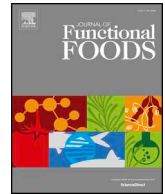




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Inulin enriched durum wheat spaghetti: Effect of polymerization degree on technological and nutritional characteristics

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ABSTRACT

The flours coming from four ancient Sicilian durum wheat cultivars were used to produce spaghetti enriched with 4% inulin at two degree of polymerization (DP): cardoon roots (CRI, high DP) and chicory (CHI, low DP). The influence of DP on quality, sensory properties, glycaemic index (GI) of enriched spaghetti was investigated; further the release of inulin after simulated *in vitro* digestion was also assessed. The DP affected most of the sensorial attributes of pasta as overall quality score (OQS), cooking loss and optimal cooking time. Among the cultivars, Timilia and Margherito maintained the OQS scores after inulin addition. Both the presence and the different DP of inulin did not influence the GI values, except a decrease recorded for Timilia spaghetti. Finally, in all the studied cultivars, DP significantly affected the inulin release in the digestive tract highlighting the highest amount of CRI inulin in solid fraction important for potential prebiotic effect.

1. Introduction

Food fortification with dietary fibres, micronutrients, antioxidants, vitamins or minerals is an important challenge to improve food quality and ensure health benefits in human disease (Radovanovic et al., 2015). Dietary fibre, as defined by American Association of Cereal Chemists (AACC, 2000), represents the edible part of plants or analogous carbohydrates that play a key role in human health for their special features: resistance to digestion and absorption in the human small intestine with complete or partial fermentation by the microflora in the colon, and the production and absorption of fermentation end products like short chain fatty acid and lactate (Dhingra, Michael, Rajput, & Patil, 2012; Roberfroid, 2005; Shoab et al., 2016; Stephen et al., 2017). The development of dietary fibre-enriched foods permits to obtain products with functional properties (Bustos, Pérez, & León, 2011). Among the dietary fibres widely used in a variety of foods, inulin represents a noteworthy ingredient; it is a fructose polymers belonging to a class of highly water-soluble fibres. Its chemical structure consists by a linear or branched fructose polymers with one or more β -linked fructose. Sometimes, the last fructose can be linked with glucose, using α -(1–2) bond. The presence of these bonds, together with the lack of

enzymes deputed to the fructans hydrolysis, avoid the digestion and absorption of inulin in the human small intestine as a dietary carbohydrate (D'Antuono, Di Gioia, Linsalata, Roskopf, & Cardinali, 2018). Some inulin sources, mainly used in the food industry are chicory and Jerusalem artichoke (Foschia, Peressini, Sensidoni, Brennan, & Brennan, 2015). The inulin from chicory is mainly composed by a combination of fructose oligomers and polymers, with units ranging from 2 to 60 (Shoab et al., 2016). Among the high molecular weight inulin (200 fructose units) is important to mention inulin from artichoke (external fructans, roots, and flowers) and from heads and roots of wild cardoon (Hellwege, Czapl, Jahnke, Willmitzer, & Heyer, 2000; Raccuia, Melilli, & Tringali, 2004). Compared to digestible carbohydrates, inulin gives only 25–35% energy and is widely used as sugar replacer in the processed foods (Shoab et al., 2016). Moreover, inulin for its “neutral taste” does not add off-flavor or aftertaste and can be used as a substitute for butter or margarine in bakery products, dairy products, frozen foods, and condiments (D'Antuono et al., 2018). The mixture water inulin produces a gelatinous solution with a creamy texture able to substitute up to 100% fats into foods (Franck, 2002). The interest of inulin as functional foods is increasing for its potential beneficial effect on human health mainly related to the degree of polymerization (DP)

Abbreviations: DP, Degree of polymerization; CRI, cardoon roots inulin; CHI, chicory inulin; CAP, Senatore Cappelli; MAR, Margherito; RUS, Russello; TIM, Timilia; OQS, overall quality score; OCT, optimal cooking time; DG, aqueous small intestinal digesta; PT, solid fraction pellet; GI, glycaemic index

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and branches. Inulin belongs to the group of FODMAP (Fermentable Oligo-, Di-, Monosaccharides and Polyols), carbohydrates able to counteract constipation improving consequent illness, because its colonic digestion draws water into the last part of intestine. It also promotes the growth of microflora in digestive tract and is considered as an appropriate ingredient to prepare low caloric foods for diabetics and to manage blood sugar levels (Shoib et al., 2016). The promoting health effect of inulin could be combined with low glycaemic value foods to obtain functional foods. Pasta, for its compact structure characterised by very close protein network entrapping starch granules and protecting them from the hydrolytic activity of digestive enzyme, is defined as low or medium glycaemic index food. Therefore, pasta digestion induces a gradual rise in blood glucose levels and consequent slow inulin release, with potential benefits for both healthy or diabetic consumers (Augustin, Franceschi, Jenkins, Kendall, & La Vecchia, 2002; Brennan & Tudorica, 2008; Foschia et al., 2015). In this contest, the promoting health effect of inulin could be combined with the low glycaemic value of pasta to obtain a functional food, because the interactions starch-inulin could further slow the starch digestion and thus lower the glycaemic response. Thus, is needed to set up correctly the amount and the type of functional ingredient (inulin) to maintain a food quality and improve nutritional value (Rakhesh, Fellows, & Sissons, 2015). Several studies have demonstrated that the inulin fortification can modify negatively the structure of pasta compromising the acceptability by the consumers and the nutritional aspects (Bustos et al., 2011; Padalino et al., 2017). Other authors (Rakhesh et al., 2015), shown that inulin influenced the starch-protein matrix, allowing more rapid access of the cooking water, gelatinizing the starch without affecting its swelling. Padalino et al., 2017 have studied the effect on quality and chemical composition of whole-meal durum wheat spaghetti enriched with inulin at two different DP, and from two different vegetable sources. The authors shown that the sensory characteristics of pasta were affected by inulin molecular weight and its interactions with gluten matrix during pasta formation were significantly modified by polymerization degree. Sicily is one of the few areas of Southern Europe where it is still possible to find landraces of cereals and durum wheat. The changes in the UE agricultural policy, the introduction of low input agricultural systems, and the greater consumer desire for food quality have reawakened interest in local typical food productions (Gallo et al., 2010). Durum wheat landraces have been recognized to have different contents in health-promoting phytochemicals (Lo Bianco, Siracusa, Dattilo, Venora, & Ruberto, 2017) and vitamins (Bognanni, Gallo, Di Stefano, & Melilli, 2020).

