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Effect of apple by-product as a supplement on antioxidant activity and quality parameters of pasta

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Summary

In this study, pasta was prepared by replacement of durum semolina (DS) with 10% and 15% of apple peel powder (APP). The control sample was pasta made from 100% DS. Total phenolic content (TPC), antioxidant capacity (AOA) and quality parameters of pasta were determined before and after cooking. Extraction of phenolics with methanol and ethanol, assisted with ultrasound (15 min) and by stirring (with magnetic stirrer for 1 h) was also compared. Results showed that addition of APP increased cooking loss and the amount of absorbed water of the pasta samples. Hardness and adhesiveness of pasta samples was decreased with APP addition. Pasta with added APP had significant higher levels of TPC and AOA compared with pasta without addition of APP. The highest TPC (1.4 g of gallic acid equivalents/kg) was in raw pasta with addition of 15% APP from which phenolics were extracted with ultrasound assisted extraction and methanol. Regarding AOA, the highest antioxidant activity (0.8 mg gallic acid equivalents/100g) was also in raw pasta with 15% APP, from which phenolics were extracted by stirring and ethanol. In cooked pasta, the levels of TPC and AOA, were significantly lower compared to raw pasta ($p \leq 0.05$). Sensory evaluation showed that there was no significant difference between DS and DS + 10% APP. Apple peel powder (APP) could be a food by-product, conveniently used as additive, for delivering health-promoting bioactive compounds such as polyphenols.

Keywords: apple peel powder, pasta quality, antioxidant capacity, phenolic content

Introduction

Consumers have a growing interest in foods containing bioactive components (such as polyphenols, dietary fibers, vitamins etc.) that may be beneficial for health (Doxastakis et al., 2007). One approach to increase the consumption of these components is to add them to popular foods, such as bread or pasta. Pasta is an important staple food widely consumed across the world and was among the first food to be authorized by the FDA (Food and Drug Administration) as a good vehicle for the addition of nutrients (Chillo et al., 2008; Gallegos-Infante et al., 2010). However, pasta enriched with fruit bioactive compounds, among which antioxidants play a very important role, have received little attention to date. The search for new antioxidants from fruit and vegetable materials has taken a very high attention in the last decade. Many research groups put their focus on the utilization of antioxidants from plants and agro-industrial by-products, putting this topic at the vanguard of research. Several studies have been carried out to improve nutritional properties of pasta by adding amaranth flour, cassava starch, cassava bagasse (Fiorda et al., 2013), barley β-glucan concentrate (Chillo et al., 2011), white lupin protein (Doxastakis et al., 2007), carboxymethylcellulose and pregelatinized corn starch (Chillo et al., 2007) but also supplements such as artichoke, asparagus, pumpkin, zucchini, tomato, carrot, broccoli, spinach, eggplant and fennel (Padalino et al., 2013), all very rich in polyphenols. Polyphenols have various biological functions, such as antioxidant, anti-inflammatory and anti-cancer activities that can protect human organism (Boyer and Liu 2004; Xiuzhen et al., 2007; Hyson, 2011; Thondre et al., 2011; Habauzi and Morand, 2012). Apples are known as a good source of phenolic compounds (Wolf et al., 2003; Denis et al., 2013), especially the peel (McGhie et al., 2005; Valdenegro et al., 2010). In addition, apple peel is richer in dietary fiber, and minerals than other edible parts of this fruit (Biedrzycka et al., 2008).

The way to incorporate apple peel, one of the by-products of apple manufacturing, as a healthy food ingredient in human diet, could provide many health benefits. However, there is limited information on the potential for incorporation of apple peel in pasta and its contribution to total antioxidant activity (AOA), total phenolic content (TPC) and quality of the final product. Therefore, the objective of this research was to evaluate the impact of cooking on total polyphenols, and antioxidant activity of pasta with different amount of apple peel powder (APP). Also, the objective was to investigate the effect of two extracting solvents and methods on total polyphenols and antioxidant activity, as well as to evaluate the influence of APP addition on pasta quality such as optimal cooking time (OCT), cooking loss, water uptake, textural parameters and sensory analysis (Chillo et al., 2011).

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Materials and methods

Raw material. Durum semolina (DS) used in this study was purchased from the Gatti d.o.o. company from Zagreb, and the actual apple cultivar, 'Granny Smith' (GS) was from Agricultural Institute (Osijek, Croatia).

