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OPTIMIZATION OF RICE-FIELD BEAN GLUTEN-FREE PASTA IMPROVED BY THE ADDITION OF HYDROTHERMALLY TREATED RICE FLOUR

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ABSTRACT

Rice and field bean semolina was used to obtain protein- and fiber-enriched gluten-free pasta. The tests covered the effect of pre-gelatinized rice flour used as a gluten-free pasta improver. A central composite design was applied involving the water hydration level and pre-treated rice flour level. Instrumental analyses of pasta (cooking loss, water absorption capacity, texture, hydration index, pasting and thermal properties, and microstructure) were carried out to assess the impact of experimental factors. The results showed that the application of hydrothermally treated rice flour improved the cooking and textural characteristics of pasta. The optimum recipe contained 5.845 g of pregelatinized rice flour and 59.266 mL of water, both selected based on the desirability function approach with the value of 0.775 corresponding to the optimum pasta properties.

Keywords: central composite design, gluten-free, pasta making, quality, starch, texture

1. INTRODUCTION

Semolina from durum wheat is a preferred substrate for the manufacture of pasta products. The quality characteristics of pasta such as texture, cooking properties, color, and sensory properties are under the influence of several conditions (SISSONS, 2008; PETITOT *et al.*, 2010). Both raw material quality and technological process have a major impact on the final product quality (DE NONI and PAGANI, 2010). Gluten protein plays an important role in determining the quality of pasta. Wheat semolina pasta products are required to exhibit the best quality (MARTI and PAGANI, 2013).

However, wheat pasta is not recommended for people with celiac disease because their immune system has a disorder in which the sentinel lesion is on enteropathy triggered by the ingestion of gluten proteins (LEFFLER *et al.*, 2017). It is known that the only possible therapy is a lifetime gluten-free diet, during which the damage to the intestinal mucosa regresses and the patient's well-being improves considerably. To ensure the manufacture of proper quality gluten-free (GF) foodstuffs, the proposed formula must be of such quality characteristics that resemble conventional pasta.

The degree of difficulty in producing GF products is closely associated with the technological role of gluten in the food-system (MARTI and PAGANI, 2013; LARROSA *et al.*, 2016). Although the demand for better-tasting, better-textured, and healthier GF products offers great market opportunities for food manufacturers, the replacement of gluten functionality still presents a major technological challenge (CHILLO *et al.*, 2007; SOZER, 2009; LOUBES *et al.*, 2016). Having that in mind, pre-treated flour could be used as the improving agents and key components to offer the effect of gluten in GF pasta (MARTI and PAGANI, 2013).

Moreover, improvement of the functional properties of starches is an important step in the manufacturing of GF pasta. To achieve this objective, natural treatment of flour through the passage of heating and cooling cycles can be applied. This passage facilitates the forming of a rigid network based on retrograded starch, which gives the dough a suitable texture (CHILLO *et al.*, 2009; MARTI *et al.*, 2010; CABRERA-CHÁVEZ *et al.*, 2012). The change in the organization of starch molecules by the application of physical and thermal treatment has been widely approved as a natural material with high food safety. The aim of this treatment is to promote the functional properties of starch without destroying its granular structure and, thus, lift any legislative limits as to its quantity in food (BEMILLER, 1997; ZAVAREZE and DIAS, 2011). The dominating properties of pregelatinized flour resulting from starch modifications under different treatments are associated with the re-organization of starch molecules in GF dough means better quality characteristics of the final product compared with those in durum semolina pasta.

In addition, pre-gelatinized rice flour was used as a major component and bulking or thickening agent in several food preparations (MOORE *et al.*, 1984; LAI and CHENG, 2004). Besides, as demonstrated by YOENYONGBUDDHAGAL and NOOMHORN (2002), the thermal treatment of rice flour improves the quality characteristics of rice noodles. HORMDOK and NOOMHORM (2007) applied the HMT (heat-moisture treatment) and the ANN (annealing) of rice starch for noodle preparation and obtained rice noodles with the desired features.

In many countries, celiac patients suffer from the lack of GF products, which makes it difficult for them to follow the recommended diet. One of the methods to improve the situation of celiac patients is to design new gluten-free products (GIUBERTI *et al.*, 2016; BOUREKOUA *et al.*, 2016). Also, as GF products are less available and less diverse, as well as being more expensive than gluten-rich food products, there is a strong urge to develop GF products that are technologically enhanced as well as economically sustainable. In

addition, there are fewer studies and information about the manufacture of traditional laminated pasta and its acceptability by consumers. Moreover, the supplementation of rice-based laminated pasta with leguminous is envisaged in order to improve the nutritional value of the proposed formulation (GIUBERTI *et al.*, 2016) and enhance the machinability of dough as well as the final quality of pasta products. Field bean is part of the leguminous group which is widely produced and consumed in the Mediterranean area and has an excellent potential to be used in ever new recipes because of its high nutritional quality. This plant is a rich source of fiber and protein and supplements the protein intake from cereals and tubers for a more beneficial amino acid balance (MICARD *et al.*, 2010; BENATALLAH *et al.*, 2012; CARACCIOLO *et al.*, 2016). Also, proteins of field bean have such functional properties as solubility, water retention capacity, foaming capacity as well as producing an emulsifying effect that plays an important role in food formulation and processing (DAKIA *et al.*, 2007; ROY *et al.*, 2010; BOYE *et al.*, 2010).

In addition, the need to achieve the appropriate quality and the possibility of production of more natural products for consumers with celiac disease are strong arguments behind the idea of a new formula of GF rice-field bean laminated pasta. For these reasons, the application of natural hydrothermal treatment of rice flour has been proposed.