In this study the effects of the addition of inulin at different DP on the quality, sensory properties and glycaemic index of four wholemeal spaghetti produces by ancient Sicilian cultivars of durum wheat was evaluated. Moreover, in order to assess how the DP could influence the permanence of inulin in small intestine, an *in vitro* gastrointestinal digestion was also performed.

2. Materials and methods

2.1. Raw material

Inulin was extracted and characterized from roots of *Cynara cardunculus* L. var. *atilis* DC, line CDL, cropped in the experimental field of Assoro (EN. 37°30'54" N; 14°16'26" E, 279 m. a.s.l.) located in the internal hilly area of Sicily during the year 2015. The harvest time was chosen on the basis of inulin metabolism (synthesis and breakdown of the polymer) in cardoon. In previous studies it was demonstrated plants maximize yields in long DP inulin before flowering, after that inulin breakdown follows to supply energy for heads development and achenes ripening (Melilli & Raccuia, 2013; Raccuia & Melilli, 2010). In the laboratory of CNR-ISAFOM located in Catania was performed the extraction, purification and characterization of inulin in order to calculate the DP and for spaghetti preparation. The moisture content of a

representative sample of roots, was measured after drying the plant material to a constant weight in a thermo-ventilated drying oven at 105 °C. Fresh roots (consisting of both primary and secondary roots) was washed in cold tap water, scraped and ground to a fine powder. The processes of extraction and purification are described in Padalino et al. (2017). Briefly 100 g of the original homogenate was diluted with water and put in a boiling water bath for 30 min. After cooling to room temperature, the extract was filtered and centrifuged at 3000g for 5 min. The inulin extracted was precipitated at 0 °C overnight. The supernatants were removed and inulin was washed with distilled water and precipitated at 0 °C overnight. The washing process was repeated until inulin was white. After the 5th cycle of washing, solutions of inulin were injected in HPAEC PAD to follow the purity. When the baseline of chromatograms appeared clear (threshold accepted under 10 nC) and the inulin colour, determined by colorimeter Minolta CR 400, had L* values upper than 85, inulin has been accepted for purification. Further, inulin was lyophilized in petri dishes and used for pasta production. The moisture content in lyophilized inulin was determined in a thermo-ventilated oven at 105 °C, resulting less than 0.5 g/100 g of fresh weight. Inulin characterization and quantification was performed following the method of Padalino et al. (2017). The maximum degree of polymerization recorded was, in this plant phenological phase, 80 fructose Units, with a mean DP of 50 fructose units. The data confirmed than what reported for cardoon in previous works (Melilli et al., 2020; Raccuia & Melilli, 2010).

Commercial inulin that is manufactured by ORAFTI (Tienen, Belgium) as Raftilose® Synergy 1 is an oligofructose (DP < 10) enriched inulin. This is a 1/1 mixture of long chain and short chain fractions of inulin extracted from chicory roots (*Cichorium intybus*). Inulin is made by a set of linear chains of fructose molecules, with a DP ranging between 3 and 65. It can be fractionated into a slowly fermentable long-chain fraction (DP ranging from 10 to 65, average 25) or in a rapidly fermentable fraction made of oligofructose (DP ranging from 3 to 8, average 4). Synergy1 is a mixture of both fractions, and has a higher amount of long chains relative to the native product.

2.2. Spaghetti preparation

Spaghetti was produced with 4 wholemeal durum wheat flour: cv Senatore Cappelli (CAP), cv Margherito (MAR), cv Russello (RUS) and cv Timilia (TIM). Chemical composition of the wholemeal flours is reported in Table 1.

For each cv, pasta without inulin was used as control (CTRL), by using the operating conditions described in Padalino et al. (2017). Wholemeal flour was mixed with water adding 4% (w/w) of the different types of inulin: inulin extracted from cardoon roots (CRI) and commercial inulin from chicory (CHI) (Orafti®). In order to ensure the solubility of the inulin powder, they were previously dissolved in water. The dough was extruded into spaghetti shape (30 cm in length × 1.70 mm).

