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Manufacture and characterization of gluten-free spaghetti enriched with vegetable flour

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ABSTRACT

The manufacture and characterization of gluten-free spaghetti based on maize flour and different vegetable flours (artichoke, asparagus, pumpkin, zucchini, tomato, yellow pepper, red pepper, green pepper, carrot, broccoli, spinach, eggplant and fennel) were addressed in this study. The screening of the vegetable flours showed that homogeneity, color, fibrous, taste and odor were the parameters that have most influenced the overall quality of the dry spaghetti. The spaghetti added with yellow pepper flour was chosen for further analysis because of its highest sensory quality; in contrast, it recorded low carotenoids content due to the high temperature of the drying process (cycle named as HTDC). Therefore, an optimization of the drying cycle was performed (lower temperature) on the yellow pepper flour (cycle named as LTDC) that resulted in an increase of the carotenoids content. Although the spaghetti with low temperature yellow pepper flour had a higher cooking loss and lower instrumental hardness when compared to the spaghetti made with only maize flour (CTRL) it however had a significantly higher protein and dietary fiber content. Moreover there was no significant difference in the amount of glucose released during in *vitro* digestion for this spaghetti sample with respect to the CTRL sample.

1. Introduction

Celiac disease is a permanent intolerance to gluten. Celiac disease relates specifically to the composition of the storage proteins present in many common cereals such as wheat, rye, barley and oat, which are harmful for the sensitive consumers (Hill et al., 2005). Unfortunately, the gluten-forming proteins are fundamental for the production of a great variety of foods, including pasta, which is generally made from durum wheat. Durum wheat proteins are characterized by a typical viscoelastic behavior that allows good networking of the matrix and optimal dough formation during the mixing and extrusion phases, and are also mostly responsible for the quality attributes of cooked pasta (Feillet and Dexter, 1996). The increased prevalence of celiac disease has lead to an increased demand for gluten-free products, thus products not containing wheat, rye, barley or spelt wheat. Maize is recommended as a safe food for celiac patients since it possesses no gluten and can be used in the production of pasta (Schoenlechner et al., 2010). The rising need for these convenience products from gluten-free cereals (maize, millet, sorghum) is a big challenge for food research and development, because the network forming ability of gluten needs to be substituted by other means, in order to achieve products with satisfying quality (Schoenlechner et al., 2010). However, concern has been raised over the long term dietary habits and food choices of celiac patients on a strict gluten-free diet, as results from a number of studies indicate an unbalanced intake of carbohydrates, protein, and fat, as well as limited intake of certain essential nutrients in celiac subjects compared to a control group of people on a normal diet (Thompson et al., 2005). Therefore, the addition of functional ingredients could improve the nutritional quality of gluten-free foods. Recent studies have focused on the production of spaghetti gluten-free based on maize flour and oat bran enriched with β-glucan (Padalino et al., 2011). In particular, the incorporation of vegetables and fruits into gluten-free extruded products that deliver physiologically active components could create a major opportunity for food processors to provide (for the celiac consumer) healthy dietary fiber-enriched products, which are currently lacking in the gluten-free market (Stojceska et al., 2009).

Foods of plant origin are good sources of functional substances and major chemical components: artichoke (polyphenolic compounds), carrot and pumpkin (dietary fiber and carotenoids), broccoli and cauliflower (minerals, vitamin, dietary fiber, thiazolidine, sulforaphane), spinach and zucchini (iron and folic acid), eggplants (dietary fiber and minerals), fennel (phytoestrogens), asparagus (minerals, vitamins, amino acids and dietary fiber, flavonoids, carotenoids, oligosaccharides and rutin) (Holmes and

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Kemble, 2009). Yellow pepper as tomato, is rich in vitamin C, which increases the body's resistance to external agents, helps fight free radicals to prevent cell degeneration and hence beneficial to the aging process and also has a key role in the process of cell renewal (Nirmal et al., 2010). Therefore they may be considered as valuable ingredients for industrial manufacture of functional foods that have been associated with low incidence and mortality rates of cancer and heart diseases in addition to a number of other health benefits (Velioglu et al., 1998).

This study focused on the manufacture and characterization of gluten-free spaghetti based on maize flour and different types of vegetable flours. To this aim, the study was organized in two subsequent steps: in the first experimental step different vegetable flours (artichoke, asparagus, pumpkin, zucchini, tomato, yellow pepper, red pepper, green pepper, carrot, broccoli, spinach, eggplant and fennel) were added to maize flour. In the second experimental step the spaghetti added with yellow pepper flour was chosen because of its highest sensory quality. Moreover, the yellow pepper flour was produced with two different drying processes at high and low temperature (HTDC and LTDC). Subsequently, the effect of the LTDC yellow pepper flour on the cooking and texture properties of the spaghetti samples along with chemical and rheological analysis were examined.

2. Material and methods

2.1. Raw material

The heat-treated maize flour was bought from Agostini mill (Montefiore dell'Aso, Ascoli Piceno, Italy), whereas all the vegetables flours were purchased from Farris farm (Troia, Foggia, Italy). The monoglycerides added to the pasta formulation was bought from Natural World (Lugo, Ravenna, Italy).

The investigated vegetable flours such as artichoke, asparagus, pumpkin, zucchini, tomato, yellow pepper, red pepper, green pepper, carrot, broccoli, spinach, eggplant and fennel were dried at high temperature (HTDC). Only the yellow pepper flour was dried at low temperature (LTDC) and used for further analysis (Table 1a). The particle size of the vegetable flours were <500 μm and the fibre content was also evaluated and listed in Table 1b.

2.2. Flour drying process

The commercial drying cycle (HTDC) of the vegetable flours was made in two-steps. In the first step the fresh vegetable was dried at a temperature of 110-68 °C for 70 min; in the second step the vegetable was dried at a temperature of 75-55 °C for 220 min. Furthermore only the yellow pepper flour was also dried with a drying cycle at constant temperature of 65 °C for 460 min (LTDC) in order to reduce the thermal damage of the high temperature. The optimization of the drying cycle was firstly performed on a laboratory scale; thereafter the information was transferred to the Farris farm (Troia, Foggia, Italy) that proceeded to industrial scale production.

Table 1b
Fiber content of the investigated vegetable flours.