The main objective of this study was to investigate the effect of the addition of hydrothermally treated rice flour as an improver in the manufacture of GF laminated pasta from rice supplemented with field bean semolina (RFBS). The optimization of water and pre-gelatinized rice flour (PGRF) levels needed to ensure the improvement of selected properties of GF pasta products was done by means of the response surface methodology (RSM) and following the desirability function approach (DFA).

2. MATERIALS AND METHODS

2.1. Raw materials

Cereal and leguminous semolina used for making GF laminated pasta was of rice and field bean. Rice (*Oryza sativa*) semolina was provided by Lubella Sp. z o. o. S.K. (Lublin, Poland). Field bean dehulled seeds (*Vicia faba minor*) were applied as supplement for improvement of the protein content and amino acids balance in GF pasta. Field bean seeds were purchased from Alamir Company (Albehera, Egypt) and ground. All semolina used in GF laminated pasta had particle sizes between 200 and 500 μ m. Rice flour (Lubella Sp. z o. o. S.K., Lublin, Poland) with particles smaller than 200 μ m was used for pregelatinization.

2.1.1 Chemical composition analysis

The raw materials and pasta samples were analyzed for fat, ash, and protein content using the standard procedures of the AACC methods (1995). Fat content was determined by Soxhlet extraction with n-hexane (AACC 30-10), ash by incineration in a muffle furnace at 550°C (AACC 08-01), the Kjeldahl procedure (AACC 46-10) was used for determining the protein content in three replications. Fiber was determined with the Scharrer-Kürschner method using the procedure of AOAC (2000). The method involved the acid digestion of the sample for the elimination of nutritive components; it was then flushed with ethyl alcohol and dried to dry matter. Based on that, the amount of fiber was calculated (AOAC 993.21). The measurements were performed in duplicate.

2.1.2 Hydrothermal treatment of rice flour

The heat treatment process of flour was performed according to the TangZhong method described by YVONNE (2007) and BOUREKOUA *et al.* (2016). Pre-gelatinized rice flour (PGRF) was made by mixing water and flour (1 to 5) in an aluminum pan. The mixture was then heated on a heating plate for 8-9 min and continuously stirred with a spatula until the temperature reached 65°C. Afterwards, the obtained paste was cooled down at ambient temperature for 1 h and stored in a fridge (4°C) for 24 h. Some preliminary experiments were made in a laboratory and indicated that the cooling of the slurries for 24 h at 4°C produces better cooking and textural properties when added to the pasta formula than PGRF kept for 1 h or used immediately after heating. Pre-gelatinized rice flour was added to pasta made with rice-field bean semolina by mixing with other ingredients of the formula after keeping at ambient temperature for 1 h.

2.2. Experimental design

A central composite design (CCD) was used, involving water hydration level (X_i) and pregelatinized rice flour (stored in a fridge for 24 h) level (X_i). The effects of two variables on the quality characteristics of laminated pasta were analyzed. The hydration range applied in the experimental design was determined by preliminary experiments using from 46.42 to 72.12 mL of water for 100 g of the recipe (46.42 mL/100 g is the minimum level of water necessary to make dough and 72.12 mL/100 g is the maximum level of water for obtaining sticky pasta). The level of pre-gelatinized rice flour was fixed from 0 to 11.70 g for 100 g of recipe based on the calculation of the water level added in each run. These values were incorporated into JMP software to determine the CCD matrix.

The optimization of the studied formula was carried out using the RSM. The factorial section is a 2^{2} test; the star section includes four tests. Five replicates (runs 1, 4, 5, 10, 11, Table 1) at the center of the design were used to estimate the pure error at the sum of square, for a total of $2^{2}+2^{2}+5=13$ runs.

Run	Hydration X ₁ (mL)		PGRF X ₂ (g)	
	Code	Value	Code	Value
1	0	59.26	0	5.85
2	-1	50.18	1	9.98
3	0	59.26	1.414	11.7
4	0	59.26	0	5.85
5	0	59.26	0	5.85
6	-1	50.18	-1	1.71
7	1	68.35	1	9.98
8	0	59.26	-1.414	0
9	-1.414	46.42	0	5.85
10	0	59.26	0	5.85
11	0	59.26	0	5.85
12	1	68.35	-1	1.71
13	1.414	72.12	0	5.85

Table 1. Factors, levels and code values used in the Central Composite Design (CCD) for gluten-free rice-field bean semolina (RFBS) pasta.

A statistical analysis was performed for the experimental data for each response variable in order to select an optimized recipe using the desirability function approach (DFA). The DFA is a method of multi-criteria optimization showing some relationships between several responses. The desirability factor (D) varied from 0 to 1, where 1 meant a maximum satisfaction and 0 was complete refusal (LARROSA *et al.*, 2016; BOUREKOUA *et al.*, 2016).

2.3. Pasta making

According to the previous research and recommendations (FAO, 1982; MICARD, 2010), the optimum combination of cereal and legume required to achieve an excellent nutritional balance is 65% of cereal and 35% of legume. The GF formulation (rice-field bean semolina: RFBS) studied in this work was based on a mixture in a ratio of 2:1 (w/w) of cereal and leguminous semolina in order to ensure a technological approach to processing and enable good machinability of laminated rice-based dough as well as providing a good amino acids balance (BENATALLAH *et al.*, 2012).

The hydration range applied in the experimental design was determined by preliminary experiments (46.42 to 72.12 mL for 100 g of recipe). The level of pre-gelatinized rice flour was fixed up to 11.70 g for 100 g of the recipe. Hydration and PGRF levels were expressed based on the RFBS blend. The basic recipe of RFBS consisted of 66.66 g of rice semolina, 33.33 g of field bean semolina, 2 g of salt and the amount of distilled water defined in the experimental design data. Pasta produced without the addition of PGRF (run 8) was considered as control pasta (Table 1).

The optimum recipe was determined according to the desirability method performed by JMP software version 7 (SAS, USA). The selected optimum pasta was also prepared according to the procedure adapted both for traditional and industrial production.

