COMPOSITION, TEXTURE, SENSORIAL QUALITY, AND BIOLOGICAL ACTIVITY AFTER *IN VITRO* DIGESTION OF DURUM WHEAT PASTA ENRICHED WITH CARROT WASTE EXTRACT ENCAPSULATES

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Abstract

Aim of this research was to evaluate durum-wheat pasta enriched with encapsulated carrot waste extracts in oil, obtained by freeze drying (FDE) or spray drying techniques (SDE). Five pastas (control, enriched with 10% FDE, or 10% SDE, or 20% FDE, or 20% SDE) were evaluated for furosine, carotenoids, tocols, colour, *in vitro* bioactivities, cooking performance, texture and sensory quality. The encapsulates added to the enriched pastas α-carotene (0.58-1.24 mg/kg), β-carotene (1.43-3.29 mg/kg), *cis*-β-carotene (0.51-1.11 mg/kg) and total tocols (10.9-33.6 mg/kg). The carotenes were stable and the tocols diminished (-13%) during pasta manufacturing; both decreased (2-18% and 4-15%, respectively) during cooking, but they were still more abundant in the enriched pastas. Antioxidant, anti-hyperglycaemic, anti-inflammatory and anti-proliferative activities after *in vitro* digestion of cooked pastas improved, while sensory acceptability of control and 10% enriched pastas were similar. The encapsulates addition significantly improved the nutritional and technological qualities of durum-wheat pasta.

Key words: bioactivity; carotenoids; cooking; freeze drying; *in vitro* digestion; spray drying.

1. Introduction

In the food industry, innovative technologies are focused on creating novel foods and/or functional foods which, in addition to the basic nutritional values, contain ingredients that improve health and decrease the risk of diseases. A smart approach for designing new products is by enriching traditional foods with natural additives and flavours.

Many studies demonstrated that cereal products, for their high daily consumption and versatility, represent an excellent basis for fortification with natural bioactive compounds (Wang et al. 2021). Hence, several studies suggest a partial replacement of flour with ingredients that provide better nutritional and functional properties (Hidalgo et al. 2018). Worldwide, pasta is a basic food consumed by individuals of all age groups due to its pleasant sensory attributes, low cost, and ease of preparation. The focus on pasta products has progressively shifted toward the addition of ingredients like legumes, which improve the aminoacids profile of wheat products, as well as animal and vegetable oils or flours derived from fish, insects and algae (Laus et al. 2017; Durante et al. 2019; Wang et al. 2021), which enhance the content of protein, ω-3 polyunsaturated fatty acids, or antioxidants.

Agricultural and food by-products discarded during food processing are a major global concern. Recent research has focused on the utilization of plant by-products as potential sources of bioactive compounds. Carrot (*Daucus carota* L.) waste has attracted considerable attention because of the potential health benefits of its lipophilic bioactive compounds, mainly carotenoids and tocopherols (Šeregelj, Ćetković et al. 2021). As food additives, natural carotenoids are more appealing than synthetic colours due to legislative actions and consumer concerns; additionally, their provitamin A role and their antioxidant activity are clinically associated with several health benefits including inhibition of LDL oxidation, anti-inflammatory properties, alleviation of

oxidative stress and enhanced immune response (Šeregelj, Vulić et al. 2021). However, carotenoids are susceptible to oxidation and isomerization during processing or storage because of their non-polar structure and highly unsaturated molecules, resulting in loss of bioactive properties and sensorial attributes (Taksima et al. 2015). Encapsulation within edible materials is an effective approach for the protection against degradation of sensitive compounds (e.g. carotenoids, tocols, free phenolics), ensuring their stability and long shelf-life. Additionally, encapsulation may modify solubility, improve bioaccessibility and bioavailability, modify time and/or place of release of targeted compounds (Nedović et al. 2013). Freeze drying and spray drying are among the most suitable preservation methods for carotenoids; their main advantages over other encapsulation techniques are simplicity, continuity, effectiveness, availability, and applicability (Nedović et al. 2013). Freeze drying is ideal for the encapsulation of thermosensitive substances because it elicits minimal thermal degradation reactions. On the other hand, spray drying is very attractive for the food industry because it is cheap and flexible. The coating agents (a.k.a. wall materials) play a crucial role in the encapsulation process: different wall materials include polysaccharides (starches, maltodextrins, and gum Arabic), lipids (stearic acid, mono- and diglycerides) and proteins (gelatine, casein, milk serum, soy and wheat). Structure and characteristics of each coating agent impart different physicochemical properties to the encapsulate (Mahdavi et al. 2016).

The aim of the present study was the formulation and characterization of innovative pasta with better nutritional properties. To this end, durum wheat semolina was used to prepare five types of pasta, i.e. control pasta, and pasta enriched with either 10% or 20% encapsulated carrot waste extract obtained by either freeze drying or spray drying techniques. The different pastas were

evaluated for their chemical, antioxidant, bioactive, microbiological, colour, textural, and sensorial attributes.

2. Material and methods

2.1. Materials

A single lot of carrot waste was obtained from the "Nectar" beverage industry (Bačka Palanka, Serbia). The waste was immediately packed, freeze-dried and stored at -20 °C until use. The total carotenoid content in the carrot waste extract was previously reported in the study of Šeregelj et al. (2021). In brief, in the carrot waste extract the β-carotene was predominant (45.10 mg/kg), followed by α-carotene (13.97 mg/kg), and cis β-carotene (6.56 mg/kg). The sunflower oil used for carotenoids extraction, purchased from a local supermarket, was from the oil manufacturing company "Dijamant" (Zrenjanin, Serbia). The whey protein concentrate was purchased from Olimp Laboratories (Debica, Poland). The inulin was provided by Elephant Pharma (Belgrade, Serbia). The durum wheat semolina was acquired from Molino Pagani (Borghetto Lodigiano, Italy).