Vegetable flour	SDF (%w/w)	IDF (%w/w)	TDF (%w/w)
Yellow pepper flour LTDC	8.72 ± 0.17^{g}	13.35 ± 0.43^{a}	22.07 ± 0.26^{d}
Yellow pepper flour HTDC	9.21 ± 0.12^{i}	9.88 ± 0.05^{b}	19.08 ± 0.06^{b}
Green pepper flour	10.13 ± 0.04^{l}	28.16 ± 0.08^{c}	38.29 ± 0.11^{a}
Red pepper flour	8.49 ± 0.03^{f}	29.95 ± 0.21^{d}	38.44 ± 0.18^{a}
Tomato flour	6.91 ± 0.01^{d}	14.01 ± 0.02^{e}	20.92 ± 0.01^{c}
Spinach flour	8.02 ± 0.06^{e}	34.50 ± 0.05^{f}	42.52 ± 0.11^{g}
Pumpkin flour	6.14 ± 0.01^{a}	42.94 ± 0.30^{g}	49.08 ± 0.31^{i}
Zucchini flour	6.18 ± 0.02^{a}	21.05 ± 0.17^{h}	27.23 ± 0.18^{e}
Carrot flour	8.91 ± 0.00^{h}	27.08 ± 0.03^{i}	35.99 ± 0.02^{f}
Asparagus flour	5.94 ± 0.03^{b}	46.51 ± 0.09^{l}	52.46 ± 0.09^{l}
Fennel flour	6.35 ± 0.09^{c}	32.15 ± 0.11^{m}	38.50 ± 0.01^{a}
Eggplant flour	10.33 ± 0.05^{m}	34.89 ± 0.27^{n}	45.22 ± 0.32^{h}

[a–n] Mean in the same column followed by different superscript letters differ significantly ($p < 0.05$).

2.3. Spaghetti preparation

To prepare the non-conventional dough a portion of maize flour was first pregelatinized. This was done using a steam cooker (LT50 2E Namad, Rome, Italy), where 10 L of water was mixed with 10% (w/w) of flour and heated to 80 °C for about 1 h. Subsequently, in order to prepare non-conventional pasta the pregelatinized flour was cooled to 40 °C and then the remaining maize flour (74% w/w), 1% (w/w) monoglycerides and 15% vegetable flours were added to the mixture and kneaded for 20 min.

The dough made from only maize flour (100%) was also manufactured and used as a reference (CTRL). The formulations of the investigated spaghetti samples are listed in Table 1a. The spaghetti samples were manufactured by means of a pilot plant equipped with an extruder (60VR, Namad, Rome, Italy), for the production of fresh-extruded spaghetti, and a dryer (SG600, Namad, Rome, Italy), for the production of the dry spaghetti. The extruder was equipped with a screw (30 cm in length, 5.5 cm in diameter) that ended with a bronze die (diameter hole of 1.70 mm). The screw speed was 50 rpm. The extrusion pressure was approximately 3.4 bar, whereas the temperature of the spaghetti after the extrusion was about 27–28 °C.

With regards to the drying, the process conditions applied were the following: 1 step, time 20 min at 55 °C; 2 step, time 580 min at

Table 1a
Spaghetti samples formulation.

Sample	Water (L)	Maize pregelatinized flour (% w/w)	Maize flour (% w/w)	Vegetable flour (% w/w)	Monoglycerides (% w/w)	Vegetable flour drying cycle
CTRL	10	10	89	–	1	–
P_LTYP	10	10	74	15	1	LTDC
P_HTYP	10	10	74	15	1	HTDC
Green pepper	10	10	74	15	1	HTDC
Red pepper	10	10	74	15	1	HTDC
Tomato	10	10	74	15	1	HTDC
Spinach	10	10	74	15	1	HTDC
Pumpkin	10	10	74	15	1	HTDC
Zucchini	10	10	74	15	1	HTDC
Carrot	10	10	74	15	1	HTDC
Asparagus	10	10	74	15	1	HTDC
Fennel	10	10	74	15	1	HTDC
Eggplant	10	10	74	15	1	HTDC

75 °C; 3 step, time 40 min at 60 °C; 4 step, time 20 min at 45 °C; 5 step, time 840 min at 40 °C. The pasta manufacture has been made in duplicate.

2.4. Dough rheological properties

The elongation and shear viscosity of each dough sample were investigated by means of a Rosand capillary rheometer (Malvern Instruments, Malvern, Worcester, UK) equipped with twin cylinders.

The samples used for the evaluation of the rheological properties were the flour mixture produced from the extruder without the die.

Two different lengths of dies with the same diameter (1 mm) were selected to measure the entry pressure losses. The length and pressure of the left die were 10 mm and 10 psi respectively and for the right die 0.25 mm and 50 psi respectively. The experiments were carried out at 30 °C and at shear rate between 10 and 2000 s^{-1}. The rheological behavior was studied using the following power law model that satisfactory fitted the experimental data:

$$\tau_S = K \cdot \dot{\gamma}_S^{n} \tag{1}$$

and

$$\tau_e = L \cdot \dot{\gamma}_e^{m} \tag{2}$$

where τ_s and τ_e are the shear stress [Pa] and extensional stress [kPa], K and L are the consistency indexes [$Pa \cdot s^n$ and $kPa \cdot s^n$, respectively], the $\dot{\gamma}_S$ and $\dot{\gamma}_e$ are the shear and extension rate [1/s], and n and m are the flow indices (dimensionless). The elongation and shear viscosity (η_e and η_s, respectively) were calculated on the range of shear and extension rate tested by using the following power law model (Bertuzzi et al., 2007):

$$\eta_S = K \cdot \dot{\gamma}_S^{n-1} \tag{3}$$

and

$$\eta_e = L \cdot \dot{\gamma}_e^{m-1} \tag{4}$$

The Bagley correction was applied to all data from Rosand rheometer. Three measurements of the viscosity experiment were performed on each sample.

2.5. Sensory analysis

Fresh-extruded and dry spaghetti samples were submitted to a panel of ten trained tasters in order to evaluate the sensorial attributes. The panelists were selected on the basis of their sensory skills (ability to accurately determine and communicate the sensory attributes, appearance, odor, flavor and texture of a product). The panelists were trained in sensory vocabulary and identification of particular attributes, prior to testing, by evaluating commercial conventional and non-conventional pasta. The panelists were asked to indicate color, homogeneity, resistance to break and the overall quality of the non-cooked spaghetti. The color is a measure of pleasantness of the yellow characteristic of pasta into consideration. The homogeneity is a measure of the presence of spots of a different color from that of the dough. The resistance to break is a measure of the strength with which the spaghetti is opposed to breakage. Elasticity, firmness, bulkiness, adhesiveness, odor and taste were also evaluated. The elasticity is a measure of the degree of extension of the spaghetti before the break and is evaluated by exerting on each spaghetto a slight traction in two points distant 10 cm. The firmness, the resistance of cooked pasta to compression by the teeth, was measured by compressing the spaghetti strand against the palate with the tongue. The bulkiness is a measure of the degree of jamming among the spaghetti strands and was evaluated by placing two spaghetti strands together and determining the force required for detachment. The adhesiveness is related to the formation of a surface coating made of amylose and this was evaluated by placing the spaghetti in the mouth, pressing it against the palate and determining the force required to remove it with the tongue. The odor is related to the pleasantness of the sensations perceived by olfaction whereas the taste is related to the pleasantness of the sensations perceived during mastication. A nine-point hedonic rating scale, where 1 corresponded to extremely unpleasant, 9 to extremely pleasant and 5 to satisfactory was used to quantify each attribute (Petitot et al., 2010). Each spaghetti sample was cooked at a different time and tested by the panel to estimate the optimal cooking time that was 10 min for dry samples and 3 min for fresh samples.

