Bioactive composition and sensory evaluation of innovative spaghetti supplemented with free or α-cyclodextrin chlatrated pumpkin oil extracted by supercritical CO_2

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Running title: Evaluation of pasta supplemented with pumpkin lipophilic bioactives

Abstract

The feasibility of producing durum wheat pasta enriched with a lipophilic phytocomplex, extracted using supercritical carbon dioxide (SC-CO_2), from ripe pumpkin, as free oil or as ready-to-mix oil/α-cyclodextrins (α-CDs) powder, was explored. Four types of pasta were prepared: (i) control spaghetti (S-CTRL); (ii) spaghetti supplemented with α-CDs (S-α-CD); (iii) spaghetti supplemented with pumpkin oil (S-Oil) and (iv) spaghetti supplemented with the pumpkin oil/α-CD powder (S-Oil/α-CD). The chemical, antioxidant, textural and sensory attibutes of the different pasta were evaluated and compared. S-Oil and S-Oil/α-CD spaghetti were significantly enriched with phytosterols, squalene, carotenoids, tocochromanols and unsaturated fatty acids. Spaghetti containing α-CDs were slightly improved in terms of fiber content. Oil chlatration increased the stability of some bioactives during pasta production and ameliorated poor textural and sensory characteristics of the cooked spaghetti compared with S-Oil sample. S-Oil/α-CD spaghetti might be accepted by customers, if the potential health benefits were also explained.

Keywords:

Antioxidant activity; bioactive compounds; *Durum wheat*, supplemented foods, novel ingredients, oil encapsulation, dry pasta, supercritical fluid extraction.

1. Introduction

The recent finding from nutrigenomic research, and a growing trend towards personalized nutrition, encourage development of innovative functional foods (Ilahy et al., 2018). Dry pasta is a staple food from traditional Italian cuisine that is renowned worldwide and, typically, made with unleavened dough using refined durum wheat semolina. Due to its low cost, easy preparation and pleasant sensory attributes, consumption has increased globally (Aghaei & Naeini, 2018). Coming from the wider Mediterranean diet, pasta is also an economical, easy-to-use vehicle for phytochemicals, acting as nutrition enhancers or providing specific physiological functions (e.g. theability to reduce blood cholesterol levels in hyper- and normocholesterolemic subjects of phytosterols or the role in visual function, immunity and epithelial tissue maintainance of vitamin A). As such, pasta has been the object of many supplementation strategies to improve nutritional value and functional attributes. Pasta formulations with animal and vegetable oils or flours, from fish, insects, algae, and different plant organs (leaves, fruits, seeds, etc.), show improved protein content and quality or increased ω-3 polyunsaturated fatty acids (PUFA), carotenoids and/or phenols contents as well as higher antioxidant activities than non-supplemented controls (Laus et al., 2017; Padalino et al., 2018).

Pumpkin (*Cucurbita* spp.) flesh and seeds have been subject to considerable attention in recent years, because of the potential health benefits of lipophilic, biologically active compounds, mainly carotenoids, tocochromanols, phytosterols and PUFA (Durante, Lenucci, & Mita, 2014a). Due to the high nutritional content and deep yellow-orange colour, pumpkin flour has been used to supplement other cereal-based products, including bread and pasta (Minarovičová, Lauková, Kohajdová, Karovičová, & Kuchtová, 2017). However, to obtain high quality active compounds with enhanced bioaccessibility and bioavailability, selection of the most suitable extraction technique is crucial.

Conventional extraction of lipophilic molecules employs large volumes of potentially toxic and environmentally harmful organic solvents that may remain as impurities after evaporation leading

to poor quality final products. The use of organic solvents in food processing has raised major public health, safety and environmental concerns. In contrast, supercritical carbon dioxide (SC-CO_2) represents an effective, non-toxic technology for extracting food-grade solvent-free oils from a range of plant materials. SC-CO_2 extracted oils contain the broad lipophilic phytocomplexes of the starting matrix in highly bioavailable forms (Lenucci et al., 2015). Some constituents of the phytocomplex, including liposoluble vitamins, may act as antioxidants, anti-inflammatory and anticancer agents or have a role in cell signaling and gene expression regulation, maintaining our health (Bruno et al., 2018).

The limited stability of these oils over time and/or during food processing and storage is a significant drawback in their use. Most bioactives are, in fact, highly reactive to light, oxygen, free radicals and/or susceptible to thermal degradation.

Encapsulation of plant oils in micro- or nano-particles is a promising technique to increase stability and, thus, shelf-life. Microencapsulated fish oil has been used in funtionalized spaghetti to delay oxidation and rancidity and slow development of the undesiderable taste and odour. Bread and pasta supplemented with microencapsulated Garcinia (*Garcinia cowa* Roxb. ex Choisy) fruit extracts showed good sensory and quality attributes and exhibited higher free radical scavenging activities than controls after cooking (Tolve et al., 2016).

α-Cyclodextrins (α-CDs) are biocompatible, non-toxic cyclic α-(1,4)-glucopyranose hexasaccharides derived from the enzymatic conversion of starch. Formation of complexes with specific guest molecules means α-CDs have been applied to chlatrate a variety of bioactives, including antioxidants, flavor compounds, and essential oils, contributing to their stability. α-CDs are approved as soluble dietary fibers and novel food ingredients. Furthermore, their high resistance to hydrolysis by human salivary and pancreatic amylases makes them useful to improve bioavailability and delivery of active compounds to the gastrointestinal mucosa as well as for taste (Lopez-Nicolàs, Rodriguez-Bonilla, & Garcia-Carmona, 2014).

In a previous study, we described SC-CO_2 extraction to obtain food-grade oil rich in carotenoids, tocochromanols and PUFA from the flesh (mesocarp and endocarp) and seeds [blended in a ratio of 1:1 by dry weight (dw)] of ripe pumpkin (*Cucurbita moschata* Duchesne ex Poir.) peponides ("Long of Naples") (Durante, Lenucci, D'Amico, Piro, & Mita, 2014b). Feasibility of chlatrating the oil in α-CD complexes, to improve its chemical stability and prepare ready-to-mix powdered ingredients for novel food formulations, simultaneously enriched with antioxidants and soluble dietary fibers (the α-CDs), was also demonstrated (Durante et al., 2016). Furthermore, with a final carotenoid concentration of 200–400 μg/ml, in the form of oil-in-water nanoemulsion, the oil was found to trigger activation of a "non-protective" form of autophagy, resulting delayed (40%) cell growth in the malignant human cell lines, Caco-2 and SAOs, derived from a colon adenocarcinoma and an osteosarcoma, respectively (Russo et al., 2017). Thus, the aim of this work was to take a step towards the formulation and characterization of an innovative supplemented pasta that would meet the increasing demand for foods with potential health benefits. More specifically, semolina from the durum wheat cultivar Vertola was used to prepare four types of pasta: (i) control spaghetti (S-CTRL); (ii) spaghetti supplemented with α-CDs (2.66% w/w; S-α-CD); (iii) spaghetti supplemented with SC-CO_2-extracted pumpkin oil (1.33% w/w, S-Oil) and (iv) spaghetti supplemented with pumpkin oil/α-CD inclusion complexes (3.99% w/w, S-Oil/α-CD) (Supplementary fig.1). The chemical, antioxidant, textural, and sensory attributes of these pasta were evaluated and compared.

2. Materials and Methods

2.1 Chemicals

High purity standards for the quali-quantitative determination of fatty acids (myristic, palmitic, stearic, arachidic, palmitoleic, oleic, linoleic and linolenic), phenolic acids (vanillic, *p*-coumaric, ferulic and sinapic), phytosterols (brassicasterol, campesterol, β-sitosterol, stigmasterol), anhydrous butter fat BCR-519 (triglycerides), diglycerides, monoglycerides, squalene and tocopherols (α- and γ-forms) were purchased from Sigma–Aldrich (Milan, Italy). The standard for carotenoids (lutein,

α-carotene, β-carotene, β-cryptoxanthin, 9-*cis*- and 13-*cis*-β-carotene) and tocotrienols (α- and γ-forms) were purchased from CaroteNature (Lupsingen, Switzerland) and Cayman chemicals (Ann Arbor, MI, USA), respectively. The total dietary fibre, total starch and resistant starch assay kits were purchased from Megazyme International (Bray, Ireland). Alipack 200 high purity food grade E290 CO_2 (>99%) for supercritical fluid extraction was purchased from Sapio (Monza, Italy). CAVAMAX® w6 food α-CDs were kindly provided by IMCD Italy SpA (Milano, Italy). All other analytical grade chemicals and solvents were purchased from Sigma–Aldrich (Milan, Italy).