2.2. Carrot waste extraction and encapsulates preparation

Freeze-dried carrot waste was mixed with sunflower oil (1:10 w/v) at 25 °C by stirring with a B800E high-speed blender for 30 min (Gorenje, Velenje, Slovenia), using time shifts of 10 min blend and 5 min pause to avoid heating. The mix was then centrifuged at 4000 rpm for 10 min with a Lace 24 centrifuge (Colo Lab Experts, Novo Mesto, Slovenia), the supernatant was recovered and was stored at 4 °C in a dark glass bottle, wrapped in foil, for further use.

The carrot waste extract was encapsulated by two different techniques, i.e. freeze drying and spray drying, according to the best conditions determined in a previous study (Šeregelj, Ćetković et al. 2021). The optimal wall materials (100% whey protein for freeze drying; 71% whey protein and 29% inulin for spray drying) were prepared as follows: for freeze drying, the wall material was mixed with distilled water at 60 °C in a 1:2 (w/v) ratio and stirred until the temperature reached 30 °C, while for spray drying the wall material was mixed with distilled water in a 1:8 (w/v) ratio following the same procedure. The wall material-carrot extract in oil solutions (100 g/60 mL) were homogenized at 11000 rpm for 3 min at room temperature. The first formulation was kept overnight at -80 °C and then freeze-dried at -40 °C for 48 h with a Christ Alpha 2-4 LSC (Martin Christ, Germany); the freeze-dried encapsulates (FDE) were stored at -20 °C until further use. The second formulation was spray-dried using a mini B-290 (Büchi Labortechnik, Switzerland) at an inlet temperature of 130 °C and an outlet temperature of 65 ± 2 °C. The spraying air flow rate and rate of liquid feed were 600 L/h and 8 mL/min, respectively. The spray-dried encapsulates (SDE) were stored at -20 °C until further use.

2.3. Pasta manufacturing

The pasta was prepared in a small-scale industrial pilot plant (Mac30, Italpast, Parma, Italy) equipped with pre-kneading tank, kneading tank, vacuum-pressurized extrusion cylinder thermostated at 20 °C and die for short-pasta format (macaroni). The control pasta dough was prepared from 2.5 kg of durum wheat semolina and the water needed to reach 32% humidity; the carrot waste enriched pastas were prepared by substituting semolina with 10% or 20% FDE or SDE, and then adding the water needed to reach 32% humidity. The ingredients were mixed at 50 rpm for 2.5 min at room temperature, transferred to the extrusion tank, further kneaded under

126 vacuum at 30 rpm for 1.0 min and extruded at 80 atm pressure. The pasta was dried following an
127 18-hour diagram at 75% relative humidity and with a 60 °C maximum peak temperature.
128
129 *2.4. Pasta cooking*
130 To assess cooked pasta quality, 50 g of each macaroni type were boiled in 0.5 L deionised
131 boiling water. The pasta was prepared at the optimal cooking time (TOC), previously determined
132 according to the Method 66-50.01 (AACC International) by squashing a cut-open macaroni
133 between two thin glass plates at different cooking times: the pasta was considered cooked when
134 the white, opaque core (non-gelatinised starch) disappeared.
135
136 *2.5. Chemical analysis of ingredients, raw and cooked pasta*
137 *2.5.1. Moisture and protein*
138 Moisture content was determined according to Method 44-15.02 (AACC International); protein
139 content, expressed as g/100 g dry matter (DM), was measured by the Kjeldahl method
140 (conversion factor 6.38 for encapsulates and 5.75 for durum wheat semolina and pasta)
141 according to Official Methods 925.31 (AOAC, 2000).
142
143 *2.5.2. Furosine*
144 Furosine content, a heat damage index, was determined by HPLC as follows (Hidalgo et al.
145 2006): 400 mg of sample were hydrolysed with 8 mL of 8 N HCl under nitrogen at 110 °C for 23
146 h, purified by solid-phase extraction (SPE) with a C18 cartridge (Sep-pak, Millipore, Ballerica,
147 MA, USA) and injected in a HPLC apparatus consisting of two 510 HPLC pumps, a 680
148 automated gradient controller, and a 490 programmable multiwavelength detector (Millipore

149 Waters, Milford, MA). Operative conditions were as follows: a C8 furosine-dedicated column
150 (250 x 4.6 mm, Alltech Italia S.R.L., Milan, Italy); column temperature, 35 °C; detection
151 multiwavelength 280 nm; mobile phase (A) 0.4% acetic acid in water, (B) 0.3% potassium
152 chloride in solvent A; flow rate, 1.2 mL/min. The elution gradient, expressed as proportion of
153 eluent B, was: initial condition, 2% for 13.5 min; from 2 to 50% in 7 min, 50% for 1 min; from
154 50 to 2% in 1.5 min, 2% for 10 min.
155
156 *2.5.3. Carotenoids and tocols*
157 The carotenoid and tocol extracts were obtained from all the raw materials and pasta samples
158 after saponification (Hidalgo & Brandolini, 2010). Carotenoids were recovered in
159 methanol:dichloromethane (50:50 v/v), filtered through a 0.2 μm PTFE and quantified by
160 reverse-phase HPLC as described by Alfieri et al. (2014). The operating conditions were: column
161 Grace-Vydac 201TP54C18, 250 x 4.6 mm, 5 mm (Hesperia, CA, USA); precolumn Vydac
162 201TP54C18, 7.5 x 4.6 mm, 5 mm (Grace, Deerfield, IL, USA); mobile phase,
163 methanol:tetrahydrofuran stabilized with 0.1% butylated hydroxytoluene (95:5, v/v); flow rate, 1
164 mL/min; pump Waters 510 (Millipore, Milford, MA, USA). Carotenoids were detected at 445
165 nm, using a Waters 996 series photodiode array detector (Millipore, Milford, MA, USA),
166 controlled by the software Millenium 32 Cromatography Manager (Waters Chromatography
167 Division, Millipore, Milford). The wavelength range used was 200-600 nm. Peaks were
168 quantified at 445 nm using β-carotene as external standard. Tocols were dissolved in
169 hexane:isopropyl alcohol (99.0:1.0 v/v), filtered through a 0.2 μm PTFE and analysed by normal-
170 phase HPLC (Rodríguez et al. 2021). The following system and operating conditions were used:
171 Alltima SI column, 250 x 4.6 mm, 5 mm (Alltech Associates Inc., Deerfield, IL, USA); Alltima

