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REGULAR ARTICLE

EFFECT OF THE ADDITION OF COMMON BEAN FLOUR ON THE COOKING QUALITY AND ANTIOXIDANT CHARACTERISTICS OF SPAGHETTI

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ABSTRACT

Pasta is a nutritionally unbalanced food, due to its low fat and fiber and low value of its protein. It is considered an adequate vehicle for food supplementation with minerals, proteins and other healthy components such as bioactive compounds present in common beans. The effect of composite pasta (wheat – common bean; 30 % w/w) on the cooking quality (optimal cooking time, cooking loss, weight loss, firmness, color), total phenolic content, antioxidant capacity by DPPH and ORAC assays and phenolic acid profile was investigated. According to the quality parameters, pasta added with bean flour was less hard with respect to the pasta made from durum wheat. The total phenolic content and antioxidant capacity by DPPH and ORAC assays were higher in the pasta with common bean flour than in the pasta control. Also, more phenolic acids were identified in cooked pasta containing common bean flour as analyzed by HPLC.

Keywords: Antioxidant; Common beans; Cooking; Polyphenols; Spaghetti

INTRODUCTION

Pasta is considered a functional food for its low content of fat and sodium. Semolina is the raw material most suitable for making pasta, because of its proteins, which are ideal for the development of the dough and preventing disintegration of the pasta during cooking (**Felliet and Dexter, 1996**). However, wheat pasta is a nutritionally unbalanced food (**Antognelli, 1980**), due to its low protein content (< 15%) and its relatively deficient in lysine, an essential aminoacid (**Shogren *et al.*, 2006**). Wheat pasta is considered an adequate vehicle for food supplementation with minerals, proteins and other healthy components (**Borneo and Aguirre, 2008**). Recent investigations on nutraceutical properties of common bean (*Phaseolus vulgaris*) have highlighted the importance of this crop on human diet for its high protein content, minerals, vitamins and complex carbohydrates, which makes this food a good source of nutrients (**Rocha-Guzmán *et al.*, 2007**).

In the last years, it has been reported that the antioxidant compounds present in foods at low concentrations that can help prevent cell damage, cancer, inflammation, aging and atherosclerosis (**Biglari *et al.*, 2008**) caused by free radicals throughout the body.

Common beans are a rich source of phytochemicals, including phenolic compounds, which have a significant impact on the prevention and treatment of chronic degenerative diseases such as diabetes mellitus, due to its antioxidant properties (**Machado *et al.*, 2008**).

There are many studies about the increase of nutritional quality of pasta by the addition of several ingredients as legumes (**Zhao *et al.*, 2005; Torres *et al.*, 2007; Gallegos-Infante *et al.*, 2010a,b**), but they lack information on the antioxidant capacity of the pasta. **Prabhasankar *et al.*, (2009)** reported antioxidant capacity for pasta fortified with edible Japanese seaweed. **Gallegos-Infante *et al.*, (2010b)**, reported furosine and total phenolic content in spaghetti, but did not report antioxidant capacity. **Boroski et al., (2011)** reported total phenolic content and IC_{50} by DPPH assay for pasta formulated with oregano and carrot leaves.

The objective of the present study was to evaluate the effect of adding common bean flour to semolina on the culinary quality and antioxidant characteristics of cooked spaghetti.

MATERIAL AND METHODS

Raw Material

The semolina was acquired from the Beleño mills, SA de CV (León Gto., México). Common bean cultivar was "Bayo Victoria", donated by INIFAP Guadiana (harvesting season 2008-2009).

Beans were cleaned and cooked in water using a pressure cooker (All American, Model 921 21 Hillsville, VA, USA) in a 1:4 ratio for 60 min at 15 psi, according to the methodology proposed by Rocha-Guzman and others, (2007). Cooked beans were dried in a tray dryer (Standard Industries, Inc., Fargo, ND, USA). Once dried, they were ground in a Moulinex food processor DAC 511. The obtained flour was sieved through 80 mesh (0.180 mm) and stored in hermetic plastic bags at 4°C.

