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Effect of fortification with parsley (*Petroselinum crispum* Mill.) leaves on the nutraceutical and nutritional quality of wheat pasta

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ABSTRACT

This study examines the nutraceutical (phenolics content, antioxidant activity, biological activity) and nutritional potential (starch and protein digestibility) of wheat pasta supplemented with 1–4% of powdered parsley leaves. Compared to the control, the potentially bioaccessible fraction of pasta fortified with 4% parsley leaves was characterized by 67% increased phenolics content, a 146% higher antiradical ability and 220% additional reducing power. Elevation of these parameters in fortified pasta was accompanied by an augmentation of its antiproliferative effect on carcinoma cells, which confirms their biological relevance. Supplementation of pasta had no significant effect on starch digestibility, while negatively affecting protein digestibility (a reduction by about 20% for pasta with a 4% supplement). Electrophoretic and chromatographic analyses indicated the presence of phenolic interactions with proteins and/or digestive enzymes. Fortification improved the nutraceutical and nutritional potential of the studied pasta; however, the final effect is made by many factors, including phenolics–food matrix interactions.

1. Introduction

Pasta is a commonly consumed cereal-based food. It is conventionally manufactured with wheat flour as the primary ingredient (Khan, Yousif, Johnson, & Gamlath, 2013). The high content of complex carbohydrates makes it a valuable source of energy in human nutrition (Borneo & Aguirre, 2008). Pasta is low in sodium and fat, and is recognized as a moderate source of proteins and vitamins (Borneo & Aguirre, 2008). Additionally, its convenience and palatability make it a popular dish all over the world (Padalino et al., 2014). Conventional pasta is a rather poor source of physiologically active compounds; thus, fortification is used to increase the prohealthy quality of this product (Borneo & Aguirre, 2008; Boroski et al., 2011). Food fortification is defined as the addition of one or more components, whether or not they are normally contained in the food, for the purpose of correcting and/or improving a biological activity of newly designed food products (Świeca, Sęczyk, Gawlik-Dziki, & Dziki, 2014).

Many studies have been performed aimed at expanding the nutritional and nutraceutical quality of wheat pasta. So far, wheat pasta has been enriched with phenolic-rich ingredients, such as buckwheat flour and bran (Biney & Beta, 2014), sorghum flour (Khan et al., 2013), wakame (Prabhasankar et al., 2009), oregano and carrot leaves (Boroski et al., 2011), dry amaranth leaves flour (Borneo & Aguirre, 2008), elderberry juice concentrate (Sun-Waterhouse, Jin, & Waterhouse, 2013) and pea flour (Padalino et al., 2014). The above studies show that supplementation of pasta leads to multiple changes in the nutritional, nutraceutical, technological properties and consumer acceptance of these products.

Epidemiological studies indicate that a diet rich in polyphenols has a protective potential against various chronic diseases associated with oxidative damage, such as diabetes, cancer and cardiovascular diseases (Khan et al., 2013). The effectiveness of polyphenols as antioxidants is mainly linked with their ability to inhibit lipid radical formation, disrupt the propagation of chain auto-oxidation reactions, suppress singlet oxygen, reduce hydrogen peroxides to stable compounds, chelate transition metal ions, inhibit endogenous pro-oxidative enzymes and activate endogenous antioxidant enzymes (Carocho & Ferreira, 2013).

Parsley (*Petroselinum crispum* Mill.) is a popular culinary vegetable native to the countries of the Mediterranean region. Nowadays, it is cultivated all over the world and widely used as a flavoring and aromatic food additive (Díaz-Maroto, Pérez-Coello, & Cabezudo, 2002; Zhang, Chen, Wang, & Yao, 2006). Besides this, it has been discovered that the bioactive constituents of parsley exhibit a wide range of pharmacological

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properties, including antioxidant, hepatoprotective, brain protective, anti-diabetic, analgesic, spasmolytic, immunosuppressant, anti-platelet, gastroprotective, cytoprotective, laxative, estrogenic, diuretic, hypotensive, antibacterial and antifungal activity (Farzaei, Abbasabadi, Reza, Ardekani, & Rahimi, 2013). Additionally, parsley is used in folk medicine for the treatment of different diseases (Farzaei et al., 2013). Furthermore, some in *vitro* and *in vivo* studies indicate that parsley leaves are a good source of antioxidants that exhibit different mechanisms of action (Farzaei et al., 2013; Fejes et al., 2000; Nielsen et al., 1999). Flavonoids — apigenin and its glycosides — are the main phenolics compounds of parsley and these can be found in relatively large amounts in the leaves (Pápay et al., 2012). According to Patel, Shukla, and Gupta (2007), apigenin is a promising chemopreventive agent with multidirectional mechanisms of action in cancer prevention and therapy. Apigenin exhibits anti-proliferative potential and abilities to induce cell-cycle arrest and apoptosis, to prevent against oxidation, to induce detoxification enzymes, to regulate the host immune system and to modulate cellular signaling (Czyż, Madeja, Irmer, Korohoda, & Hülser, 2005; Patel et al., 2007).

The primary focus of this study is the evaluation of the nutraceutical and nutritional potential of wheat pasta supplemented with powdered parsley leaves. For this purpose, phenolics content, antioxidant activity, antiproliferative activity *in vitro* and main nutrient (starch, proteins) digestibility of control and parsley-supplemented pasta was determined. Additionally, special attention was paid to the interaction between bioactive compounds and the food matrix and their effect on pasta quality.