SI guard column 7.5 x 4.6 mm, 5 mm (Alltech Associates Inc., Deerfield, IL, USA); mobile phase, hexane:ethyl acetate:acetic acid (97.3:1.8:0.9, v/v/v); flow rate, 1.6 mL/min; pump L-2130 Elite LaChrom (VWR, Hitachi, Japan); fluorimetric detector Jasco 821 FP Intelligent Spectrofluorometer (Japan) at excitation-emission wavelengths of 290 nm and 330 nm, respectively; connected to a computer with the software Empower 2 (Waters Chromatography Division, Millipore, Milford) through the Waters e-SAT/IN module. Peaks were quantified using α-tocopherol, β-tocopherol, and γ-tocopherol as external standards. The tocotrienols were quantified using the standard curves of their corresponding tocopherol. The results are reported as mg/kg on dry matter basis (DM). All these analyses were performed three times.

2.6. Antioxidant capacity of cooked pasta

Antioxidant and bioactive potential of cooked pasta samples was investigated after *in vitro* simulated gastrointestinal digestion, performed according to the procedure proposed by Minekus et al. (2014). Digestates were immediately frozen at -80 °C and freeze-dried. The antioxidant capacity was assessed following three different methods: β-carotene bleaching antioxidant capacity (BCB) assessed as outlined by Al-Saikhan et al. (1995), reducing power (RP) according to Oyaizu (1986), and superoxide anion (SOA) following Gironés-Vilaplana et al. (2012). The tests were performed on hexane extracts and the results were expressed as millimoles of Trolox equivalent (TE)/100 g of digested sample.

2.7. Bioactive potential of cooked pasta

The α-glucosidase inhibitory potential was used to assess the antihyperglycemic activity (AHgA), using the method reported by Tumbas Šaponjac et al. (2014). *In vitro* anti-

inflammatory activity (AIA) was determined by protein denaturation bioassay, using egg albumin, according to the method by Ullah et al. (2014).

Human cell lines MRC-5 (normal foetal lung fibroblasts) and HT-29 (colon adenocarcinoma) were used for the estimation of the antiproliferative effects of digested pasta samples at a 250-1500 mg/mL mass concentration, following the guidelines of the sulfohodamin B (SRB) assay (Skehan et al., 1990). Cell lines were grown in Dulbecco's modified Eagle medium (DMEM; PAA Laboratories GmbH, Pashing, Austria) with 4.5% glucose, supplemented with 10% heat-inactivated foetal calf serum (FCS; PAA Laboratories GmbH, Pashing, Austria), 100 IU/mL of penicillin and 100 µg/mL of streptomycin (Galenika, Belgrade, Serbia). The cell lines were grown attached to the surface, cultured in 25 cm^3 flasks (Corning, New York, USA) at 37 ºC in a high humidity atmosphere with 5% CO_2, and sub-cultured twice a week. Single cell suspension was obtained using 0.1% trypsin (Serva, Heidelberg, Germany) with 0.04 % EDTA. All these analyses were performed three times.

2.8. Microbiological assessment

To determine the microbiological profile, each pasta sample was tested by the following ISO methods: ISO 4833-1:2013 (aerobic mesophilic bacteria), ISO 21527-2:2008 (yeast and moulds), ISO 21528-2:2017 (*Enterobacteriaceae*), ISO/DIS 6888-1:2018 (*Staphylococcus* aureus) and ISO 6579-1:2017 (*Salmonella* spp.). All the analyses were performed three times.

2.9. Colour

The colour of dry and cooked pasta samples was measured using a Minolta Chromameter (Model CR-400, Minolta Co., Osaka, Japan) with a CR-A33b attachment. All the samples were

illuminated with D65-artificial daylight (10˚ standard angle). Three random readings were recorded on the levelled surface of ten macaroni. The results are expressed in the CIE LAB space as *L** (lightness; 0 = black, 100 = white), *a** (+a=redness, −a=greenness) and *b** (+b=yellowness, −b=blueness) values.

2.10. *Cooking performance*

Cooking loss, determined in double by heating the cooking water to dryness at 105 °C overnight, was expressed as percentage of dry pasta weight. Weight increase index was calculated as the ratio of the volumes of cooked and uncooked pasta samples. Water absorption was expressed as percent weight gain during cooking with respect to the weight of uncooked pasta.

2.11. Texture

Surface stickiness of cooked pasta was determined with a texture analyser (TA.XT Plus, Exponent Stable Micro System, Godalming, Surry, UK) using a compression-type probe, firmness-stickiness rig (HDP/PFS). After removing surface moisture with a paper towel, a single cooked sample was placed on a raised platform and retained with an aluminium plate. A rectangular aluminium probe, attached to a 30 kg load cell, compressed the sample (test speed 1.5 mm/s). Once good contact between probe and pasta was achieved (trigger force 20 g, holding time at maximum compression 3 s), the probe was withdrawn to measure pasta stickiness. Measurements were performed in triplicate.