2.2 Pumpkin matrix preparation, SC-CO_2 oil extraction and oil chlatration into a-CDs

Certified pumpkin (*Cucurbita moschata* Duch., cv Lunga di Napoli) seeds, purchased from Emanuele Larosa sementi s.r.l. (Andria, Ba, Italy), were sown (200 cm within the row and 250 cm between rows) in open fields (Località Pittuini, Nardò, 40° 27’ 65” ′N latitude, 17°91′87″ E longitude) in southern Italy during June 2015. Fully ripe pumpkin peponides were harvested during September 2015, when the stem of the plant began to shrivel. Dehydrated pumpkin powder was prepared from the rind and flesh of ripe peponides, according to the protocol developed by Durante et al. (2014b) using a pilot scale system (Farris s.r.l., Orsara di Puglia, Foggia, Italy). The seeds were vacuum-dried at 60°C and ground to 35-mesh (500 μm) using a laboratory mill (ZM200, Retsch GmbH, Haan, Germany). The dehydrated pumpkin powder and milled seeds were blended in a ratio of 1:1 (w/w), vacuum-packaged in food grade oxygen impermeable plastic bags and stored in a freezer at -20°C until SC-CO_2 extraction. Pumpkin oil was extracted using a Spe-ed SFE system (Applied Separations, Allentown, PA, USA) and chlatrated into CAVAMAX® w6 food α-CDs as previously described (Durante et al., 2016).

2.3 Pasta making

Certified durum wheat (*Triticum durum* Desf.) semolina of the cultivar Vertola, kindly provided by Dr. Di Miceli (University of Palermo, Italy), was used for pasta production. This semolina is particularly suitable for supplementation with lipophilic bioactive compounds, due to its very low endogenous lipoxygenase (LOX) activity (0.062 ± 0.002 EU/g), as described by Pasqualone et al. (2016).

S-CTRL pasta was prepared using just semolina (2500 g), while S-α-CD, S-Oil and S-Oil/α-CD spaghetti were obtained adding 65 g of CAVAMAX® w6 food α-CD powder, 32.5 g of SC-CO_2 extracted pumpkin oil or 97.5 g of lyophilized Oil/α-CD complexes to 2435 g, 2467.5 g and 2402.5 g semolina, respectively. In order to reach a dough moisture of 40%, on average 600 mL tap water was added. Amounts of α-CD, oil and oil/α-CD were determined with the purpose of significantly contributing to recommended daily allowances (RDAs) of A and E vitamins and suggested functional dose of phytosterols (EFSA, 2015a,b; Ogbe, Ochalefu, Mafulul, & Olaniru, 2015). The ingredients were processed into spaghetti using a NAMAD extruder (Namad Impianti, Rome, Italy) under the following conditions: 15 min kneading; 0,1 MPa chamber vacuum; 30°C die temperature; 42 rpm extruder auger speed. The dough was extruded through a Teflon-coated spaghetti die (diameter 1.65 mm) and dried (AFREM, Lyon, France) for 20 h, using a low temperature drying program (T max = 58°C) with linear decrease of the relative humidity into the drier from 85% to 70% during the entire drying process. The temperature was increased linearly from 50°C to 58°C in 60 min, then decreased from 60°C to 50°C in 60 min. The pasta were incubated at 50°C until the end of the drying cycle before being cooled from 50 to 40°C in 60 min and maintained for 1 hour at 40°C. The pasta making process was repeated twice.

2.4 Cooking performance

The spaghetti were cooked in boiling tap water (1:10 w/v) with no salt, according to the AACC method 66-50.01 (AACC, 1999). The optimum cooking time (OCT) was evaluated according to

D'Egidio, Mariani, Nardi, Novaro, & Cubadda (1990) and determined as when the white central core of the pasta just disappeared when squeezed between two glasses.

Water absorption (A) was calculated based on increase weight of the pasta at the OCT and determined as: A= (W-W0)/W0*100, where W and W0 were the weight of cooked and raw pasta, respectively.

Total organic matter (TOM) of pasta (surface material released from cooked spaghetti after exhaustive rinsing) was determined using the method described by D'Egidio et al. (1990). TOM values > 2.1 g/100 g denote low quality pasta, between 2.1 and 1.4 g/100 g good quality pasta, and <1.4 g/100 g very good quality pasta.

2.5 Proximate composition

The proximate composition of the semolina and pasta was performed in triplicate. The data are expressed as percentage on a dry weight (dw) basis.

Protein content was determined by the Official Method 46-30.01 (AACC, 1999) using a Leco FP 528 Nitrogen/Protein Analyser (St. Joseph, MI, USA), calibrated with EDTA at a standard nitrogen concentration (9.57%) and the conversion factor N x 5.7.

Ash and gluten index were determined according to the Official Methods 08-01.01 (AACC, 1999) and 38-12.02 (AACC, 2000), respectively.

Total dietary fibres, total starch and resistant starch were determined according to the Official Methods 985.29, 996.11 and 2002.02 (AOAC, 2006), respectively.

Moisture was measured just before chemical analyses on 3 g of milled sample using a Sartorius MA35 thermobalance (Muggio, Monza-Brianza, Italy) at 120°C.

2.6 Pasta quality parameters

Digital callipers (0-150 mm) were used to determine the diameter (maximum and minimum) of cooked spaghetti. The colour of semolina and raw pasta was evaluated using a Chroma Meter CR-

400 Tristimulus colorimeter (Minolta, Milan, Italy) and described with the CIELab colour space coordinates L^* (lightness), a^* (red-green chromaticity), and b^* (yellow-blue chromaticity), and the CIE Standard Illuminant D_{65}. 'Browness' was expressed as 100-L, as typical for cereal products. The instrument was calibrated using a standard white plate. Cooked pasta firmness was determined according to AACC method 66-50.01 (AACC, 1999) using a TA-XT2 texture analyzer with a spaghetti tensile rig code A/LKB-F (Stable Micro System, Godalming, UK).

2.7 Sensory evaluation

After cooking, the pasta was cooled for 10 min before proceeding. Sensory evaluation was performed by a panel of five trained assessors (2 males and 3 females, aged 45–55 y), according to D'Egidio et al. (1990). The assessors evaluated three textural characteristics: bulkiness (adhesion of pasta strands to each other), stickiness (amount of material adhering to the spaghetti surface) and strengthness (resistance to chewing by the teeth). Each descriptor was scored from 10 to 100. The scores for bulkiness and stickiness were: 10-20 = very high, 21-40 = high, 41-60 = rare, 61-80 = minimal and 81-100 = absent; those for strengthness were 10-20 = absent, 21-40 = rare, 41-60 = sufficient, 61-80 = good and 81-100 = very good. The score for each sensorial component was the average of three individuals' assessments. The overall judgment was calculated as the arithmetic mean of scores from each descriptor. Samples were blind-labeled with random codes to avoid bias.

2.8 Scanning electron microscopy (SEM) imaging of pasta

Transverse cross sections of raw and cooked pasta were obtained with a sharp razor blade and critical-point-dried (K850 Critical Point Drier, Quorum Technologied LTD, Ashford, UK) using liquid CO_2. Samples were mounted on carbon adhesive stubs and gold coated with a Balzers SCD 040 sputter coater (BAL-TEC AG, Balzers, Lichtenstein; thickness of gold layer: 40nm). Microstructure observation (2000x magnification) were carried out by a ZEISS EVO HD 15 SEM

(Carl Zeiss Microscopy GmbH, Ober- kochen, Germany) operating under high-vacuum at an accelerating voltage of 20 keV.

2.9 Determination of isoprenoid, soluble and insoluble-bound phenolic compounds

Cooked spaghetti were dried to constant weight using a Christ ALPHA 2-4 LSC freeze-dryer (Martin ChristGefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Raw and cooked spaghetti were ground to 35-mesh size (500 μm sieve) using a laboratory mill (ZM200, Retsch GmbH, Haan, Germany). The resulting powders were vacuum-packaged in food grade oxygen impermeable plastic bags and stored at -20°C until analysis.

Isoprenoids (tocochomanols and carotenoids) were extracted from semolina, raw and cooked pasta as described by Panfili, Fratianni, & Irano (2003) with slight modifications. Briefly, triplicate aliquots (1 g) of each sample were suspended in 60% w/v KOH (2 ml), 95% v/v ethanol (2 mL), 1% w/v NaCl (1 mL) and 0.05% w/v BHT in acetone (5 mL) and incubated at 60°C for 30 min. After alkaline hydrolysis and cooling, the sample was diluted with 1% w/v NaCl (15 mL) and extracted with 9:1 v/v *n*-hexane/ethyl acetate (15 mL, three times). The extracts (upper phases) were collected, dried, re-dissolved in ethyl acetate (100 μL); pumpkin oil (0.1 g) was dissolved directly in ethyl acetate (1 mL). All samples were filtered (0.45 μm syringe filter, Millipore Corporation, Billerica, MA, USA) and assayed by HPLC-DAD according to Durante et al. (2017). Absorbance was measured at 475 nm and 290 nm for carotenoids and tocochromanols, respectively.

