Research Article

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Impact of xanthan gum addition on phenolic acids composition and selected properties of new gluten-free maize-field bean pasta

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Abstract: Replacing the gluten network to produce high quality pasta is a great technological challenge. One of known solutions to the problem is the addition of xanthan gum. This paper focuses on the possibility of obtaining a new type of gluten-free maize-field bean pasta and explores the characteristics of phenolics content, antioxidant activity, cooking quality, textural and thermotropic behavior as well as the microstructure of pasta products with the various levels of added xanthan gum. The obtained results revealed that 0.25, 0.50 and 0.75% addition of xanthan gum to pasta did not have significant influence on its phenolics content and antioxidant activity, whereas 1.00% addition caused a decrease in the tested parameters. On the other hand, the opposite effect of gum addition on the cooking quality, texture characteristic and microstructure was

observed. The addition of xanthan gum to the formulation improved pasta quality while reducing the leaching of its components into the cooking water. Pasta prepared with 1.00% xanthan gum showed the lowest cooking loss, the highest firmness, and the lowest adhesiveness. These results revealed a significant influence of xanthan gum content on pasta properties as confirmed by the thermal analysis and SEM microstructure observations.

Keywords: High performance liquid chromatography-mass spectrometry; Gluten-free pasta; *Vicia faba minor*; Xanthan gum; Phenolic compounds.

1 Introduction

Gluten, a mixture of plant proteins (gliadin and glutenin), has been in the focal point of dietary studies for several years. This is due to the fact that gluten is regarded as contributing to celiac disease, problems with the digestive track and with the absorption of important nutrients [1]. According to statistics, the number of celiac disease cases is on the rise. A solution to this problem can be gluten-free products containing maize instead of other gluten-rich cereals. This approach to diet is already in place, yet, in many cases, to replace this ingredient poses a great technological challenge. The absence of gluten has a marked influence on the structure and quality of final products. It results in changes of crispiness, moisture absorption and other characteristic features for selected products. Hence, the industry and the world of science make every effort to improve the technology of gluten-free production in order to preserve the most important qualities and distinguishing features of new food ranges that are intended to be safe for people with celiac disease.

The food industry manufactures numerous types of gluten-free products, among them cereals, cookies,

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snacks, etc. Today, intensive studies are being pursued on daily food products such as pasta. This product, due to its nutritional and sensory qualities, is a traditional food consumed around the world [1, 2]. Generally, the two basic ingredients of pasta are wheat flour and/or semolina and water. In order to enhance the quality or nutritional value of pasta, other ingredients, such as milk, eggs, vegetables, etc. are added [3]. Nonetheless, the essential ingredient is appropriate flour that ensures good quality delivered to consumers. The most important factor responsible for pasta structure is gluten as it has a direct impact on the cooking properties. Although this ingredient contributes substantially to the form of pasta, some consumers who suffer from celiac disease are made to avoid this traditional food. With this end in view, manufacturers and scientists make attempts to modify pasta composition. The most often used solution is to substitute wheat flour for maize, that is, a gluten-free plant [4, 5, 6]. Among the advantages of corn flour, there are the yellow color, bland taste, and easy digestion [7, 8]. Still, the absence of gluten in the proceeded material create serious technological challenges [9]. One of the possible solutions to this problem is to add hydrocolloids (xanthan gum, guar, carrageenan) that replaces gluten in pasta successfully [8, 10, 11]. These substances are widely used as food additives since they can improve such qualities as density, emulsification, water absorption, structure stabilization, or gelling. Apart from the food industry, hydrocolloids are popular in the textile, cosmetic, pharmaceutical, or oil industries, which is debatable but accepted by the general public [12].

Considering the high demand for gluten-free products, as well as for foodstuffs enriched with valuable nutritional and natural additives, much attention is attached to daily food products intended for people suffering from celiac disease and those seeking gluten-free diets to lose weight [8]. One of interesting solutions to the problem of absence of gluten is the addition of the *Vicia faba* (the family *Fabaceae*). The *Vicia faba* is known for the high content of vitamins, antioxidants, fiber, starch, protein and other nutritional components. The beans feature in the Mediterranean cuisine as well as in the cooking of North Africa, Asia, and Latin America [13-15]. Therefore, this plant can serve as a valuable additive to enrich gluten-free pasta [13, 15].

This paper discusses the studies of the effect of different concentration of xanthan gum on polyphenols content and antioxidant activity as well as on the cooking quality, textural and thermal characteristics and the microstructure of a new type of gluten-free maize-field bean pasta.

2 Material and methods

Maize semolina (*Zea mays* L.) with the particle size below 500 μm was provided by PZZ Lubella Sp. z o. o. Sp. K. (Lublin, Poland). Its approximate composition was as follows (in g per 100 g): 14.5 moisture content, 0.1 ash, 3.1 fat, 6.5 protein, 2.2 fiber. Field bean seeds (*Vicia faba minor*) (Alamir Company, Al Behera, Egypt) were dehulled and ground to flour (particles smaller than 500 μm) in the laboratory grinder LMN-100 (TESTCHEM, Radlin, Poland). The approximate composition of the field bean flour was as follows (in g per 100 g): 10.15 moisture content, 2.37 ash, 1.29 fat, 31.2 protein, 11.03 fiber). The standards of phenolic acids were delivered by Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). All the chemicals were of chromatographic grade. LC grade methanol (MeOH) was purchased from J.T. Baker (Phillipsburg, USA). The Millipore Direct-Q3 purification system (Bedford, MA, USA) was used to prepare LC grade water.

