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Pasta added with chickpea flour: chemical composition, In vitro starch digestibility and predicted glycemic index
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Abstract

Pasta was prepared with durum wheat flour mixed with chickpea flour at two different levels and its chemical composition, in vitro starch digestibility and predicted glycemic index were assessed. Protein, ash, lipid, and dietary fiber content increased while total starch decreased with the chickpea flour level in the composite pasta, all in accordance to the composition of the legume flour. Potentially available starch decreased and resistant starch (RS) increased by adding chickpea flour to the pasta. The main indigestible starch component in composite spaghetti was the fiber-associated RS, representing up to 50% of total RS levels. The starch hydrolysis index (HI) decreased as chickpea flour in the pasta increased, reflecting the slow and low digestion of the starch in the leguminous ingredient. Predicted glycemic index was lower in spaghetti added with chickpea flour than in durum wheat-control pasta. Pasta added with chickpea flour might be a dietetic alternative for people with low-calorie requirements.

Keywords: Glycemic index, chemical composition, pasta, chickpea, resistant starch, dietary fiber

INTRODUCTION

In the past, food was only thought of as something having taste, aroma, color, texture, contributing to the general nutritional status. Actually, most consumers recognize additional categories of foods, including functional foods and nutraceuticals (Burdock et al., 2006). Nutraceuticals are food or food ingredients that have specific physiological effects. The nutraceutical concept was originally defined as "a food or parts of food that provide medical or health benefits, including the prevention and/or treatment of disease" (DeFelice, 1995). Nutraceuticals may range from isolated nutrients, dietary fiber supplements to genetically engineered foods, herbal products and processed products such as cereals, pasta, soups, and beverages (Andlauer and Fürst, 2002). Both low-carbohydrate and slowly-digested carbohydrate food products are considered nutraceuticals. Pasta is considered a slowly digestible starch food, a feature governed by the particular physical characteristics of the product (Granfeldt, 1994). Additionally, pasta may be easily added with other ingredients such as vitamins, flavors,
colorants, dietary fiber supplements, proteins, etc. with the objective to increase its nutraceutical characteristics.

Grain legumes are a valuable source of protein (18-25 %, dry basis) and carbohydrates (50-60 %, dry basis), with starch (22-45 %, dry basis) and non-starch polysaccharides (dietary fiber) as predominant fractions, and a small but significant amount of oligosaccharides (Bravo et al., 1998; Hoover and Zhou, 2003; Tharanathan and Mahadevamma, 2003). In several countries, legumes have increasingly been used in dietetic formulations to prevent diabetes and cardiovascular diseases, lowering blood cholesterol levels and reducing colon cancer risk (Brand et al., 1990).

Native legume starches are less easily digested than native cereal or root starches (Socorro et al., 1989). Several studies have been published on the in vitro and in vivo digestion of starch in legumes and the resistant starch (RS) formation during cooking and storage of legumes-based food products (Bravo et al., 1998; Bravo et al., 1999; Hoover and Zhou, 2003; Jenkins et al., 1982; Osorio-Díaz et al., 2005; Tovar et al., 1992a; Tovar and Melito, 1996; Velasco et al., 1997). The high RS content in legumes explains, at least partly, why the starch digestion rate and therefore the release of glucose into the blood stream are slower after the ingestion of legumes, resulting in reduced glycemic and insulimemic postprandial responses in comparison with cereal grains or potatoes (Jenkins et al., 1982; Tovar et al., 1992a; Tovar et al., 1992b). Legumes have also high amounts of dietary fiber in a form that confers high mechanical resistance to the cell walls, thus minimizing the seed disintegration during cooking (Melito and Tovar, 1995; Tovar et al., 1992a; Tovar et al., 1992b; Würsch et al., 1986). This, along with the presence of certain antinutrients, may also account for the slow and relatively low digestibility of starch in legumes. On the other hand, it is now believed that food proteins are not only a source of amino acids, but also they may play bioactive roles by themselves and/or can be the precursors of biologically active peptides with several physiological functions (Duranti, 2006; Nilsson et al., 2004). Due to the high protein content and the presence of some antinutritional compounds of proteinaceous character (such as lectins, trypsin, chymotrypsin and amylase inhibitors), positive effects of legume protein on human health beyond classical nutritional properties are expected (Duranti, 2006).