In the first step, the ingredients were mixed for 15 min at 25°C using a KitchenAid mixer, model kPM5 (St. Joseph, Michigan, USA). The obtained dough was rounded, divided into balls of 50 g and covered with a sealed plastic wrap and then rested at 25°C for 1 h to let the starch hydrate. The dough was molded and passed through the reduction rolls of a pasta machine Marcato Ampia type 150 (Campodarsego, Italy) for four times from each pass and in all directions to produce a uniform dough sheet. The roller gap was maintained at 5 for the last sheeting. The final thickness of each dough sheet was 1.5 mm as determined with a caliper. Finally, pasta samples were dried in an oven with air circulation at 40°C for 4 h until it reached the final moisture content below 12%. They were stored in sealed plastic bags at room temperature.

2.4. Determination of pasta quality

2.4.1 Cooking quality

The cooking quality was determined according to the Approved Method 66-50 (AACC, 2000) with the modifications proposed by CHILLO *et al.*, (2007). In brief, 10 g of dried pasta was boiled in 300 mL of distilled water for an optimal cooking time (OCT), dripped for 3 min and weighed. The cooking water was evaporated by drying at 105°C overnight. The residue was weighed and the cooking loss (CL) was reported as a percentage of dry sample weight before cooking. The water absorption capacity (WAC) was expressed as a percentage increase of pasta weight after cooking compared with the weight of uncooked pasta. The measurements were performed in triplicate.

2.4.2 Hydration properties

The hydration properties of the optimum pasta and control sample were determined at ambient temperature designating the water absorption index (WAI), water solubility index (WSI), and swelling power (SP). Ground pasta samples (0.7 g) were mixed in centrifugal tubes with 7 mL of distilled water. After 5 min of rest, the samples were hydrated for 10 min and mixed every minute for uniform reconstitution, followed by 10 min, 21°C) Centrifuge centrifugation (15000 rpm, in T24 (VEB MLW MEDIZINETECHNIK, Leipzig, Germany). The supernatant was dried to constant weight at 105°C. The tests were carried out in four replications. The WAI and WSI calculations were made according to the method described by WÓJTOWICZ and MOŚCICKI (2014). SP was calculated as proposed by LAI and CHENG (2004).

2.4.3 Textural characteristics

The texture characteristics of pasta were achieved using the universal testing machine Zwick/Roell BDO-FB0.5 TH (Zwick GmbH& Co., Ulm, Germany). The cutting force (N) of single cooked strand of the optimum and control pasta was evaluated in five independent replications with a Warner-Bratzler blade (60 mm long, 3 mm thick, double face truncated at 45°), as described by WÓJTOWICZ and MOŚCICKI (2014). The measurements were carried out with a test speed of 8.33 mm/s using a 0.5 kN working head.

OTMS Ottawa cell was used for the evaluation of firmness, stickiness and cohesiveness of cooked pasta samples from each run of experimental design and for the optimum pasta properties (MARTINEZ *et al.*, 2007). For firmness (F) (N), stickiness (S) (mJ) and cohesiveness (mJ) of cooked pasta products, a double-compression test was applied. 50 g of cooked and drained pasta was put in the OTMS 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.

2.4.4 Pasting properties

The pasting performance of hydrothermally treated slurries were determined after 1 h of cooling at room temperature and after 24 h storage at 4°C. The properties were adjusted according to BOUASLA *et al.* (2017) using a Brabender Micro-Visco AmyloGraph (Brabender OHG, Duisburg, Germany) under the constant measurement conditions: speed 250 rpm, sensitivity 235 cmg, and heating rate 7.5°C/min. The following characteristics were considered: pasting temperature (°C), temperature at the beginning of viscosity increase; initial viscosity (mPas), cold viscosity at 30°C; peak viscosity (PV) (mPas), the highest viscosity during the heating cycle; breakdown (BD) (mPas), corresponding to the difference between the PV and the viscosity at the end of the holding period at 93°C and an index of viscosity minus the viscosity at the end of the holding period at 93°C and an index of viscosity increase during the cooling cycle; final viscosity (mPas), i.e. viscosity reached at the end of the cooling period.

2.4.5 Thermal characteristics

For calorimetric measurements, the differential scanning calorimeter DSC Star System (Mettler Toledo AG, Greifensee, Switzerland) was used. The calibration of the instrument was done by means of the indium standard before the evaluation of the sample. All the measurements were performed under nitrogen gas atmosphere. The instrument was

controlled with Star system. Ground samples of dried optimum and control pasta (3-5 mg) with a granulation below 250 μ m were precisely weighed in aluminum crucibles with a pin (volume 40 μ L). The sample-encapsulating press was used for the sealing of powder-filled pans. The samples were thermally inserted into the instrument, equilibrated at 25°C for 10 min and then heated at the rate of 10°C/min up to 180°C. An empty aluminum pan was used as the reference in all recorded thermograms. During the measurement, the temperature was controlled with an accuracy of ± 0.1 °C by means of the high precision thermoregulation system TC 100 MT (Peter Huber Kältemaschinenbau AG, Germany) (BOUREKOUA *et al.*, 2017).

Heat flow during the whole process of sample heating was recorded. The following features of thermogram were analyzed: onset temperature (T_o) , peak temperature corresponding to the maximum heat flow (T_o) and endset temperature (T_o) . In order to compare the length of the transition, the difference between endset and onset temperatures $(T_c = T_c - T_o)$ was calculated. The gelatinization enthalpy (ΔH) expressed per weight of dry sample (J/g) was assessed by integrating the area between the thermogram and the baseline under the peak. All thermal parameters were calculated using the evaluation mode of the Star system. Thermal scans were performed in three independent replicates.