In order to evaluate the effect of the investigated vegetable flours on the organoleptic quality of the maize spaghetti, the sensorial attribute percentage change, $\Delta SA\%$, was introduced and calculated as follows (Mastromatteo et al., 2012):

$$\Delta SA\% = \frac{SA^{\mathrm{CTRL}} - SA^{\mathrm{VegFl}}}{SA^{\mathrm{CTRL}}} \cdot 100 \tag{5}$$

where SA^{CTRL} is the sensorial attribute value of the dry spaghetti, uncooked and cooked, without vegetable flours (CTRL) and SA^{VegFl} is the sensorial attribute value of the spaghetti added with vegetable flour. In particular, the change in the sensory parameter value, such as color, homogeneity, taste, elasticity, firmness, resistance to break, bulkiness, adhesiveness and overall quality, was calculated with respect to the CTRL sample. It is worth noting that the greater the positive value of $\Delta SA\%$ the further the sensory quality is from the control sample (CTRL).

2.6. Carotenoid determination

2.6.1. Chemicals

All chromatographic solvents were high-performance chromatography (HPLC) ultra-gradient grade and were purchased from Carlo Erba (Milan, Italy). Ammonium acetate was purchased from Sigma (Milan, Italy). β-carotene, lutein and zeaxanthin were purchased from Extrasynthese (Genay, France) assay ≥ 95%.

2.6.2. Extraction method

The carotenoids were extracted as described by Sun et al. (2007) with slight modifications. 10 g of yellow pepper flour were mixed with 50 mL of dichloromethane and the mixture was gently stirred for 20 min at 35 °C. The extract was centrifuged at 5000 × g for 10 min at 4 °C (Eppendorf 5804 R, Italy) and the supernatant was transferred to a clean flask. The residue was mixed with another 50 mL of dichloromethane to repeat the extraction. The resulting supernatant was combined with the previous one. This operation was repeated 5 times. The combined supernatants were evaporated to dryness under vacuum at 35 °C and the residue was dissolved in 3 mL of dichloromethane, filtered through a 0.45 μm syringe filter (Teknokroma PTFE 0.45 μm) and then used for HPLC analyses.

2.6.3. High-performance liquid chromatography

The carotenoids were separated and quantified by a HPLC as described by Sun et al. (2007). The HPLC used was an Agilent 1200 apparatus (Agilent Technologies, USA) consisting of a LC ChemStation 3D system controller, degasser, binary pump solvent delivery, auto sampler, column oven and DAD detector system. The column used for this separation was C_{18} Aqua 5 μ 200A (150 × 2.00 mm) and 5 μm particles diameter (Phenomenex, Milan, Italy). 10 μL samples of extract or calibration standards were injected directly into the column. The mobile phase consisted of 30% ammonium acetate 1 M in methanol (eluent A) and methanol (eluent B). The elution program was as following: at 0 min 5% B, 25 min 95% B, 40 min 95% B at the flow rate of 0.5 mL/min. Detection was performed by monitoring the absorbance signals at 450 nm. The retention times of carotenoids were identified using the UV–visible spectra of pure reference standards. The extractions were carried out in duplicate and the analyses were carried out in triplicate. The calibration curves obtained by injecting standard solutions containing β-carotene, lutein and zeaxanthin were characterized by a correlation coefficient $r^2 > 0.988$. The same procedure was also applied on uncooked and cooked spaghetti samples.

2.7. Chemical determination

Dry spaghetti samples were ground to fine flour on a Tecator Cyclotec 1093 (International PBI, Milano, Italy) laboratory mill (1 mm screen –60 mesh). Moisture content (%) was measured according to AACC method 44-19. Protein content (%N × 6.25) was analyzed with the micro Kjieldahl method according to the Approved Method 46-13 (AACC, 2000). Total dietary fiber (TDF), soluble-water fiber (SDF) and insoluble-water fiber (IDF) contents were determined by means of the Total Dietary Fiber Kit (Megazyme) based on an enzymatic gravimetric procedure of the AACC approved method 32-07 (2000). All nutritional analyses of the flour and spaghetti samples were made in triplicate.

2.8. Spaghetti cooking quality

2.8.1. Optimal cooking time

The optimal cooking time (OCT) was evaluated every 30 s during cooking by observing the time of disappearance of the core of the spaghetti by squeezing it between two transparent glass slides according to the AACC approved method 66-50 (2000). The time at which the core completely disappeared was taken as the OCT.

2.8.2. Cooking loss

The cooking loss, the amount of solid substance lost to cooking water, was determined according to the AACC approved method 66-50 (2000). 10 g sample of spaghetti was cooked at OCT in 300 mL of boiling distilled water. The cooking water was collected in an aluminum vessel, placed in an air oven at 105 °C and evaporated until a constant weight was reached. The residue was weighed and reported as a percentage of the starting material.

2.8.3. Swelling index and water absorption

The swelling index of cooked pasta (grams of water per gram of dry pasta) was determined according to the procedure described by Cleary and Brennan (2006). Spaghetti strands were cut into equal lengths of 40 mm. 10 g sample of this spaghetti was then accurately weighed, cooked at OCT and dried at 105 °C until a constant weight was reached. The swelling index was expressed as (weight of cooked spaghetti) – (weight of spaghetti after drying)/(weight of spaghetti after drying). Moreover, the water absorption of drained pasta was also determined as (weight of cooked spaghetti) – (weight of raw pasta)/(weight of raw pasta). Three measurements were performed for each analysis and the mean values were obtained.

2.8.4. Hardness and adhesiveness

For each test, three spaghetti strands (40 mm-length) were cooked at OCT. After cooking, the spaghetti samples were gently blotted and submitted to hardness and adhesiveness analysis, by means of a Zwick/Roell model Z010 Texture Analyzer (Zwick Roell Italia S.r.l., Genova, Italia) equipped with a stainless steel cylinder probe (2 cm-diameter). The three samples were put side by side on the lower plate and the superior plate was moved down onto the spaghetti surface. The hardness (mean maximum force, N) and adhesiveness (mean negative area, Nmm) were measured. Six measurements for each spaghetti sample were performed. Trial specifications were as follows: Pre-load of 0.3N; Load Cell of 1 kN; percentage deformation of 25%; crosshead speed constant of 0.25 mm/s.