Pasta preparation. Pasta was produced from durum semolina and APP (by 10 and 15% semolina replacement). Apple peel powder was added to pasta in order to increase the levels of polyphenols and antioxidant activity. Durum semolina was mixed with APP in a plastic bowl with a metal stirrer. Mixture was transferred into minipress (Fimar, Italy) and water content of the mixture was set to 36% by adding tap water. The conditions applied were the following: temperature of water 25 °C, kneading time 10 min. Dough was extruded in laboratory minipress through *fettuccine* die (8 mm), and air dried at room temperature for about 24 h. Pasta made from only durum semolina (100%) was also produced and used as a reference (DS).

Apple peel powder (APP) was produced from GS apples. Apple peel was removed with peeler (Adamo lo sbuccia, Italy), 1-2 mm thickness and dried in laboratory lyophilisator (Christ Freeze Dryer, Gamma 2-20, Germany). Drying conditions were as follows: freezing temperature -55 °C; the temperature of sublimation -35 °C to 0 °C; and the vacuum level 0.220 mbar. The temperature of isothermal desorption varied from 0 °C to 22 °C under the vacuum of 0.060 mbar. Freeze-drying lasted about 24 h until the total solids content was 94-98 %. Freeze-dried apple peel was powdered using a grinder (Končar 130 Watt) in order to obtain apple peel powder. APP was stored in hermetically closed glass dish, and kept at room temperature.

Pasta cooking quality. A 100 g sample of *fettuccine* was placed into 1000 ml boiling tap water with 5 g of sodium chloride. Optimal cooking time (OCT) was evaluated by observing the time of disappearance of the core of the *fettuccine* strand during cooking (every 30 sec), by squeezing the *fettuccine* between two transparent glass slides. The cooking loss (the amount of solid substance lost to cooking water) was determined according to the modified method by Chillo et al. (2008). Cooking water (100 g) was collected in a glass beaker and evaporated until a constant weight was reached. The residue was weighed and expressed as a mass of solids released during cooking. Amount of absorbed water was expressed in grams as the mass of water absorbed by 100 g cooked *fettuccine* samples. Pasta volume increase was calculated as the ratio of the volume of cooked pasta and raw pasta.

Sensory evaluation. The properties of the dried pasta, external shape and appearance were examined visually and elasticity by breaking *fettuccine* strand and examining the breakage surface. The maximum score for dried pasta properties was 20 points, and for cooked pasta properties (cooking loss, water uptake, volume increase) the maximum score was 40 points. Odour, stickiness/resilience, consistency and taste were evaluated by a panel of 7 trained assessors and the maximum score was 40 points. The panellists were selected in a preliminary session and were experienced in the products and terminology. The cooking quality is defined as high if pasta reaches 90-100 points, good if it reaches 80-89 points, satisfactory 70-79 points, low below 70.

Hardness and adhesiveness were determined on texturometer TA.XT2 Plus, Stable Microsystems. The *fettuccine* were cooked (10 g sample in 100 ml tap water with 0.5 g of sodium chloride) for their previously determined optimal cooking times, drained and left to rest for approximately 30 min modified method by Fiorda et al. (2013). Texture analysis included double compression of pasta samples to 40% of their thickness with a 10 mm aluminium cylindrical probe. Recording speed was 0.5 mm/s. The hardness (mean maximum force, g), and adhesiveness (mean negative area, gs) of cooked pasta samples were measured. Five measurements for each *fettuccine* sample were performed.

Colour of uncooked (raw), dried and cooked *fettuccine* was determined by Minolta Chroma Meter (Model CR-300, Minolta Co., Osaka, Japan). The instrument was calibrated using white tile and colour was expressed using CIE-Lab scale. The L* is the measure of the brightness (lightness) from black (0) to white (100). The a* is the function of the red-green difference. Positive a* indicates redness, negative a* indicates greenness. The b* is the function of the green-blue difference. Positive b* indicates yellowness, negative b* indicates blueness. Each colour data represents the mean of five replicates.

Extraction of polyphenols for the determination of TPC and AOA was performed as follows. Dried pasta (1 g) was fragmented using a food processor Braun Multiquick Professional 600 Watt Turbo mill and extracted with 10 mL of various solvents - 1% HCl methanol, and 1% HCl ethanol. The samples were extracted at room temperature in two ways, with magnetic stirring (1 h) and assisted with ultrasounds (15 minutes) in order to obtain the pasta extract. After freeze – drying cooked pasta followed earlier described extraction procedure for dried pasta.

Total polyphenol content was measured by using a modified colorimetric Folin-Ciocalteu method (Ough and Amerine 1988). A volume of 2 mL of the pasta extract was mixed with 10 mL of (1:10 v/v with water) Folin-Ciocalteu reagent (Kemika, Zagreb, Croatia) and

8 mL of 7.5% solution of sodium carbonate. The colour was developed in 120 min, and the absorbance was read at 765 nm by spectrophotometer (Jenway 6300, Bibby Scientific, UK). The measurements were performed in triplicates for each sample and the average value was interpolated on a gallic acid calibration curve and expressed as g of gallic acid equivalents per kg of sample (g GAE/kg).