Extraction and analyses of soluble and insoluble-bound phenolic compounds from semolina and raw pasta was carried out as described previously (Laddomada et al., 2015). Triplicate aliquots (250 mg) of each sample were extracted twice with 80% v/v ethanol (1 mL) in a sonic bath for 10 min and centrifuged at 4000 x g for 15 min. The combined supernatants, containing the soluble phenolic fraction, were collected, taken to dryness, and hydrolyzed with 2 M NaOH (400 μL) for 4 h. The insoluble-bound phenolic acids were extracted from the 4000 x g pellets by hydrolysis with 2 M NaOH (5 mL) for 4 h, followed by centrifugation at 4000 x g for 15 min. Samples were acidified to

pH 2.0 with 12 M HCl and extracted twice by partition with ethyl acetate (500 and 800 µL for soluble and insoluble-bound phenolic acids, respectively). The upper organic layer, obtained after 5 min centrifugation at 8000 x g, was collected, evaporated, dissolved in 80% ethanol (100 µL), and passed through a 0.45 µm syringe filter before beeing assayed using HPLC-DAD at 280, 295 and 320 nm to quantify the phenolic acids. Peaks were identified by comparing retention times and UV-Vis spectra to those of authentic isoprenoid and phenolic standards.

2.10 Lipid composition, fatty acids, phytosterols and squalene analysis

Lipids were extracted from triplicate aliquots (1 g) of semolina, raw and cooked pasta. Briefly, samples were suspended in 1:2:1.8 v/v/v chloroform/methanol/H_2O (28.8 mL), incubated overnight at 4°C and centrifuged at 6000 x g for 5 min. The supernatant was added with 5 mL chloroform and 6 mL H_2O, vortexed and centrifuged for 10 min at 600 x g. The organic phase was evaporated and stored at -20°C until analysis.

For lipid analysis, aliquots (100 mg) of the oil and dried extracts were solubilized in 200 µL of hexane; 5 µL of 2.5 mg/mL myristic-d_{27} acid solution were added to 5 µL of the hexanoic solution and evaporated to dryness. Then, 20 µL of a 20 mg/mL solution of methoxyamine hydrochloride were added and the mixture agitated at 30°C for 90 min. The methoxyaminated samples were derivatized with 90 µL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) containing 1% trimethylchlorosilane (TMCS) at 37°C for about 60 min. The derivatized samples were analyzed using a 5977E GC-MS system (Agilent Technologies, USA).

Samples (1 µL) were introduced in a split/splitless injector in the splitless mode at 325°C and separated on a DB-1HT column (15 m length, 0.25 mm ID and 0.1 µm film thickness), preceded by a 1 m 0.25 mm deactivated precolumn; temperature programming was 1 min at 50°C, 10°C/min until 350°C, 14 min at 350°C. Helium was used as carrier gas with a costant pressure of 0.11 MPa. The temperature of the interface was 350°C. Mass spectrometric parameters were set with electron impact ionization energy of 70 eV, ion source temperature of 230°C, and MS quadrupole

temperature of 150°C. The MS system was routinely set in scan mode from 50 to 800 *Da*. Peaks were identified by comparing retention times and mass spectra to those of the standards. For triglycerides quantification, the reference anidrous butter fat BCR-519 was used.

Fatty acids were derivatized according to the German Society for Fat Research (DGF – C-VI 11a) method (Lange, 2000). Briefly, 0.1 g aliquots of pumpkin oil or the entire lipidic extract from each sample were dissolved in 0.5 M NaOH methanolic solution (3 mL), incubated at 100°C for 5 min and cooled at room temperature. After adding 2.0 mL of boron trifluoride (12% w/v) in methanol, the solution was heated at 100°C for 30 min and cooled in ice. Esterified fatty acids were partitioned into hexane (1 mL) by vigorous vortex-stirring (30 s) followed by the addition of 1 mL 0.6% w/v NaCl. The organic upper phase was collected, and the extraction repeated with hexane (1 mL). Hexane extracts were combined and concentrated to a final volume of 1.0 mL.

Fatty acid methyl esters (FAMEs) were assayed using a 5977E GC/MS system (Agilent Technologies, USA) equipped with a DB-WAX column (60 m, 0.25 mm i.d., 0.25 mm film thickness, Agilent), as described by Durante et al. (2016).

Phytosterols and squalene were determined (three independent replicates) after hot saponification of the lipidic extracts and 0.1 g of pumpkin oil, as described by Anastasopoulos, Kalogeropoulos, Kaliora, Kountouri, & Andrikopoulos (2011) with slight modifications. A methanolic solution (2 mL) of 0.5 M KOH was added to each sample. The mixture was incubated at 90°C for 15 min and then methylated with 14% w/v boron trifluoride in methanol (1.5 mL) for 2 min at 90ºC. Five mL of sodium chloride solution (1%, w/v) were added and the nonsaponifiable fraction partitioned into 2 mL hexane by vortexing and centrifuging at 4000 x g for 10 min. Hexane extract was collected and dried. After the addition of 100 µL of 1 mg/mL 5-α-cholestane (internal standard), the samples were derivatized to trimethylsilylethers (TMS ether) by the addition of 125 µL *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (with 1% TMCS) and 125 µL of pyridine at 70ºC for 20 min. Samples were assayed using a 5977E GC-MS system (Agilent Technologies, USA).

Compounds were separated on HP-5ms column (30 m, 0.25 mm i.d., 0.25 mm film thickness, Agilent) under the following operative conditions: 99.999% pure helium as carrier gas, flow-rate 1.0 mL/min, injector temperature 250°C, split ratio 15:1, column temperature 250°C 0-5 min, then 250-300°C at 2°C/min. The instrumental conditions used for the MS detector were as follows: transfer line temperature 280°C, detector voltage 70 eV, acquisition mode scan range from 50 to 600 m/z. Solvent delay time was set at 2 min and the total running time was 32 min. Phytosterols and squalene were identified and quantified based on authentic standard solutions.

2.11 Determination of the hydrophilic, lipophilic and total antioxidant activity of raw pasta

Hydrophilic, lipophilic and total antioxidant activities (HAA, LAA, and TAA, respectively) of raw pasta were evaluated using both oxygen radical absorbance capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC) assays according to Laus et al. (2017). Hydrophilic and lipophilic antioxidants were extracted from 0.5 g of each milled sample (three independent replicates) with 100% methanol or 100% acetone, respectively, at 4°C with constant shaking (300 rpm) for 20 h. Samples were centrifuged at 8800 x g for 7 min. Supernatants were recovered and used to assess HAA or LAA. Antioxidant activities, expressed as µmol Trolox equivalents/100 g of pasta fresh weight (TE/100 g fw), were determined on the basis of a Trolox dose-response calibration curve. ORAC was performed using an Infinite M-200 microplate reader (Tecan Group Ltd., Männedorf, Switzerland) and Corning Costar 96-well clear round bottom plates (Corning Inc., New York, NY, USA). Differences between area under the fluorescence decay kinetic curve (AUC) of each sample and the AUC of the blank were used to quantify antioxidant activity.

TEAC values were assayed using a UV-1800 Spectrophotometer (Shimadzu, Kyoto, Japan) and by plotting the percentage decrease in absorbance at 734 nm, as a function of the standard Trolox concentration.

2.11 Calculation of the maximum theoretical enrichment values of supplemented spaghetti

The maximum theoretical enrichment of supplemented spaghetti (S-MTE), expressed as mg compound/100 g pasta, were calculated as the sum of each compound in amounts of semolina (98.67 g) and pumpkin oil (1.33 g) blended to prepare 100 g of supplemented semolina, and considering an approximate semolina-to-pasta conversion factor (CF) of 1.04, according to the following formula:

S-MTE= (mg compound in 98.67 g of semolina + mg compound in 1.33g of oil)*CF

2.12 Statistical analysis

The results, expressed as mean ± standard deviation of three independent experiments (n = 3), were compared by one-way ANOVA followed by the Tukey's *post hoc* method, using SigmaStat 11.0 (Systat Software Inc., Chicago, IL, USA). Differences between means were considered significant at 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1. Chemical and proximate characterization of the starting materials

Table 1a shows the chemical characterization of pumpkin oil used for pasta preparation. The oil was composed mainly of triglycerides (64.1 g/100 g), diglycerides (10.2 g/100 g) and free fatty acids (7.4 g/100 g) with a prevalence of PUFA (42.3%), mono-unsaturated fatty acids (MUFA; 30.7%) and saturated fatty acids (SFA; 27.0%). Linoleic acid (C18:2 n-6) contributed about 42% of the total identified fatty acids, followed by oleic (C18:1 n-9; ~30%), palmitic (C16:0; ~17%) and stearic (C18:0; ~9%) acids. The oil also contained several nutritionally beneficial compounds including phytosterols (16.74 g/100 g), tocochromanols (399.0 mg/100 g), carotenoids (179.5 mg/100 g) and squalene (159.4 mg/100g).