2.3. Color

Color was evaluated in spaghetti before and after cooking. Pasta colour data were collected with the use of a Chroma Meter (Minolta CR

Table 1
Chemical composition (g/100 g) of durum wheat flours used.

	CAP	MAR	RUS	TIM
Fats	2.10	2.50	0.70	1.80
Carbohydrates	66.3	67.3	71.2	68.0
Proteins	12.7	12.6	11.7	12.0
Fiber	7.74	6.11	6.00	5.50

Cultivars: Senatore Cappelli (CAP), Margherito (MAR), Russello (RUS), Timilia (TIM).

– 400, Milan, Italy) as previously described by Melilli, Tringali, and Raccuia (2016). The colorimeter was calibrated using the manufacturer's standard white plate ($L^* = 96.55$; $a^* = -0.35$; $b^* = -0.16$).

2.4. Sensory analysis

Dry spaghetti samples were submitted to a panel of fifteen trained tasters (six men and nine women, aged between 28 and 45) in order to evaluate the sensory attributes. The panelists were selected on the basis of their sensory skills (ability to accurately determine and communicate the sensory attributes such as appearance, odor, taste and texture of a product). The panelists were also trained in sensory vocabulary and identification of particular attributes by evaluating durum wheat commercial spaghetti (ISO 11036, 7304). They were asked to indicate color and resistance to break of uncooked spaghetti. Elasticity, firmness, bulkiness, adhesiveness, fibrous nature, color, odor and taste were evaluated for cooked spaghetti (Padalino et al., 2017). To this aim, a nine-point scale, where 1 corresponded to *extremely unpleasant*, 9 to *extremely pleasant* and 5 to the *threshold acceptability*, was used to quantify each attribute (Petitot, Boyer, Minier, & Micard, 2010). On the basis of the above-mentioned attributes, panelists were also asked to score the overall quality (OQS) of the product using the same scale.

2.5. Spaghetti cooking quality

The optimal cooking time (OCT), the cooking loss and the amount of solid substance lost into the cooking water were evaluated according to the AACC-approved method 66-50 (2000). The swelling index of cooked pasta was determined according to the procedure described by Cleary and Brennan (2006). The swelling index was expressed as following:

$$\frac{(\text{weight of cooked spaghetti}) - (\text{weight of spaghetti after drying})}{(\text{weight of spaghetti after drying})}$$

The water absorption of drained pasta was also determined as following:

$$\frac{(\text{weight of cooked spaghetti}) - (\text{weight of raw pasta})}{(\text{weight of raw pasta})}$$

Three measurements were performed for each analysis, and the mean values were calculated.

2.6. Cooking losses

On 1 mL of residual water of the cooking test, the amounts of inulin eventually released by pasta was determined in HPAEC-PAD, using the same method described for inulin extracted from roots. Only the content of fructose was considered for the calculation, because of the glucose released by starch during acid hydrolysis could interfere with the free glucose of the long inulin chain.

2.7. In vitro glycaemic index of inulin enriched spaghetti

In vitro glycaemic index estimation of enriched spaghetti was performed following the method described by Tazart, Lamacchia, Zaidic, and Harosa (2016). Briefly, 0.1 g of cooked spaghetti were firstly digested with pepsin (Sigma P7000; 0.4 g/g of cooked spaghetti) in HCl-KCl buffer (pH 1.5) for 1 hr. Then, α -amylase (Sigma A3176; 14 mg/g of cooked spaghetti) solution in Tris-Maleate buffer (pH 6.9) was added. The samples were incubated for 3 h and aliquots of 1 mL each at 0, 20, 40, 60, 90, 120 and 180 min were taken and incubated at 100 °C for 5 min to inactivate the enzyme. After centrifugation (10,000g at 4 °C), 0.5 mL of each supernatant was incubated with amyloglucosidase (Sigma 10115; 5 mg/g of cooked spaghetti) in acetate buffer (pH

4.75) for 45 min. After treatment with digestive enzymes, the glucose released was quantified spectrophotometrically (Varian Inc. Cary 50) at 510 nm with a commercially available enzymatic kit (D-Glucose Assay Procedure, K-GLUC 02/18, Megazyme) based on glucose oxidase/peroxidase enzymatic system. The predicted glycaemic index (GI) was calculated using the empirical formula described by Yaman, Sargin, and Mızrak (2019):

$$GI = 39.71 + 0.549HI$$

where HI is the hydrolysis index obtained from the ratio between the area under the hydrolysis curve of the test sample and the area obtained for white bread used as reference.

2.8. In vitro digestion process of inulin enriched spaghetti

To evaluate the potential effect of polymerization degree on inulin release of enriched spaghetti in small intestine during digestion process, the three stages of *in vitro* digestion model was performed following the D'Antuono et al. (2016). This procedure mimics the physiological three steps of digestive process (oral, gastric and small intestine). All the samples previously described (CTRL, CHI, CRI) were cooked at their OCT and 6 g of cooked spaghetti were subjected to *in vitro* digestion process. After oral, gastric and small intestinal phases, samples were centrifuged at 4500 rpm for 10 min for obtaining the aqueous small intestinal digesta (DG) and solid fraction pellet (PT). Each experiment was carried out in triplicate.

2.9. Inulin extraction and quantification

The analysis of free sugars (glucose, fructose and sucrose) and inulin quantification as total fructose after acid hydrolysis were carried out on cooked spaghetti before digestion (T0), and on two fractions of digested spaghetti, DG and PT.