2.12. Sensory evaluation

240 Sensory acceptance tests were performed in the laboratory by a semi-trained sensory panel (4
241 male, 11 female, 23 to 45 years old) that consisted of staff members from the Institute of Food
242 Technology, University of Novi Sad. All panellists were screened for sensory acuity of their
243 senses and have previous experience in hedonic testing of various pasta products. Although
244 lower number of participants may not be used to draw representative statistical conclusions,
245 however they can provide initial insights into observed variations in analysed sensory properties
246 of the pasta samples (Höglund et al., 2018). Pasta was cooked in distilled boiling water (100 g of
247 spaghetti in 1 L water) until optimal cooking time was reached. Cooked pasta was drained and
248 portion of approximately 40 g was served in plastic cups covered with lids in randomised
249 balanced order to each participant within 15 min after cooking. The samples were coded with
250 three digits random numbers. Low sodium water that was used for palate cleansing between
251 samples. Pasta samples were evaluated for colour, taste, flavour, texture and overall preference
252 using the 9-point hedonic scale (1 - extreme dislike, 5 - neither like nor dislike, 9 - extreme like)
253 as described by Lawless and Heymann (2010). Before testing, all participants were questioned
254 for possible food allergies and signed a written consent to participate in the study. The study was
255 approved by the Ethics Committee of Institute of Food Technology in Novi Sad, University of
256 Novi Sad, Serbia (Ref. No. 175/I/14-3).
257
258 *2.13. Statistical analysis*
259 One-way analyses of variance (ANOVA) were performed to assess the differences among
260 samples for all the traits analysed. When significant differences were detected, Fisher's least
261 significant differences (LSD) at $p \leq 0.05$ were computed. To evaluate the compounds degradation
262 during processing, a mass balance calculation was performed considering the relative content of

raw materials. To determine the effect of cooking, the compounds contents in dry and cooked pasta were compared. The data are presented as mean ± standard deviation (SD). All analyses were performed using the STATGRAPHICS® Centurion XVI v16.2.04 statistical program (Statpoint Technologies Inc., Warrenton VA, USA). The average values and standard deviations were computed using the Excel program (Microsoft® Office Excel 2007).

3. Results and discussion

3.1. Chemical composition of semolina and encapsulated carrot waste extracts

Table 1 reports the chemical composition of the durum wheat semolina and of the two encapsulated carrot waste extracts (FDE and SDE). The protein content of semolina was 11.51 g/100 g DM, in line with existing literature values (Brandolini et al. 2015). Significantly superior protein contents were observed in the carrot waste encapsulates, because their wall materials were made from whey protein (FDE) or a blend of whey protein and inulin (SDE). The furosine levels of semolina was in the interval of variation reported for different wheat flours (3.5 - 14.8 mg/100 g protein; Hidalgo and Brandolini 2011). The high furosine values of FDE and SDE were also due to the presence of whey protein and inulin, whose preparation included thermal treatments. The FDE encapsulates (obtained at low temperatures, i.e. -80 °C) had higher furosine levels than the SDE encapsulates (synthesized at high temperatures, i.e. 130 °C) because the former was encapsulated with whey proteins, while the latter included 71% whey proteins and 29% inulin. Hence, the composition was more important than the encapsulation conditions.

A high carotenoid content is a valued semolina quality trait. The main carotenoid in durum wheat semolina was the xanthophyll lutein, which represented 94.5% of the total, in agreement with Brandolini et al. (2015); another, scarcer, carotenoid was zeaxanthin (Table 1). On the other

hand, β-carotene was the most abundant carotenoid in carrot waste encapsulates, followed by α-carotene and *cis*-β-carotene. No significant differences for α- and β-carotene were observed between FDE and SDE, while *cis*-β-carotene was marginally higher in the SDE encapsulates, possibly for the different drying conditions.

The main tocol in semolina was β-tocotrienol (76.0% of total), followed by α-tocotrienol (12.1%), α-tocopherol (10.6%), and β-tocopherol (1.3%). A similar profile was reported by Laddomada et al. (2015); they also reported that the amount of β-tocotrienol varied significantly between cultivars, while the other compounds exhibited only limited variation. Conversely, carrot waste encapsulates were characterized by the prevalence of α-tocopherol (94.1% and 94.8% of total tocols for FDE and SDE, respectively), the most biologically active form of vitamin E, which is very abundant in the sunflower oil used for carrot waste extraction. The other homologues identified in the carrot waste encapsulates were β-tocopherol and γ-tocopherol. Da Silva et al. (2020) reported higher α-tocopherol content in sunflower oil enriched with β-carotene from carrots. Generally, tocopherol content depends on genotype and meteorological conditions, and is highly correlated to temperature.

3.2. Chemical characterization of raw and cooked pasta

The chemical characterization of raw and cooked pasta is reported in Table 2. The characteristics of the ingredients led to significant differences between the control pasta and the pasta enriched with FDE and SDE; on the other hand, no major differences were evidenced between pastas with the same FDE and SDE percentage additions.

The furosine concentration in the raw control was lower than the results reported by Brandolini et al. (2018) for pasta dried at low temperatures. In the pasta enriched with encapsulated carrot

waste extract, the furosine content increased with augmenting percentages of FDE and SDE. Overall, the SDE-enriched samples showed lower heat damage than those enriched with FDE. Nevertheless, even the furosine content of the 20% FDE encapsulate-enriched pasta were in the lower range of similar products dried at high temperatures (Marti et al. 2017).