The phytosterol fraction was made up mainly of β-sitosterol (15.6 g/100 g); stigmasterol, campesterol, and brassicasterol were present in low amounts. In the USA and EU, phytosterols added to a variety of food products are generally recognized as safe. Their ingestion, however,

should not exceed 3 g/day, because there is no evidence of health benefits at higher doses and there might be undesirable effects (Ogbe, Ochalefu, Mafulul, & Olaniru, 2015).

Tocopherols were more abundant than tocotrienols; α- and γ- were the only isomers detected in the oil, with the latter largely (6-10 fold) exceeding the former. Squalene content was within ranges (about 95–1192 mg/100 g oil) reported in the literature for pumpkin seed oil (Ogrodowska, Tańska, & Brandt, 2017). Several studies have indicated that squalene is a highly effective oxygen-scavenging agent and its biological action has been described in detail elsewhere (Lou-Bonafonte et al., 2018). β-Carotene was the most abundant carotenoid (86.5 mg/100 g oil) followed by α-carotene (70.6 mg/100 g) and lutein (16.2 mg/100 g). The total amounts of tocochromanols and carotenoids in the oil were lower than reported previously (Durante et al., 2016); differences were also evident in the qualitative profiles of these compounds. This variability is probably related to different preparations of the pumpkin matrix that, in this case, besides flesh included the fruit peels (exocarp). Pumpkin peels have less carotenoids than the flesh and contain mainly pigments associated with green-tissues, such as the xantophylls β-cryptoxantin and lutein (Kim, Kim, Kim, Choi, & Lee, 2012), the amounts of which were increased in our extract compared with those obtained from a matrix prepared using only the flesh of pumpkin peponides (Durante et al., 2016).

The chemical and proximate characterization of Vertola semolina is reported in Table 1b. Total lipids contributed to approx. 0.6% of the semolina weight and comprised mainly free fatty acids (211.7 mg/100 g fw). Linoleic acid represented about 35% of the total followed by palmitic (33.6%) and stearic (21.7%) acids; Beleggia et al. (2011) have reported similar profiles. Vertola semolina was also characterized by a high total phytosterols content (135.3 mg/100 g fw), which agreed with Normén et al. (2002), but much higher than the approx. 20 mg/100 g dw reported by Beleggia et al. (2011) in wholemeal semolina made from unspecified durum wheat cultivars from Apulia (Italy).

As expected, phenolic acids were the main bioactive non-nutrient compounds. More than 90% occurred in the insoluble form, as cell wall bound ferulic acid (24.31 mg/100g fw). The remaining were soluble and comprised essentially sinapic (55.6%) and ferulic (27.0%) acids. Our results fell

within the range of values reported for whole-wheat flour (Laddomada et al., 2017) and were similar to those of monovarietal durum wheat semolina milled from Duilio caryopsides (Nicoletti, Martini, De Rossi, Taddei, D'Egidio, & Corradini, 2013).

Tocochromanols and carotenoids (only lutein) were detected in small amounts (1.71 mg/100 g and and 0.21 mg/100 g, respectively) and similar to literature values (Laddomada et al., 2015). Gluten, yellow (b*) and brown (100-L*) indices were 60.51, 19.97 and 10.69, respectively, within the good quality range for semolina. Gluten index is an important parameter to measure semolina quality and has gained wide acceptance as a method for defining whether gluten quality is weak (< 30), normal (from 30 to 80) or strong (>80) (Oikonomou, Bakalis, Rahman, & Krokida, 2015). Furthermore, a yellowish color is highly appreciated by consumers of durum wheat pasta and, therefore, the yellow index of semolina should be high, while brown index should be low, allowing the perception of a brilliant and luminous color in the final product (Giannone et al., 2018).

Ash (0.91%) was within the limits imposed by the FAO/WHO Codex Alimentarius for semolina (FAO/WHO, 1995) while dietary fiber content was 3.12%, slightly lower than average values (3.6%) reported in the USDA Food Composition Database (https://ndb.nal.usda.gov/ndb/search/list, accessed on 20/07/2018).

3.2. Bioactive compounds, antioxidant activity and lipid profiles of raw pasta

Concentrations of bioactives (carotenoids, tocochromanols, phytosterols and squalene) and antioxidant activities (HAA, LAA and TAA) of S-CTRL, S-α-CD, S-Oil and S-Oil/α-CD spaghetti are reported in table 2. As expected, no differences were evidenced between S-CTRL and S-α-CD samples, while S-Oil and S-Oil/α-CD spaghetti were significantly ($p<0.05$) enriched in carotenoids, tocochromanols and phytosterols. In both samples, total carotenoid concentrations were at least 9.8-fold that of S-CTRL, with S-Oil/α-CD spaghetti showing a small, but significant, increase in total carotenoid concentration (2.34 mg/100 g fw) with respect to S-Oil pasta (2.06 mg/100 g fw). Lutein

was detected in all samples: in S-CTRL and S-α-CD its concentration was almost identical to that found in semolina, while in both S-Oil and S-Oil/α-CD spaghetti lutein was more than double control values. In these samples, β-carotene and α-carotene were the most abundant carotenoids, followed by 9-*cis* and 13-*cis*-β-carotene isomers. Unespectedly, β-cryptoxanthin, although present in the oil at higher concentrations than 13-cis-β-carotene, was not detected in any pasta samples. Comparing data recorded for S-Oil with the maximum theoretical enrichment values of supplemented spaghetti (S-MTE), a substantial and differential loss was observed for total carotenoids (21%), α-carotene (39%), β-carotene (37%), and β-cryptoxanthin (100%), but not for lutein, 9-cis- and 13-*cis*-β-carotene isomers (Table 2). This might be related to the different susceptibilities of carotenoids to degradation during processing (mixing, extrusion and drying). Hidalgo, Brandolini, & Pompei (2010) have reported carotenoid losses (48%) during the kneading-extrusion phase, while drying did not induce significant changes. The authors also demonstrated that each carotenoid was affected differently, depending on processing conditions and characteristics of the semolina, particularly LOX activity. Thus, low LOX activity Vertola semolina, and the presence of other bioactive compounds (eg. tocochromanols) exerting protective or synergistic effects on carotenoid oxidation, might have a role in protecting endogenous and exogenous pigment degradation. When the oil was supplemented in the α-CD chlatrated form, the percentage loss of total carotenoids, particularly α- and β-carotene, was substantially reduced (10%, 27% and 30%, respectively), suggesting that α-CD complexation preserves carotenoids, as reported by Durante et al. (2016).

The quali-quantitative characterization of tocochromanols evidenced remarkable differences among pasta samples. Supplementation with the oil, either free or chlatrated, increased strongly (≥ 39-fold) tocochromanols concentrations in the pasta compared with S-CTRL, overcoming almost entirely the loss of this important class of compounds during processing, which was clearly evident from the comparison of tocochromanol profiles for S-CTRL and S-α-CD with that of semolina. In both pasta

types, α-tocopherol was the only form detected, at an almost negligible concentration, while in S-Oil and S-Oil/α-CD samples, γ-tocopherol was the most abundant, as is typical of many foods, followed by α-tocotrienol and α-tocopherol, reconstituting the biochemical complexity of semolina, at least in part, which was lost during pasta processing.

Phytosterol loss from semolina was about 57% in S-CTRL and in S-α-CD pasta, much higher than the values (23-30%) reported by Beleggia et al. (2011). In S-Oil and in S-Oil/α-CD spaghetti, phytosterol content was three-fold higher than in S-CTRL and S-α-CD pasta. S-Oil and S-Oil/α-CD samples were characterized by the presence of squalene (approx. 1.7 mg/100 g fw), which was absent in S-CTRL and S-α-CD pasta.

No significant differences were detected among the four types of pasta with regard soluble and polymer bound phenolic acids (Supplementary table 1).