Concerning T0, 10 g of dry spaghetti cut into pieces of 8 cm were boiled in 100 mL of water for the established OCT, dried on filter paper and shredded. Then, 5 g of cooked spaghetti were extracted in 50 mL of water at 100 °C for 30 min and subsequently centrifuged at 3000g. A fraction (1 mL) of obtained supernatant was diluted to 10 mL, filtered at 0.45 μm and analysed in HPLC for the free sugars quantification. Simultaneously, other 10 mL were subjected to acid hydrolysis with 100 μL of 3 N HCl for 2 h at 70 °C, then the sample was cooled, filtered at 0.45 μm , diluted fifty times and analysed in HPLC for inulin quantification as total fructose. The PT instead was extracted in 50 mL of water for 30 min at 100 °C, centrifuged at 3000g, and the supernatant was analysed following the same procedure before and after acid hydrolysis.

Further, DG samples were analysed without extraction for the free sugar quantification, and for inulin determination an aliquot of 10 mL was hydrolysed following the procedure above described.

The amount of fructose from inulin was calculated as follow:

$$F_i = F_t - F_f - F_s$$

where: F_i = fructose from inulin, F_t = total fructose after hydrolysis, F_f = fructose free and F_s = fructose from sucrose before hydrolysis. Inulin content (I) was calculated considering the correction for the glucose moiety of inulin and for the loss during hydrolysis, according to the procedure described by Steegmans, Iliens, & Hoebregs, 2004.

$$I = 0.995 F_i$$

The carbohydrate analysis was carried out with HPLC Dionex DX500 system equipped with GP50 gradient pump, ED40 Electrochemical Detector in Pulsed Amperometric Detection (PAD) and Dionex Peaknet 5.11 chromatographic Software. The chromatographic separation of sugars was obtained by a Dionex CarboPac PA1 column, and CarboPac PA1 guard column in isocratic mode with elution of 150 mM NaOH at flow rate of 1 mL/min.

2.10. Statistical analysis

Data were submitted to the Bartlett's test for the homogeneity of variance and then analysed using analysis of variance (ANOVA). Means were statistically separated on the basis of Duncan's test, when the 'F' test of ANOVA for treatment was significant at least at the 0.05 probability (CoHort Software, CoStat version 6.451). For *in vitro* digestion, data were expressed as mean \pm SD of 3 replicates of each sample for each cultivar used for spaghetti preparation. The statistical differences among the four cultivars were evaluated with One Way Analysis of Variance (ANOVA) followed by All Pairwise Multiple Comparison Procedures (Holm-Sidak method). The statistical differences within the same cultivars between CHI and CRI spaghetti for *in vitro* digestion and glycaemic index were evaluated by *t*-test. All the statistical analysis were performed by SigmaPlot 12.0 (SigmaPlot™ Exact Graphs and Data Analysis, Systat Software, San Jose, CA, USA)

3. Results and discussion

3.1. Color, sensory attributes and cooking quality

The use of inulin fibre in pasta, as well as the cultivar of durum wheat used, could strongly influence the organoleptic characteristic of this product affecting the consumer acceptability. The color indices were mainly influenced by cultivars both in uncooked and cooked spaghetti. In particular, for uncooked spaghetti, the percentage of total variation resulted 70% (L*), 99% (a*) and 94% (b*) (Table 2). The type of inulin did not influence the yellow index (b*); after cooking process, the type of inulin used affected the b* index with a total variation of 35.6%, while L* and a* resulted influenced mainly by cultivar of wholemeal flours (Table 2).

For all the cultivars, averaged, for type of inulin, the cooking process increased the L* value, and decrease the yellow index. RUS maintained the best yellow index after inulin addition (Table 3).

The sensory attributes of cooked spaghetti samples were determined and showed in Table 4. Averaged for used cultivars, the CTRL had an OQS of 6.47, the addition of CHI inulin decreased the score at 5.84, while statistically differences have not been recorded between CTRL and CRI (6.09).

The ANOVA (Table 2) showed that elasticity was mainly influenced by inulin (I) and interaction of Cvs \times I. Long DP inulin improved this attribute. The inulin addition was mainly reflected on bulkiness and

adhesiveness and OQS sensations. In particular, out of cultivar, all the scores resulted higher than the threshold of acceptability, and the best results were obtained using CRI. On average of cultivars, inulin addition improved or maintained elasticity, firmness, bulkiness, adhesiveness, color, odor and the OQS scores. Within the same cvs, RUS with both CHI and CRI addition and CAP with CHI addition showed a decline of OQS, respect to its relative CTRL (Table 4), confirming the previous data obtained by Padalino et al. (2017). The smallest OQS was due mainly to an increase in the adhesiveness and bulkiness of the samples (lower scores). TIM and MAR maintained the OQS scores after inulin addition. Finally, there were no effects of inulin addition on odor and taste scores. Also Aravind, Sissons, Fellows, Blazek, and Gilbert (2012) reported that the odor and taste score were not changed upon addition of inulin with respect to the CTRL sample (100% durum wheat).