Spaghetti processing

Semolina was mixed with 30% of bean flour; the spaghetti was prepared according to procedure described by **Walsh *et al*., (1971)**. One Kg of mixed flour was hydrated to obtain 32% moisture, and extruded through an 84-strand, teflon-coated spaghetti die (0.157-cm opening), using a semi-commercial laboratory extruder (Demaco, Melbourne, Fl, USA).

The processing conditions were as follows: dough temperature, 40°C; extruder auger speed, 25 rpm; and chamber vacuum, 46 cm Hg. The extruded spaghetti was dried at 70°C by 12 h and a relative humidity of 83% with the maximum temperature described by **Yue *et al.*, (1999)**. The pasta control was made with 100% semolina.

Spaghetti color

The color of dried spaghetti was measured in a Minolta Color Difference Meter (Model CR310, Minolta Camera Co., Osaka, Japan), using the Hunter scale *L, a, b. L* values measured dark to white (0 to 100); *a* values, redness when positive and greenness when negative; and *b* values, yellowness when positive and blueness when negative. Results were expressed as color differential (ΔE) between control (pasta with no common bean flour) and the substituted spaghetti, calculated as follows:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Where *ΔL* was calculated as: L sample – L control. Δ*a* was calculated as: **a** sample – **a** control. Δ*b* was calculated as: **b** sample – **b** control.

Results were the means of independent duplicate determinations (**Setiady *et al*., 2007**).

The optimal cooking time

Optimal cooking time was determined using the Approved Method 66-50 (**AACC, 1992**). Spaghetti (10 g) was cooked in 300 mL of boiling water. After 8 min of cooking, strands of spaghetti straps were removed from the boiling water at 30 s and squeezed between 2 pieces of clean plastic. Optimum cooking time corresponded to the disappearance of the white color in the core of the spaghetti.

Cooked weight, cooking loss, and cooked firmness

Cooking quality of spaghetti was determined using the Approved Method AACC 66-50 (**AACC, 1992**). Spaghetti (10g) was cooked to optimum in 300 mL of boiling water. Cooked weight (g) was the weight of cooked spaghetti after being allowed to drain for 2 min.

Cooking loss (% total solids weight) was measured by evaporation of the cooking water to dryness under a forced-air at 110°C overnight. Cooked firmness was measured with a Texture analyzer (Model TA-XT2, Texture Technology Corp., Scarsdale, NY, USA). Cooked firmness was determined by the work (g.cm) required to shear 4 cooked strands of spaghetti.

Antioxidant characterization

Spaghetti was cooked to optimum cooking time, drained, and stored in airtight containers in a freezer at -20°C for subsequent lyophilization. Cooked and dried samples were passed through a mill and sieved through a mesh 60 (0.160mm) (IKA Works Inc., Wilmington, NC., USA).

Total phenolic content (TPC), radical DPPH scavenging activity capacity, oxygen radical absorbing capacity assay (ORAC), and the phenolic acid profile in the ground raw materials and dried and cooked pastas were analyzed.

Sample extraction

Ground samples (20 g) in triplicate were dissolved in 70 % aqueous acetone (200mL) for 3 h at room temperature. Subsequently, the crude extracts were concentrated under vacuum in a rotary-evaporator at 40°C. The extracts were freeze-dried. The extractions were performed in three replicates for each sample.

Determination of total phenolic content

Total phenolic content (TPC) was determinated by Folin-Ciocateu´s assay, according to **Singleton *et al.*, (1999)**, using gallic acid (GA) as the standard. Briefly, in a 96-wells microplate were added 100 µL of extract, 200 µL of Folin-Ciocalteu reagents solution and 800µL of 7% $NaCO_3$. The mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 765nm against destilled water as a blank. Total phenolic content was expressed as gallic acid equivalents (mg of GAE/g sample) using the calibration curve of gallic acid. Linearity range of the calibration curve was of 50 µM to 2.5 mM (r=0.99).