HAA and LAA in the four pasta types (Table 2) were determined using the ORAC and TEAC methods. No significant differences were found among samples with respect to HAA, which was always much higher than LAA. S-Oil/α-CD pasta had the highest LAA values, 1.4- and 4.7-fold higher than those of S-CTRL, respectively, with significant differences between S-Oil and S-Oil/α-CD samples. Similar increases were reported by Pasqualone et al. (2016) using an oleaginous extract prepared, using SC-CO_2, from wheat bran to fortify pasta.

Oil supplementation, either free or chlatrated, more than doubled total lipid contents of the pasta and was associated with significantly increased triglycerides, diglycerides, monoglycerides, and free fatty acids, compared with S-CTRL and S-α-CD samples (Tab. 3). MUFA and PUFA percentages were also increased at expense of SFA. Accordingly, S-CTRL and S-α-CD pasta were higher in palmitic, stearic and myristic acids than S-Oil and S-Oil/α-CD, in which the relative percentages of linoleic, linolenic and oleic acids were all improved. Because of the high n-6 content of pumpkin oil, the ratio n-6 to n-3 also rose from 20.86:1 (S-CTRL) to 25.03:1 (S-Oil) determining a further shift from the ideal ratio of 4:1.

3.3. *Proximate composition and technological pasta quality attributes*

Table 4 reports the proximate compositions, color attributes, cooking performances, textural and sensory parameters of control and supplemented spaghetti; factors indicated as the most important for pasta quality and consumer acceptance. No significant differences were detected among the pasta types with regard of proteins, which ranged between 13.35% and 13.49%, except for the S-Oil/α-CD pasta, where a slight but significant reduction (12.87%) was observed, possibly due to a dilution effect of proteins because of Oil/α-CD powder addition to semolina. Supplementation significantly reduced pasta ash content from 0.94% of S-CTRL to 0.79% of S-Oil. Intermediate values were obtained for S-α-CD and S-Oil/α-CD (0.90% and 0.89%, respectively), which were not statistically different. Protein and ash values were in agreement with data commonly reported for durum wheat pasta including those reported by Laus et al. (2017) for pasta supplemented with lipophilic (tocochromanols, carotenoids) or hydrophilic (phenols) compounds extracted from durum wheat bran by super and sub critical fluid extraction, respectively. Addition of α-CDs diluted slightly (-4% compared to S-CTR) total starch concentrations in the raw spaghetti. Pasta samples (100 g dw) had more than 3 g total fiber, corresponding to approximately 10% of the RDA for an adult (EFSA, 2010). Interestingly, compared to S-CTRL and S-Oil, total dietary fibers and resistant starch were significantly increased following addition of α-CDs, regardless of whether these were complexed with the oil. The differences were both significant in the S-α-CD sample (+23% and +61%, respectively), while significance was only observed for resistant starch in the S-Oil/α-CD pasta (56% increase).

The diameter before cooking was similar for all pasta and was in the range 1.53-1.69 mm. Yellow (b^*) and red (a^*) indices were significantly higher in S-Oil and S-Oil/α-CD samples, due to the orange color of the oil, while the brown index (100-L^*) was statistically lower in S-CTRL, S-Oil and S-Oil/α-CD compared to the S-α-CD pasta. However, no significant differences in brown index were obeserved between S-CTRL and S-Oil.

With regard to the cooking properties, supplementation with α-CDs and pumpkin oil (free or chlathrated) substantially increased TOM to values generally associated to low pasta quality. Nevertheless, it affected positively (increased) OCT and scarcely influenced water absorption. The mechanisms by which the different types of supplementation may act, to determine increases in TOM, are probably different. In the case of S-Oil pasta, pumpkin oil components (e.g. charged lipids, mono- and diglycerides) may act as emulsifiers, forming complexes with amylose during cooking, leading to leaching as described by Bustos, Perez, & Leon (2015), while direct leaching of α-CDs into the cooking water might explain the higher TOM of S-α-CD. S-Oil/α-CD complexes are less soluble in water than simple α-CDs (Durante et al., 2016), but some of the oil could be released during cooking and, thus, the intermediate TOM value of S-Oil/α-CD pasta may be as a result of an equilibrium between reduced α-CD leaching and loss of emulsified gelatinized starch.

The firmness of cooked oil supplemented spaghetti was significantly reduced compared with S-CTRL and S-α-CD pasta, although chlatration of the oil in α-CDs led to partial recovery.

With regard to the sensory evaluation, all pasta had good scores for strengthness. The highest scores for bulkiness, stickiness, and overall judgment were registered for S-CTRL. Bulkiness and stickiness were “rare” in S-α-CD and S-Oil/α-CD pasta but “high” in S-Oil, suggesting a positive role for oil chlatration in affecting both descriptors. Accordingly, S-α-CD and S-Oil/α-CD pasta had an overall score higher than S-Oil.

3.4. SEM observations of raw and cooked pasta

SEM observations of raw S-CTRL, S-α-CD, S-Oil and S-Oil/α-CD pasta (Fig. 1i) revealed a well-developed protein matrix with starch granules deeply embedded in the gluten network, with few differences among samples. Imprints of missing starch granules (starch shadows) were also evident in all samples. Small cracks were present in the protein-starch matrix of S-CTRL and S-α-CD spaghetti, likely due to tension within the pasta dough during drying and/or shrinkage during preparation. The presence of cracks appeared reduced in S-Oil and S-Oil/α-CD pasta, likely due to

the lubricating action of the oil during extrusion and pasta drying, as previously suggested by Pasqualone et al. (2016).

Differences at the microstructural level were more evident after cooking (Fig. 1ii). Starch granules of S-CTRL spaghetti appeared swollen and embedded in a complex and filamentous protein network. S-Oil pasta did not show evident microstructural differences compared to S-CTRL. Conversely, supplemetation of semolina with durum wheat bran oil at a higher concentration (5.5%) reduced starch swelling by interfering with pasta hydration during cooking (Pasqualone et al., 2016), suggesting a correlation between oil concentration and alterations to cooked pasta microstructures. A more compact structure was observed in S-α-CD and S-Oil/α-CD pasta, where partially-swelled starch granules were deeply embedded in an amorphous matrix, indicating the presence of α-CDs affected gluten organization negatively. The effect of CD supplementation on wheat flour dough characteristics are complex and not well investigated. However, Kim & Hill (1984) reported that 1.5% β-CD addition increased swelling and solubility of wheat starch granules during gelatinization, inhibited enzymatic starch granule hydrolysis by binding to non-catalytic sites of α-amylase, disrupted the amylose-lipid complex formation, and formed inclusion complexes with lipids. Further studies are required in this regard to evaluate the effects of α-CDs supplementation on the technological and biochemical properties of pasta.

3.5. Quali-quantitative profiles of the main bioactives in cooked pasta

Table 5 reports the quali-quantitative profiles for the main bioactive compounds in cooked pasta samples. S-Oil and S-Oil/α-CD pasta had substantially higher contents of phytosterols, tocochromanols, squalene and carotenoids, compared to S-CTRL and S-α-CD samples. With regard to each class of compounds, although a reduction was observed in total phytosterols and carotenoids (not tocochromanols), probably due to thermal hydrolysis, cooking affected only marginally the phytochemical profiles of pasta when compared to the corresponding raw sample. Interestingly,

total carotenoids in S-Oil/α-CD cooked pasta were significantly higher than in S-Oil. The increment (16.9%) was higher than in raw pasta (13.6%), suggesting a protective role for α-CD chlatration on degradation of carotenoids, even during cooking.

Although vitamin A and E deficiency is rare in developed countries, most populations do not meet the RDAs (700 μg/dayand 15 mg/day for vitamin A and E, respectively) (EFSA, 2015a,b). Considering 100 g dw of cooked S-Oil and S-Oil/α-CD pasta provided, respectively, 8.86 and 10.41% of the RDA for vitamin A and 2.99 and 1.83% of RDA for vitamin E, supplemented spaghetti may contribute to improve vitamin status. Furthermore, a 100 g dw serving of cooked S-Oil or S-Oil/α-CD pasta provided approximately 5% to 8% of the funtional dose of phytosterols (1.5-2.4 g/day). A daily intake within the suggested range has been reported to reduce plasma total and LDL-cholesterol on average by 7 to 10.5% by inhibiting absorption of cholesterol through displacement in mixed micelles (Ogbe, Ochalefu, Mafulul, & Olaniru, 2015). Obviously, further studies are required to assess the effective bioaccessibilities and bioavalabilities of S-Oil/α-CD pasta nutrients and bioactives, as well as the functional potential of the supplemented product.