The pastas enriched with 10% FDE/SDE had about 23% higher carotenoid contents than the control sample, while those enriched with 20% FDE/SDE increased the carotenoids contents about 63%. Lutein and zeaxanthin, originating from semolina, were detected in all the samples and decreased when the semolina was partially replaced by FDE or SDE. On the other hand, the encapsulates boosted the content of α-carotene, β-carotene and *cis*-β-carotene. The FDE-enriched pastas were slightly richer in α- and β-carotene than those with SDE. A mass balance showed that the carotenes from the encapsulates were very stable during the pasta-making process, while the xanthophylls from semolina suffered an average degradation of 27.1%. Cooking reduced carotenoids content in all cases, but the loss was inferior for the SDE-containing samples and the control; the carotenes from encapsulates were more susceptible to degradation than the xanthophylls from semolina (19.4% vs. 10.0%, respectively). In fact, carotenoid loss during traditional pasta-making happens mainly during the kneading phase (Hidalgo & Brandolini, 2010), while is modest during cooking (Brandolini et al. 2018). Overall the enriched pastas showed a significantly higher carotenoid content than the control even after cooking.

The addition of the encapsulated carrot waste extract significantly improved the tocol content of the pastas. In fact, those with 10% FDE or SDE had 32.6% and 38.6% higher tocol contents, respectively, than the control sample, while those enriched with 20% FDE or SDE increased them by 100.3% and 88.7%, respectively. The homologues with the greatest increase were α- and β-tocopherol, particularly in pasta enriched with 20% of FDE. Cooking led to minimal tocol

losses, particularly in the 10% encapsulate enriched pasta. In general, some carrot waste extract in oil remains non-encapsulated on the particle surface, hence the higher amounts of non-encapsulated carotenoids and tocols in pasta formulation led to greater reduction during the thermal process. The different homologues had diverse stability: α-tocopherol showed an average degradation of 13.4%, β-tocopherol of 28.4, α-tocotrienol of 11.9% and β-tocotrienol of only 2.6%. Hidalgo and Brandolini (2010) observed a 29.7% tocols degradation during the mixing/extrusion phase of low-temperature pasta making. Like for the carotenoids, the decrease was inferior in the SDE-added samples compared to the FDE-added ones. It should be remembered that the consumption of foods rich in tocopherols has been associated with delayed cellular ageing, reduced risks of cardiovascular diseases and regression of several cancers in cell culture (Aguirrezábal et al. 2015).

3.3. Bioactive properties of cooked control and enriched pasta

The antioxidant capacity, along with the antihyperglycemic, anti-inflammatory, and cytotoxic activities of the different pastas, are presented in Table 3.

The antioxidant capacity of the samples showed that pasta enrichment with encapsulated carrot waste extract in oil led to a significant increase in antioxidant capacity, and that the increase was proportional to the encapsulates concentration. Similarly, several authors found that the enrichment of pasta with lipophilic antioxidants such as carotenoids, tocopherols and tocotrienols led to an improvement of antioxidant properties (Laus et al. 2017; Durante et al. 2019).

The antihyperglycemic activity, recorded as potential to inhibit the α-glucosidase, increased with augmenting levels of carrot waste encapsulates in pasta. The highest inhibitory activity was observed in 20% FDE pasta (26.64%) and 20% SDE pasta (24.06%). Recent reports indicate that

many plants and their by-products have the potential to inhibit this enzyme. For example, Jo et al. (2011) described the efficacy of *Schisandra chinensis* berry pulp extract against α-glucosidase, while Cam et al. (2014) described a similar potential for pomegranate peel extracts. The most used drugs for managing inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), however these synthetic products are characterized by drawbacks like toxicity and gastric irritation, leading to the formation of gastric ulcers; furthermore, the symptoms often relapse after discontinuation (Vulić et al. 2019). Therefore, natural agents that potentially prevent the production of auto-antigens in some inflammatory and arthritic diseases is a crucial approach for anti-inflammatory drug development. Interestingly, the increase in anti-inflammatory activity followed the augment of encapsulate levels in the pasta insomuch that the samples with 20% FDE showed 26.6% inhibition, while those with 20% SDE expressed 20.0% inhibition. Cell growth activity represents possible growth stimulation (proliferation) that could be induced by the investigated active substance in tested food products. In these experiments, under conditions given in Material and methods section, Human cell lines MRC-5 (normal foetal lung fibroblasts) and HT-29 (colon adenocarcinoma) were seeded in order to estimate of the antiproliferative effects of digested pasta samples at a 250-1500 mg/mL mass concentration. Therefore, the cell growth activity of the 10% enriched pastas were not different from the control (IC_{50} >1250 μg/ml) and for both cell lines the highest activity was obtained by the 20% SDE pasta (IC_{50}=732.94 μg/ml and 605.61 μg/ml in in HT-29 and MRC-5 cell lines, respectively). The pasta with 20% encapsulated carrot waste extract showed a better activity to MRC-5 than to HT-29 cell line. The multi-endpoint bioassays in human cell lines used in this study, based on whole-cell response, are powerful indicators of metabolic, biochemical, and genetic alterations that arise under the influence of the compounds evaluated (Liu, 2004). Liu (2004) hypothesized that

the synergistic effects of phytochemicals are responsible for their potent antioxidant and anticancer activities of fruits and vegetables, and that the benefits of diets rich in those foods should be attributed to the complex mixture of compounds present.

3.4. Microbiological assessment of control and enriched pasta

The semolina represents a suitable environment for the growth of various microorganisms, which can origin from wheat itself (and in our situation, from the waste utilised for microencapsulation) or by contaminants during the production and manipulation processes. All the enriched pasta samples, as well as the control, were microbiologically safe to consume because the results (Table 4) agree with the microbiological limits established by the European legislation for foodstuffs (EU, 2005). In fact, the number of aerobic and mesophilic bacteria was always largely below the allowable threshold; additionally, both the *Staphylococcus aureus* and *Enterobacteriaceae* were below the detection limit, suggesting that the pasta production process was hygienically adequate. One of the most dangerous foodborne pathogens, *Salmonella* spp., was not detected. The very low presence of yeasts and moulds indicate that raw materials, water, encapsulates, and equipment were minimally contaminated and therefore were suitable for pasta production. No significant differences in microorganisms presence were observed between the control and the carotenoid-enriched pastas, implying that the addition of bioactive compounds did not foster changes in the microbiological profile of dry pasta products.