2.1 Preparation of gluten-free pasta

Maize flour was mixed with field bean flour in 2/1 (w/w) ratio. The gluten-free pasta formulation consisting of 200 g of maize/field bean blend, 4 g of salt and 110 mL of water (set during a preliminary study) was mixed continuously for 2 min. Xanthan gum was dispersed in cold water and added to the recipe in the amount of 0.25, 0.50, 0.75 and 1.00% as a maize/field bean flour blend replacement. The control pasta was prepared without gum addition. The ingredients were kneaded for 15 min using KitchenAid kPM5 (St. Joseph., Michigan, USA). The obtained dough was rested for 1 h at room temperature covered with plastic wrap. The dough was sheeted through reduction rolls of the Marcato Ampia type 150 pasta machine (Campodarsego, Italy) four times upon each pass and in all directions to produce a sheet with 1.5 mm thickness measured with a caliper. The final shape of the tested pasta was formed by cutting rolls with a 5 mm distance. Pasta samples were dried at 40°C for 4 h in an air oven and stored dry in plastic bags at room temperature.

2.2 Chemical analyses

2.2.1 Extraction procedure

Extraction in an ultrasonic bath (UAE – ultrasound-assisted extraction) was performed in accordance with a procedure described earlier [16]. Two grams of minced

samples were transferred quantitatively to flat-bottom flasks with conical ground joint. Then, 40 mL of ethanol was added and the process of extraction was started, using thermostated ultrasonic bath (40 min at the temperature of 60°C, the power of 320W and the ultrasound frequency of 33 kHz). Filtered extracts were collected to beakers. 40 mL of ethanol was added to the reminder, afterwards the extraction was repeated. The obtained extracts were combined and evaporated under fume hood until dry. The residues were dissolved in 5 mL of methanol.

2.2.2 LC-ESI-MS/MS analysis of phenolic compounds

The chromatographic analysis (high-performance liquid chromatography and electrospray ionization mass spectrometry, HPLC-ESI-MS/MS) was conducted according to the methodology described by Oniszczuk et al. [17]. The extracts were analyzed in the Agilent 1200 Series HPLC (Agilent Technologies, USA) containing a binary gradient solvent pump, degasser, autosampler, and column oven. The samples were dosed to the Zorbax SB-C18 column (2.1 x 50 mm, 1.8 µm particle size) at 25°C, in 3 µL injections. Gradient method was used with mobile phases: water with 0.1% HCOOH (A) and methanol with 0.1% HCOOH (B). The flow rate was 400 µL/min and the gradient was as follows: 0-1 min – 5%B, 2-4 min – 20%B, 8-9.5 min – 70%B, 11.5-15 min - 5%B.

The data on analytical results and parameters and the correlation coefficient for calibration curves, as well as the detection limit and quantification limit values for phenolic acids, were described earlier [17].

2.2.3 Radical-scavenging activity of the analyzed extracts

The antioxidant activity of the extracts was determined by means of the colorimetric DPPH method [16]. In order to determine the activity, 0.1 mM methanolic DPPH solution was prepared. A reference sample was prepared by mixing 2.5 mL of the solution and 0.5 mL of methanol. An appropriate extract measurement sample was prepared by mixing 2.5 mL of DPPH and 0.5 mL of the extract. Absorbance changes were recorded directly after the addition of the extract at the wave length of 517 nm at room temperature. The absorbance was measured every 5 min for half an hour. This approach allows to monitor changes of absorbance over the time and when plateau will be reached. Each measurement was repeated three times, and the final result is the average of the replications.

The antioxidant activity was calculated with the following formula:

$$\% \text{ DPPH radical scavenging ability} = \frac{A_0 - A_1}{A_0} \times 100 [\%]$$

where, A_0 - absorbance of the reference sample, A_1 - absorbance of the sample with tested extracts.

2.2.4 TLC-DPPH test of extracts

In order to determine active antioxidants in the examined extracts, the TLC-DPPH test was performed. In this case, the analyzed extracts and a solution of the standard substance (rutin, 0.5 mg/mL) were applied onto chromatographic plates. The procedure was discussed earlier by Oniszczuk et al. [16]. In the first stage, a pasta sample with the addition of xanthan gum was developed in the mobile phase (ethyl acetate:water:acetic acid (8:1:1 v/v/v) in vertical chambers (DS II, Chromdes, Lublin, Poland). Next, the plates were dried, immersed in a freshly prepared 0.1% (w/v) methanolic DPPH solution and scanned with a flat-bed scanner (Lide 50, Canon) every 10 min for over an hour. The test was performed in triplicate. The Sorbfil TLC Videodensitometer software (Sorbpolymer, Russia) was employed for the analysis of obtained results [16].

2.3 Determination of pasta quality

2.3.1 Cooking quality

The cooking quality was determined according to the 66-50 Approved Method [18]. Twenty five grams of dry pasta was cooked in 250 mL of boiling water over the optimum cooking time and flushed with 150 mL of cold water. Collected cooking and rinsing water was completely evaporated by drying overnight at 105°C. The amount of the residue was weighed, and the cooking loss (CL) was calculated as the percentage of dry pasta sample weight.

The water absorption capacity (WAC) was calculated along with the increasing pasta weight after cooking against pasta dry weight [11, 19]. The measurements were performed in triplicate.

2.3.2 Textural characteristics

The texture of cooked pasta was tested using the universal testing machine Zwick/Roell BDO-FB0.5 TH (Zwick GmbH & Co., Ulm, Germany) equipped with a 0.5 kN working head. The OTMS Ottawa cell was used to test firmness (F) and adhesiveness (A) of the control sample and pasta with various xanthan gum addition [20, 21]. A double-compression test was applied to evaluate firmness (N) and adhesiveness (mJ). Fifty grams of cooked and drained pasta was placed in a testing chamber and compressed with a test speed of 3.3 mm/s. The *testXpert*® 10.11 software was used to record data in three independent replications.