4. Conclusions

Supplementation of semolina with SC-CO_2 extracted pumpkin oil, free or chatrated into α-CDs, allowed preparation of spaghetti with improved phytosterols, squalene, carotenoids, tocochromanols and unsaturated fatty acids contents, providing a reasonable proportion of the RDA for A and E vitamins, as well as increased lipophilic antioxidant capacity. α-CD pumpkin oil complexation increased slightly pasta fiber content. Further, it increased the stability of some bioactive components during pasta production and ameliorated firmness of cooked spaghetti compared to S-Oil sample. Oil supplementation affected negatively TOM, firmness, bulkiness and stickiness values compared to S-CTRL; nevertheless, the overall sensory evaluation was satisfactory for the S-Oil/α-CD pasta. Although the cost of S-Oil/α-CD spaghetti would be higher than typical commercial pasta, we believe it could have potential appeal to consumers, because of

the good balance of bioactive qualities and hedonistic features and could represent an innovative supplemented products to address specific physiological or pathological needs.

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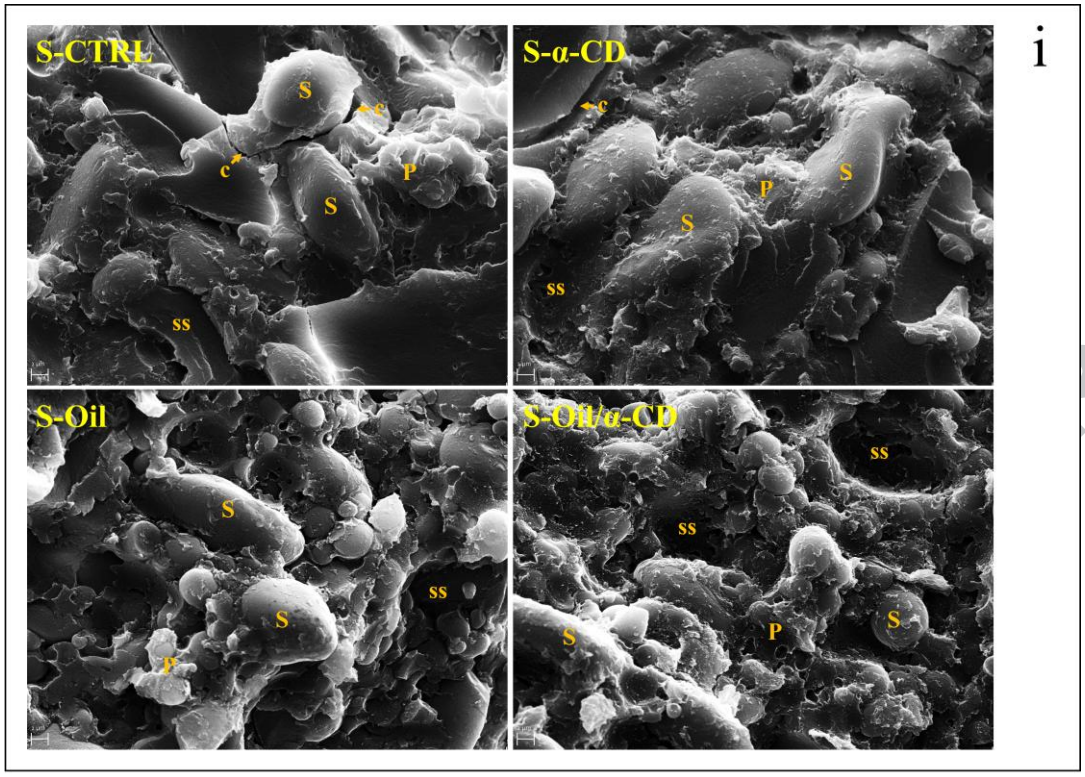
Figure Captions

Fig. 1: SEM micrographs of cross sections of raw (i) and cooked (ii) spaghetti. S-CTRL, control spaghetti; S-α-CD, spaghetti supplemented with α-cyclodextrins; S-Oil, spaghetti supplemented with SC-CO_2 extracted pumpkin oil; S-Oil/α-CD, spaghetti supplemented with the pumpkin oil/α-CD powder. S, starch granules; ss, starch shadows; p, protein network; c, craks. Magnification 2000x.

Supplementary fig. 1: Macroscopic appearance of control (S-CTRL) and supplemented spaghetti. Pasta was supplemented with α-cyclodextrins (S-α-CD), SC-CO_2 extracted pumpkin oil (S-Oil) and with pumpkin oil/α-CD inclusion complexes (S-Oil/α-CD).


S-CTRL
S-α-CD
S-Oil
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i
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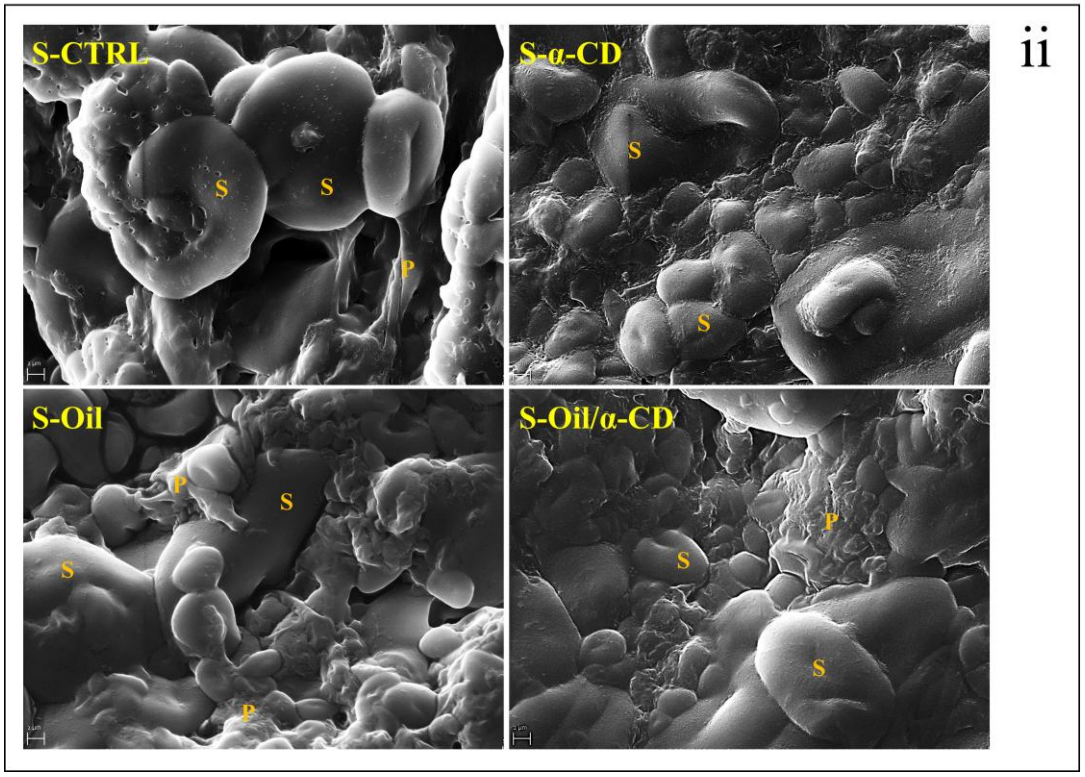
S-CTRL
S-α-CD
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ii
S
P

Table 1. Chemical characterization of SC-CO_2 extracted pumpkin oil (**a**) and semolina (**b**).

a

	Oil
Lipids (g/100 g)	
Triglycerides	64.1±0.6
Diglycerides	10.2±1.0
Monoglycerides	0.38±0.02
Free Fatty acids	7.4±0.6
Total	*82.1±2.2*
Fatty acid composition (%)	
Myristic (C14:0)	0.30±0.02
Palmitic (C16:0)	17.1±1.9
Stearic (C18:0)	9.0±0.9
Arachidic (C20:0)	0.64±0.03
Palmitoleic (C16:1)	0.30±0.01
Oleic (C18:1 n-9)	30.4±1.1
Linoleic (C18:2n-6)	41.7±1.2
Linolenic (C18:3 n-3)	0.58±0.02
SFA	27.0
MUFA	30.7
PUFA	42.3
PUFA/SFA	*1.56*
Phytosterols (g/100 g)	
Brassicasterol	0.16±0.01
Campesterol	0.32±0.06
Stigmasterol	0.66±0.08
β-Sitosterol	15.6±2.3
Total	*16.74±2.45*
Tocochromanols (mg/100 g)	
α-Tocopherol	21.8±0.8
γ-Tocopherol	216.3±9.8
α-Tocotrienol	22.2±1.9
γ-Tocotrienol	138.7±2.8
Total	*399.0±15.3*
Squalene (mg/100 g)	159.4±17.5
Carotenoids (mg/100 g)	
Lutein	16.2±0.4
α-Carotene	70.6±0.5
9-cis-β-Carotene	4.6±0.1
β-Carotene	86.5±0.6
13-*cis*-β-Carotene	0.50±0.01
β-Cryptoxanthin	1.13±0.03
Total	*179.5±1.6*