Radical DPPH scavenging activity

The method of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical for the determination of free radical scavenging was used, according to the methodology of **Chen and Ho (1995)**, with slight modifications. Briefly, 10 µL of sample were placed in a 96-well microplate, and added with 190 µL of DPPH to each sample. The mixture was shaken vigorously for 1 min and left to stand for 30 min at room temperature in darkness. Thereafter, the absorbance for the sample (A_{sample}) was measured at 517nm against a blank of ethanol. A negative control ($A_{control}$) was taken after adding DPPH and the solvent of the extraction.

The calibration curve was performed with a standard solution of Trolox at the concentrations of 1, 0.75, 0.5, 0.125, 0.0625 and 0.03125 mM (r=0.99). Water was used as a blank.

The free radical scavenging capacity of the sample was expressed as mM of Trolox equivalents per gram of dry sample (mM Trolox / g sample). The samples were analyzed in triplicate.

Oxygen Radical Absorbing Capacity Assay

The ORAC assay was performed according to the methodology of **Prior *et al*., (2003)** and **Wu *et al*., (2004)** and modified by **Xu and Chang (2007)**. A 96-well microplate was loaded with 20 µL of sample, Trolox (standard) and PBS solution like a blank; then the plate was sealed with parafilm, incubated in the same fluorometer (FLOUstar OPTIMA Microplate Reader) at 38°C for 30 min, and subsequently covered, removed, and incubated for 10 additional min. To each well, 200 µL of fluorescence solution and 20 µL of 3.2 mM AAPH (2,2´-azobis dihydrocloride), were added to initiate the reaction. The kinetics was measured by fluorescence changes immediately to an excitation of 485 nm and an emission of 520 nm. The final ORAC values were calculated using linear regression curve of Trolox standard or sample and the area under the fluorescence decay curve. The results were expressed as micromoles of Trolox equivalent per gram of dry sample (mol TE / g).

HPLC

The qualitative analysis of free phenolic acids was performed in a 1200 Agilent Series HPLC, according to the methodology by **Xu *et al*., (2007)** with modifications. A 4.6 mm × 250 mm, 5 µm, Zorbax Stablebond Analytical SB-C18 column (Agilent technologies, Rising Sun, MD) was used for separation at 40°C. Elution was performed using mobile phases A (0.1% TFA aqueous solution) and B (100% methanol) and the flow rate was set to 0.7 mL/min. The solvent gradient in volumetric ratios was as follows: B was increased from 5% to 30% in 50 min, and held for 15 min. Then B was increased to 100% at 67 min, and held for 10 min to clean up the column. At last B was decreased to 5% and held for 10 min.

Statistical analysis

Data were analyzed by ANOVA test and post hoc comparison of means($p<0.05$) by Tukey test V.7.0 (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Quality evaluation: physical and cooking characteristics

The physical and cooking characteristic of the spaghetti are shown in Table 1. The optimum cooking time decreased by the addition of common bean flour ($p<0.05$). This result was similar to those reported by **Gallegos-Infante *et al.*, (2010a)**, who reported a time of 8 min in pasta with 30% of common bean, by **Torres *et al.*, (2007)**, who reported lower cooking time in pasta added with germinated pigeon pea, and similar to data by **Boroski *et al.*, (2011)** in pasta formulated with the addition of carrot leaf and/or oregano leaf.

Table 1 Physical characterization of the spaghetti

Sample (Spaghetti)	Optimum cooking time (min)	Cooking weight (g)	Cooking loss (%)	Firmness (g/cm)	Color (% ΔE)
Spaghetti control	10:00±0.01[a]	30.01 ± 0.33[a]	5.43 ± 0.25[a]	3.98 ± 0.16[a]	----------
Spaghetti with 30% of bean	8:45±0.01[b]	27.50 ± 0.33[b]	8.23 ± 0.25[b]	2.86± 0.16[b]	20.7 ± 2.96

Data are expressed as mean ± standard deviation (n=3). Means in columns with different letter are statistically different ($p<0.05$)

The cooking losses (leaching to the cooking water) increases significantly in the spaghetti with 30% common bean, in accordance with **Zhao *et al.*, (2005)**, who found that the addition of legumes, as green pea, yellow pea, chickpea and lentils to semolina increased the cooking loss, Thus, high cooking loss implicates spaghetti with lower quality. Similar effect was observed by **Torres *et al.*, (2007)** in pasta with germinated pigeon pea, attributing losses to the structural changes in the protein network because of the partial substitution of wheat protein by legume protein.