2.5. Sensory evaluation

The sensory assessment was carried out on the optimal selected pasta and control sample. Pasta products were cooked in the optimum time and drained and placed in warm conditions until testing. The samples were served in a random order on a white ceramic plate to a panel of 55 untrained consumers (25-45 years old, 26 females and 29 males) who were the habitual consumers of pasta and were familiar with the definitions and references. They evaluated the products for appearance, taste, flavor, consistency, and stickiness on a 5-point scale (1 = poor, 5 = good) (WÓJTOWICZ and MOŚCICKI, 2014). For the preliminary tests of taste, five independent sessions were organized; each session involved a panel of 11 untrained consumers who were familiar with the terminology related to pasta (ISO 11036). For the final analysis, a single session was performed with the same pasta cooking conditions involving the presentation of the sample and specific environmental conditions. The results and observations of final sensory analysis were taken into account.

The overall acceptability was evaluated by the same panel, and each pasta sample was assessed using a verbal nine-point hedonic scale. The ratings were converted into numerical scores, where 1 was dislike extremely, 5 neither like, nor dislike, and 9 as like extremely. GF pasta was considered acceptable if the mean score for the overall quality was above 5 (WÓJTOWICZ and MOŚCICKI, 2014; BOUASLA *et al.*, 2017).

2.6. Pasta microstructure

The pictures of dry optimum and control pasta were taken using a scanning electronic microscope (SEM). A piece of dry pasta, attached to a carbon disc with a silver tape was sprayed with gold in the K-550X vacuum sublimator (Emitech, Ashford, England). The electron microscope VEGA LMU (Tescan, Warrendale, USA) was used to observe the surface and cross-section of the samples at different magnifications (×200, ×600 for surface, and ×200, ×600, ×1500 for cross-sections). The accelerating voltage of 30 kV was applied.

2.7. Statistical analysis

A second order complete polynomial equation was applied to fit the behavior of each measured variable as a function of dough composition (MYERS *et al.*, 2009; LARROSA *et al.*, 2016) by using the JMP software version 7 (SAS, USA). The models were used to determine response surfaces in Statistica, version 10 (StatSoft. Inc., USA). A one-way ANOVA analysis of variance was employed for the assessment of the effect of water (X_1) and pre-gelatinized rice flour (X_2) on dependent variables (Y) with a 0.05 significance level. The model proposed for each response was:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{11} X_1 X_1 + b_{22} X_2 X_{21} b_{12} X_1 X_2$$

where: b_0 is the value of the fitted response at the center point of design, that is (0,0); b_1 and b_2 are the linear regression terms; b_{11} , b_{22} are the quadratic regression terms; and b_{12} is the cross-product regression term (interaction coefficients). Optimization was performed with JMP, version 7 to find the best formula of GF rice-field bean pasta with the DFA method. To compare the measured parameters (cooking and textural parameters, hydration index, pasting and thermal properties) between the optimized recipe and the control pasta, the results were analyzed with the ANOVA test at the level of significance of p<0.05 followed by Fisher's LSD using Statistica 10.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

Table 2 shows the chemical composition of the raw materials and pasta samples. As expected, protein, fat, ash, and fiber increased significantly with the addition of field bean to rice-based pasta (p<0.05) because of the leguminous supplementation of rice flour (GIMENEZ *et al.*, 2012; GIUBERTI *et al.*, 2015; GIUBERTI *et al.*, 2016).

Table 2. Proximate composition of raw materials and gluten-free pasta samples (g/100 g).

	Protein	Fat	Ash	Fiber
Rice	6.72±0.02 ^c	0.14±0.02 ^c	0.42±0.026 ^c	1.24±0.03 ^c
Field bean	31.40±0.52 ^a	1.38±0.07 ^a	2.53±0.12 ^ª	10.88±0.18 ^ª
Rice pasta	6.90±0.02 ^c	0.13±0.04 ^c	0.37±0.05 ^c	1.31±0.03 ^c
Rice-field bean pasta	14.92±0.03 ^b	0.61±0.03 ^b	1.15±0.05 ^b	4.31±0.10 ^b

^{ac}Values followed by the same letter in the same column are not significantly different (p=0.05).

The enrichment of our formula based on rice with field bean semolina causes an increase of the protein content by 116% and the fiber level in the recipe by 229%. Celiac consumers have a low intake of dietary fiber (BOUASLA *et al.*, 2017), so the proposed formula could be beneficial if having an increased fiber content. Legumes supply an important dose of dietary fiber and protein with a good amino acids balance, and the supplementation of cereals with legumes could enhance the nutritional value of the final product.

3.1.1 Pasting performance of PGRF

The pasting properties of PGRF were investigated by the measurements through a viscoamylograph test. The viscograms of raw flour and PGRF were analyzed for comparison as shown in Fig. 1.



Figure 1. Pasting profile of native rice flour, and PGRF after 1h and 24 h of preparation and storage. Legend: (—) temperature profile; (—) rice flour; (—) treated rice 24 h; (- - - -) treated rice 1 h.