The aim of this study was evaluate the influence of chickpea flour level on the chemical composition, starch digestibility and predicted glycemic index of composite legume-wheat pasta.

MATERIALS AND METHODS

Chickpea flour preparation

Sun-dried chickpea seeds were provided by Sonora University, México. Seeds were ground using a commercial grinder (Mapisa Internacional S.A. de C.V., México, D.F.) to pass a US no. 50 sieve (300 µm) and stored at room temperature (25 ºC) in a glass container.

Chemical composition of chickpea and durum wheat flours

Moisture content was determined by gravimetric heating (130 ± 2 ºC for 2 h) using a 2-3 g sample. Ash, protein, fat, and dietary fiber were analyzed according to AACC methods 08-01, 46-13, 30-25, and 32-05, respectively (AACC, 2000). Dietary fiber (DF) was tested using the 985.29 AOAC method (AOAC, 1999). Total starch content was analyzed enzymatically after dispersion of the sample in 2 N KOH, according to Goñi et al. (1997). These analyses were carried out in triplicate.

Preparation of pasta

Pasta was prepared by mixing two levels of durum wheat flour and chickpea flour 80:20 and 60:40, respectively. Control pasta was prepared with 100 % durum wheat flour. The control sample and blends were added with enough NaCl solution (2 %, w/v) to achieve complete hydration of the flour. Mixing was done with a mixer (N50, Hobart, North York, Canada) for 4.5 min using a low speed (speed position 1 of the mixer). The mixed dough was sheeted through the rolls of a pasta machine with gap setting at 30 mm. After the first pass, the pasta sheet was folded and passed twice through the rollers at this same setting. The dough sheet was cut through no. 12 cutting rolls into strips of approximately 30 cm in length, with 0.3 × 0.2 cm cross-section. The resulting spaghetti strip was immersed in boiling water for 12 min after being allowed to drain for 2 min. All samples were frozen in liquid nitrogen, freeze dried and stored at room temperature in sealed plastic containers.

Chemical composition of pasta

The same methods described for the assessment of chemical composition of chickpea and durum wheat flours were used. Spaghettis were analyzed after cooking and draining, as for eating.

In vitro digestibility tests

Potentially available starch content was assessed following the multienzymatic protocol reported by Holm et al. (1986). The sample (300 mg, dry basis) was suspended in 20 mL of distilled water and incubated with α-amylase (Termamyl® Novo A/S, Copenhagen) in a boiling water bath for 20 min. This mixture was then diluted to 100 mL with distilled water. To 0.5 mL of this suspension, amyloglucosidase (Roche, Indianapolis, IN) and 0.1 mol/L Na acetate buffer, pH 4.75 (1 mL) were added. The mixture was incubated for 30 min at 60 ºC, diluted to 10 mL with distilled water, and analyzed for glucose using glucose oxidase peroxidase assay (SERAPAK® Plus, Bayer de México, S.A. de C.V., Edo. de México). Resistant starch
was measured by two protocols: (1) resistant starch associated to fiber (RSAF or RS3; Tovar, 2001) content was measured as starch remnants in dietary fiber residues, according to the so called “Lund method” as modified by Saura-Calixto et al. (1993). (2) The method proposed by Goñi et al. (1996) was employed to estimate the total amount of indigestible starch (comprising RS2, RS3 and part of RS1 fractions; Tovar, 2001). In brief, removal of protein with pepsin P-7012 (Sigma Chemical Co., St. Louis, MO) at 40 °C, 1 h, pH 1.5, incubation with amylase A-3176 (Sigma Chemical Co., St. Louis, MO) at 37 °C for 16 h to hydrolyze digestible starch, treatment of the insoluble residue with 2 M KOH, incubation with amylglucosidase A-7255 (Sigma Chemical Co., St. Louis, MO) at 60 °C, 45 min, pH 4.75, and determination of glucose using a glucose oxidase peroxidase assay (SERA-PAK® Plus, Bayer de México, S.A. de C.V., Estado de México).