b

	Semolina	
Lipids (mg/100 g fw)		
Triglycerides	82.4±7.1	
Diglycerides	108.2±9.1	
Monoglycerides	27.3±1.0	
Free Fatty acids	211.7±14.1	
Total	*429.6±31.3*	
Fatty acid composition (%)		
Myristic (C14:0)	1.5±0.7	
Palmitic (C16:0)	33.6±1.6	
Stearic (C18:0)	21.7±3.4	
Arachidic (C20:0)	nd	
Palmitoleic (C16:1)	nd	
Oleic (C18:1 n-9)	6.4±0.9	
Linoleic (C18:2n-6)	35.0±4.1	
Linolenic (C18:3 n-3)	1.84±0.09	
SFA	56.8±5.7	
MUFA	6.4±0.9	
PUFA	36.8±4.2	
PUFA/SFA	0.65	
Phytosterols (mg/100 g fw)		
Brassicasterol	nd	
Campesterol	11.6±1.2	
Stigmasterol	21.6±2.3	
β-Sitosterol	102.1±8.3	
Total	*135.3±11.8*	
Phenolic acids (mg/100 g fw)	**Soluble**	**Bound**
Vanillic acid	0.32±0.02	0.47±0.06
p-Coumaric	0.12±0.02	0.54±0.19
Ferulic acid	0.68±0.11	24.31±0.21
Sinapic acid	1.40±0.10	nd
Total	*2.52±0.25*	*25.32±0.45*
Tocochromanols (mg/100 g fw)		
α-Tocopherol	0.85±0.08	
γ-Tocopherol	0.30±0.06	
α-Tocotrienol	0.57±0.01	
Total	*1.71±0.08*	
Carotenoids (mg/100 g fw)		
Lutein	0.21±0.01	
Ash (%)	0.91±0.01	
Total fiber (%)	3.12±0.02	
Gluten index	60.51±4.14	
Yellow index (b^*)	19.97±0.31	
Brown index (100-L^*)	10.69±0.01	

Data represent the mean ± standard deviation of three independent replicates (n = 3). nd, not detected; fw, fresh weight.

Table 2. Composition of the main bioactive compounds (carotenoids, tocochromanols and phytosterols) and antioxidant activity of control (S-CTRL) and supplemented raw spaghetti. Pasta was supplemented with α-cyclodextrins (S-α-CD), SC-CO_2 extracted pumpkin oil (S-Oil) and with pumpkin oil/α-CD inclusion complexes (S-Oil/α-CD). The maximum theoretical enrichment values of pasta (S-MTE) is also reported.

	Pasta				
	S-CTRL	**S-α-CD**	**S-Oil**	**S-Oil/α-CD**	**S-MTE**
Carotenoids (mg/100 g fw)					
Lutein	0.21±0.02^{b}	0.29±0.08^{b}	0.57±0.01^{a}	0.64±0.03^{a}	0.43±0.02^{c}
α-Carotene	nd	nd	0.57±0.01^{c}	0.69±0.06^{b}	0.94±0.01^{a}
9-*cis*-β-Carotene	nd	nd	0.05±0.01^{a}	0.06±0.01^{a}	0.06±0.00^{a}
β-Carotene	nd	nd	0.73±0.01^{b}	0.80±0.05^{b}	1.15±0.01^{a}
13-*cis*-β-Carotene	nd	nd	0.14±0.03^{a}	0.15±0.01^{a}	0.01±0.00^{c}
β-Cryptoxanthin	nd	nd	nd	nd	0.02±0.00
Total	*0.21±0.02^{d}*	*0.29±0.08^{d}*	*2.06±0.07^{c}*	*2.34±0.1b^{c}*	*2.61±0.05^{a}*
Tocochromanols (mg/100 g fw)					
α-Tocopherol	0.08±0.01^{c}	0.14±0.03^{c}	0.31±0.04^{b}	0.37±0.02^{b}	1.14±0.09^{a}
γ-Tocopherol	nd	nd	2.34±0.04^{b}	2.62±0.19^{b}	3.18±0.19^{a}
α-Tocotrienol	nd	nd	0.47±0.20^{b}	0.44±0.04^{b}	0.87±0.04^{a}
γ-Tocotrienol	nd	nd	nd	nd	1.85±0.04
Total	*0.08±0.01^{c}*	*0.14±0.03^{c}*	*3.12±0.28^{b}*	*3.43±0.25^{b}*	*7.04±0.36^{a}*
Phytosterols (mg/100 g fw)					
Campesterol	6.3±0.1^{c}	6.4±0.1^{c}	12.1±0.8^{b}	13.9±0.1^{a,b}	15.6±1.9^{a}
Stigmasterol	13.4±0.1^{c}	13.5±0.1^{c}	26.1±0.7^{b}	27.9±0.5^{b}	30.1±1.3^{a}
β-Sitosterol	36.9±0.5^{c}	38.8±1.1^{c}	134.4±6.3^{b}	133.8±1.3^{b}	308.2±38.8^{a}
Total	*56.6±0.7^{c}*	*58.7±1.3^{c}*	*172.6±7.8^{b}*	*175.6±1.9^{b}*	*353.9±43.0^{a}*
Squalene (mg/100 g fw)	nd	nd	1.8±0.6^{a}	1.7±0.2^{a}	2.1±0.2^{a}
Antioxidant activity - ORAC (μmol TE/100 g fw)					
HAA	74.3±0.8^{a}	70.6±2.7^{a}	75.0±2.6^{a}	77.1±4.2^{a}	-
LAA	23.7±0.1^{c}	23.4±0.5^{c}	29.9±0.5^{b}	33.4±0.5^{a}	-
TAA	98.0±0.9^{c,b}	94.0±3.2^{c}	105.0±3.1^{b,a}	110.5±4.7^{a}	-
Antioxidant activity - TEAC (μmol TE/100 g fw)					
HAA	88.7±6.5^{a}	89.0±8.2^{a}	93.4±8.1^{a}	86.9±5.8^{a}	-
LAA	6.2±2.0^{c}	7.0±1.3^{c}	18.4±2.5^{b}	29.1±5.4^{a}	-
TAA	94.9±8.5^{a}	96.0±9.5^{a}	111.8±10.6^{a}	116.0±11.2^{a}	

Values are expressed as mg/100 g fw and represent the mean ± standard deviation of three independent replicates (n = 3). Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple comparison procedures (Tukey post hoc test, $P < 0.05$). Different letters denote significant differences among samples. nd, not detected; ORAC, oxygen radical absorbance capacity; TEAC, trolox equivalent antioxidant capacity; HAA, hydrophilic antioxidant activity; LAA, lipophilic antioxidant activity; TAA, total antioxidant activity; TE, trolox equivalents.

Table 3. Lipids and fatty acids composition of control (S-CTRL) and supplemented raw spaghetti. Pasta was supplemented with α-cyclodextrins (S-α-CD), SC-CO_2 extracted pumpkin oil (S-Oil) and with pumpkin oil/α-CD inclusion complexes (S-Oil/α-CD).

	Pasta			
	S-CTRL	**S-α-CD**	**S-Oil**	**S-Oil/α-CD**
Lipids (mg/100 g fw)				
Triglycerides	77.3±11.5^{b}	65.4±9.4^{b}	359.6±23.3^{a}	403.8±85.4^{a}
Diglycerides	113.0±64.3^{b}	83.9±13.5^{b}	250.4±48.2^{a}	250.7±25.6^{a}
Monoglycerides	32.8±9.9^{c}	31.5±5.3^{c}	250.7±23.8^{a}	119.3±11.3^{b}
Free fatty acids	293.6±27.7^{b}	192.8±53.6^{b}	367.7±93.6^{a}	465.0±62.1^{a}
Total	*516.7±113.4^{b}*	*373.6±81.8^{b}*	*1228.4±188.1^{a}*	*1238.8±184.4^{a}*
Fatty acids composition (%)				
Myristic acid	1.20±0.28^{a}	1.27±0.22^{a}	0.97±0.19^{a}	1.18±0.49^{a}
Palmitic acid	37.2±0.4^{a}	37.0±0.3^{a}	27.3±0.5^{b}	26.9±1.00^{b}
Stearic acid	26.1±0.7^{a}	26.5±0.4^{a}	15.6±0.8^{b}	15.5±2.4^{b}
Oleic acid	5.4±0.2^{b}	5.3±0.1^{b}	13.1±1.6^{a}	13.1±0.3^{a}
Linoleic acid	28.8±1.0^{b}	28.6±0.5^{b}	41.3±2.1^{a}	41.6±4.0^{a}
Linolenic acid	1.38±0.08^{b}	1.34±0.08^{b}	1.65±0.04^{a}	1.72±0.08^{a}
SFA	64.5±1.4^{a}	64.8±0.9^{a}	43.9±1.5^{b}	43.6±3.9^{b}
MUFA	5.4±0.2^{b}	5.3±0.1^{b}	13.1±1.6^{a}	13.8±2.0^{a}
PUFA	30.2±1.08^{b}	29.9±0.5^{b}	42.9±2.1^{a}	43.3±4.1^{a}
PUFA/SFA	0.47	0.46	0.98	0.99
n6/n3	20.86	21.34	25.03	24.19

Values, expressed as mg/100 g fw or as relative percentage (%), represent the mean ± standard deviation of three independent replicates (n = 3).

Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple comparison procedures (Tukey post hoc test, $P < 0.05$).

Different letters denote significant differences among samples.

SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 4. Proximate composition, color attributes, cooking performances, textural parameters and sensory evaluation of control (S-CTRL) and supplemented spaghetti. Pasta was supplemented with α-cyclodextrins (S-α-CD), SC-CO_2 extracted pumpkin oil (S-Oil) and with pumpkin oil/α-CD inclusion complexes (S-Oil/α-CD).

	S-CTRL	S-α-CD	S-Oil	S-Oil/α-CD
Proximate composition				
Protein (%)	13.49±0.02^{a}	13.35±0.16^{a}	13.26±0.03^{a}	12.87±0.33^{b}
Ash (%)	0.94±0.01^{a}	0.90±0.01^{b}	0.79±0.01^{c}	0.89±0.01^{b}
Total starch (%)	78.89±1.55^{a}	75.80±0.10^{b}	78.91±0.46^{a}	75.59±0.53^{b}
Total fiber (%)	3.13±0.01^{b}	3.86±0.18^{a}	3.17±0.06^{b}	3.48±0.40^{a,b}
Resistant Starch (%)	0.36±0.03^{b}	0.58±0.04^{a}	0.41±0.01^{b}	0.56±0.01^{a}
Diameter (mm)	1.53-1.60	1.60-1.63	1.54-1.69	1.54-1.65
Color characteristics				
Yellow index (b*)	33.10±0.23^{c}	29.56±0.32^{d}	58.82±0.36^{b}	61.17±0.39^{a}
Red index (a*)	0.87±0.13^{c}	0.48±0.09^{d}	4.44±0.19^{b}	6.31±0.07^{a}
Brown index (100-L*)	43.40±0.39^{c}	45.01±0.42^{a}	43.69±0.29^{b}	41.42±0.40^{d}
Cooking performances				
OCT (opt. cook. time) min	8.30±0.8^{a}	10.36±1.04^{a}	10.15±1.21^{a}	10.30±1.34^{a}
TOM (g/100 g pasta)	1.63±0.03^{d}	4.12±0.03^{a}	3.07±0.04^{c}	3.34±0.04^{b}
Water absorption (g/100 g pasta)	147.5±15.0^{a}	141.7±14.1^{a}	149.7±14.7^{a}	158.7±16.1^{a}
Textural parameters				
Firmness (TA.XT) (Kg)	0.284±0.001^{a}	0.281±0.001^{a}	0.260±0.001^{c}	0.270±0.005^{b}
Sensory evaluation				
Bulkiness	70	60	40	50
Stickiness	80	60	40	50
Strengthness	65	75	65	65
Overall judgment	72	65	49	55

Values represent the mean ± standard deviation of three independent replicates (n = 3). Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple comparison procedures (Tukey post hoc test, $P < 0.05$).

Table 5. Composition of the main bioactive compounds (carotenoids, tocochromanols and phytosterols) of control (S-CTRL) and supplemented cooked spaghetti. Pasta was supplemented with α-cyclodextrins (S-α-CD), SC-CO_2 extracted pumpkin oil (S-Oil) and with pumpkin oil/α-CD inclusion complexes (S-Oil/α-CD).

	Pasta			
	S-CTRL	**S-α-CD**	**S-Oil**	**S-Oil/α-CD**
Carotenoids (mg/100 g dw)				
Lutein	0.24±0.03^{b}	0.21±0.05^{b}	0.42±0.02^{a}	0.53±0.02^{a}
α-Carotene	nd	nd	0.42±0.04^{a}	0.50±0.05^{a}
9-*cis*-β-Carotene	nd	nd	0.05±0.01^{a}	0.05±0.01^{a}
β-Carotene	nd	nd	0.54±0.09^{a}	0.61±0.05^{a}
13-*cis*-β-Carotene	nd	nd	0.11±0.02^{a}	0.12±0.01^{a}
Total	*0.24±0.03^{c}*	*0.21±0.05^{c}*	*1.54±1.8^{b}*	*1.8±0.14^{a}*
			(% RDA Vit. A =8.86)	*(% RDA Vit. A =10.41)*
Tocochromanols (mg/100 g dw)				
α-Tocopherol	nd	0.11±0.01^{c}	0.45±0.10^{a}	0.27±0.03^{b}
γ-Tocopherol	nd	nd	3.03±0.23^{a}	3.02±0.38^{a}
α-Tocotrienol	nd	nd	0.35±0.07^{a}	0.49±0.63^{a}
Total	*nd*	*0.11±0.01^{b}*	*3.83±0.40^{a}*	*3.78±0.79^{a}*
		(% RDA Vit. E= 0.76)	*(% RDA Vit. E =2.99)*	*(% RDA Vit. E =1.83)*
Phytosterols (mg/100 g dw)				
β-Sitosterol	5.2±0.1^{c}	5.1±0.2^{c}	111.7±6.5^{b}	121.5±2.2^{a}
Squalene (mg/100 g dw)	nd	nd	1.5±0.1^{a}	1.6±0.1^{a}

Values, expressed as mg/100 g dw, represent the mean ± standard deviation of three independent replicates (n = 3). Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple comparison procedures (Tukey post hoc test, $P < 0.05$), different letters denote significant differences among samples. nd = not detected.

Supplementary table 1. Profiles of soluble and polymeric bound phenolic acids of control (S-CTRL) and supplemented spaghetti. Pasta was supplemented with α-cyclodextrins (S-α-CD), SC-CO_2 extracted pumpkin oil (S-Oil) and with pumpkin oil/α-CD inclusion complexes (S-Oil/α-CD).

Pasta	Phenolic acids (μg/g fw)	
	Soluble	**Bound**
S-CTRL		
Vanillic acid	0.29±0.03	0.61±0.05
p-Coumaric	0.11±0.03	0.62±0.11
Ferulic acid	0.37±0.02	27.28±5.65
Sinapic acid	0.87±0.10	nd
Total	*1.64±0.18*[a]	*25.81±5.80*[a]
S-α-CD		
Vanillic acid	0.29±0.01	0.56±0.03
p-Coumaric	0.12±0.01	0.66±0.06
Ferulic acid	0.33 ±0.08	30.31±3.28
Sinapic acid	0.90±0.09	nd
Total	*1.65±0.18*[a]	*31.52±3.37*[a]
S-Oil		
Vanillic acid	0.33±0.01	0.55±0.02
p-Coumaric	0.13±0.01	0.55±0.01
Ferulic acid	0.36±0.02	24.60±0.43
Sinapic acid	0.97±0.04	nd
Total	*1.78±0.07*[a]	*25.70±0.45*[a]
S-Oil/α-CD		
Vanillic acid	0.33±0.01	0.57±0.02
p-Coumaric	0.15±0.03	0.62 ±0.05
Ferulic acid	0.50±0.5	29.02±2.23
Sinapic acid	1.06±0.12	nd
Total	*2.04±0.17*[a]	*30.22±2.30*[a]

Values are expressed as mg/100 g fresh weight (fw) and represent the mean ± standard deviation of three independent replicates (n = 3). Data were submitted to one-way analysis of variance (ANOVA), differences among groups were detected using multiple comparison procedures (Tukey post hoc test, $P < 0.05$). Different letters denote significant differences among samples. nd = not detected.

Highlights

- Supercritical CO_2-extracted pumpkin oil was rich in lipophilic bioactive compounds
- Pumpkin oil/α-cyclodextrin complex was a ready-to-mix powder apt to supplement pasta
- Supplemented pasta was mostly enriched in carotenoids, tocochromanols and squalene
- Cyclodextrin oil chlatration increased carotenoid stability during pasta cooking
- Supplemented pasta provided a substantial proportion of RDA for A and E vitamins