3.5. Colour of the control and enriched pasta

Table 5 shows the colour profile of raw and cooked pasta samples. The encapsulated carrot waste extract addition led to distinct differences, which persisted after cooking. In raw pasta, the

401 replacement of semolina flour with FDE and SDE caused a slightly increase of lightness *(L**),
402 and yellowness (*b**), while greenness (*a**) was reduced. The yellow colour of the control pasta
403 was due to the presence of carotenoids in semolina, while the red intensity increase was
404 ascribable to the carrot waste encapsulates, rich in β-carotene. The samples enriched with SDE
405 were characterized by a more intensive yellow colour.
406 The same effects were observed after cooking the pasta, because the addition of the encapsulates
407 in general increased all three colour parameters; the only exceptions were the *b** values of the
408 SDE-added pasta, which were lower or not different from those of the control pasta. Gull et al.
409 (2015) observed a yellowness increase in cooked pasta from millet flour and carrot pomace
410 powder and suggested that the change may be due to swelling and conversion of pigments during
411 cooking.
412
413 *3.6. Cooking performance, texture and sensory attributes of control and enriched pasta*
414 A change in the formulation of pasta, in particular the addition of protein, can significantly affect
415 its properties. Table 6 reports the cooking performance, textural and sensory properties of the
416 control pasta and of the pasta enriched with encapsulated carrot waste extract. For all
417 microencapsulate-enriched pasta the addition of carrot waste encapsulates led to an increase of
418 the optimal cooking time from 13 min (control) to 14 min (10% FDE and 10% SDE), and to 15
419 min (20% FDE and 20% SDE), while also decreasing cooking loss, weight increase index and
420 water absorption, probably due to the presence of whey proteins in the formulation. Additionally,
421 the FDE-enriched pasta has a smaller dry residue than the SDE-enriched one, likely for its higher
422 whey protein content. Cooking loss represents the solids (hydrosoluble molecules, such as starch
423 and microelements) lost into the cooking water and is one of the most important pasta quality

parameters, insomuch that a low cooking loss indicates good pasta quality. The quality of protein and the formation of a continuous protein network are essential for carbohydrates entrapment and for obtaining good cooking quality pasta. Our results agree with previous reports showing that pasta cooking loss is significantly decreased by the addition of protein-abundant encapsulates with whey protein carrier, as noticed for example by Duda et al. (2019) for their pasta with protein-rich cricket powder.

According to Dick and Youngs (1988), the weight of high-quality pasta increases threefold after cooking. In our experiments, the enriched pasta registered lower water absorption compared to the control, with a weight increase index ranging from 2.7 (10% SDE pasta) to 3.0 (control pasta, 10% and 20% FDE pasta). Desai et al. (2018) reported similar results and hypothesised that the lower water absorption and starch swelling were a consequence of protein powder competition for water. An additional explanation for our results is that inulin, a component of the SDE coating agent, is highly hydrophilic and preferentially absorbs water, thus inhibiting starch swelling and altering pasta structure (Tudorica et al. 2002).

Pasta cooking quality can be also evaluated by its stickiness. The addition of FDE or SDE led to a drastic decrease in this trait compared to the control sample. The protein network which entraps the starch granules may restrict water absorption, thus preventing starch leaching and decreasing stickiness (Lamacchia et al. 2007). Hence, the largely inferior stickiness recorded in the FDE and SDE enriched pastas may be attributed to the addition of the whey-protein-rich encapsulates, but also to the sunflower oil used in the extraction of bioactive compounds from carrot waste, which probably acted as a lubricant and favoured the separation of the aluminium probe from the cooked pasta.

Sensory evaluation showed that different percentages of encapsulate strongly modified the sensory attributes of the pasta. The panelists demonstrated a clear preference for the pasta with less carrot waste encapsulate. Therefore colour, texture, taste, flavour and overall acceptability of the 10% enriched pasta were comparable to those of the control, but the pasta with 20% FDE or SDE showed significantly lower scores for all these attributes, probably because of the high carrot waste extracted in oil delivered by the encapsulates.

4. Conclusions

Enrichment of durum wheat semolina pasta with encapsulated carrot waste extract in oil enabled the obtention of a final product with improved carotenoid and tocol contents. In durum wheat pasta, the encapsulates boosted the content of carotenes and tocopherols. Besides the increased stability of the bioactive compounds during pasta preparation, the enrichment also augmented optimal cooking time and decreased cooking loss, weight increase index, water absorption and pasta surface stickiness. Overall sensory acceptability of 10% enriched pasta was satisfactory and comparable to those of the control durum pasta. Considering a single portion (85 g pasta, cooked), the 10% FDE and 10% SDE provided 15% and 17% of vitamin A equivalent as well as 23% and 25% of vitamin E (α-tocopherol) Recommended Daily Allowance, respectively.

Disclosure of interests

The authors report no conflict of interest

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621 **Table 1.** Protein (g/100 g DM), furosine (mg/100 g protein), carotenoid and tocol (mg/kg DM)
622 content (mean±SD; n=3) of durum wheat semolina, freeze dried carrot waste encapsulate and
623 spray dried carrot waste encapsulate.