Spaghetti with 30 % of common bean flour showed a decrease in firmness ($p<0.05$) in comparison with the spaghetti control. **Malcolmson *et al.*, (1993)**, found that protein level greatly affected firmness, compressibility and cooking loss of optimally cooked spaghetti, whereas elasticity was related to drying temperature. Lower firmness implicates spaghetti with lower quality. **Rayas-Duarte *et al.*, (1996)** reported that using buckwheat and amaranth flours in pasta, there was a diminishing value in firmness but an increase with lupin flour.

This could be related to the inclusion of insoluble fiber; at higher inclusion of fiber, the pasta structure became more porous (**Petitot *et al*., 2009**), which could be reflected in the firmness.

Results obtained for color change indicated a relative difference of (20.7 ± 2.96). This difference was associated with the use of common bean flour, which produced a darker color. **Setiady et al., (2007)**, indicates that the actual consumer not is affected by the darker color in pasta, because the consumers relate this phenomenon with more natural products. The obtained results are agreed with those obtained by **Gallegos-Infante *et al*., (2010b)** for pasta with common bean flour. As well, **Setiady *et al*., (2007)** found that the use of different ingredients in pasta noodles enhanced the color change.

Antioxidant characterization

Total phenolic content (TPC)

The results for the antioxidant evaluation are shown in Table 2. The common bean flour had the highest phenolic content (1.93±0.02 mg GAE/g). The blend of semolina - common bean flours showed an increase of total phenolic content in comparison with semolina, in proportion to the addition of common bean flour. There are no differences in total phenolic contents among crude spaghetti with common bean flour, cooked spaghetti with common bean flour and blend semolina – common bean flours. The semolina had the lowest content of total phenolic content with a TPC increase in the raw spaghetti. However, the TPC decreased during the cooking process to reach the original level of semolina, four times lower than the present in cooked spaghetti with 30% common bean flour. In general, the values of TPC found in the present work were lower than those reported by **Gallegos-Infante *et al*., (2010b)** for pasta made with semolina and common bean flour, respectively. However, comparing the TPC values of spaghetti control versus cooked spaghetti with common bean flour, the relative increase in TPC was more relevant in the present work than that reported by these authors. The differences could be related to the different harvesting seasons, and differences in agriculture practices of the common beans that produced different phenolic composition in the beans.

Table 2 Antioxidant characterization of raw materials and spaghetti

Sample	Total Phenolic Content (mg gallic acid /g)	DPPH (µmol Trolox/g)	ORAC (µmol Trolox/g)
Bean flour	1.93±0.02[a]	2.73±0.01[a]	83.61±0.01[a]
Semolina	0.14±0.001[d]	0.14±0.03[c]	18.25±0.05[g]
Semolina – bean flour	0.53±0.006[b]	1.51±0.25[b]	47.64±0.01[b]
Cooked spaghetti control	0.14±0.001[d]	0.15±0.05[c]	29.66±0.03[e]
Cooked spaghetti with common bean flour	0.49±0.07[b]	1.26±0.10[b]	39.64±0.02[c]

Data are expressed as mean ± standard deviation (n=3). Means in columns with different letter are statistically different ($p < 0.05$)

DPPH

The common bean flour showed the highest value of DPPH test (2.73 ± 0.01 µmol Trolox /g) (Table 2). The uncooked spaghetti with common bean flour did not show differences with cooked spaghetti with common bean flour and the blend semolina-common bean flour. Furthermore, the value of DPPH test in cooked spaghetti with common bean flour (1.26 µmol Trolox /g) was nine times higher than the value showed by the cooked spaghetti control (0.15 µmol Trolox /g). **Prabhasankar *et al.*, (2009)**, studied pasta supplemented with Indian Brown seaweed and reported an increase in antioxidant activity measured by DPPH test related to the cooking process.