Starch hydrolysis index of products "as eaten" (chewing/dialysis test)

The in vitro rate of starch hydrolysis was assessed following the protocol developed by Granfeldt et al. (1992). Samples of each product containing 1 g of available starch were tested. Six healthy subjects participated in the chewing phase of the experiments, which was followed by pepsin digestion and further incubation with porcine pancreatic amylase in a dialysis bag. The reducing amyloysis products appearing in the dialysate were measured colorimetrically, and expressed as maltose equivalents. Data were plotted as hydrolysis degree vs. time curves and the Hydrolysis Index (HI) was calculated as the area under the curve (0-180 min) for the test product expressed as a percentage of the corresponding area for white bread, chewed by the same person. The average HI was calculated from the six digestions replicates run for each sample and means were compared by the Mann-Whitney test, using statistical program (SPSS v. 2.03, Chicago, IL).

The predicted Glycemic Index (pGI) was calculated from HI values, using the empiric formula proposed by Granfeldt (1994): \( pGI = 0.862 \text{HI} + 8.198 \), for which the correlation coefficient \( (r) \) was 0.026 \( (P<0.00001) \).

**Statistical Analysis**

Results are presented as mean ± SEM (standard error of means). Chemical properties were analyzed in quadruplicate in a completely randomized design. In vitro digestibility test of cooked spaghettis were measured in duplicate. A commercial software program (Sigma Stat ver. 2.03, Jandel Corporation, San Rafael, CA) was used to conduct two-way analysis of variance (ANOVA) for determining significant differences among means. Statistically significant differences \((P>0.05)\) among means were determined using the Tukey multiple comparison procedure.

**RESULTS AND DISCUSSION**

**Chemical composition**

The chickpea flour presented the highest protein content (23.7 %) and durum wheat flour meanwhile the control pasta showed the lowest levels (13.7 %, Table 1). Evidently, the protein content was improved in the pasta added with chickpea flour, a change that varied according to the legume/wheat ratio. As most legumes, chickpeas are considered a good protein source; values recorded here are in the same range as other reported elsewhere: 20.7 % (Zhao et al., 1996), 22.0 % (Sabanis et al., 2006), 23.14 % (Zhao et al., 2005), and 24.0 % (Iqbal et al., 2006). The composite spaghettí protein contents (Table 1) resemble those found in recent studies, where pasta added with chickpea flour presented protein contents that ranged between 13.0 and 18.36 % (Sabanis et al., 2006; Zhao et al., 2005). The protein level in chickpea-added pasta might be considered significant for the nutraceutical properties attributed to legume proteins (Duranti, 2006). The generally accepted high ash content of legumes was confirmed, since a 2.7 % level was determined for the chickpea flour used in this work (Table 1). Similar or somewhat higher ash levels...
were reported by Sabanis et al. (2006) (2.4 %), Zhao et al. (2005) (3.0 %), and Iqbal et al. (2006) (3.6 %). Legumes are characterized by high contents of minerals, which depend on the species and cultivar and also on the characteristics of the soil where the plants are grown. The relatively low ash content of durum wheat (0.75 %) was similar to that reported in various semolina types (0.94 %, Sabanis et al., 2006; 0.79 %, Yalla and Manthey, 2006). As expected, ash contents of the composite spaghetti resembled the ingredients proportion used (Table 1). A similar situation has been observed by Goñi and Valentin-Gamazo (2003) and Sabanis et al. (2006). The crude fat content in chickpea flour was 4.4 %; higher values were reported in flours from other chickpea cultivars (5.2 %, Iqbal et al., 2006; 5.69 %, Zhao et al., 2005). Durum wheat flour had a lower lipid level (2.58), which is appreciably higher than in another semolina type (1.6 %, Yalla and Manthey, 2006). The lipid content in the chickpea-containing pasta also followed the legume proportion used for the preparation (Table 1). The chickpea flour showed a total dietary fiber (TDF) content of 19.1 %, a value that was higher than those reported in other chickpea cultivars (14.33 %, Rosin et al., 2002; 15.27 %, Saura-Calixto et al., 2000). The control pasta presented 9.73 % TDF, which is higher than that recorded in other studies for white spaghetti (2.91 %, Rosin et al., 2002; 6.36 %, Saura-Calixto et al., 2000). TDF values increased as the chickpea content in the mixture (Table 1) rose to levels similar to those reported in chickpea-added pasta (9.41 %, Goñi and Valentin-Gamazo, 2003). Dietary fiber in these preparations is important due to its functional effects in the gut. For instance, viscous fiber-containing foods may elicit low postprandial glycemic responses due to delayed glucose absorption (Bravo et al., 1999; Jenkins et al., 2000). The influence of the method of pasta preparation cannot be discarded. The reduction of AS content in the control pasta (Table 1) was slightly higher than that reported in chickpea flour of other cultivars (59.8 %) (Rosin et al., 2002). Control spaghetti exhibited higher TS level (71.03 %) than the chickpea flour; hence, addition of chickpea flour resulted in reduced TS values compared to the control pasta (Table 1). Decreased TS levels in the composite spaghetti may be of use in the dietary management of particular subjects.