The effects of both types of inulin on spaghetti technological properties are shown in Table 5. The cooking loss in spaghetti samples were mainly related to the used cultivars (79% of total variation Table 2). Averaged for inulin types, CAP attained the highest loss (7.13%), due mainly to the addition of CHI (7.54%). CAP enriched with CRI had similar value of cooking loss of its CTRL. These results could be also attributed to the higher fiber content (7.74 g/100 g) of the flours vs. the others cvs (about 6 g/100 g). Data listed in Table 5 also highlight a rise of the cooking loss in spaghetti samples added with CHI inulin, as compared to CTRL sample. In fact, CHI samples showed the highest cooking loss values within the same cultivar. It is possible that some inulin at low DP could be leaching from the pasta during cooking, which would contribute to the cooking loss, as also reported by Roberfroid (2005) and Foschia et al. (2015).

Interaction Cvs \times I was observed for swelling index (Table 2). Statistical differences have not been recorded among type of inulin, but we can note that a decrease was observed in all the Sicilian cultivars of durum wheat after inulin addition (Table 5). Data reported by Aravind et al. (2012) showed that samples fortified with high molecular weight inulin (high polymerization degree) recorded no changes in swelling index, pasta water absorption, cooking loss and firmness at amount \leq 10% with respect control sample, probably due to the high fibre content of wholemeal flour and polymer addition.

In general, the OCT decreased with the wholemeal flour substitution with inulin, and it can be attributed to gluten dilution. Concerning the water absorption and swelling index, there were no changes in these parameters for samples enriched with caroon inulin (Table 5). Specifically, CHI recorded a significant decrease in swelling index and water

Table 2
Sum of square (SS) and percentage of variation (%) of spaghetti samples quality traits in relation to the studied factors.

Trait	Cvs			Inulin Type (I)			Cvs \times I		
	SS	%	P	SS	%	P	SS	%	P
L* Uncooked	244.0	70.3	***	96.9	27.9	***	6.0	1.7	***
a* uncooked	214.4	98.9	***	1.8	0.8	ns	0.5	0.2	ns
b* uncooked	558.5	94.4	***	3.9	0.7	ns	29.1	4.9	ns
L* cooked	82.5	86.8	***	7.1	7.5	*	5.4	5.7	ns
a* cooked	104.6	99.2	***	0.6	0.6	ns	0.2	0.2	***
b* cooked	16.4	61.4	ns	9.5	35.6	***	0.8	3.0	ns
Elasticity	0.29	8.41	ns	0.56	16.23	**	2.60	75.36	***
Firmness	4.12	63.19	***	0.60	9.20	**	1.80	27.61	*
Fibrous	11.40	16.03	***	11.60	16.32	***	48.10	67.65	***
Bulkiness	1.60	22.44	***	3.60	50.49	***	1.93	27.07	**
Adhesiveness	2.03	36.45	***	2.96	53.14	***	0.58	10.41	ns
Color	1.98	59.10	**	0.67	20.00	*	0.70	20.90	ns
Odor	34.00	31.78	***	15.00	14.02	***	58.00	54.21	***
Taste			ns			ns			ns
OQS	0.13	4.21	ns	1.00	31.93	**	2.00	63.86	**
Cooking Loss (%)	11.80	79.73	***	1.90	12.84	***	1.10	7.43	*
Swelling Index	0.14	46.67	***			ns	0.16	53.33	**
Water Absorption	617	38.95	**	492	31.06	**	475	29.99	ns
OCT	7.46	22.34	***	20.80	62.29	***	5.13	15.36	**

Table 3
Color indices detected on uncooked and cooked samples.

Pasta	MAR			RUS			CAP			TIM		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
<i>Uncooked spaghetti</i>												
CTRL	48.0	5.6	25.8	53.4	3.6	15.7	52.2	3.3	15.7	42.4	8.1	24.0
CHI	48.9	4.9	29.7	52.6	4.9	15.9	44.7	5.8	26.5	45.3	7.3	26.5
CRI	43.6	6.4	25.8	43.4	7.1	24.4	40.2	6.3	20.4	43.1	6.6	23.3
Means	46.8	5.6	27.1	49.8	5.2	18.7	45.7	5.1	20.9	43.6	7.3	24.6
<i>Cooked spaghetti</i>												
CTRL	57.0	0.2	16.7	61.2	0.6	18.2	57.5	-0.2	15.7	55.5	1.5	16.5
CHI	59.9	0.0	17.5	58.4	1.4	18.0	58.1	0.7	16.5	55.6	1.7	17.0
CRI	58.8	1.2	17.6	55.9	1.0	16.2	52.3	1.0	13.4	56.1	1.3	14.8
Means	58.6	0.5	17.3	58.5	1.0	17.5	56.0	0.5	15.2	55.7	1.5	16.1

absorption as compared to the CTRL sample, probably due to the competitive activity of inulin with the starch for water during pasta formation. The cooking quality results could be related to the amounts of fructose detected on water after the cooking process (Table 6). The CTRLs had similar fructose amounts. CHI inulin was less retained than CRI in all samples. Among cultivars, CAP followed by RUS gave the best results in terms of fructose loss, especially with CRI inulin. MAR and TIM, traditionally used mainly for bread production, due to their gluten matrix, release higher inulin amounts.