The results showed that changes in viscosity depended on the treatment conditions. The Brabender viscograms showed an increase in the IV of pre-gelatinized rice flour after 1 h and 24 h of preparation (54 mPas, 52 mPas, respectively) as a confirmation of partial gelatinization of starch under a hydrothermal treatment compared to untreated rice flour (17 mPas). The high IV for PGRF was attributed to a modification in the molecular organization of the starch during treatment, which resulted in the loss of granule integrity and breaking of the order of crystallinity of starch (LAI and CHENG, 2004; MARTI et al., 2013). The hydrothermal treatment of rice flour exhibited a significant lowering of PV for both treatments (370 mPas and 407 mPas, respectively, for treated rice for 1 h and 24 h of storage) compared to the raw material (606 mPas). The decrease in PV might be related to the limited swelling capacity and its effect was the increase in PT (78.2°C and 78.1°C, respectively, for treated rice flour for 1 h and 24 h) compared to untreated rice flour (70°C). This corresponds with the increased temperature needed to gelatinize undamaged starch granules, as found by HORMDOK and NOOMHORM (2007). Moreover, the hydrothermal process induced the viscosity reduction during the cooling stage; the same behavior was observed in several studies (ADEBOWALE et al., 2005; HOOVER and VASANTHAN, 1994; JACOBS et al., 1995; STUTE, 1992; BOUREKOUA et al., 2016). After treatment, the progression and continuation of retrogradation of the starch molecules during storage will take place, which leads to a significant (p<0.05) increase in the viscosity of treated rice flour 24 h after the beginning of storage (827 mPas) compared to treated rice flour after 1 h (655 mPas). This phase is commonly described as the setback region and is related to the retrogradation and reordering of starch molecules. All these observations indicated that a

hydrothermal treatment may affect the granule rigidity and starch molecular reassociation. Therefore, various treatment conditions applied would give the possibility to obtain a different form of pre-gelatinized flour with different pasting properties that should be used for various applications.

3.2. The effect of pre-gelatinized rice flour on the laminated GF pasta quality

3.2.1 Verification of the fitted models

For each model, linear, quadratic and interaction effects were calculated. Regression coefficients are shown in Table 3. Adequacy of the model was verified by estimating the coefficient of determination (R^2) and the lack-of-fit test.

 Table 3. Regression coefficients for the predictive models for cooking loss, water absorption capacity, firmness and stickiness.

Model term	CL (%)	WAC (%)	F (N)	S (mJ)
X ₁	-0.3115	+0.0829	-0.0073*	-0.0086*
X ₂	-0.1631	-0.0755	-0.2998	-0.0028*
X ₁ X ₁	+0.4966	+0.4503	-0.0280*	+0.1112
X ₂ X ₂	+0.0056*	-0.0323*	-0.0192*	+0.0009*
X ₁ X ₂	+0.6541	+0.7510	+0.6851	-0.0149*
Lack of fit	NS	NS	NS	NS
F	3.87 ^{NS}	3.39 ^{NS}	6.05 ^{NS}	14.96 ^{NS}
R ²	0.73	0.71	0.81	0.91

CL: cooking loss, WAC: water absorption capacity, F: firmness, S: stickiness, X_1 : water, X_2 : PGRF, X_1X_1 , X_2X_2 : quadratic coefficients, X_1X_2 : interaction between coefficients,

 \hat{F} : variance Ficher-Snedecor, R: coefficient of determination,

^{NS}: not significant (p>0.05),

* significant at $p \le 0.05$.

The statistical analysis proved that the fitting models were adequate because they yielded satisfactory values of R², and the lack-of-fit test was not significant for all responses. If the values of a predicted model are not significantly different from real values, and the lack-of-fit is not significant, the model is adequate (GOUPY, 2013). The results of the discussed experiment confirmed that the model was adequate to envisage the responses of CL, WAC, F and S.

3.2.2 Cooking quality

The CL and WAC represent the crucial parameters of the cooking quality of pasta. The effect of a range of water and PGRF on these responses was shown on Fig. 2A and 2B, respectively. The CL of pasta samples ranged from 9.35 to 14.26%. The CL decreased with an increased addition of PGRF up to the incorporation level of 7 g/100 g, which raised the maximum incorporation as its quadratic effect was positive (p<0.05, Table 3). Moreover, the chart (Fig. 2A) and the data in Table 3 demonstrate the absence of the linear and quadratic effect of water on the value of CL.

As reported in a paper presented by BOUASLA *et al.* (2017), a cooking loss below 10% indicates good quality pasta. Moreover, MARTI *et al.* (2013) reported a cooking loss for pasta ranging between 3.5 and 11.3%; CHAM and SUWANNAPORN (2010) found the CL of rice noodles varying from 6.5 to 10.25%; and BOUASLA *et al.* (2017) presented the range of 3.5 - 5.93% of the CL for extruded GF pasta. According to these values, it could be concluded that pasta with an addition of PGRF has sufficient cooking quality as a GF product.



Figure 2. Response surface of CL (A) and WAC (B) of gluten-free RFBS pasta depend on hydration level and PGRF amount.

As starch is the decisive component of GF pasta, its applicability can be noticeably enhanced by a convenient re-organization (BEMILLER, 1997). The cooking quality of pasta could be improved by the addition of treated flour containing an appropriate amount of modified starch (HORMDOK and NOOMHORM, 2007). The hydrothermal treatment of rice flour discussed in this study probably leads to the formation of a permanent and solid network of retrograded starch within gelatinized starch, which may produce the effect of lesser solubilization of amyloceous components and, therefore, results in a less cooking loss, as found also by RESMINI and PAGANI (1983). Recently, MARTI and PAGANI (2013) announced that the incorporation of 50% of treated rice flour improved the characteristics of pasta in terms of cooking properties. As hypothesized by the above authors, pre-gelatinized flour could perform the role of a binding agent leading to the establishment of a new network around starch granules, thus increasing their resistance to cooking stress, as suggested by PAGANI (1986).

The WAC of RFBS laminated pasta ranged from 215.83 to 255.00% (Fig. 2B). The WAC grew with an increasing addition of PGRF up to the incorporation level of 6 g/100 g, which decreased with the maximum incorporation as its negative quadratic effect (p<0.05, Table 3). In addition, the chart (Fig. 2B) showed that the WAC of the samples increased with an increasing amount of water.