	Durum wheat semolina	FDE	SDE
Protein	11.51±0.27^{c}	47.53±0.81^{a}	35.08±0.32^{b}
Furosine	7.33±0.45^{c}	435.12±8.03^{a}	363.14±3.65^{b}
α-carotene	nd^{b}	5.49±0.18^{a}	5.45±0.17^{a}
β-carotene	nd^{b}	18.48±0.05^{a}	18.08±0.12^{a}
cis β-carotene	nd^{c}	5.01±0.13^{b}	5.84±0.13^{a}
Lutein	4.85±0.04^{a}	nd^{b}	nd^{b}
Zeaxanthin	0.28±0.01^{a}	nd^{b}	nd^{b}
Total carotenoids	5.13±0.04^{b}	28.97±0.36^{a}	29.37±0.42^{a}
α-tocopherol	3.54±0.02^{c}	229.65±0.27^{a}	216.24±5.91^{b}
β-tocopherol	0.44±0.11^{b}	10.06±0.60^{a}	8.99±0.52^{a}
γ-tocopherol	nd^{c}	4.27±0.24^{a}	2.89±0.30^{b}
α-tocotrienol	4.06±0.12^{a}	nd^{b}	nd^{b}
β-tocotrienol	25.43±0.44^{a}	nd^{b}	nd^{b}
Total tocols	33.47±0.47^{c}	243.98±0.56^{a}	228.13±5.09^{b}

624 FDE, freeze dried encapsulate; SDE, spray dried encapsulate. nd: not detected. Different letters
625 in the same row indicate significant differences among samples at $p \leq 0.05$.

626

627 **Table 2.** Protein (g/100 g DM), furosine (mg/100 g protein), carotenoid and tocol (mg/kg DM) content
628 (mean±SD; n=3), as well as percentage of loss during cooking, of raw and cooked control pasta and
629 pasta enriched with encapsulated carrot waste extract.

	Control	10% FDE	10% SDE	20% FDE	20% SDE
Raw pasta					
Furosine	75.62±8.54^{e}	292.09±12.01^{c}	216.57±22.49^{d}	421.71^{e}±16.18^{a}	383.08±4.54^{b}
α-carotene	nd^{e}	0.74±0.01^{c}	0.58±0.01^{d}	1.24±0.05^{a}	1.00±0.10^{b}
β-carotene	nd^{e}	1.71±0.05^{c}	1.43±0.003^{d}	3.29±0.25^{a}	2.67±0.31^{b}
cis β-carotene	nd^{c}	0.62±0.05^{b}	0.51±0.04^{b}	1.11±0.01^{a}	1.05±0.12^{a}
Lutein	3.61±0.41	3.17±0.05	3.38±0.21	2.78±0.14	2.92±0.04
Zeaxanthin	0.29±0.10	0.14±0.03	0.19±0.02	0.12±0.01	0.14±0.03
Total carotenoids	3.90±0.31^{d}	6.39±0.03^{c}	6.09±0.24^{c}	8.53±0.45^{a}	7.78±0.45^{b}
α-tocopherol	3.91±0.26^{e}	18.55±0.57^{d}	20.13±0.51^{c}	40.50±0.20^{a}	36.89±1.00^{b}
β-tocopherol	2.54±0.42^{b}	4.50±0.06^{a}	3.17±0.30^{b}	4.97±0.79^{a}	4.87±0.40^{a}
γ-tocopherol	nd	nd	nd	nd	nd
α-tocotrienol	3.67±0.32^{a}	2.38±0.13^{bc}	2.80±0.01^{b}	2.38±0.10^{bc}	2.23±0.07^{c}
β-tocotrienol	23.44±1.46^{a}	19.07±0.07^{b}	20.43±0.47^{b}	19.37±0.43^{b}	19.34±0.38^{b}
Total tocols	33.56±1.81^{d}	44.50±0.69^{c}	46.53±0.68^{c}	67.22±1.12^{a}	63.34±1.04^{b}
Cooked pasta					
α-carotene	nd^{c}	0.50±0.01^{b}	0.56±0.05^{b}	0.86±0.06^{a}	0.80±0.13^{a}
β-carotene	nd^{c}	1.17±0.003^{b}	1.37±0.20^{b}	2.28±0.30^{a}	2.13±0.16^{a}
cis β-carotene	nd^{c}	0.52±0.03^{b}	0.46±0.02^{b}	1.04±0.10^{a}	0.94±0.08^{a}
Lutein	3.63±0.22^{a}	3.08±0.07^{abc}	3.41±0.33^{ab}	2.66±0.20^{c}	2.85±0.19^{bc}
Zeaxanthin	0.18±0.01	0.13±0.02	0.16±0.01	0.12±0.02	0.12±0.01
Total carotenoids	3.81±0.23^{d}	5.41±0.08^{c}	5.95±0.61^{b}	6.95±0.68^{a}	6.85±0.59^{a}
α-tocopherol	2.92±0.09^{c}	16.91±1.88^{b}	19.31±0.07^{b}	31.79±1.28^{a}	32.99±2.34^{a}
β-tocopherol	2.67±1.55	2.63±0.17	2.44±0.05	4.00±0.74	2.59±0.61
γ-tocopherol	nd	nd	nd	nd	nd
α-tocotrienol	2.97±0.11^{a}	2.25±0.27^{b}	2.72±0.08^{a}	1.91±0.04^{b}	2.02±0.24^{b}
β-tocotrienol	21.41±0.97^{a}	19.40±0.34^{bc}	20.25±0.02^{ab}	19.28±0.27^{bc}	18.03±0.47^{c}
Total tocols	29.97±0.38^{c}	41.18±1.97^{b}	44.71±0.22^{b}	56.97±1.71^{a}	55.63±2.43^{a}
Cooking loss					
Carotenoid (%)	2.3	15.6	1.6	17.6	12.8

Tocol (%)	10.7	7.4	3.9	15.2	12.2

630 FDE, freeze dried encapsulate; SDE, spray dried encapsulate. nd: not detected. Different letters in
631 the same raw indicate significant differences among samples in the same row at $p \leq 0.05$.

632

Table 3. Bioactive potential (mean±SD; n=3) of raw and cooked control pasta and pasta enriched with encapsulated carrot waste extract.