3.2. Predicted glycaemic index

Although the quantification of glycaemic index (GI) in foods using *in vivo* methods was standardized by the Food and Agriculture Organization/World Health Organization (FAO/WHO. Food and Agriculture Organization/World Health Organization, Food products determination of the Glycemic Index (GI) and relevant classification, International Organization for Standardization, 2008 (GI) and relevant classification, International Organization for Standardization, 2008, 2008), *in vitro* assays are more advantageous alternative for the low costs and the less time required. The *in vitro* methods are based on the comparison between the carbohydrate digestibility of the sample with the control (whitebread) over time. The GI of the samples is calculated using the hydrolysis index (HI) value in the formula proposed by Goñi, Garcia-Alonso, and Saura-Calixto (1997) and results are well correlated

Table 5

Cooking quality of spaghetti. Different letters in each column indicate statistical differences at $P < 0.05$ (Duncan's test).

Cvs	Inulin	Cooking Loss (%)	Swelling Index	Water Absorption	OCT (min)
MAR	CTRL	5.95	1.72	140.00	10.50
	CHI	6.08	1.66	135.00	8.50
	CRI	5.65	1.68	142.00	8.50
RUS	CTRL	6.56	1.88	149.00	11.00
	CHI	7.16	1.71	137.00	9.00
	CRI	6.25	1.77	139.00	8.50
CAP	CTRL	6.98	1.80	139.99	9.50
	CHI	7.54	1.70	134.00	8.00
	CRI	6.87	1.72	134.00	8.00
TIM	CTRL	5.32	1.72	134.00	10.00
	CHI	5.89	1.65	130.00	8.00
	CRI	5.74	1.58	128.00	8.00
Mean Cvs					
	CTRL	6.20b	1.78 a	142.7 a	10.25 a
	CHI	6.67 a	1.68 a	134.0b	8.38b
	CRI	6.13b	1.69 a	135.7b	8.25b
Mean Inulin types					
	MAR	5.89c	1.69b	139.0 a	9.00 a
	RUS	6.66b	1.79 a	141.7 a	9.50 a
	CAP	7.13 a	1.74 a	136.0b	8.50b
	TIM	5.65c	1.65b	130.7c	8.00b

Table 4

Sensorial attributes of all cooked spaghetti. Different letters in each column indicate statistical differences at $P < 0.05$ (Duncan's test).

Cvs	Inulin	Elasticity	Firmness	Fibrous	Bulkiness	Adhesiveness	Color	Odor	Taste	OQS
MAR	CTRL	5.68	5.60	4.50	6.60	6.70	7.20	7.70	7.65	6.26
	CHI	5.48	5.16	4.82	5.95	5.75	7.05	7.71	7.48	6.06
	CRI	6.08	5.73	4.83	6.68	6.50	7.04	7.27	7.38	6.32
RUS	CTRL	6.00	6.75	5.75	7.20	6.00	7.50	8.00	7.25	7.00
	CHI	5.64	6.23	4.75	5.59	5.51	6.94	7.66	7.09	5.64
	CRI	6.05	6.11	4.79	5.91	5.69	7.03	7.60	7.14	5.79
CAP	CTRL	6.34	6.38	6.67	6.31	6.05	7.08	7.32	7.32	6.38
	CHI	5.90	6.20	0.00	5.62	5.30	7.20	7.40	7.25	5.60
	CRI	5.75	6.30	6.28	5.25	5.28	7.20	7.45	7.50	6.20
TIM	CTRL	5.65	5.98	6.02	6.34	6.06	7.17	7.50	7.24	6.25
	CHI	6.08	5.93	5.75	6.02	5.56	6.94	7.67	7.09	6.04
	CRI	6.05	5.41	4.69	6.04	5.69	7.03	7.60	7.14	6.05
Mean Cvs										
	CTRL	5.92b	6.18 a	5.73 a	6.61 a	6.20 a	7.24 a	7.63 a	7.37 a	6.47 a
	CHI	5.78b	5.88b	3.83b	5.80b	5.53b	7.03b	7.61 a	7.23 a	5.84b
	CRI	5.98 a	5.89b	5.15c	5.97b	5.79b	7.08b	7.48 a	7.29 a	6.09 a
Mean Inulin types										
	MAR	5.75a	5.50c	4.72c	6.41 a	6.32 a	7.10 a	7.56 a	7.50 a	6.21 a
	RUS	5.90a	6.36 a	5.10b	6.23 a	5.73bc	7.16 a	7.75 a	7.16 a	6.14 a
	CAP	6.00a	6.29 a	4.32 d	5.73b	5.54c	7.16 a	7.39 a	7.36 a	6.06 a
	TIM	5.93a	5.77b	5.49 a	6.13 a	5.77bc	7.05 a	7.59 a	7.16 a	6.11 a

Table 6
Fructose amounts (mg L^{-1}) in boiling water after cooking.

Cvs		Total fructose	Fructose loss
MAR	CTRL	28	
	CHI	72	44
	CRI	73	45
RUS	CTRL	27	
	CHI	53	26
	CRI	45	18
CAP	CTRL	27	
	CHI	45	18
	CRI	29	2
TIM	CTRL	23	
	CHI	66	43
	CRI	62	39

with the *in vivo* studies (Yaman et al., 2019).