### Starch hydrolysis

Available starch (AS) in control spaghetti was higher (67.5 %) than in the samples containing chickpea flour (Table 2). This pattern is in agreement with total starch levels, indicating a dilution effect by the lesser starch content of chickpea. Digestible starch in white spaghetti prepared with durum wheat flour was estimated in 71-83 % (Goñi and Valentin-Gamazo, 2003; Rosin et al., 2002), while digestible starch assessed in spaghetti containing 25 % chickpea flour was 69.9 % (Goñi and Valentin-Gamazo, 2003). The agronomic varieties of durum wheat and chickpea used in each study might be responsible of these apparent inconsistencies. However, a hypothetical influence of the method of pasta preparation cannot be discarded. The reduction of AS content in the product added with chickpea flour shows potential nutraceutical features for these preparations. Furthermore, the increase in chickpea flour level resulted in higher total resistant starch (RS) contents (Table 2). Pasta containing 40 %

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Table 2. Nutritively relevant starch fractions in cooked pasta (%). aMeans of the triplicates ± Standard error. Dry basis. Means in columns not sharing the same letter are significantly different (P<0.05).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Available starch</th>
<th>Resistant starch</th>
<th>Fiber-associated resistant starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control spaghetti (durum wheat flour)</td>
<td>67.54 ± 1.22</td>
<td>2.84 ± 0.11</td>
<td>1.67 ± 0.23</td>
</tr>
<tr>
<td>Spaghetti 80/20 (durum wheat flour/chickpea flour)</td>
<td>63.28 ± 1.58</td>
<td>3.48 ± 0.38</td>
<td>2.23 ± 0.26</td>
</tr>
<tr>
<td>Spaghetti 60/40 (durum wheat flour/chickpea flour)</td>
<td>60.42 ± 1.31</td>
<td>4.67 ± 0.23</td>
<td>2.45 ± 0.14</td>
</tr>
</tbody>
</table>

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Table 3. Hydrolysis index (HI) and predicted glycemic index (pGI) of cooked spaghetti added with chickpea flour. aHydrolysis index (HI) was related to bread = 100 % (Granfeldt et al., 1992). Values are mean of six chewing and dialysis replicates ± standard error (P<0.05). bPredicted glycemic index (pGI) = 0.862 HI + 8.198 (Granfeldt, 1994). Means in columns not sharing the same letter are significantly different (P<0.05).

<table>
<thead>
<tr>
<th>Sample</th>
<th>HI (%) a</th>
<th>pGI (%) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control spaghetti (durum wheat flour)</td>
<td>84.08 ± 4.65</td>
<td>80.68</td>
</tr>
<tr>
<td>Spaghetti 80/20 (durum wheat flour/chickpea flour)</td>
<td>72.50 ± 2.14</td>
<td>70.70</td>
</tr>
<tr>
<td>Spaghetti 60/40 (durum wheat flour/chickpea flour)</td>
<td>61.78 ± 3.01</td>
<td>61.45</td>
</tr>
<tr>
<td>White bread reference</td>
<td>100b</td>
<td>94b</td>
</tr>
</tbody>
</table>
chickpea flour presented a relatively high RS value (4.67 %), which is about 64 % higher than the control pasta product. Such an increased RS content should not be disregarded, considering the beneficial effects associated with the consumption of RS (Halmart et al., 2003). According to Goñi and Valentin-Gamazo (2003), RS contents in chickpea-wheat composite spaghettis were between 2.92 and 3.78 %, depending on the flour substitution level. Cooked legume starches have a marked tendency to recrystallize upon cooling, forming retrograded indigestible fractions (Peñalver et al., 2007; Tovar et al., 1990) that is associated to dietary fiber residues (Saura-Calixto et al., 1993; Tovar, 2001). Due to the high dietary fiber content recorded in the composite spaghettis, their fiber-associated resistant starch (FARS) was evaluated. FARS values followed the total RS content pattern, although no significant difference was found between the two legume-containing spaghettis formulas (2.2-2.5 %; Table 2). Considering that cooked whole chickpeas contain 6.4 % FARS (Peñalver et al., 2007), the level recorded for the composite pasta samples appears in accordance with the proportion of legume flour incorporated in the product. These FARS figures may be used for the calculation of starch-corrected DF values (Peñalver et al., 2007), which would result in 8.8 and 13.3 % for the 80:20 and 60:40 mixtures, respectively. It should also be pointed out that up to 50 % of the total RS measured corresponded to FARS (Table 2). Detailed compositional information on the indigestible portion of a particular food may be of importance, since diverse digestion-resistant fractions may exert different physiological effects (Halmart et al., 2003; Saura-Calixto et al., 2000; Tovar et al., 1992a). To the best of the authors’ knowledge, there is no report on the quantification of the various RS/DF fractions in legume/wheat composite pasta products.