2.9. Determination of the gelatinization degree

For the determination of the gelatinization degree (%Gel), the chemical method proposed by Wootton and Chaudhry (1980) was used. It was calculated from the colorimetric measurement of starch–iodine complex formed in aqueous suspension of sample before and after complete gelatinization of the starch. Immediately after extrusion the spaghetti samples were frozen before performing the analysis. Each frozen dough sample was divided into two sub-samples (A and B) of 2 g each. Sample A was homogenized with deionised water (100 ml) by stirring for 10 min and centrifuged at 3600 rpm (ALC Multispeed Refrigerated Centrifuge PK 12 1R, ANNITA-IIR processing and control interface). Then, duplicate aliquots (1 ml) were diluted with water to 10 ml and treated with iodine solution (0.1 ml) (prepared with 4 g potassium iodide and 1 g iodine in 100 ml of distilled water). The absorbance of these samples at 600 nm was measured with a spectrophotometer (Shimadzu UV 1700). Sample B was prepared as sample A and subsequently thermally treated in an autoclave at 135 °C for 1 h in order to reach complete gelatinization (method 76-11 AACC, 1995). Absorbance of sample B was measured at 600 nm. Absorbance was maintained between 0.3 and 0.9 by altering the aliquot sizes taken for the untreated and thermally treated samples. The degree of gelatinization (%Gel) was calculated from the ratio:

$$\%\mathrm{Gel} = (\mathrm{A_A}/\mathrm{A_B}) \times 100 \quad (6)$$

where $\mathrm{A_A}$ and $\mathrm{A_B}$ are the absorbance of the iodine complexes prepared from the aqueous suspension before and after thermal gelatinization, respectively.

2.10. In vitro digestion

The digestion was carried out as described by Chillo et al. (2011) with slight modifications. Briefly, dry spaghetti samples (5 g) were broken into 5.0 ± 1.0 cm lengths and weighed accurately. A covered boiling water bath containing 50 ml of boiling water was used to cook the spaghetti to the optimal cooking time. The spaghetti were tipped into a digestion vessel with 50 ml of distilled water and 5 ml maleate buffer (0.2 mol/L pH 6.0, containing 0.15 g $CaCl_2$ and 0.1 g sodium azide per liter) in a block at 37 °C (GFL 1092, Germany) and allowed to equilibrate for 15 min. Digestion was started by adding 0.1 ml amyloglucosidase (A 7095 Sigma Aldrich, Milan, Italy) and 1 ml of 2g/100 g pancreatin (P7545 Sigma Aldrich, Milan, Italy) in quick succession and the vessel were stirred at 130 rpm. At 0, 20, 60, and 120 min 0.5 ml of digested samples were removed for analysis of released glucose. After the 120 min sampling the digests were homogenized using an Ultraturrax (Ika werke, Germany) to convert them into slurries. The incubation continued for 1 h and 0.5 ml of digested samples was removed for analysis of released glucose. The digestion was carried out in duplicates.

2.11. Analysis of starch digest

The samples removed during digestion were added to 2.0 ml of ethanol and mixed. After 1 h, the ethanolic sub-samples were centrifuged (2000 g, 2 min) (Biofuge fresco HERAEUS, Germany) and an aliquot (0.05 ml) of the supernatant was removed. This aliquot was added to 0.25 ml amyloglucosidae (E-AMGDF, Megazyme International Ireland Ltd, 1ml/100 ml in sodium acetate buffer 0.1 mol/L, pH 5.2) for 10 min at 20 °C. 0.75 ml DNS solution (10 g 3,5-dinitrosalcylic acid, 16 g NaOH and 300 g Na–K tartarate (Sigma Aldrich, Milan, Italy) made to final volume (1 L) was then added to the tubes. The tubes were heated for 15 min in boiling water, then cooled in cold water for 1 h, after which 4 ml of water (15 °C) was added. After mixing, the reducing sugar concentration was measured colorimetrically (530 nm) using a Shimadzu UV–vis spectrophotometer (model 1700, Shimadzu corporation, Japan). Glucose standards of 10.0 mg/ml were used. The results were then plotted as glucose release (mg) per g of sample v. time. The analyses were carried out in triplicate.

2.12. Statistical analysis

The rheological and sensorial analysis and the cooking, texture and chemical properties of gluten-free spaghetti based on maize flour enriched with vegetable flours were evaluated in this study. The results of the above analysis were compared by a one-way variance analysis (ANOVA). A Duncan's multiple range test with the option of homogeneous groups ($p < 0.05$), was carried out to determine significant differences between spaghetti samples. STATISTICA 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used for this aim.

3. Results and discussion

In this study the formulation of pasta based on maize flour loaded with vegetable flours was investigated. The work was organized in two subsequent steps: the first was aimed to optimize the type of vegetable flour to be added to the formulation by sensory analysis; whereas, in the second step, the chemical and rheological analysis and the determinations of texture and cooking properties of pasta loaded with yellow pepper flour were performed.

1° Step – Optimization of the vegetable flour type

3.1. Sensory analysis of gluten-free spaghetti with different vegetable flours

In this first step, a 15% concentration of 11 different types of vegetable flours (see Fig. 1a and b) was added to the maize flour spaghetti formulation. This percentage was chosen not only because it provides an optimum additional nutritional quality but also a preliminary sensory analysis showed that 15% concentration is the optimum value required to manufacture pasta with a sensory quality score relatively close to the acceptability level. In this step only the vegetable flours produced with the commercial drying process (HTDC) were used.

The screening of the vegetable flours showed that homogeneity, color, fibrous, taste and odor were the parameters that have most influenced the overall quality of the dry spaghetti (Fig. 1a and b).

Fig. 1a and b show the results of the percent sensorial attributes change (Δ*SA%*) for all the dry uncooked and cooked spaghetti