The GI values for CTRL and enriched spaghetti with 4% of inulin at different DP are presented in Table 7. The GI values of spaghetti CTRL ranged from 63 to 75 in relation to the cultivar of whole-meal durum wheat used for pasta preparation. Concerning the effect of inulin addition, both the presence and the different DP of inulin did not affect the GI values of cooked spaghetti, with the exception of TIM. CRI inulin at high DP in TIM spaghetti significantly decreased the GI value respect to control pasta with a percentage of decrease of 6.6%. Same effect was recovered for TIM spaghetti enriched with CHI inulin, although with a lower percentage of decrease from spaghetti CTRL (1.5%, Table 7). Our results are partially in agreement with data reported by Brennan and Tudorica (2008). The authors have shown that pasta enriched with different percentage of chicory inulin (from 2.5% to 10%) did not reduce the predicted GI respect the white bread used as control. Moreover, pasta enriched with 10% inulin induced a decrease only in HI values. In fact, the authors analyzing the microstructure of inulin enriched pasta by SEM micrographs, highlighted that cooked pasta containing inulin, appeared to have similar internal structures to the pasta CTRL not related to the percentage of enrichment (2.5% or 10%). Probably the gluten and fibers complex surrounded starch granules, which were less swollen than in bread, and presenting a well maintained shape due to limited water availability (Brennan & Tudorica, 2008).

3.3. Inulin quantification in cooked spaghetti

Concerning the inulin quantification after acid hydrolysis in

Table 7

In vitro glycaemic index (GI) and percentage of decrease of GI of enriched spaghetti versus control spaghetti.

		GI	% Decrease GI vs CTRL
MAR	CTRL	72.7 ± 0.6	/
	CHI	72.9 ± 1.3	/
	CRI	74.2 ± 0.6	/
RUS	CTRL	72.1 ± 0.8	/
	CHI	71.4 ± 1.8	/
	CRI	69.6 ± 0.9	/
CAP	CTRL	65.8 ± 1.6	/
	CHI	66.8 ± 0.9	/
	CRI	64.8 ± 0.6	/
TIM	CTRL	76.2 ± 1.8	/
	CHI	75.0 ± 1.2*	1.5
	CRI	71.1 ± 0.5*	6.6

CTRL: control spaghetti; CHI enriched spaghetti with 4% inulin from chicory roots; CRI: enriched spaghetti with 4% inulin from cardoon roots.

Data are expressed as mean ± SD of 3 replicates of each sample for each whole meal durum wheat cultivar used for spaghetti preparation.

* Indicate statistical differences ($P < 0.05$) versus control within cultivar evaluated by *t*-test.

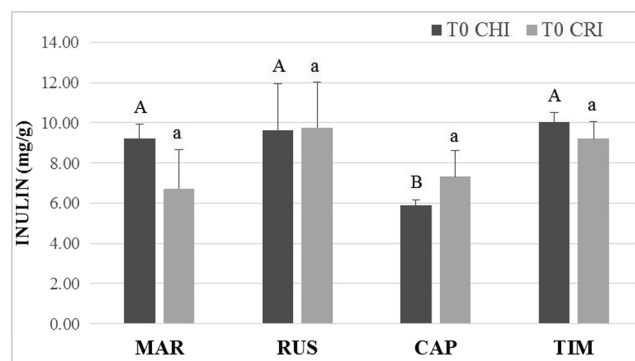


Fig. 1. Inulin quantification in enriched cooked spaghetti before *in vitro* digestion process. CHI enriched spaghetti with 4% inulin from chicory roots; CRI: enriched spaghetti with 4% inulin from cardoon roots. Data are expressed as mean ± SD of 3 replicates of each sample for each whole meal durum wheat cultivar used for spaghetti preparation. Different uppercase letters indicate statistical differences ($p < 0.05$) among CHI spaghetti of different cultivars. Different lowercase letters indicate statistical differences ($p < 0.05$) among CRI spaghetti of different cultivars.

fortified cooked spaghetti before *in vitro* digestion for CHI samples, the amount of inulin ranged from 5.88 (CAP) to 9.61 (RUS) mg/g (Fig. 1), and only CAP was significantly lower respect the other cultivars ($p < 0.05$). For the samples enriched with CRI, the levels of inulin were similar among the four cultivars and ranged from 6.73 (MAR) to 9.76 (RUS) mg/g. Instead, the DP did not affected the inulin amount in the cultivar considered. In fact, no differences in inulin levels between CHI and CRI spaghetti for each cultivar used, were found (Fig. 1). Our results on enrichment of spaghetti with inulin at different DP are in agreement with the results obtained by Aravind et al. (2012), that shown that an amount of inulin could be release from the pasta during cooking regardless of the DP.