**Starch hydrolysis index of products "as eaten"**

Enzymatic hydrolysis curves for the different pasta products are depicted in Figure 1. Hydrolysis Indices (HI) calculated from the hydrolysis curves, and corresponding predicted Glycemic Indices (pGI) are presented in Table 3. White bread, used as reference, showed a digestion value of about 50 % after 180 min, which agrees with the values reported in the original protocol by Granfeldt et al. (1992). In general, the course of hydrolysis did not vary among pasta samples during the first 120 min of the test; thereafter, the control spaghetti showed higher hydrolysis percentage than chickpea flour-containing products. Also, the preparation richer in chickpea flour resulted less easily hydrolyzed (Figure 1; Table 3). Starch-containing cells in whole cooked legumes such as chickpea, possess remarkably rigid cell walls, whose mechanical resistance persists even in boiled and mildly homogenized samples (Tovar et al., 1990; Tovar et al., 1992a; Tovar et al., 1991) which, in addition to the particular features of their starch constituents, result in low digestion rates (Björck et al., 1994; García-Alonso et al., 1998; Granfeldt et al., 1992). However, as the alternative flour used in the pasta preparation was obtained by milling of raw chickpeas, their starch moiety is likely to be in a free granular state (Tovar et al., 1991).

Hence, the physical starch-encapsulation phenomenon should not contribute to the slow digestion feature recorded. It would be interesting to further investigate the mechanisms governing the low HI in the composite spaghettis, but it may be anticipated that the physicochemical characteristics of the leguminous starch and the presence of amylase inhibitors (Hoover and Zhou, 2003) must be playing an important role.

Predicted GIs (Table 3) of spaghettis added with chickpea flour confirm that this type of process preserves the beneficial "slow digestion" features of starch in pulses (Tovar et al., 1992b; Tovar et al., 2003; Velasco et al., 1997; Würsch et al., 1986). In addition to the intrinsic properties of legume starches, their cognate viscous dietary fibers have been suggested to slow down diffusion of amylolytic products to the absorptive mucosa (Björck et al., 1994; Jenkins et al., 1987; Tovar, 1994; Würsch et al., 1986), a possibility that might therefore decrease the diffusion rate of composite pasta starch digests. The combined action of these factors results in moderate glycemic responses, as suggested for bean/tortilla mixed dishes (Sáyago-Ayerdi et al., 2005; Tovar et al., 2003).

The in vivo evaluation of experimental wheat/chickpea pasta (Goñi and Valentin-Gamazo, 2003) reported a 58.9 % GI, which is lower than present in vitro predicted values (Table 3). Further investigation of the postprandial metabolic responses to composite pasta products is therefore granted.

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**Figure 1.** Average hydrolysis curves of white bread used as reference (●), control pasta (durum wheat flour) (■), 80:20 (▲) and 60:40 (●) pastas elaborated with different durum wheat flour:chickpea flour ratio.

**Table 3.** Calculated and predicted glycemic indices of composite spaghettis.
CONCLUSIONS

Cooked spaghettis elaborated with semolina (durum wheat flour) and chickpea flour exhibited increased protein, ash, lipid, and dietary fiber contents while decreased total starch levels compared to the durum wheat control pasta. The available starch content decreased, whereas the resistant starch level increased with the addition of chickpea flour. Moreover, moderate predicted glycemic indices were found for the chickpea-added product. Present data suggest that pasta added with chickpea flour may be an alternative for people with special caloric or metabolic requirements.

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