2.3.3 Evaluation of thermotropic behavior

Heat-flow curves were collected with a DSC (Mettler Toledo AG, Greifensee, Switzerland) controlled by Stare Software. The temperature over the entire measurement period was regulated by the TC100MT Huber high-precision thermoregulation system. The indium standard was used prior to measurements to calibrate the instrument. The measurements were performed with neutral nitrogen gas using an empty measuring aluminum crucible as the reference. Weighted portions 3–5 g of powdered (<250 µm) dried pasta were placed in DSC crucibles with a pin and sealed. The measurements were carried out according to the following model: thermal equilibration at 25°C for 10 min, heating at the rate of 10°C/min up to 180°C. The ensuing thermal parameters were calculated using the evaluation mode from the Stare system: transition onset (T₀) and endset (T_c) temperatures, peak temperature (T_p), and temperature ranges (T_r = T_c–T₀). Gelatinization enthalpy (ΔH, J/g) was assessed by integrating the area under the baselined thermogram curve. ANOVA (Statistica 10, StatSoft, Inc., USA), followed by the LSD Fisher's test, was applied at P < 0.05 to assess the effects produced by the addition of xanthan gum.

2.3.4 Microstructure of dry pasta

Dry samples of the control pasta and pasta products with xanthan gum addition were evaluated for the microstructure using a scanning electronic microscope (SEM). The pasta was cut into 2 mm pieces and put on a carbon disc using a silver tape. The prepared samples were sprayed with gold in the vacuum sublimator K-550X (Emitech, Ashford, England). The surface and cross-section of the pasta samples were studied using the VEGA

LMU electron microscope (Tescan, Warrendale, USA) with the magnification of ×600. 30 kV of accelerating voltage was applied during the observations.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 LC-ESI-MS/MS analysis of phenolic compounds

One of essential parts of functional food research is the studies of the features of natural active components (i.e. antioxidants such as polyphenols) in extracts obtained from selected food products. A high content of active substances enhances the health-promoting properties of food and contributes to consumers' well-being. An example of this type of studies is the research done by Bouasla et al. of gluten-free precooked rice-yellow pea pasta [22]. Given the satisfactory results obtained by the scientists, further studies of phenolic acid content as well as of the antioxidant activity of extracts obtained from gluten-free maize-field bean pasta with xanthan gum seem well-funded.

The first step of the experiment was the extraction of polyphenols from pasta samples. The results of our previous studies indicate that the UAE easily arrives at extraction equilibrium and therefore, permits shorter periods of time, reducing the energy input. Moreover UAE can offer a high yield of analyzed compounds, simple manipulation, reduced volume of solvent, high reproducibility and meets the requirements of "Green Chemistry". The authors optimized the extraction conditions. Based on obtained results, the following conditions: two cycles for 20 min, 60°C and ethanol as the extractant, proved to be the most appropriate for the isolation of phenolic acids with the use of UAE. The next step of the study was an HPLC-MS analysis of polyphenolic extracts. Ten phenolic acids were identified (Table 1) in maize-field bean pasta with the addition of xanthan gum. These were: protocatechuic 4-OH-benzoic, vanilic, *trans*-caffeic, *cis*-caffeic, *trans*-p-coumaric, *cis*-p-coumaric, *trans*-ferulic, *cis*-ferulic, *trans*-sinapic and *cis*-sinapic acids. It should be underlined that the concentrations of the following substances: protocatechuic, *cis*-caffeic and *trans*-sinapic acids were below the limit of quantification but, at the same time, above the limit of detection. The content of polyphenolic compounds is attributed, to a large extent, to the addition of field beans to the pasta.

Table 1: Content of phenolic acids composition in corn-field bean pasta with addition of xanthan gum.

Content of xanthan gum (%)	Phenolic acids ($\mu\text{g/g}$ of dry weight)										
	protocatechiuc acid	4-OH-benzoic acid	vanillic acid	trans-caffeic acid	cis-caffeic acid	trans-p-coumaric acid	cis-p-coumaric acid	trans-ferulic acid	cis-ferulic acid	trans-sinapic acid	cis-sinapic acid
0	BQL ^b	0.359	1.308	0.192	BQL	3.812	1.654	1.381	4.739	BQL	0.475
RSD % ^a	-	1.34	3.35	2.34	-	2.53	3.57	3.78	1.26	-	3.71
0.25	BQL	0.334	1.400	0.205	BQL	3.923	1.691	1.318	4.821	BQL	0.384
RSD %	-	2.68	1.96	3.59	-	4.32	2.71	3.87	3.77	-	2.71
0.50	BQL	0.358	1.349	0.195	BQL	4.412	1.781	1.370	4.985	BQL	0.362
RSD %	-	3.27	4.22	3.23	-	3.13	4.91	2.16	3.57	-	2.48
0.75	BQL	0.412	1.408	0.241	BQL	4.121	2.093	1.350	5.126	BQL	0.421
RSD %	-	2.21	1.87	2.67	-	4.41	2.47	3.91	3.71	-	2.13
1.00	BQL	0.265	1.163	0.184	BQL	2.623	1.972	0.856	4.451	BQL	0.328
RSD %	-	1.51	2.78	4.11	-	2.94	3.22	2.29	2.91	-	3.13

^aRSD % - relative standard deviation in percent (n=3); ^bBQL - peak detected, concentration lower than the LOQ but higher than the LOD

Our findings are in the line with the research carried out by Abu-Reidah et al. [23] who identified hydrobenzoic acid derivatives (protocatechuic and vanillic acids) and hydroxycinnamic acid derivatives (caffeic, coumaric, ferulic and sinapic acids) in the studied plant. According to the cited publication, a reversed-phase UHPLC (ultra-high performance liquid chromatography) method coupled with the QTOF-MS were used to analyze dietary metabolites from a hydro-methanolic extract of *Vicia faba* seeds.