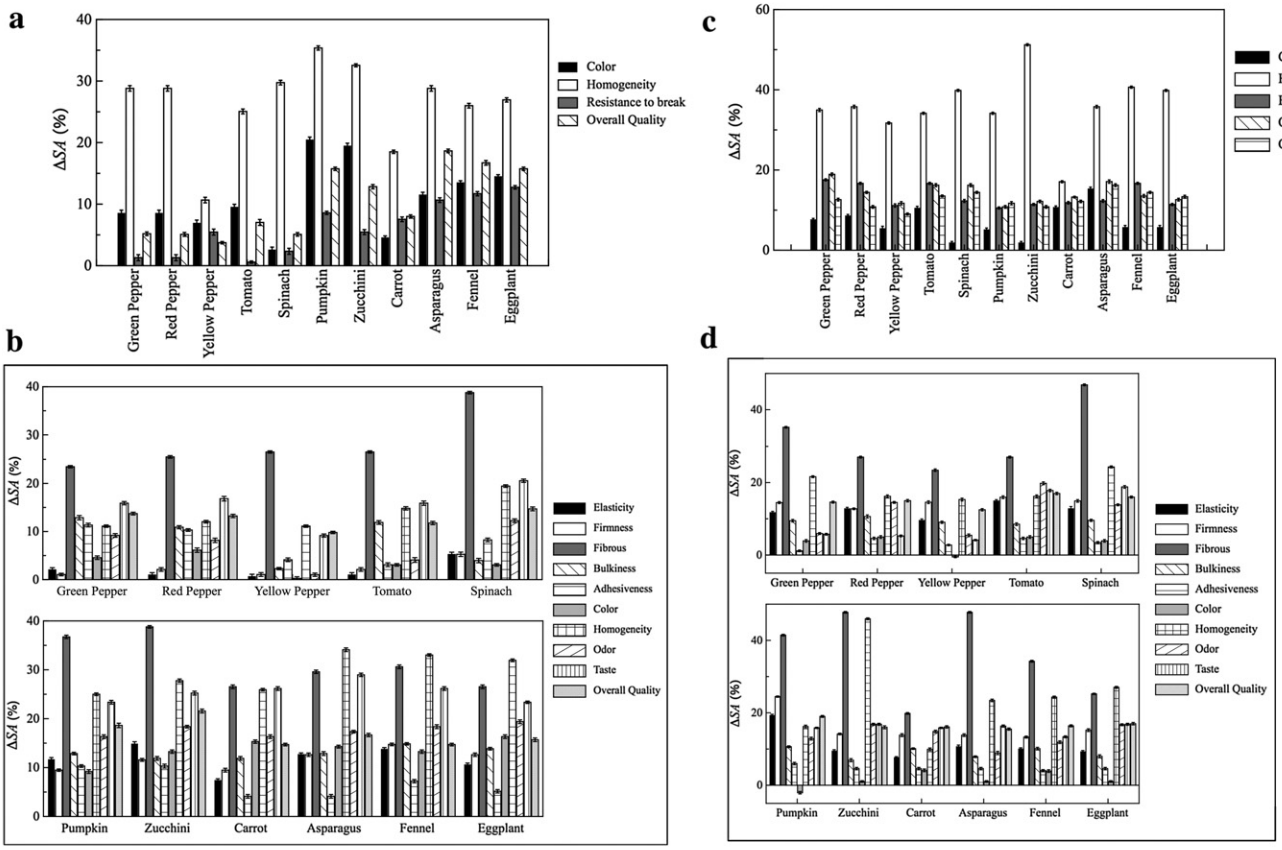

Fig. 1. Screening of the investigated vegetable flours: percent sensorial attributes (Δ*SA%*) of **a)** uncooked dry spaghetti samples, **b)** cooked dry spaghetti samples, **c)** uncooked fresh spaghetti samples, **d)** cooked fresh spaghetti samples.

samples with respect to the CTRL sample. With regards to the uncooked spaghetti, it can be noted from this table that for the overall quality, the samples added with pumpkin, zucchini, asparagus, fennel and eggplant flours produced the highest Δ*SA%* value. It can be stated that the homogeneity in general was the principal discriminating attribute for the uncooked spaghetti acceptability; for example, the Δ*SA%* for the sample with pumpkin flour was about 35%. This result is due to the very high concentration of fibers present in these flours. With regards to the samples containing the pumpkin and spinach it can be seen that the color also greatly influenced the overall quality, negatively. Whereas, the increase of the Δ*SA%* for the break resistance attribute also significantly influenced the overall quality score of the uncooked spaghetti samples added with asparagus, fennel and eggplant flours. It can be noted that the spaghetti added with the eggplants flour recorded less resistance to break compared to the CTRL (Δ*SA%* of about 12.7%). A possible explanation these findings could be that the fibers of vegetable flour bring about weaker spaghetti samples (Elleuch et al., 2011). The homogeneity decreased significantly the overall quality score (i.e. 5%) of the spaghetti sample added with the spinach flour. The incorporation of the green and red pepper flours to the spaghetti led to sensorial parameters near to that obtained with spinach and also with very good resistance to break compared to the CTRL (i.e., Δ*SA%* about 1.3% for red pepper). The high homogeneity score of the uncooked spaghetti samples added with red and green pepper flour was due to the fiber color that was very different with respect to the maize flour color. This finding was not evident in the spaghetti samples added with yellow pepper flour, as this was close in color to that of the maize flour. Results showed that the maize spaghetti loaded with yellow pepper flour recorded the lowest decrease in the sensory properties; in fact, the overall quality Δ*SA%* for this sample was about 3.7%. In particular, the sample gave an appealing orange color and the homogeneity attribute decreased significantly in comparison with the other samples (i.e. Δ*SA%* 10.6%).

With regards to the cooked samples, Fig. 1b shows also the change in the sensory parameter values with respect to the CTRL. Among all the investigated samples added with vegetable flours, results highlighted that the sample with yellow pepper recorded the lowest change for all the sensory properties apart from the fibrous property; in fact, the overall quality decrease was approximately 9.8%. This result was due principally to its pleasant orange color and its highly desirable taste and odor attributes. Whereas on the other hand the taste and color significantly decreased the overall quality score for the samples containing the red and green pepper. This could be due to the taste that was very sweet for the red pepper flour and very strong for the green pepper flour, in addition exhibited a very intense color with respect to the CTRL. The elasticity, firmness, bulkiness and adhesiveness decreased, i.e. an increase of Δ*SA%*, for almost all the samples analyzed with the exception of the spaghetti samples added with red pepper, green pepper and tomato flours. In fact, the addition of tomato flour to the spaghetti formulation produced results near to that of the spaghetti containing the red and green pepper flour with the exception of the color attribute. Regarding spaghetti samples loaded with zucchini and spinach flours, the highest change of the fibrous attribute was recorded, which negatively influenced their overall quality score. This result can be due to the fact that these two vegetables have the highest content of fibers with respect to the other vegetable flour samples. Whereas the spaghetti samples containing asparagus, fennel and eggplant resulted in organoleptic attributes values far from the reference sample, i.e. unpleasant taste and color and less homogeneous with respect to the other vegetable flour samples.

From the overall acceptability rating (Fig. 1a and b), it was concluded for this experimental stage that the spaghetti sample enriched with a 15% concentration of yellow pepper flour had better acceptance than that of the other gluten-free spaghetti samples. These results were also in agreement with those obtained from the sensory analysis of the uncooked and cooked fresh extruded spaghetti samples (Fig. 1c and d). In fact, the spaghetti with yellow pepper flour showed the lowest change of the sensorial parameters for both uncooked and cooked extruded spaghetti with respect to the control. On the basis of these results, the yellow pepper flour was chosen for the subsequent phase study.

3.2. Carotenoids content of yellow pepper flour and pasta

As reported beforehand, results from the sensory analysis showed that the most suitable vegetable flour to be used to obtain functional pasta with acceptable organoleptic properties is the yellow pepper flour. To verify the presence of the health-giving compounds derived from the yellow pepper flour, analytical investigations were carried out after the production of yellow pepper flour and on spaghetti containing the addition of the yellow pepper flour.

The results from Table 2a show that the carotenoids content in the HTDC yellow pepper flour was much lower than the LTDC flour. This low concentrations of the carotenoids in the HTDC flour was probably due to the drying process, in particular to the high temperature reached during this process as found by Zepka and Mercadante (2009). With the reduction of the temperature (LTDC), an increase in the concentration of Zeaxantin, Lutein and Beta Carotene in the investigated vegetable flour was obtained as shown in Table 2a. This could be due to the process of dehydration that determined an increase of the nutritional components in the flour with respect to the fresh pepper.