ORAC

The highest ORAC value was observed in the common bean flour (83.61µmol ± 0.01 µmol Trolox/g) (See, Table 2). The uncooked spaghetti with common bean flour showed a reduction in ORAC value (30.93 ± 0.04 µmol Trolox/g) in comparison with the semolina – bean flour blend (47.64 ± 0.01 µmol Trolox/g). Opposite behavior was observed for the

spaghetti control, with an increase in the value of ORAC in uncooked spaghetti control. However, its value (23.24 ± 0.01 µmol Trolox/g) was lower than uncooked spaghetti with common bean flour. An increase in the ORAC value in both spaghetti control and pasta with common bean flour after the cooking (29.66 ± 0.03 µmol Trolox/g and 39.64 ± 0.02 µmol Trolox/g, respectively) was observed. The cooked spaghetti with common bean flour showed higher ORAC values than spaghetti control.

It is interesting to note that in the present work there are no differences between cooked and uncooked samples with DPPH test, but there were differences using ORAC assay. The reasons might be because that DPPH and ORAC assay mechanisms are different and there are varying degrees of reactivity of the bioactive compounds present in the experimental samples.

HPLC analysis

Results about low molecular weight phenolic acids profile are shown in Table 3. Cooked samples containing common bean flour showed more phenolic compounds in comparison with cooked pasta control. Cooked spaghetti with common bean flour only did not show salicylic acid and 2-3-4-trihydroxybenzoic acid. The gallic acid concentration in common bean flour was the highest. The blend of Semolina–common bean flours showed the presence of gallic acid, protocatechualdehyde, caffeic acid, vainillin, p-coumaric and m-coumaric. Several authors indicate that caffeic acid is a very heat labile compound; our results confirmed that, since cooked pasta had decreased level of phenolic acids.

Table 3 Phenolic acids (µg/ g of sample) found in spaghetti samples by HPLC

Phenolic acids/Sample	Common bean flour	Semolina	Semolina - bean flour (70: 30 %, w/w)	Cooked spaghetti control	Cooked spaghetii made with bean flour (30% w/w)
gallic acid	836 ± 84		438 ± 13.6		677± 75.3
protocatechuic acid	63.9 ± 4.0			149 ± 20.2	323 ± 13.8
2-3-4 trihydroxybenzoic acid	582 ± 24.5		491 ± 70.3		
*p*rotocatechuic aldehyde acid	157 ± 5.5		111 ± 27.1		151 ± 58.6
p-hydroxybenzoic acid	88.6 ± 2.0				99 ± 18.4
caffeic acid	205 ± 1.9	2416 ± 476	3027 ± 570.0	694 ± 56.0	652 ± 148
Vainillin	178 ± 11.4		128 ± 17.1		113 ± 25.3
p-coumaric acid	321 ± 16.0		232 ± 35.4		203 ± 42.0
m-coumaric acid	167 ± 3.4		160 ± 7.3		77.0 ± 9.1
salicylic acid	141 ± 2.0				
chlorogenic acid					214 ± 26.4

Compound identification was confirmed by comparison with standards.

Therefore, the addition of bean flour to the dough increases the content of phenolic acids and its antioxidant capacity. These phenolic compounds have been attributed with a role as antioxidants in biological systems (**Rocha-Guzman *et al*., 2007; Xu and Chang, 2009**).

CONCLUSION

The addition of common bean flour to spaghetti, increased its total phenolic content and antioxidant capacity. This effect is more evident after the cooking process in pasta added with common bean flour. However, the pasta with 30 % (w/w) common bean flour showed some change in cooking quality in comparison with the pasta control.

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