The addition of 0.25, 0.50 and 0.75% of xanthan gum to the pasta did not significantly affect polyphenol content. However, at a concentration of 1.00% of xanthan gum, the content of phenolic acids was significantly lower. This might be due to the formation of bonds between the gum and phenolic compounds with a strong binding of polyphenols on the surface of xanthan. These complexes are difficult to break during the extraction process. Therefore, the samples with the highest levels of xanthan gum exhibit a lower yield of phenolic acids. An example chromatogram of the analyzed phenolic acids is shown in Figure 1.

3.2 TLC-DPPH test of extracts

In order to estimate the antioxidant activity of prepared samples, a TLC-DPPH dot-blot test was performed. An advantage of the method is quick evaluation of the biological activity of any examined mixtures. In the case of the discussed studies, all of the tested samples revealed a free radical scavenging activity. In the next step, the studied extracts were developed on TLC plates, followed by the post-chromatographic derivatization with a DPPH methanolic solution and the interpretation of obtained results.

As noted earlier, in order to obtain numerical data, from the TLC-DPPH test, the Sorbfil TLC Videodensitometer computer software was used. The software read each white spot, characteristic of the active antioxidant, appearing on a violet TLC-DPPH plate as peak similar to that obtained from the HPLC analysis. Rutin, a model antioxidant, was read as a standard substance. The analysis of selected extracts was based on the comparison of the total area under the peaks obtained for the whole extract with the area obtained for the standard substance [16]. The results revealed that the percentage content of xanthan gum in pasta with the addition of 0.25, 0.50 and 0.75% of this component had no impact on the free radical scavenging ability. However, at a concentration of 1.00%

Figure 1: An example of the LC-ESI-MS/MS chromatogram of analyzed phenolic acids. Description: 1 - 4-OH-benzoic acid, 2 - vanillic acid, 3 - *trans*-caffeic acid, 4 - *cis*-caffeic acid, 5 - *trans-p*-coumaric acid, 6 - *cis-p*-coumaric acid, 7 - *trans*-ferulic acid, 8 - *cis*-ferulic acid, 9 - *trans*-sinapic acid, 10 - *cis*-sinapic acid.

Figure 2: Free radical scavenging activity of extracts of corn-field bean pasta with the addition of xanthan gum towards DPPH in methanol.

Table 2: Activity of investigated samples of GF pasta with various addition of xanthan gum in relation to rutin's (0.5 mg/ mL; spot nr 5) activity.

Content of xanthan gum (%)	Activity in relation to rutin (Σ of areas under the common peak/area under rutin peak)			
	Time (min)			
	0	10	20	30
0.25	2.582 ± 0.019 ^a	2.578 ± 0.033 ^a	2.502 ± 0.022 ^a	2.450 ± 0.041 ^a
0.50	2.269 ± 0.022 ^b	2.264 ± 0.043 ^b	2.261 ± 0.011 ^b	2.251 ± 0.024 ^b
0.75	2.267 ± 0.031 ^b	2.249 ± 0.017 ^b	2.215 ± 0.041 ^b	2.201 ± 0.036 ^b
1.00	1.861 ± 0.081 ^c	1.831 ± 0.023 ^c	1.792 ± 0.023 ^c	1.805 ± 0.071 ^c

^{a-b} Means with the same superscript within columns are not significantly different ($p > 0.05$)

of xanthan gum, the antioxidant potential of pasta was significantly lower (Table 2). This result is conditioned by a lower content of polyphenols, including phenolic acids in the sample with the addition of 1.00% of xanthan gum.

It is worth mentioning that the interpretation of obtained results should include the influence of the time elapsing between the staining and documentation of changes observed on the chromatographic plates. The plates were scanned every 10 min for half an hour after the initiation of the reaction. Due to the fact that the bleaching of the plates was observed immediately after derivatization with DPPH (0 min, Table 2), the high antioxidant activity

of all extracts should be emphasized. The interpretation of obtained results revealed that the high ability to scavenge free radicals is strictly connected with the content of phenolic compounds. Additionally, the traditional process of pasta formulation proved to be appropriate for the production of high-quality functional food. According to Karkoucha et al. [24], faba seeds abound in pro-health phytochemicals, such as phenolic compounds that raise the pro-health properties of functional food. Similar studies were performed by Fernandez-Panchon et al. [25] and Luo et al. [26]. They found that the pro-health properties of field beans are strictly connected with the high level of antioxidants. Given this, maize-field bean pasta with the addition of xanthan gum can be a valuable source of natural antioxidant agents. Additionally, Karkouch et al. [24] underlined the beneficial role of the *Vicia faba* in the treatment several diseases such as Parkinson, hypertension, or renal failure.