To prove the preservation of the carotenoids content in the manufactured functional pasta added with LTDC yellow pepper flour, the concentration of the three carotenoids mentioned above, the dry spaghetti was measured before and after the cooking process; results are listed in Table 2a. It is worth noting that no significant change in the carotenoids content after cooking was found in the pasta samples enriched with yellow pepper flour whereas for the CTRL there was a significant decrease in the carotenoids content after cooking. The significant higher amount of carotenoids recorded in the LTDC pepper flour compared to the quantity measured in

Table 2a
Carotenoids content of yellow pepper flour and pasta samples added with yellow pepper flour (mg/g).

	Zeaxanthin mean ± S.D.	Lutein mean ± S.D.	Beta-carotene mean ± S.D.
Fresh yellow pepper	3.08 ± 0.18^{a}	0.06 ± 0.00^{a}	0.86 ± 0.03^{a}
HTDC yellow pepper flour	4.95 ± 0.28^{b}	0.18 ± 0.02^{b}	0.30 ± 0.07^{b}
LTDC yellow pepper flour	60.32 ± 7.90^{c}	4.75 ± 0.80^{c}	1.61 ± 1.30^{c}
Extruded uncooked spaghetti (CTRL)	2.16 ± 0.14^{b}	0.77 ± 0.08^{b}	0.05 ± 0.02^{b}
Extruded uncooked spaghetti (P_LTYP)	5.86 ± 0.23^{a}	$1.07 \pm 0.08^{a,b}$	0.76 ± 0.10^{a}
Dry cooked spaghetti CTRL	1.15 ± 0.12^{c}	0.29 ± 0.05^{c}	0.02 ± 0.01^{b}
Dry cooked spaghetti (P_LTYP)	5.60 ± 0.41^{a}	1.36 ± 0.24^{a}	0.63 ± 0.08^{a}

[a–c] Mean in the same column followed by different superscript letters differ significantly ($p < 0.05$).

the extruded uncooked and cooked dry spaghetti (P_LTYP), could be ascribed to the different ease of extraction of carotenoids from the three different food matrices.

3.3. Sensory analysis of the gluten-free spaghetti with yellow pepper flours (HTDC and LTDC)

The spaghetti samples of maize enriched with 15% HTDC yellow pepper flour and LTDC yellow pepper flour were subjected to sensorial analysis to evaluate their organoleptic properties compared to the CTRL sample. The sensorial attributes change, Δ*SA*, was calculated according to eq. (6). Regarding the uncooked fresh extruded spaghetti, the sample P_LTYP showed the lowest change of the sensory properties compared to the CTRL (data not shown). The addition of the LTDC yellow pepper flour improved the overall quality of the spaghetti. In fact, the overall quality Δ*SA*% of 3.3% was due its pleasant color (Δ*SA*% 0.6%) and odor (Δ*SA*% 1.8%). Similar results were obtained from the sensory analysis of the cooked fresh extruded spaghetti. In fact, the Δ*SA*% of the overall quality change ranged from 12% for sample P_HTYP to 8% for sample P_LTYP.

The results obtained for the dry spaghetti are shown in Fig. 2a and b. Fig. 2a shows the sensorial parameters of the uncooked dry spaghetti samples. The P_LTYP sample recorded the lowest change of the sensory properties; in fact, the overall quality decrease (Δ*SA*%) was about 1.7%. In particular, an improvement in the color attribute for the P_LTYP sample was recorded compared to the P_HTYP sample; the color changed from dark orange to a pleasant light orange. This result can be due to the fact that the drying process temperature of the fresh pepper determined significant color changes of the LTDC yellow pepper flour with respect to the process used to produce commercial flour (HTDC). In fact, the temperature is the principal parameter of the drying process that influences the color of dried product (Krokida et al., 1998).

The sensory properties of the dry cooked spaghetti samples are reported in Fig. 2b. Also in this case the spaghetti sample P_LTYP had a pleasant yellow color, agreeable taste and odor, and low adhesiveness and homogeneity compared to the P_HTYP sample. Regarding the other sensorial properties no significant differences were found between the samples. In particular, the Δ*SA*% values of elasticity, firmness, bulkiness and adhesiveness obtained for the two samples, P_HTYP and P_LTYP, were near to that of the reference sample; whereas, the fibrous Δ*SA*% value was far from that of CTRL. In fact, for P_HTYP sample, Δ*SA*% was about 26.5% and about 18% for P_LTYP. This result could be due to the fact that the pepper flour had higher content of dietary fiber compared to the maize flour; therefore the spaghetti samples were more fibrous (Elleuch et al., 2011).

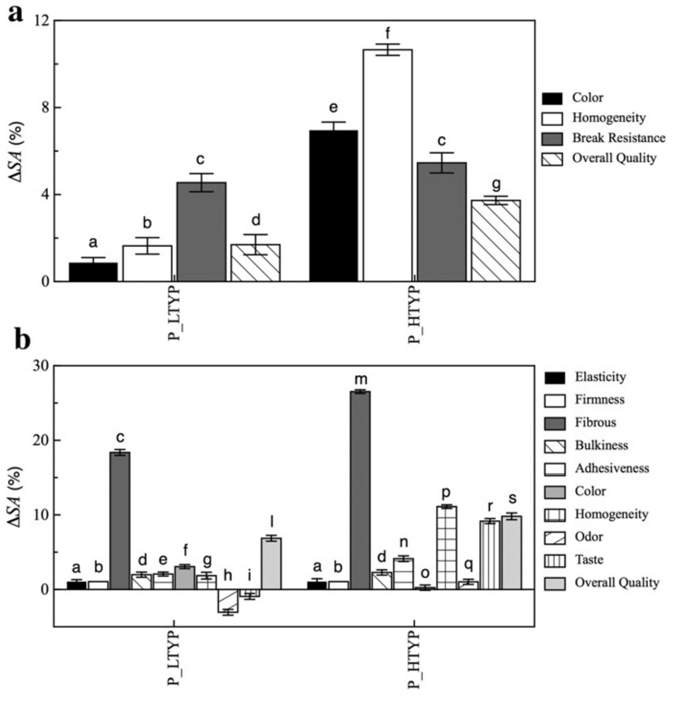

Fig. 2. The percent sensorial attributes (Δ*SA*%) of the dry spaghetti samples with the yellow pepper flour (HTDC and LTDC) **a)** uncooked and **b)** cooked.