	Control	10% FDE	10% SDE	20% FDE	20% SDE
			Antioxidant activity (μmol TE/100g)		
BCB	9.81±0.30^{e}	19.90±0.71^{c}	17.75±0.30^{d}	26.39±0.16^{a}	24.88±0.12^{b}
RP	4.83±0.00^{e}	6.29±0.43^{c}	5.94±0.22^{d}	8.65±0.00^{a}	7.16±0.03^{b}
SOA	25.06±1.21^{c}	35.56±1.92^{b}	36.63±1.12^{b}	43.76±2.23^{a}	41.24±1.52^{a}
			Antihyperglycemic activity (%)		
AHgA	16.20±0.61^{d}	20.06±0.16^{c}	17.46±1.09^{d}	26.64±0.62^{a}	24.06±0.01^{b}
			Antinflammatory activity (%)		
AIA	10.16±1.014^{d}	19.85±0.20^{b}	13.79±1.16^{c}	25.45±0.22^{a}	20.00±0.31^{b}
			Antiproliferative activity (IC50; μg/ml)		
HT-29	>1250^{a}	>1250^{a}	>1250^{a}	1075.71±64.76^{b}	732.94±14.14^{c}
MRC-5	>1250^{a}	>1250^{a}	>1250^{a}	716.22±15.73^{b}	605.61±52.23^{c}

FDE: freeze dried encapsulate; SDE: spray dried encapsulate; BCP: β-carotene bleaching; RP: reducing power; SOA: superoxide anion. Different letters in the same row indicate significant differences among samples at $p \leq 0.05$.

Table 4. Microbiological quality (log CFU/g; mean±SD; n=3) of control pasta and pasta enriched with encapsulated carrot waste extract.

Microbiological parameters	Control	10% FDE	10% SDE	20% FDE	20% SDE
Aerobic and sporogenic bacteria	1.20 ± 0.21	1.20 ± 0.11	1.30 ± 0.00	1.00 ± 0.31	1.40 ± 0.70
Yeasts and moulds	< 1	< 1	< 1	< 1	< 1
Enterobacteriaceae	< 1	< 1	< 1	< 1	< 1
Staphylococcus aureus	< 1	< 1	< 1	< 1	< 1
Salmonella sp.	nd*	nd	nd	nd	nd

* nd – not detected

Table 5. Values of the CIELab colour parameters *L**, *a** and *b** (mean±SD; n=3) of raw and cooked control pasta and pasta enriched with encapsulated carrot waste extract.

Sample	Raw pasta			Cooked pasta		
	*L**	*a**	*b**	*L**	*a**	*b**
Control	74.86±1.54^{b}	-4.85±0.14^{c}	24.06±2.10^{c}	56.97±0.82^{c}	-1.26±0.35^{d}	36.88±1.36^{c}
10% FDE	76.79±1.27^{a}	-3.46±0.20^{b}	24.79±1.66^{c}	58.42±0.84^{bc}	0.44±0.25^{c}	39.50±0.90^{b}
10% SDE	75.31±1.31^{ab}	-3.82±0.25^{b}	30.32±2.54^{a}	65.21±1.39^{a}	0.11±0.21^{c}	34.46±1.35^{d}
20% FDE	76.80±1.54^{a}	-0.99±0.41^{a}	27.81±1.21^{b}	59.5±2.46^{b}	2.63±0.37^{a}	44.95±2.28^{a}
20% SDE	76.81±1.49^{a}	-1.34±0.63^{a}	30.75±1.73^{a}	67.20±1.86^{a}	1.73±0.50^{b}	36.84±1.51^{c}

FDE: freeze dried encapsulate; SDE: spray dried encapsulate. Different letters in the same column indicate significant differences among samples at $p \leq 0.05$.

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Table 6. Cooking performance, texture properties and sensory attributes (mean ± SD; n=3) of control pasta and pasta enriched with different concentrations (10 % or 20%) of encapsulated carrot waste extract.

	Control	10% FDE	10% SDE	20% FDE	20% SDE
		Cooking performance			
Optimal cooking time (min)	13	14	14	15	15
Cooking loss (%)	4.62±0.00^{a}	3.85±0.10^{c}	4.41±0.00^{a}	3.54±0.01^{d}	4.21± 0.21^{b}
Weight increase index	3.04±0.10^{a}	3.04±0.10^{a}	2.74±0.11^{b}	3.03±0.10^{a}	2.93± 0.00^{b}
Water absorption (%)	144.12±1.10^{a}	136.92±1.61^{b}	126.94±1.52^{c}	137.34±1.23^{b}	128.62± 1.31^{c}
		Texture properties			
Stickiness (g)	48.61±9.02^{a}	4.21±0.38^{b}	3.93±0.31^{b}	2.54±0.25^{c}	2.33± 0.40^{c}
		Sensory attributes			
Colour	7. 14±0.69^{ab}	7.71±0.95^{a}	3.86±1.57^{cd}	5.29±2.43^{bc}	2.86± 1.07^{d}
Texture	7.43±1.13^{a}	6.57±1.40^{a}	6.29±1.38^{a}	3.29±0.49^{b}	3.43± 0.98^{b}
Taste	6.71±0.76^{a}	7.14±0.69^{a}	7.00±0.82^{a}	2.43±1.40^{b}	2.14± 1.07^{b}
Flavour	6.57±1.13^{a}	6.86±1.07^{a}	6.86±1.35^{a}	2.29±1.50^{b}	1.86± 1.07^{b}
Overall acceptability	6.86±1.35^{a}	7.43±0.79^{a}	6.29±1.60^{a}	2.86±1.58^{b}	1.86± 0.90^{b}

FDE: freeze dried encapsulate; SDE: spray dried encapsulate. Different letters in the same row indicate significant differences among samples at $p \leq 0.05$.