The WAC is a primordial measured parameter, which depends on the fragments of damaged starch and the fragility of its granules (BOUASLA *et al.*, 2017). The observed water uptake dynamics suggests that hydrothermal treatment conditions of rice flour in our study might possibly promote a less hydrophilic structure of starch resulting in low

water absorption, as suggested by MARTI *et al.* (2013). In addition, HORMDOK and NOOMHORM (2007) reported that rice starches thermally treated by both HMT and ANN slightly reduced the WAC of composite noodles. Moreover, the low WAC indicates a poor quality of cooked pasta due to chewy texture (WANG *et al.*, 2012).

3.2.3 Textural characteristics

The textural properties of pasta are related to the ability to maintain consistency after cooking (LARROSA *et al.*, 2016). The response surface obtained for the texture measurement showed that firmness ranged from 216.50 to 344.72 N (Fig. 3A). The chart showed an increased firmness of samples along with the increasing amount of PGRF up to the incorporation level of 7 g/100 g, which decreased with the maximum incorporation as its negative quadratic effect (p<0.05, Table 3). However, the chart (Fig. 3A) showed a decreased firmness of samples with the increased amount of water as its negative linear and quadratic effect (p<0.05, Table 3).

As demonstrated by the results of BOUASLA *et al.* (2017), firmness of extruded gluten-free pasta supplemented with legumes ranged between 199.5 and 326.5 N. MARTI *et al.* (2013) reported that firmness of rice pasta ranged from 188 to 902 N., ZHAO *et al.* (2005) regarded firmness as work required to shear 4 cooked strands of spaghetti that ranged between 5.72 to 8.64 g/cm. The firmness characteristic of pasta can be evaluated with various methods, so the results are difficult to compare.



Figure 3. Response surface of firmness (A) and stickiness (B) of gluten-free RFBS pasta depend on hydration level and PGRF amount.

The response surface in Fig. 3B for RFBS pasta showed the stickiness values varying from 9.10 to 25.65 mJ. The chart demonstrated decreased stickiness of samples as the amount of PGRF and water increased. Moreover, the addition of treated rice represents a negative linear effect, a positive quadratic effect and a positive interaction effect between the two variables (water and PGRF). Also, water induced a negative linear effect on this parameter (p<0.05, Table 3).

Ĥeat treatment of rice flour seems to generate a higher regulated crystalline area in starch granules; this induced a lesser leaching of amylose fractions and, consequently, low

stickiness. Moreover, the modification and re-organization of starch macromolecules as a result of the applied treatment gives rise to the formation of a rigid network. Consequently, the strengthened starchy matrix could protect the amyloceous components from increased leaching and, thus, giving an improved texture as exhibited by the low CL values and high firmness.

The hydrothermal treatment discussed above partially eliminates the swelling of granules by slowing down gelatinization and enhancing the stability of starch paste (HOOVER and VASANTHAN, 1994; HORMDOK and NOOMHORM, 2007) and, thus, enhancing the aptitude of cooking and consistency of rice pasta (YOENYONGBUDDHAGAL and NOOMHORN, 2002; MARTI *et al.*, 2013). In addition, RAINA *et al.* (2005) showed that the application of pre-gelatinized rice flour activated the starch arrangements, which guaranteed a good textural quality of both uncooked and cooked rice pasta. FIORDA *et al.* (2013) also reported a significant enhancement and elevated firmness of pasta after the incorporation of pre-gelatinized flour made from blends of cassava starch and bagasse, and the level of pre-gelatinized flour was the most significant component when it comes to pasta stickiness.

3.3. Optimization and characteristics of optimum GF pasta

3.3.1. Cooking and textural quality

The optimum recipe was determined based the desirability method performed by JMP, version 7 (SAS, USA). Desirability is a useful approach for the optimization of multiple responses in order to select the best recipe. The main objective of optimization was to determine the levels of variables (X_1, X_2) that produce the best characteristics of GF pasta. Good quality of pasta is a combination of high water absorption, low cooking loss, and good texture parameters (high firmness and low stickiness) (BRUNEEL *et al.*, 2010). The criteria used to obtain individual desirability functions, predicted responses and real results are presented in Table 4.

Table 4. The criteria used to obtain desirability functions, predicted responses and individual desirability functions (di).

Parameter	Objective	Predicted response	Individual desirability values (di)	Experimental response
CL (%)	Minimum	9.53 ^a	0.956	9.49 ^a
WAC (%)	Maximum	237.49 ^a	0.603	234.14 ^a
F (N)	Maximum	338.09 ^a	0.891	339.85 ^ª
S (mJ)	Minimum	9.14 ^a	1	9.05 ^a

CL: cooking loss, WAC: water absorption capacity, F: firmness, S: stickiness,

Experimental responses with the same superscript than predictive response indicate there are not significant differences (p>0.05).

For optimization, the global desirability was selected, and the DFA method for selection of the optimum recipe involved the CL and S as minimized, the WAC and F as maximized. Based on such a desirability approach, the optimum amounts were found to be 5.845 g of PGRF and 59.266 mL of water for 100 g of RFBS with a value of 0.775. A hydration level is another key factor to be considered in order to obtain good quality pasta. This may be directly related to the fact that hydrating goes with formulation having an impact on the textural properties of pasta. Moreover, in the optimized to achieve the acceptable

quality of pasta. It was demonstrated that the insufficient water content produces streaky and/or flaky dough sheet surfaces, resulting in softer texture of cooked pasta. Using the above optimum levels of water in the formulation has also adverse quality effects, as dough will be stickier to handle and difficult to sheet tending to produce poor quality finished pasta. The cooking behavior, textural parameters (cutting force, firmness, stickiness and cohesiveness) of the optimized and control GF pasta are shown in Table 5.