As far as the overall quality values are concerned, data suggests that the addition of the yellow pepper flour obtained using the

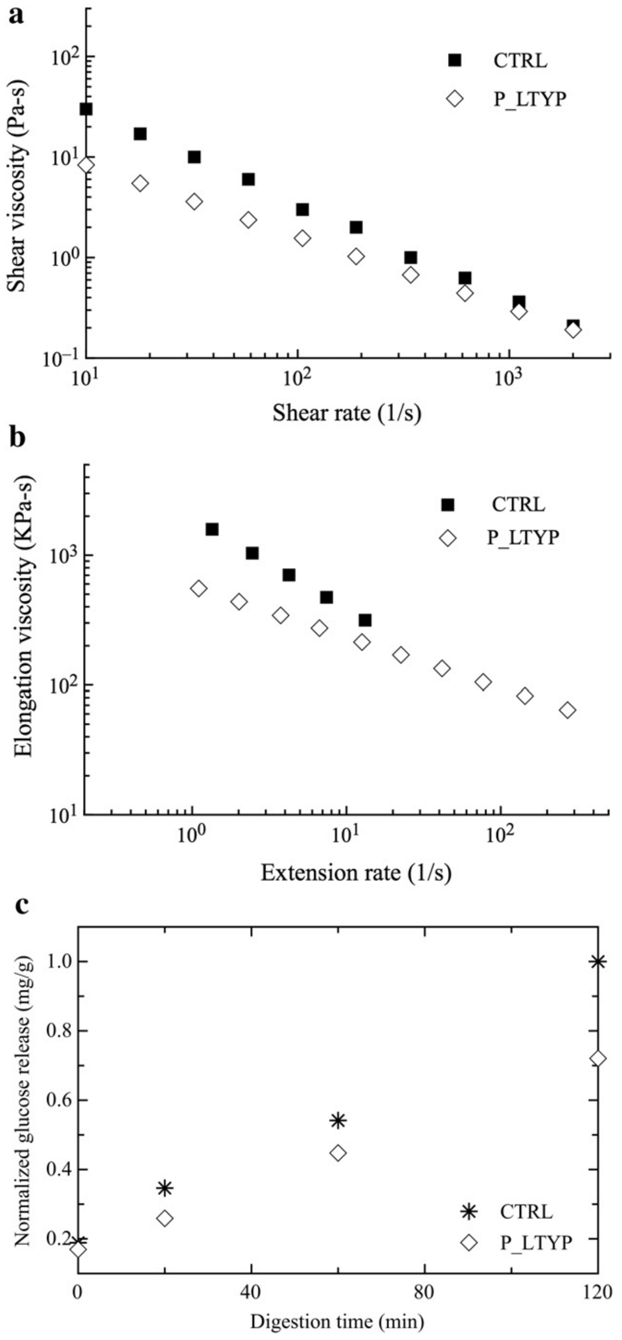

Fig. 3. Rheological behavior of the dough samples. **a**) Shear viscosity as a function of shear rate; **b**) Elongation viscosity as a function of extension rate. **c**) – Normalized glucose release vs digestion time for the investigated spaghetti samples.

Table 2b
Values of the consistency indices (L and K) and the flow indices (m and n) obtained by fitting eqs. (1) and (2) to the experimental data.

	L	m	K	n
CTRL	1957.52^{a}	0.2921^{a}	253.87^{a}	6.56E-02^{a}
P_LTYP	575.56^{b}	0.2111^{b}	38.90^{b}	2.62E-01^{b}
Semolina	755.48^{c}	0.3496^{c}	47.08^{b}	0.3681^{c}

$^{a-c}$ Mean in the same column followed by different superscript letters differ significantly ($p < 0.05$).

more efficient drying process (LTDC) improved the organoleptic properties of the investigated functional pasta.

Step 2: Characterization of gluten-free spaghetti with LTDC yellow pepper flour

In this last experimental step, the spaghetti samples P_LTYP were subjected to rheological, chemical and nutritional analysis; the textural and cooking properties were also evaluated to further characterize this spaghetti formulation.

3.4. *Rheological analysis*

A capillary rheometer was used in this work to determine extensional and shear viscosity, which is a measure of the resistance of a fluid that is being deformed, by either shear stress or extensional stress. In fact, during the extrusion process, levels of extensional and shear viscosity sufficient to allow the additives to bind to the compounds of the flour (protein and starch) and to then develop a structure able to improve the mechanical properties of the spaghetti are necessary.

Fig. 3a,b shows shear and elongation viscosity as a function of the shear rate and extension rate, respectively. As can be inferred from the above figure, the trend is typical of pseudoplastic materials. In fact, shear and elongation viscosity values decline with the shear and extension rate. The pseudoplastic behavior of a polymeric system can be explained as follows (Brydson, 1981): if the system of asymmetric particles, which are initially randomly dispersed, is subjected to shear, the particles tend to align themselves with the major axis, in the direction of shear, thus reducing the viscosity. The degree of alignment is a function of the deformation rate. At low shear rate, there is only a slight departure from randomness but at higher shear rate particles are almost completely oriented (George et al., 1996). From Fig. 3a,b it can be seen that the CTRL sample had the higher values of elongation and shear viscosity with respect to the P_LTYP sample at low shear rate. Coherently, the consistency indices K and L (Table 2b), obtained by fitting eqs (1) and (2) to the experimental data, decreased in the sample with pepper flour. In fact, the CTRL sample recorded the highest values of both the consistency indices K and L (Table 2b). Moreover, the P_LTYP sample showed rheological properties near to those of the semolina sample. In particular, the consistency index K of P_LTYP was statistically similar to that of semolina sample (Table 2b); whereas, the consistency index L recorded a value statistically different from the semolina sample even though it had the same order of magnitude.

Aravind et al. (2012) observed a significant reduction in pasta viscosity by incorporation of soluble fibers, which is consistent with a competition between the dietary fibers and starch for the available water, inhibiting starch pasting and retrogradation, and/or disruption of the starch/protein matrix. In this case, most probably the pepper flour dietary fibers interfere with the pregelatinized maize starch.

Moreover the pepper flour could contain components such as sugars that affect the process of gelatinization of starch (Prokopowich and Biliaderis, 1995). Torley and van der Molen (2005) also found that many food products contain a mixture of sugar sources that affect the gelatinization of starch. In fact, the spaghetti samples with yellow pepper flour recorded a high gelatinization degree (54.1%) with respect to the CTRL (47.9%). Also the dough viscosity is directly influenced by the amount of pregelatinized maize starch in the feed materials (Hsieh et al., 1991). Therefore, the incorporation of the yellow pepper flour and the consequent decreasing of the amount of maize flour used decreases the quantity of pregelatinized starch available to structure the dough and also decreases the firmness of dough.

3.5. *Chemical composition*

In Table 3a the chemical parameters such as protein, moisture, SDF, IDF and TDF of the maize and yellow pepper flours as well as of the spaghetti samples CTRL and P_LTYP were listed. As can be seen in the above table, the yellow pepper flour presented a statistically different nutritional composition compared to the maize flour. In particular, the yellow pepper flour showed the lowest protein content and the highest dietary fiber content compared to the maize flour. The yellow pepper flour also recorded the highest content for both IDF and SDF than that of the maize flour. This result can be due to the fact that vegetables and fruits appear to be superior sources of dietary fiber compared to cereals (Elleuch et al., 2011). As a consequence, the spaghetti enriched with the yellow pepper flour showed a significant increase of insoluble and soluble fibers than the CTRL sample. Regarding the protein content, a negligible even though statistically significant increase in the P_LTYP sample compared to the CTRL sample was observed.