Antioxidant activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity was measured by using the Brand-Williams et al. (1995) method. According to the method, 0.2 mL of the pasta extract was diluted with methanol (2 mL), and 1 mL of DPPH solution (0.5 mM) was added. After 15 min, the absorbance was measured at 517 nm. The results were expressed as milligrams of gallic acid equivalents per 100 g of sample. Additional dilution was needed if the measured DPPH value was over the linear range of the standard curve.

Statistical analysis. All measurements were done in triplicate and data were expressed as mean ± standard deviation. The experimental data were subjected to an one-way analysis of variance (ANOVA) and Fisher's LSD were calculated to detect significant difference ($p \leq 0.05$) between the mean values. Statistical analyses were performed with the statistical program MS Excel (Microsoft Office 2007 Professional).

Results and discussion

The optimal cooking times for the *fettuccine* samples are reported in Table 1. The OCT of DS and DS + 10% APP was 7.3 min, and for DS + 15% APP OCT was 6.2 min. Differences in OCT between DS + 15% APP and other samples are probably a result of physical disruption of the gluten matrix (Chillo et al., 2011). The disruption of the gluten matrix could also explain the increase of cooking loss (CL) in samples with addition of APP (Table 1). Incorporation of APP into pasta decreases its protein content. During cooking, a weak or discontinuous protein matrix results in a protein network that is too loose and permits a greater amount of leaching during starch granule gelatinization, causing an increased CL (Chillo et al., 2011). Results showed no significant difference among volume of dried and cooked pasta samples (Table 1). Increase of water absorption with APP addition could be explained by higher fiber and pectin content. These results are in agreement with the work of Stampfli et al. (1996) who determined that hydrocolloids are widely used in food products to improve moisture retention and control water mobility. Sensory evaluation of pasta cooked to optimal cooking time showed that sample with 15% APP got lowest points among samples. This was due to different taste, odour and colour of the samples with APP addition in comparison to control sample made from durum semolina. Considering stickiness/resilience and consistency there are no significant differences among samples, however odour and taste showed significant difference between DS and DS + 15% APP. DS + 15% APP showed the lowest total score among samples (Table 1).

Table 1. Quality of dried and cooked pasta samples

Quality parameters	**DS**[A]	**DS+10% APP**	**DS+15% APP**
Volume of dried pasta (cm^3)	80	80	80
Optimal cooking time (min)	7.3	7.3	6.2
Cooking loss (%)	9,4	9,9	11.5
Amount of absorbed water (g)	128.7	131.1	132.3
Volume increase	2.5	2.5	2.6
Volume of cooked pasta (cm^3)	200	200	210
Points			
External shape of dried pasta	5	5	5
Appearance of dried pasta	10	10	10
Elasticity of dried pasta	5	5	0
% cooking loss	25	25	25
Amount of absorbed water	5	5	5
Volume increase	5	5	5
Sensory evaluation			
Odour of cooked pasta	10^{a}	9.7^{ab}	9^{b}
Stickiness/resilience of cooked pasta	9.5^{a}	9.5^{a}	9.5^{a}
Consistency of cooked pasta	9^{a}	8.5^{a}	7.8^{a}
Taste of cooked pasta	10^{a}	9.5^{ab}	8.2^{b}
Total score	93.5	92.2	84.5

[A]DS – durum semolina; APP – apple peel powder; One-way variance analysis was performed to evaluate the statistical difference between the values. Mean in the same row followed by different superscript letters differ significantly ($p \leq 0.05$).

Textural properties of the cooked pasta samples to the optimal cooking time in terms of hardness and adhesiveness are shown in Table 2. The adhesiveness value for DS + 10% APP and DS + 15% APP was not significant different from that the DS. Regarding the hardness parameter, the DS sample was harder compared to the DS + 10% APP and the DS + 15% APP samples. According to Padalino et al. (2013) this may be due to the higher fiber content in the samples with APP addition. Hardness and cooking loss results suggest that the incorporation of APP prevents the formation of the starch and gluten network and therefore negatively influences the cooking quality of the pasta.

Table 2. Textural properties of the cooked pasta samples

	Hardness (g)	Adhesiveness (gs)
DS[A]	1328±201[a]	138±26[a]
DS+10%APP	1109±125[b]	122±35[a]
DS+15%APP	1066±52[b]	92±6[a]

[A]DS – durum semolina; APP – apple peel powder. Mean in the same column followed by different superscript letters differ significantly ($p \leq 0.05$).