Parameters	Optimum pasta	Control pasta			
Cooking quality					
Cooking loss (%)	9.49±0.03 ^a	14.26±0.01 ^b			
Water absorption capacity (%)	234.14±7.21 ^a	217.11±0.10 ^b			
Textural parameters					
Cutting force (N)	1.21±0.03 ^a	0.62 ± 0.03^{b}			
Firmness (N)	339.85 ± 0.03^{a}	295.50±1.12 ^b			
Stickiness (mJ)	9.05±0.07 ^a	25.00±0.5 ^b			
Cohesiveness (mJ)	28.14±0.15 ^a	14.12±0.15 ^b			
Pasting properties					
Pasting temperature (°C)	77.50±0.04 ^a	73.80±0.13 ^b			
Initial viscosity (mPa s)	18.00±0.10 ^a	14.00±0.01 ^b			
Peak viscosity (mPa s)	195.00±0.02 ^a	256.00±0.01 ^b			
Final viscosity (mPa s)	351.00±0.03 ^a	456.00±0.26 ^b			
Setback (mPa s)	160.00±0.07 ^a	205.00±0.02 ^b			
Breakdown (mPa s)	2.05±0.05 ^a	5.00±0.07 ^b			
Hydration properties					
WAI (g/g)	2.93±0.07 ^a	1.15±0.21 ^b			
WSI (%)	7.38±0.13 ^a	10.85±0.13 ^b			
SP (-)	3.22±0.95 ^a	5.60±0.01 ^b			
Thermal characteristics					
<i>T</i> _o (°C)	50.80±0.04 ^a	46.93±0.11 ^b			
<i>Τ</i> _ρ (°C)	102.84±0.00 ^a	95.58±0.00 ^b			
T_c (°C)	171.57±0.02 ^a	169.76±0.24 ^b			
T_r (°C)	120.77±0.01 ^a	123.76±0.03 ^b			
ΔH (J/g)	123.64±1.09 ^a	180.70±1.23 ^b			

 Table 5. Characteristics of optimum and control gluten-free RFBS.

^aMeans with the same superscript within line are not significantly different (p > 0.05).

As described by BRUNEEL *et al.* (2010), good quality pasta should be characterized by the low CL and stickiness, the high WAC and firmness. For commercial durum wheat spaghetti, they reported that the cooking loss ranged between 3.9 and 6.1%, stickiness between 0.2 and 119.9 mN/mm, water absorption capacity between 1.83 and 2.13 g/g dm, and firmness between 5.8 and 7.8 N/mm.

The addition of hydrothermally treated rice flour reduced the CL significantly (9.49%) and improved the WAC (234.14%) of optimum pasta, and these were found comparable or superior to the control pasta (14.26% of CL, 217.11% of WAC, respectively) (p<0.05). For texture measurements, the optimized pasta exhibited higher and better values of cutting

force (1.21 N), F (339.85 N) and cohesiveness (28.14 mJ) with lower S (9.05 mJ) than the control pasta (0.62 N, 295.5 N, 14.12 mJ, 25.00 mJ, respectively). The presented results of GF pasta are difficult to compare with the durum pasta properties. A good and firm structure of pasta means less quantity of substances going into cooking water (PADALINO *et al.*, 2013). A hydrothermal treatment of rice flour might stimulate the variation in the physical and chemical properties of starch. These modifications of starch organization are responsible for the new macromolecular structure, resulting in reduced stickiness and cooking loss and an improved texture.

3.3.2 Pasting properties

Integrity of starch granules is commonly investigated by measurements of the pasting behavior of cereals before and after modification under specific treatment conditions. The results of pasting parameters presented in Table 5 show some significant differences in the pasting characteristics of optimized formula (PT, IV, PV, FV, BD and Set) in comparison with the control pasta without PGRF (p<0.05). Higher PT, IV and lower PV, FV, BD and Set were noted for the optimum pasta. The observed differences can be explained by the modification and alteration of rigidity of treated starch granules (MARTI et al., 2010). The reported increase in pasting temperature, a decrease in PV, FV and BD may be attributed to the alterations of the pasting properties in treated flour possibly due to the production of bonds between the chains in the amorphous regions of starch granules as well as modification of crystallinity during the hydrothermal treatment, as reported previously by other authors (ADEBOWALE and LAWAL, 2002; CHUNG et al., 2009; HORMDOK and NOOMHORM, 2007; OLAYINKA et al., 2008; ZAVAREZE and DIAS, 2011). Additionally, the low PV of the optimum pasta may ensue from the limited starch swelling capacity (HAGENIMANA et al., 2006). The strengthening of intra-granular bonds means that more heat is required for the structural disintegration of starch and formation of paste (OLAYINKA et al., 2008; ZAVAREZE and DIAS, 2011). A reduction in BD after thermal treatment indicates a higher stability of starch exposed to continuous heating, as reported by ADEBOWALE et al. (2005). Moreover, the lowest recorded value of BD may indicate that PGRF may have a good potential as a food improver for food exposed to heat and mechanical treatment. Setback (Set) indicating the retrogradation tendency was apparently lower in the optimized recipe than in the control (p<0.05). Retrogradation is influenced by the amount of extracted amylose, the size of granules, and the presence of stiff, nondisintegrated swollen granules (LAN *et al.*, 2008).

Additional amylose–amylose and/or amylopectin–amylopectin chain interactions may occur upon the applied hydrothermal treatment, which can result in the cut-down of amylose extraction and decrease of retrogradation. A similar phenomenon of the modifications of pasting parameters of heat-moisture treated potato starch was reported by STUTE (1992). The reported alterations in pasting characteristics indicated some degree of re-association of starch molecules during the hydrothermal treatment. Moreover, the high IV of sample incorporated with PGRF indicates a good WSI, consistent with the value of hydration parameters shown in Table 5. This is combined with the effect of heat treatment on the properties of pre-gelatinized rice flour and dextrinization of starch molecules to a high extent, as reported by LAI and CHENG (2004). Upon heating, the bonding force within starch granules can affect the swelling behavior, which explains the rapid increase in viscosity. Moreover, the starch hydration properties were greatly affected by the thermal treatment as a consequence of macromolecular disorganization and degradation (NAKORN *et al.*, 2009; MARTI *et al.*, 2013).

