g)	29.4	26.4	26.8
Total Non starch polysaccharides (g)	3.8	4.6	4.8
Soluble Non starch polysaccharides (g)	1.9	2.0	2.3
Insoluble Non starch polysaccharides (g)	1.9	2.6	2.5
Total fat (g)	0.8	0.6	0.5
Total protein (g)	4.7	6.1	6.3
Available lysine content (g)	0.10	0.26	0.21

DW-LT: 100% durum wheat pasta dried at low temperature; F-LT:35% faba-bean pasta dried at low temperature; F-VHT: 35% faba-bean pasta dried at very high temperature

Table 2: *In vitro* digestibility of carbohydrates in cooked pasta

Pasta	Dry matter (%)	RS (g/100g w.b.)	Total available carbohydrates (g/100g w.b.)	RAG (% available carbohydrates)	SAG (% available carbohydrates)
DW-LT	32.8	0.58 ± 0.01 $_a$	27.8± 0.3 $_a$	67.0± 0.7 $_a$	32.4±0.7 $_a$
F-LT	33.7	0.88 ± 0.04 $_b$	25.6± 0.3 $_b$	64.9± 3.2 $_a$	34.4± 3.2 $_a$
F-VHT	33.3	0.95 ± 0.04 $_b$	24.3± 0.1 $_c$	53.0± 0.4 $_b$	46.3± 0.4 $_b$

DW-LT: 100% durum wheat pasta dried at low temperature; F-LT: faba-bean containing pasta dried at low temperature; F-VHT: faba-bean containing pasta dried at very high temperature.

RS: Resistant Starch (expressed as glucose equivalent); RAG: Rapidly Available Glucose; SAG: Slowly Available Glucose. w.b.: wet basis.

Values are presented as mean ±SD

$_{a,b,c}$ *Mean values within a column with different superscript letters differ significantly (p<0.05) (ANOVA followed by Student's Test).*

Table 3: Blood glucose and insulin responses during 120 min after the ingestion of glucose solution, DW-LT, F-LT and F-VHT.

Samples	Glucose solution	DW-LT	F-LT	F-VHT
Glycemia				
iAUC (mM.min)	223.5± 14.8 $_a$	104.9 ± 12.6 $_b$	96.0 ± 15.4 $_b$	103.2 ± 14.4 $_b$
iPeak max (mmol/L)	3.5± 0.3 $_a$	1.8 ± 0.2 $_b$	1.9±0.2 $_b$	1.9± 0.2 $_b$
Peak time (min)	39± 5 $_a$	40 ± 6 $_a$	41± 6 $_a$	33± 3 $_a$
GI (%)	100 $_a$	52.3 ± 7.0 $_b$	41.9± 5.7$_b$	49.4± 6.8 $_b$
GP (min/mM)	34.6± 5.9 $_a$	65.8 ± 5.7 $_b$	56.0± 5.5 $_b$	56.5± 4.7 $_b$
GPI (%)	100 $_a$	210.3± 26.6 $_b$	174.9± 20.6 $_b$	179.0± 21.4 $_b$
Insulin				
iAUC (nM.min)	20.5± 1.8 $_a$	8.5± 1.2 $_b$	9.6± 1.0 $_b$	9.9± 1.4 $_b$
iPeak max (nmol/L)	0.32± 0.08 $_a$	0.13± 0.02 $_b$	0.17± 0.03 $_b$	0.23± 0.07 $_a$
Peak time (min)	50± 2 $_{ab}$	58± 8 $_a$	49± 11 $_{ab}$	32± 1 $_b$
II (%)	100 $_a$	45.7± 6.8 $_b$	50.1± 5.2 $_b$	55.0± 8.6 $_b$
IP (min/nM)	461± 222 $_a$	1276± 209 $_c$	925± 145 $_b$	857± 119 $_b$
IPI (%)	100 $_a$	300.3± 43.2 $_c$	218.0± 30.2 $_b$	207.7± 29.5 $_b$

DW-LT: 100% durum wheat pasta dried at low temperature; F-LT: faba-bean containing pasta dried at low temperature; F-VHT: faba-bean containing pasta dried at very high temperature

iAUC: incremental area under the curve; iPeak max: maximum incremental peak of concentration; GI, Glycemic Index; GP, Glycemic Profile; GPI, Glycemic Profile Index; II, Insulinemic Index; IP, Insulinemic Profile; IPI, Insulinemic Profile Index. Data are presented as means±SEM (n=15 except for DW-LT where n=14).

$_{a,b,c}$ *Mean values within a line with unlike superscript letters were significantly different (p<0·05).* Linear mixed model (fixed effect: type of preloads; random effect: volunteers) followed by Tuckey's test.