3.3 Radical-scavenging activity of extracts

The data collected from the spectrophotometric analyses of the samples showed a high DPPH radicals scavenging potential in all pasta samples; yet, at a concentration of 1.00% of xanthan gum, the antiradical ability was slightly lower, which can probably be attributed to the strong binding of polyphenols on the surface of xanthan gum (Figure 2). In the case of the spectrophotometric analysis, the highest antioxidant activity of the tested samples was observed after 30 min from reaction initiation, but it is worth underlining that a satisfactory high ability to

Table 3: Quality parameters and thermal properties of gluten-free pasta with various addition of xanthan gum

Parameters	Xanthan gum levels (%)				
	0	0.25	0.50	0.75	1.00
CL (%)	17.86 ± 0.16 ^a	15.23 ± 0.13 ^b	14.00 ± 0.07 ^c	12.38 ± 0.15 ^d	10.67 ± 0.08 ^e
WAC (%)	246.36 ± 2.30 ^c	282.66 ± 1.55 ^b	327.46 ± 2.80 ^a	329.40 ± 1.60 ^a	329.88 ± 1.78 ^a
F (N)	321.00 ± 2.64 ^e	370.00 ± 2.08 ^d	408.66 ± 1.52 ^c	431.66 ± 2.88 ^b	456.41 ± 1.23 ^a
A (mJ)	25.25 ± 0.08 ^a	19.16 ± 0.05 ^b	15.50 ± 0.06 ^c	12.09 ± 0.04 ^d	10.38 ± 0.07 ^e
T ₀ (°C)	43.14 ± 0.11 ^e	46.72 ± 0.19 ^d	47.14 ± 0.09 ^c	49.38 ± 0.24 ^b	50.42 ± 0.13 ^a
T _p (°C)	90.31 ± 0.15 ^e	96.22 ± 0.15 ^d	97.13 ± 0.09 ^c	99.46 ± 0.14 ^b	100.02 ± 0.07 ^a
T _c (°C)	165.74 ± 0.10 ^e	168.44 ± 0.32 ^d	169.40 ± 0.32 ^c	170.71 ± 0.18 ^b	172.14 ± 0.10 ^a
T _r (°C)	122.60 ± 0.08 ^e	121.72 ± 0.10 ^d	122.26 ± 0.07 ^c	121.33 ± 0.34 ^b	121.72 ± 0.22 ^a
ΔH (J/g)	181.04 ± 0.11 ^e	198.57 ± 0.45 ^d	200.29 ± 0.16 ^c	211.13 ± 0.11 ^b	213.66 ± 0.46 ^a

CL: cooking loss, WAC: water absorption capacity, F: firmness, A: adhesiveness

^{a-e} Means with the same superscript within line are not significantly different ($p > 0.05$).

scavenge free radicals was observed immediately after the beginning of the measurement.

The outcomes of the research demonstrated that maize-field been pasta with the addition of xanthan gum contains a high level of phenolic acids and, therefore, has a great potential as a source of natural antioxidants. According to available data, the consumption of field beans is beneficial to human health. This is due to the complex mixture of health-related phytochemicals containing phenolic compounds and to their antioxidant effects [25, 26]. The study also confirms that legumes are excellent sources of dietary antioxidants capable of reducing risks of lifestyle and chronic diseases.

3.4 Cooking quality

For consumers of gluten-free pasta products, cooking loss (CL) and the water absorption capacity (WAC) are some of the key determinants of pasta quality [11]. The results of CL and WAC tests in the control pasta and samples with various levels of xanthan gum are shown in Table 3. A significant decrease ($p < 0.05$) was reported of cooking loss along with the increasing level of xanthan gum compared with the control pasta. The CL values decreased from 15.23% if 0.50% of the gum was applied to 10.67% for the gum level of 1.00%. The use of xanthan gum in the formulation reduced the leaching of pasta components into the cooking water. This is owing to the strong network formation between gum and starch, with a strong binding of starch granules on the surface of xanthan gum. Consequently, there is an improved cooking quality of

pasta in terms of resistance to disintegration and a reduced release of components during cooking, as suggested by Chauhan et al. [11] and Kaur et al. [27]. They confirmed the phenomenon of a significant decrease of the leaching of solids into the cooking water as the level xanthan gum in rice pasta increased. In addition, these observations are in line with a study by Yalcin and Basman [28] who reported a positive effect of rice noodles supplementation with xanthan gum and a significant decrease of CL indicating a good noodles quality. Susanna and Prabhasankar [29] also reported a decrease of CL when hydrocolloids (guar gum) were added to gluten-free pasta.

The degree of pasta hydration can be measured as the water absorption capacity index [11]. The WAC of all tested gluten-free pasta with various levels of xanthan gum was higher than in the control pasta (246.36%) and ranged from 282.66 to 329.88%. Insignificant ($p > 0.05$) variation was shown in the WAC of the tested pasta with a different amount of xanthan gum ranged from 0.50 to 1.00% (Table 3), but some significant differences were observed between the control pasta and when 0.25% of the gum was used in the recipe. The effect of gum use in the recipe might be the result of the hydrophilic behavior of gums in general as suggested by Kaur et al. [27] Yalcin and Basman [28] confirmed a higher water absorption in noodles supplemented with xanthan gum.

3.5 Textural characteristics

The textural properties of pasta are related to the ability to maintain consistency after cooking, and they are reported

as the most important and crucial quality aspects of pasta products [30, 31]. The results of the effect of hydrocolloid addition on the textural characteristics of gluten-free pasta containing different levels of xanthan gum and of the control pasta are presented in Table 3. The control pasta formulated with a maize and field bean flour blend showed lower firmness (321.00 N) than gluten-free pasta enriched with variable amounts of xanthan gum (ranged from 370.00 to 456.41 N). The use of gum revealed significant effects ($p < 0.05$) on selected textural properties of the tested pasta. Among all the tested gluten-free pasta, the samples with the addition of 1.00% of xanthan gum showed the highest firmness (456.41 N). The results gathered in our study showed the binding effect of water-soluble starch by hydrocolloids, which improved the texture of gluten-free pasta. Moreover, interactions between the proteins of field beans used in the formulation and gum may occur as a result of ionic charges and, consequently, enhance the pasta structure. These findings are aligned with a study by Kovacs and Vamos [32] and Raina et al. [33] who observed increased cohesiveness and a strong gluten network in rice pasta texture improved by the addition of hydrocolloids.