3.4. In vitro digestion process of inulin enriched spaghetti

The results of *in vitro* digestion process of inulin-enriched spaghetti are shown in Fig. 2. The inulin quantification was performed on aqueous (DG) and solid fraction (PT) of digested spaghetti without inulin (CTRL) and on spaghetti fortified with 4% of CHI and 4% CRI inulins, respectively. The results have shown that there is an effect of DP on inulin release during digestion process regardless the cultivar of wholemeal durum wheat used. The release of CHI inulin (low DP) in aqueous fraction was higher than CRI inulin (high DP) in all the cultivars studied, although the differences were not statistically significant except for MAR. These results confirmed what was already described for the inulin loss during cooking process underlining that physicochemical properties of inulin are linked to the DP (Foschia et al., 2015; Roberfroid, 2005). On the contrary, the amount of CRI inulin was statistically higher ($p < 0.05$) than the amount of CHI inulin in the solid fraction of digested spaghetti for all the considered cultivars, thus making a greater amount of this soluble fibre available for fermentation by the intestinal microflora. Probably, the β -configuration of inulin makes it resistant to the hydrolytic activity of the enzymes of the human upper digestive tract. In fact, almost 90% of the inulin passes to the colon where can be selectively fermented by the beneficial bacteria of the large intestine and producing short-chain fatty acids (like acetate, propionate, and butyrate), lactate, bacterial fuel and gases (Morreale, Benavent-Gila, & Rosella, 2019; Shoab et al., 2016). This feature makes inulin able to be classified as prebiotic dietary fibre, although there is no a specific defined dose for applying a health claim (Morreale et al., 2019).

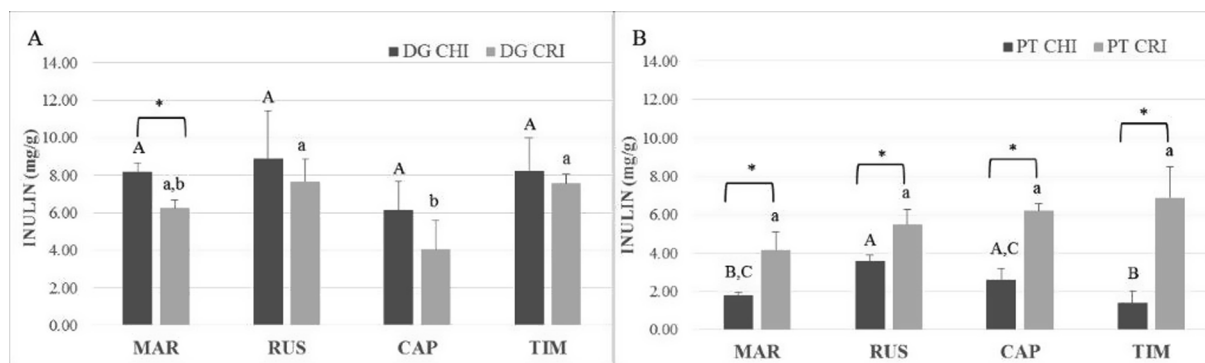


Fig. 2. Inulin quantification in enriched cooked spaghetti after *in vitro* digestion process. CHI enriched spaghetti with 4% inulin from chicory roots; CRI: enriched spaghetti with 4% inulin from cardoon roots. A: inulin amounts in aqueous fraction (DG); B: inulin amounts in solid fraction (PT). Data are expressed as mean \pm SD of 3 replicates of each sample for each whole meal durum wheat cultivar used for spaghetti preparation. Different uppercase letters indicate statistical differences ($p < 0.05$) among CHI spaghetti of different cultivars. Different lowercase letters indicate statistical differences ($p < 0.05$) among CRI spaghetti of different cultivars. * Indicate statistical differences ($P < 0.05$) between CHI and CRI spaghetti within the same cultivar evaluated by t-test.

4. Conclusion

Four ancient Sicilian durum wheat cultivars, RUS, CAP, TIM, MAR, were used to produce inulin enriched spaghetti. In particular, 4% of inulin at two different DP were used, the cardoon roots inulin (CRI high DP) and the commercial inulin from chicory (CHI, low DP). The inulin enrichment permits to obtain spaghetti characterized by OQS value within the acceptability threshold (> 5) and higher for CRI respect CHI spaghetti. Among cultivars, TIM and MAR maintained the OQS scores after inulin addition. The cooking loss in spaghetti samples were mainly related to the used cultivars with an increase into spaghetti enriched with CHI inulin, as compared to CTRL sample. Noteworthy to underline that CAP and RUS gave the best results in terms of cooking quality also as fructose release, especially with CRI inulin. Instead MAR and TIM, traditionally used for bread production and due to their gluten matrix, release higher inulin amounts. Both the presence and the different DP of inulin did not have an effect on GI values of cooked spaghetti, with the exception of TIM. TIM spaghetti enriched with CRI inulin showed a significantly reduction of GI value respect to control pasta. Same effect was recovered for TIM spaghetti enriched with CHI inulin, although with a lower effect compared to CTRL. Moreover, in all the studied cultivars, the DP significantly affected the inulin release in the digestive tract highlighting a higher amount of CRI inulin than that CHI inulin, in solid fraction of digested spaghetti. The here showed results highlighted that the high polymerization degree inulin, not modifying the technological and sensorial properties of spaghetti, could allow their consume as functional food. In fact, in order to demonstrate the potential functionality as prebiotic of inulin enriched spaghetti, further studies will evaluate the influence of the polymerization degree on the viability and the metabolic activity of selected bacteria by *in vitro* gut microbiota model.

5. Ethics statement

The research did not include any human subjects and animal experiments

CRediT authorship contribution statement

Antonella Garbetta: Investigation, Data curation, Writing - original draft. **Isabella D'Antuono:** Investigation, Data curation, Writing - original draft. **Maria Grazia Melilli:** Conceptualization, Methodology, Writing - original draft. **Carla Sillitti:** Investigation. **Vito Linsalata:** Investigation. **Salvatore Scandurra:** Investigation. **Angela Cardinali:** Conceptualization, Data curation, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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