Colour is an important quality parameter of pasta. It results from the desirable yellow component and the undesirable brown component. Colour measurements of uncooked (fresh), dried and cooked pasta samples are presented in Table 3. Considering the L* parameter (brightness), it decreased in uncooked, dried and cooked pasta samples with the APP addition, same as the a* parameter (redness). The b* parameter (yellowness) increased with the APP addition in uncooked, dried and cooked pasta samples. Yellowness and brightness are correlated both to the pigment concentration and to enzymatic reactions, whereas redness is generally related to non-enzymatic browning (Doxastakis et al., 2007).

Table 3. Colour of pasta enriched with apple peel powder

	L*	**a***	**b***
Uncooked Pasta			
DS[A]	77.7 ± 0.3[a]	-1.4 ± 0.1[a]	28.5 ± 0.4[c]
DS + 10% APP	74.6 ± 0.3[b]	-1.9 ± 0.2[b]	33.0 ± 1.5[b]
DS + 15% APP	72.7 ± 0.7[c]	-2.3 ± 0.2[c]	34.5 ± 0.8[a]
Dried Pasta			
DS	84.2 ± 0.2[a]	-1.7 ± 0.0[a]	20.2 ± 0.5[a]
DS + 10% APP	80.4 ± 0.7[b]	-1.7 ± 0.1[a]	21.1 ± 0.8[a]
DS + 15% APP	79.5 ± 0.1[b]	-1.7 ± 0.1[a]	20.9 ± 0.7[a]
Cooked Pasta			
DS	75.3 ± 0.6[a]	-3.1 ± 0.2[b]	25.2 ± 1.4[b]
DS + 10% APP	68.5 ± 1.7[b]	-0.4 ± 0.6[a]	26.3 ± 1.5[b]
DS + 15% APP	67.6 ± 1.2[b]	-0.1 ± 0.3[a]	29.0 ± 1.8[a]

[A]DS – durum semolina; APP – apple peel powder; Data are the means ±standard deviations (n=5). One-way variance analysis was performed to evaluate the statistical difference between values. Mean in the same column followed by different superscript letters differ significantly ($p \leq 0.05$).

The total polyphenol contents of DS and DS + APP were examined and presented in Figure 1A and 1B. As the results showed, TPC are proportionally increased by addition of APP to pasta. The extraction yield of the extracts highly depends on the solvent polarity, which determines both quantitatively and qualitatively the extracted phenolic compounds (Sineiro et al., 2008). The phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in solvents. The use of an alcoholic solution provides satisfactory results for the extraction process (Perva-Uzunalic et al., 2006). However, in this case extraction solvent based on methanol produced extracts with higher yield compared to extracts produced with ethanol. Extraction with magnetic stirring and extraction assisted by ultrasound was compared. Slightly higher values were obtained in extracts obtained by means

of ultrasound assisted extraction. The highest polyphenol content was in sample DS + 15% APP followed by DS + 10% APP and DS, which were extracted by ultrasound assisted extraction and methanol, (1.4, 1.0, 0.4 g/kg, respectively). All extracts were free-radical inhibitors but methanol extract was more potent than ethanol regardless of the extraction method.

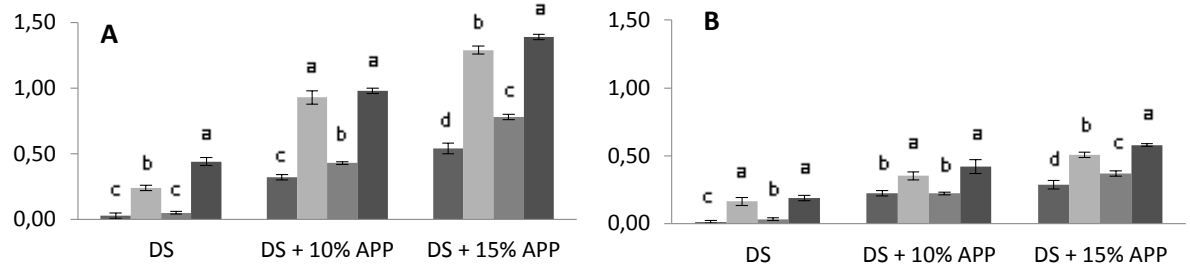

Fig. 1. Total phenol content (g/kg) of dried (A) and cooked (B) pasta. DS – Durum semolina; DS + 10% APP - Durum semolina with 10% of apple peel powder; DS + 15% APP - Durum semolina with 15% of apple peel powder. stirrer EtOH; stirrer MeOH; ultrasound EtOH; ultrasound MeOH. Within the same sample, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD).