Adhesiveness, reflected in product adhesion to teeth during consumption, is an undesirable characteristic of pasta products [11]. Instrumental measurement of adhesiveness was done by the separation of the tested product from the Ottawa chambers surface. The control pasta showed the highest adhesiveness (25.25 mJ) compared with gluten free pasta (19.16, 15.50, 12.09 and 10.38 mJ) enriched with different levels of xanthan gum (0.25, 0.50, 0.75 and 1.00%, respectively). Significant differences were observed in adhesiveness when gum addition increased from 0.50 to 1.00% ($p < 0.05$). The addition of xanthan gum resulted in the creation of a continuous protein matrix resulting in a rigid protein network which prevents the excessive leaching of substance during cooking, which reduces pasta adhesiveness. Padalino et al. [34] similarly reported lower adhesiveness of gluten-free spaghetti if hydrocolloids were added.

3.6 Thermotropic characteristics of pasta

The differential scanning calorimetry (DSC) is useful in the examination of starch gelatinization as it detects the temperature of the different stages of this process. The onset temperature (T_o) indicates the beginning of the gelatinization process, and the peak temperature corresponds to the maximum heat flow (T_p), thus indicating the temperature of main phase transition, and

the endset temperature indicates the end of the process of gelatinization (T_c). The difference between endset and onset temperatures is the information about the length of the gelatinization process. The thermal parameters of the gelatinization process of pasta enriched with the different levels of xanthan gum and of the control pasta measured by the DSC are presented in Table 3. The obtained parameters significantly varied between the control sample and recipes with xanthan gum added in different quantities ($p < 0.05$). As shown in Table 3, the addition of hydrocolloid (xanthan gum) caused a significant increase of onset, endset and peak temperatures as the level of xanthan gum was rising from 0.25 to 1.00%. The obtained results also showed that the use of xanthan in the formulations raised the value of these parameters ($T_o = 50.42$, $T_c = 172.14$ and $T_p = 100.02^\circ\text{C}$ for pasta with 1.00% xanthan gum) as compared with the control (43.14, 165.74 and 90.31°C , respectively).

These results demonstrate that a limited level of solvent plasticization of the amorphous regions due to the addition of gum to the recipe raises gelatinization temperature and requires more heat energy for starch granule swelling and initiation of gelatinization [35]. Apart from the above, the increase in the fraction of gum in pasta alignment is accompanied by the increase in enthalpy as a characteristic of starch melting. Such a phenomenon may be explained by the positive association between hydrocolloids and starch, which results in the reduction of starch chain mobility followed by higher energy requirements for starch phase transition, as noted by Zhou et al. [36] and Larrosa et al. [35].

3.7 Pasta microstructure

Microscopic pictures of pasta products are shown in Figure 3. They represent the surface and cross-section of dry pasta. The application of xanthan gum as an additive improved the surface uniformity as compared with the control sample. It was observed that the increasing amount of gum had a positive effect on the surface structure, and the agglomerates of starch granules combined with xanthan gum can be easily observed (Figure 3a, c, e, g, for samples with 0.25, 0.50, 0.75 and 1.00% of xanthan gum, respectively). They are not present on the surface of the control pasta (Figure 3i). These more compact structures observed on the surface of pasta supplemented with xanthan gum confirm the results for the textural properties, such as increased hardness and lower adhesion (Table 3).

Figure 3: SEM pictures of the corn-field bean pasta surface and cross-section, respectively (magnification x600): a, b – pasta with 0.25 % of xanthan gum, c, d– pasta with 0.50 % of xanthan gum, e, f– pasta with 0.75 % of xanthan gum, g, h– pasta with 1.00 % of xanthan gum, i, j – control pasta.

The internal structure of the control sample, as shown in Figure 3j, exhibits singular starch granules with some empty holes inside the pasta tread. This can explain the easier leaching of pasta components during cooking and lower hardness of the control maize-field bean pasta. Field beans are protein-rich, so some part of starch granules was fixed inside the protein matrix (Figure 3j). The application of xanthan gum leads to the development of a more compact internal structure with some visible agglomerates of starch granules embedded in the fibrous protein-gum matrix, which was observed even if 0.25% of the gum was added (Figure 3b). The level of 0.50% of gum addition was enough to achieve a more dense and resistant structure of gluten-free pasta (Figure 3d) compared with that observed for the control sample. Similar observations about the effect of xanthan

gum were reported by Susanna and Prabhasankar [29]. They clearly showed starch molecules encapsulated within the xanthan network having an impact on reduced starch leaching during cooking. Increasing the amount of xanthan gum in the maize-field bean pasta recipe led to the development of a smoother and more consistent internal structure with partly swollen starch granules embedded inside the protein-hydrocolloid matrix. Only few unbonded components were observed in the cross-section pictures when 0.75 and 1.00% of xanthan gum was applied in the recipe (Figure 3f and 3h, respectively). Basically, xanthan gum plays a significant role in the formation of a stable structure of gluten-free pasta at least if added in the amount of 0.50% or more. But some negative effects of a higher amount of xanthan gum have

to be considered as it may lower the antioxidant activity of gluten free maize-field bean pasta products.