2. Materials and methods

2.1. Chemicals

Pancreatin, α-amylase, pepsin, bile extract, amyloglucosidase, invertase, dinitrosalicylic acid, Folin–Ciocalteau reagent, ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), potassium ferricyanide, RPMI-1640 medium, Fetal Bovine Serum (FBS), Bradford reagent were purchased in Sigma–Aldrich (St. Louis, MO, USA) company. All others chemicals were of analytical grade.

2.2. Parsley leaf supplement preparation

Parsley (*P. crispum* Mill. var. Omega) leaves were obtained from the Experimental Agricultural Station of the University of Life Sciences in Lublin, Poland. Parsley leaves were washed, rinsed, and air dried in the dark at 30 °C (SML30, Poland) until constant weight was obtained. A residual moisture of 8 g/100 g was present in the final dried samples. Dried leaves were milled and sifted (Thyr, Saskia, Germany) to produce parsley leaf flour (flour passed through 0.25 mm sieve). Parsley leaf flour was stored in air-tight dark plastic boxes until needed for pasta production.

2.3. Pasta preparation

Semolina wheat flour (Radix-bis, Rotmanka, Poland) was purchased from a local store. Spaghetti pasta flour was prepared with wheat flour and different concentrations of powdered parsley leaves (0% – C, 1–4%, P1–P4, respectively; w/w). For each formulation, pasta flour and distilled water (flour: water, 2.5:1, w/w) were mixed using a domestic blender (Kitchen Aid, Mod K5SSWH) for 5 min to obtain homogeneous dough. This dough was formed and cut in a pasta machine (Pasta machine, Kitchen collection, Mod 20171, Chillicothe, OH, USA). Spaghetti pasta (about 2.5 mm thickness, 40 mm length) was dried in a laboratory dryer (SML30, Poland) at 40 °C. A residual moisture of 12 g/100 g was present in the final dried pasta samples. Dried pasta (100 g) was cooked in 1000 mL of boiling distilled water. Optimum cooking time was determined by using the 66–50 (AACC, 2000) method and this was achieved at 7.5 min (white core in the pasta was still present but disappeared after squeezing between two glass plates). After cooking, the pasta was drained and cooled at room temperature. The cooked pasta was frozen at −20 °C and lyophilized in a laboratory freeze drier (Labconco FreeZone, Kansas City, MO, USA). Then, the freeze-dried pasta was milled and sieved to pass through a 0.25 mm sieve. The ground samples were stored in darkness at −20 °C.

2.4. Extraction procedure

2.4.1. PBS buffer extracts (PBS)

Powdered samples of pasta (2 g) or parsley leaves (2 g) were extracted for 1 h with 15 mL of phosphate buffer saline (NaCl 137 mmol/L, KCl 2.7 mmol/L, Na_2HPO_4 10 mmol/L, KH_2PO_4 1.8 mmol/L, pH 7.4) The extracts were centrifuged (6800×*g*, 20 min.) and extraction procedure was repeated. Extracts were combined and stored in darkness at −20 °C until analysis.

2.4.2. Chemical extracts (CHEM)

Powdered samples of pasta (2 g) or parsley leaves (2 g) were extracted for 1 h with 15 mL of 20 mM hydrochloric acid in methanol: acetone: water solution (30:30:40; v/v/v; pH = 2). The extracts were centrifuged (6800×*g*, 20 min.) and extraction procedure was repeated. Extracts were combined and stored in darkness at −20 °C until analysis.

2.4.3. Extracts after simulated gastrointestinal digestion (DE)

In vitro digestion was performed as described by Świeca, Baraniak, and Gawlik-Dziki (2013). Simulated saliva solution was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, and 8 g NaCl and 100 mg of mucin in 1000 mL of distilled water. The solution was adjusted to pH = 6.75 and α-amylase (E.C. 3.2.1.1.) was added to obtain 200 U/mL of enzyme activity. For gastric digestion 300 U/mL of pepsin (from porcine stomach mucosa, pepsin A, EC 3.4.23.1) was prepared in 0.03 mol/L NaCl, pH = 1.2. Further, simulated intestinal juice was prepared by dissolving 0.05 g of pancreatin (activity equivalent 4 × USP) and 0.3 g of bile extract in 35 mL 0.1 mol/L $NaHCO_3$.

Pasta samples were subjected to simulated gastrointestinal digestion as follows: 5 g of powdered pasta or parsley leaves were homogenized in a stomacher laboratory blender for 1 min to simulate mastication in the presence of 15 mL of simulated salivary fluid; and subsequently, the samples were shaken for 10 min at 37 °C. The samples were adjusted to pH = 1.2 using 5 mol/L HCl; and subsequently, 15 mL of simulated gastric fluid was added. The samples were shaken for 60 min at 37 °C. After digestion with the gastric fluid, the samples were adjusted to pH = 6 with 0.1 mol/L of $NaHCO_3$ and then 15 mL of a mixture of bile extract and pancreatin were added. The extracts were adjusted to pH = 7 with 1 mol/L NaOH and finally 5 mL of 120 mmol/L NaCl and 5 mL of mmol/L KCl were added to each sample. Once prepared, the samples were submitted for *in vitro* digestion for 120 min, at 37 °C in darkness. Thereafter, samples were centrifuged 20 min 5000×*g*. Supernatants (gastrointestinally digested extracts – DE) and pellets were separated and used for further analysis.

2.5. Phenolics analysis

2.5.1. Total phenolics content

The amount of total phenolics was determined using Folin–Ciocalteau reagent (Singleton & Rossi, 1965). To 0.5 mL of the sample, 0.5 mL H_2O, 2 mL Folin–Ciocalteau reagent (1:5 H_2O) were