According to literature durum semolina phenolic profile consists of protocatechuic, p-hydroxybenzoic, gentistic, caffeic, vanillic, chlorogenic, syringic, p-coumaric, and ferulic acids (Onyeneho and Hettiarachchy 1992; Thondre et al., 2011). Some of these phenolic acids are responsible for antioxidant activity of DS. By adding apple peel, pasta was enriched with additional polyphenol groups (flavonols, flavan-3-ols, dihydrochalcones) of which the main compounds are: epicatechin, procyanidin B2, phloretin xyloglucoside, phloridzin, and chlorogenic acid (Łata and Tomala 2007; Matthes and Schmitz-Eiberger 2008; Wojdyło et al., 2008; Neveu et al., 2010; Ceymann et al., 2012). Most of the newly added polyphenolic compounds have greater antioxidant activity especially epicatechin, procyanidin B2 and quercetin glycosides (Ki Won et al., 2003). The results of DPPH radical scavenging suggest a proportional increase of AOA by the addition of APP (Figure 2A). Regarding influence of extraction solvent and extraction method, a better AOA was measured in samples extracted with a magnetic stirrer, however there was no significant difference between the solutions. The highest AOA was in DS + 15% APP followed by DS + 10% APP and DS (0.7, 0.3, 0.2 mg gallic acid equivalents/100g, respectively).

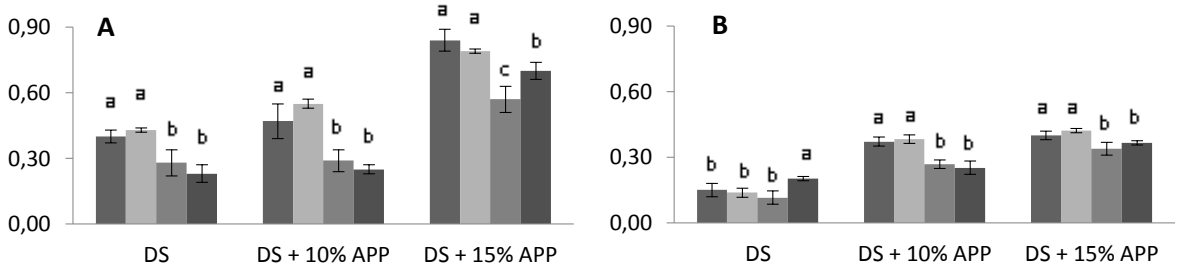

Fig. 2. Antioxidant activity measured with DPPH method (mg gallic acid equivalents/100g) of dried (A) and cooked (B) pasta. DS – Durum semolina; DS + 10% APP - Durum semolina with 10% of apple peel powder; DS + 15% APP - Durum semolina with 15% of apple peel powder. stirrer EtOH; stirrer MeOH; ultrasound EtOH; ultrasound MeOH. Within the same sample, means followed by different letters are significantly different at $p \leq 0.05$, (ANOVA, Fisher's LSD).

Cooking significantly decreased TPC ($p \leq 0.05$) in all samples (Figure 1B), however results showed that DS + 15% APP after cooking still had a higher level of TPC than dried DS. Regarding extraction solvent and method used for extraction the same trends were observed in both dried and cooked pasta. As well as in dried pasta, higher AOA of cooked pasta was measured in samples extracted with a magnetic stirrer. After cooking, there was a significant reduction of AOA ($p \leq 0.05$) of DS + 15% APP while AOA of DS + 10% APP preserve most of its activity compared to dried DS + 10% APP (0.5 and 0.4 mg gallic acid equivalents/100g, respectively).

Conclusions

Results showed that addition of APP increased cooking loss and the amount of absorbed water of the pasta samples. Sensory attributes were decreased with APP addition. Hardness and adhesiveness were decreased with APP addition. APP addition had significant influence on the colour of the samples. APP addition decreased L* and a* and increased b* values. However, this study showed that addition of APP significantly increased total polyphenol content and AOA of pasta compared with pasta without addition of APP. Extraction assisted with ultrasound proved to be a superior method for polyphenol extraction. The highest polyphenol content was in sample DS + 15% APP which was extracted with a ultrasound assisted extraction and methanol. Regarding antioxidant activity, better AOA was measured in samples extracted with a magnetic stirrer and ethanol. The highest AOA was also in pasta with addition of DS + 15% APP. This could mean that the extraction assisted by ultrasounds affects polyphenol ability for reduction of free radicals. It was also shown that after cooking, this pasta still contained higher levels of polyphenols compared with dried pasta without addition of APP.

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