4 Conclusions

As pointed above, gluten intolerance is a major dietary problem and to develop new gluten-free products is a real challenge. As commonly known, maize is one of the fundamental gluten-free components used in bread, crisps, snacks, and pasta products. The studies discussed above address (i) a new and potentially much-demanded product: gluten-free maize-field bean pasta and (ii) the influence of the addition of xanthan gum on phenolic acids, the antioxidant activity and selected quality features of the said pasta. The results obtained revealed that the addition of 0.25, 0.50 and 0.75% of xanthan gum to the pasta did not have any significant impact on the polyphenols content, whereas 1.00% addition of xanthan gum significantly lowered the content of phenolic acids. The phenolic content in the studied pasta exhibited an antioxidant activity. In the case of the TLC-DPPH test, similarly to some previous findings, an increasing addition of xanthan gum to the studied pasta in the amount up to 0.75% did not significantly influence the free radical scavenging activity. Unfortunately, 1.00% addition of the aforementioned component caused a significant depletion of the antioxidant activity. Another important feature of pasta is its cooking quality and textural characteristics. The obtained results showed that the application of xanthan gum in the formulation reduced the leaching of components and lowered the CL. Moreover, gluten-free pasta prepared with the addition of 1.00% of xanthan gum showed the highest firmness. Increasing the amount of xanthan gum increased endothermic enthalpy compared with the control pasta. A microstructure analysis confirmed the formation of a more compact internal structure of gluten-free pasta with added xanthan gum, visible both in the surface and the cross-section, compared with the control pasta. Taking the above into account, a low addition of xanthan gum does not have a significant influence on the biological activity properties of pasta whereas the addition of 1 % can produce some disturbance in its healthy qualities.

Abbreviations

CL- cooking loss
DPPH- 2,2-diphenyl-1-picrylhydrazyl
DSC- differential scanning calorimetry

HPLC-ESI-MS/MS- high-performance liquid chromatography and electrospray ionization mass spectrometry

LC- liquid chromatography

SEM- scanning electron microscope

TLC- thin layer chromatography

UAE – ultrasound-assisted extraction

WAC- water absorption capacity

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References

- [1] Martínez M.L., Marín M.A., Gili R.D., Pencì M.C., Ribotta P.D., Effect of defatted almond flour on cooking, chemical and sensorial properties of gluten-free fresh pasta, *Int. J. Food Sci. Technol.*, 2017, 52, 2148-2155.
- [2] Petitot M., Abecassis J., Micard V., Structuring of pasta components during processing: impact on starch and protein digestibility and allergenicity, *Trends Food Sci. Technol.*, 2009, 20, 521-532.
- [3] Kaya E., Tuncel N.Y., Tuncel N.B., Utilization of lentil, pea, and faba bean hulls in Turkish noodle production, *J. Food Sci. Technol.*, 2018, 55, 1734-1745.
- [4] Oniszcuk A., Wójtowicz A., Oniszcuk T., Olech M., Nowak R., Wojtunik K., Klimek M., Krawczyk W., Hajnos M., Extruded corn gruels containing linden flowers: quantitation of phenolic compounds and selected quality characteristics, *Open Chem.*, 2015, 13, 1209-1217.
- [5] Oniszcuk A., Oniszcuk T., Wójtowicz A., Wojtunik K., Kwaśniewska A., Waksmundzka-Hajnos M., Radical scavenging activity of extruded corn gruels with addition of linden inflorescence, *Open Chem.*, 2015, 13, 1101-1107.
- [6] Wójtowicz A., Zalewska-Korona M., Jabłońska-Ryś E., Skalicka-Woźniak K., Oniszcuk A., Chemical characteristics and physical properties of functional snacks enriched with powdered tomato, *Polish J. Food Nutr. Sci.*, 2018, 68, 251-261.
- [7] Fabian C., Ju Y.H., A review on rice bran protein; its properties and extraction methods, *Crit. Rev. Food Sci. Nutr.*, 2011, 51, 816-827.
- [8] Phongthai S., D'Amico S., Schoenlechner R., Homthawornchoo W., Rawdkuen S., Effects of protein enrichment on the properties of rice flour based gluten-free pasta, *LWT-Food Sci. Technol.*, 2017, 80, 378-385.
- [9] Heo A., Jeon S., Lee S., Utilization of *Lentinus edodes* mushroom β -glucan to enhance the functional properties of gluten-free rice noodles, *LWT-Food Sci. Technol.*, 2014, 55, 627-631.
- [10] Sozer N., Rheological properties of rice pasta dough supplemented with proteins and gums, *Food Hydrocoll.*, 2009, 23, 849-855.
- [11] Chauhan A., Saxena D.C., Singh S., Effect of hydrocolloids on microstructure, texture and quality characteristics of gluten-free pasta, *Food Measure*, 2017, 11, 1188-1195.