3.6. *Spaghetti cooking quality*

The cooking properties of the CTRL and P_LTYP spaghetti samples are shown in Table 3b. From this table it emerges that the optimum cooking time for the CTRL spaghetti sample was 10 min whereas 7 min for the spaghetti in base of yellow pepper flour. This result could be due to the fact that the incorporation of yellow pepper flour decreased the quantity of gelatinized starch, and then it determined a loss of continuity of the protein-starch network. In fact, the absence of the continuity of the protein-starch network seems to facilitate the water diffusion through the spaghetti matrix, reducing the time that the water needs to reach the spaghetti centre during cooking. Chillo et al. (2007) found that the absence of gluten network in the spaghetti in base of amaranthus flour influenced the optimum cooking time.

Table 3a
Chemical composition of the flours and dry uncooked spaghetti samples.

	Protein (%)	Moisture (%)	SDF (%)	IDF (%)	TDF(%)
Maize Flour	5.0^{a} ± 0.03	11.8^{a} ± 0.02	0.2^{a} ± 0.005	2.8^{a} ± 0.12	2.9^{a} ± 0.12
LTDC Yellow Pepper Flour	3.1^{b} ± 0.28	13.5^{b} ± 0.13	8.7^{b} ± 0.17	13.3^{b} ± 0.43	22.1^{b} ± 0.26
CTRL	6.0^{a} ± 0.09	10.7^{a} ± 0.02	0.36^{a} ± 0.01	3.7^{a} ± 0.02	4.1^{a} ± 0.03
P_LTYP	6.4^{b} ± 0.008	9.0^{b} ± 0.01	0.87^{b} ± 0.02	4.5^{b} ± 0.01	5.4^{b} ± 0.03

$^{a-b}$ Mean in the same column followed by different superscript letters differ significantly ($p < 0.05$).

Table 3b
Optimal cooking time, cooking loss, swelling index, hardness and adhesiveness of the dry cooked spaghetti samples.

	OCT (min)	Cooking loss (%)	Swelling index	Water absorption (%)	Hardness (N)	Adhesiveness (Nmm)
CTRL	$10.0^{a} \pm 0.01$	$7.8^{a} \pm 0.74$	$1.7^{a} \pm 0.40$	$141^{a} \pm 27$	$12.0^{b} \pm 1.05$	$0.76^{a} \pm 0.10$
P_LTYP	$7.0^{b} \pm 0.02$	$12.0^{b} \pm 0.44$	$2.2^{a} \pm 0.54$	$131^{a} \pm 8.6$	$9.6^{a} \pm 0.73$	$0.85^{a} \pm 0.13$

$^{a\text{-}b}$ Mean in the same column followed by different superscript letters differ significantly ($p < 0.05$).

The quantity of solids going into water during cooking is a determinant factor of the spaghetti quality and more textured spaghetti leads to lower cooking loss. As a reference, the cooking loss of commercial pasta based on durum wheat is about 4.5%. Table 3b highlights that the spaghetti samples in base of yellow pepper flour had higher cooking losses (7.8%) with respect to the CTRL sample (12%). This result could be due to the fact that the incorporation of the yellow pepper flour reduced the quantity of gelatinized starch by causing high cooking losses for the P_LTYP spaghetti sample. In fact, Tam et al. (2004) found that the starch gelatinization can help reduce cooking loss.

Pagani et al. (1986) observed that the quality of protein and the formation of a continuous protein network are very important in the entrapment of carbohydrates in order to obtain pasta of good cooking quality; whereas, Tudorică et al. (2002) found that the increased cooking loss was due to the disruption of the protein-starch network. In addition, the yellow pepper flour could also be lost in the cooking water; however, this assumption requires further analysis.

The swelling index of pasta is an indicator of the water absorbed by the starch and proteins during cooking, which is utilized for the gelatinization of starch and the hydration of protein. Results listed in Table 3b indicate that there are no significant differences between the P_LTYP and CTRL samples for the swelling index and water absorption. Therefore, the incorporation of the yellow pepper flour to the pasta formulation did not seem to have any effect on these two cooking parameters.

Table 3b shows the textural properties of the cooked spaghetti to the optimal cooking time in terms of hardness and adhesiveness. The adhesiveness value for the P_LTYP spaghetti sample was not statistically different from that CTRL. Regarding the hardness parameter, this value was statistically lower in the P_LTYP sample compared to the CTRL. This may be due to the higher fiber content in the P_LTYP sample. In fact, the hardness and cooking loss results suggest that the incorporation of pepper flour prevents the formation of the starch network and therefore negatively influences the cooking quality of the pasta. Moreover these results may be related to the rheological data, where the incorporation of the yellow pepper flour and the corresponding decrease of the quantity of pregelatinized starch available to structuring the dough resulted in less hard pasta.

3.7. *In vitro digestion*

Fig. 3c shows the normalized glucose release vs time digestion for the investigated samples. The glucose release values were normalized in order to take into account for the different starch content in the two samples. The values were evaluated by dividing each glucose release value for that of the CTRL sample measured at 120 min.

Significant differences in glucose release rates were observed at 20, 60 and 120 min of digestion between the two samples studied (P_LTYP and CTRL) (Fig. 3c). In particular, the amount of glucose released from the P_LTYP spaghetti sample was statistically lower than that of CTRL sample. This result could be due to the fact that the spaghetti with yellow pepper flour had a higher content of dietary fiber respect to the CTRL sample. In fact, addition of dietary fiber can further reduce the *in vitro* glycaemic response of pasta and introduce additional health benefits (Yokoyama et al., 1997). Moreover, Tudorică et al. (2002) found that the reduction in the digestibility of pasta added with soluble fibers of barley can be explained by the changes in the microstructure of cereal based products (Tudorică et al., 2002), and the limitation of water availability for starch gelatinization due to the hydration of soluble non-starch polysaccharides.

4. Conclusion

The optimization of the formulation of gluten-free functional spaghetti based on maize flour and different types of vegetable flours was addressed in this study. Results indicated that yellow pepper flour gives the best results among the tested vegetable flours from a sensory point of view. Moreover, the LTDC yellow pepper flour had higher content of carotenoids compared to the HTDC yellow pepper flour. The effect of the LTDC yellow pepper flour on the cooking and textural properties of the spaghetti samples along with chemical and rheological properties were also examined. Regarding the rheological properties, the incorporation of yellow pepper flour caused differences in the viscoelastic properties. The chemical composition improved in the spaghetti enriched with the yellow pepper flour. In particular, the spaghetti added with the yellow pepper flour had the highest dietary fiber content respect to the CTRL sample.

The cooking loss was higher in the spaghetti with pepper flour with respect to the CTRL, while the instrumental hardness was visibly lower. Moreover with respect to the CTRL, the addition of yellow pepper flour did not influence glucose release during *in vitro* digestion of P_LTYP spaghetti. Further clinical experiments are required to ascertain if similar trends can be also observed *in vivo*.

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