- [12] Hayrabolulu H., Demeter M., Cutrubinis M., Güven O., Şen, M., Radiation induced degradation of xanthan gum in aqueous solution, *Radiat. Phys. Chem.*, 2018, 144, 189-193.
- [13] Göl Ş., Doğanlar S., Frary A., Relationship between geographical origin, seed size and genetic diversity in faba bean (*Vicia faba* L.) as revealed by SSR markers, *Mol. Genet. Genomics*, 2017, 292, 991-999.
- [14] Torres A.M., Avilan C.M., Gutierrez N., Palomino C., Moreno M.T., Cubero J.L., Marker assisted selection in faba bean (*Vicia faba* L.), *Field Crops Res.*, 2010, 3, 243-252.
- [15] Purves R.W., Khazaei H., Vandenberg A., Quantification of vicine and convicine in faba bean seeds using hydrophilic interaction liquid chromatography, *Food Chem.*, 2018, 240, 1137-1145.
- [16] Oniszczuk A., Olech M., Oniszczuk T., Wojtunik K., Wójtowicz A., Extraction methods, LC-ESI-MS/MS analysis of phenolic compounds and antiradical properties of functional food enriched with elderberry flowers or fruits, *Arab. J. Chem.*, 2016, (in press), DOI : <https://doi.org/10.1016/j.arabjc.2016.09.003>
- [17] Oniszczuk T., Oniszczuk A., Gondek E., Guz L., Puk K., Kocira A., et al., Active polyphenolic compounds, nutrients contents and antioxidant capacity of extruded fish feed containing purple coneflower (*Echinacea purpurea* (L.) Moench.), *Saudi J. Biol. Sci.*, 2019, 26, 24-30.
- [18] AACC International. Approved Methods of the American Association of Cereal Chemistry, In: 11th Ed. Methods 66-50, Pasta and Noodle Cooking Quality- Firmness, AACC International: Saint Paul, 2000.
- [19] Chillo S., Laverse J., Falcone P., Del Nobile M., Effect of carboxymethylcellulose and pregelatinized corn starch on the quality of amaranthus spaghetti, *J. Food Eng.*, 2007, 83, 492-500.
- [20] Wójtowicz A., Mościcki L., Influence of legume type and addition level on quality characteristics, texture and microstructure of enriched precooked pasta, *LWT-Food Sci. Technol.*, 2014, 59, 1175-1185.
- [21] Martínez C., Ribotta P., Leon A., Anon C., Physical, sensory and chemical evaluation of cooked spaghetti, *J. Texture Stud.*, 2007, 38, 666-683.
- [22] Bouasla A., Wójtowicz A., Zidoune M.N., Olech M., Nowak R., Mitrus M., et al., Gluten-free precooked rice-yellow pea pasta: effect of extrusion-cooking conditions on phenolic acids composition, selected properties and microstructure, *J. Food Sci.*, 2016, 81, 1070-1079.
- [23] Abu-Reidah I.M., del Mar Contreras M., Arráez-Román D., Fernández-Gutiérrez A., Segura-Carretero A., UHPLC-ESI-QTOF-MS-based metabolic profiling of *Vicia faba* L. (*Fabaceae*) seeds as a key strategy for characterization in foodomics, *Electrophoresis*, 2014, 35, 1571-1581.
- [24] Karkouch K., Tabbene O., Gharbi D., Mlouka M.A.B., Elkahoui S., Rihouey C., et al., Antioxidant, antityrosinase and antibiofilm activities of synthesized peptides derived from *Vicia faba* protein hydrolysate: A powerful agents in cosmetic application, *Ind. Crops Prod.*, 2017, 109, 310-319.
- [25] Fernandez-Panchon M.S., Villano D., Troncoso A.M., Garcia-Parrilla M.C., Antioxidant activity of phenolic compounds: from *in vitro* results to *in vivo* evidence, *Crit. Rev. Food Sci. Nutr.*, 2008, 48, 649-671.
- [26] Luo Y., Li J., Xu C., Hao Z., Jin X., Wang Q., Impact of processing on *in vitro* bioavailability of phenols and flavonoids and antioxidant activities in faba bean (*Vicia faba* L.) and azuki bean (*Vigna angularis* L.), *Legume Res.*, 2014, 37, 492-499.
- [27] Kaur N., Sharmal S., Yadav D.N., Bobadel H., Singh B., Quality characterization of brown rice pasta supplemented with vital gluten and hydrocolloids, *Agric. Res.*, 2017, 6, 185-194.
- [28] Yalcin S., Basman A., Effect of gelatinization level, gum and transglutaminase on the quality characteristics of rice noodles, *Int. J. Food Sci. Technol.*, 2008, 43, 1637-1644.
- [29] Susanna S., Prabhaskar P., A study on development of gluten free pasta and its biochemical and immunological validation, *LWT-Food Sci. Technol.*, 2013, 50, 613-621.
- [30] Larrosa V.J., Lorenzo G., Zaritzky N.E., Califano, A.N, Response surface methodology to assay the effect of the addition of proteins and hydrocolloids on the water mobility of gluten-free pasta formulations, In: Gutiérrez-López G., Alamilla-Beltrán L., del Pilar Buera M., Welti-Chanes J., Parada-Arias E., Barbosa-Cánovas G., (Eds.), *Water Stress in Biological, Chemical, Pharmaceutical and Food Systems. Food Engineering Series*, Springer, 2015.
- [31] Bruneel C., Pareyt B., Brijs K., Delcour J.A., The impact of the protein network on the pasting and cooking properties of dry pasta products, *Food Chem.*, 2010, 120, 371-378.
- [32] Kovacs E., Vamos E., Changes of the composition of protein fractions in wheat dough systems made with and without emulsifiers, *Mitt. Gebiete Lebensm Hyg.*, 1993, 84, 630-639.
- [33] Raina C.S., Singh S., Bawa A.S, Saxena D.C., Textural characteristics of pasta made from rice flour supplemented with proteins and hydrocolloids, *J. Texture Stud.*, 2005, 36, 402-420.
- [34] Padalino L., Mastromaeo M., De Vita P., Ficco D., Del Nobile M., Effects of hydrocolloids on chemical properties and cooking quality of gluten-free spaghetti, *Int. J. Food Sci. Technol.*, 2013, 48, 972-983.
- [35] Larrosa V., Lorenzo G., Zaritzky N., Califano A., Improvement of the texture and quality of cooked gluten-free pasta, *LWT - Food Sci. Technol.*, 2016, 70, 96-103.
- [36] Zhou Y., Wang D., Zhang L., Du X., Zhou X., Effect of polysaccharides on gelatinization and retrogradation of wheat starch, *Food Hydrocoll.*, 2008, 22, 505-512.