3.3.3 Hydration properties

Starch solubility is the result of amylose leaching from starch during swelling as dissociating from and diffusing out of granules. This process represents a transition from the ordered to disordered structure of starch granules that occurs during heating in the presence of water (ZAVAREZE and DIAS, 2011). Macromolecular disorganization and degradation is the result of alteration of starch hydration properties by the thermal treatment (NAKORN et al., 2009). The hydration properties differed significantly between the optimized and control recipe (p<0.05) – the results are shown in Table 5. A significant increase of the WAI in the optimum sample as compared to the control results probably from a greater possibility of exposed hydrophilic groups to create bonds with water molecules during the formation of gel. In addition, this is the result of partial starch gelatinization occurring during the treatment, as suggested by LAI and CHENG (2004). Moreover, the reduction in the SP and WSI were observed compared to the control sample without PGRF. WADUGE et al. (2006), JACOBS et al. (1995) and HOOVER and VASANTHAN (1994) explained that a decrease in the SP may result from the increased crystallinity and interactions between amylose and amylopectin molecules. The authors also showed that this phenomenon could be attributed to enhanced intra-molecular bonds and modifications of the crystalline structure of starch.

In addition, GOMES *et al.* (2005) described the effect of increased molecular organization on the reduction of hydration properties of starch. Also, the same group suggested that the reduction in the solubility of annealed starch was due to the enhancement of the formation of bonds between amylose and amylopectin or between amylopectin molecules, inhibiting the extraction of starch granules. The hydrothermal treatment applied in our work probably leads to the re-organization of the structure attributed to the changes in starch granule rigidity under treatment, which entails other specific interactions between starch molecules (HOOVER and MANUEL, 1996; LAI, 2001) such as the formation of more ordered double helical amylopectin side-chain clusters and amylose–lipid complexes located within granules. A similar phenomenon of decreased swelling power and solubility was reported in other studies (HOOVER and MANUEL, 1996; OLAYINKA *et al.*, 2008).

3.3.4 Thermal properties

The differential scanning calorimetry (DSC) is useful in the characterization 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, peak temperature corresponds to the maximum heat flow (T_o), thus indicating the temperature of main phase transition, and the endset temperature indicates the end of the process of gelatinization (T_o). The difference between endset and onset temperatures is the information about the length of the gelatinization process. The thermal parameters characterizing the gelatinization process of the optimum and control pasta measured by the DSC are presented in Table 5.

The obtained parameters significantly varied between the control and optimized recipe (p<0.05). As shown in Table 5, the optimum pasta was characterized by a higher onset, endset and peak temperatures (T_r =50.80, T_r =171.57 and T_r =102.84°C) as compared to the control (46.93, 169.76 and 95.58°C, respectively). This indicates that a higher temperature is needed to start the gelatinization process, and that the main transition occurs at a higher temperature. On the other hand, a significant decrease in the length of transition (gelatinization process) was observed – as indicated by the shortening of T_r distance in the optimum sample.

Crystalline and amorphous transitions influence the shape of experimental curves representing the gelatinization process (CHAM and SUWANNAPORN, 2010). The analysis of thermal results showed some distinctive changes in the heat-treated internal granular structure of starch, which revealed a higher stability. Similarly, the hydrothermal treatment of rice flour admixed to pasta produced a more homogenous structure of starch crystallite during melting, swelling and hydration and resulted in the formation of new kinds of starch crystallites displaying dissimilar heat stability, as indicated by HORDMOK and NOOMHORM (2007). Besides, the neighboring amorphous region can govern the melting temperatures of starch crystallite indirectly. An addition of treated rice flour to pasta would increase the stability of amorphous regions during crystallite melting. As a consequence, a higher temperature is required to melt crystallites of PGRF. Increased T_{u} , T_{u} and T_{c} can be explained by the structural changes of starch granules and the associated interactions between amylose or amylose-lipid (HOOVER and VASANTHAN, 1994). ADEBOWALE *et al.* (2009) found that for starch granules, the hydration and swelling of the amorphous regions is driven by gelatinization, which involves the melting of crystalline regions and double helices. During the swelling of the amorphous regions, stress is induced on the crystalline regions as well as on the polymer chains and the former is removed from the starch crystallite surface. Subsequently, treated starches require a higher temperature for the swelling and breaking of crystalline areas leading to the increased T_{u} , T_{v} , and T_{v} , which was also confirmed in our study.

As an accompanying effect, a decrease in enthalpy of the temperature-induced gelatinization process was observed in the optimum sample (123.64 J/g) as compared to the control (180.70 J/g). It is obvious that the reduction in ΔH following the hydrothermal treatment indicated partial gelatinization of some fractions of molecules having smaller heat stability, as suggested by STUTE (1992) and HORDMOK and NOOMHORM (2007). Also, the decrease in ΔH can be interpreted as a presence of disorganized double helices of starch granules revealing a crystalline and non-crystalline character under the treatment conditions. Thus, some reductions in the fraction of unbend and melted double helices during gelatinization would be noticed after treatment.

3.4. Sensory attributes

The sensory characteristics of cooked GF pasta are reported in Table 6. The sensory evaluation showed no significant difference (p>0.05) in taste, color and flavor between the GF control and the optimum pasta. The obtained results showed that the optimum recipe pasta was significantly better with the highest scores for appearance and stickiness.

	Appearance ¹	Color ¹	Flavor ¹	Taste ¹	Stickiness ¹	Overall acceptability ²
Control pasta	3.75±0.34 ^b	4.18±0.21 ^a	3.45±0.34 ^ª	4.40±0.43 ^a	3.17±0.40 ^b	4.42±0.33 ^b
Optimum pasta	4.45±0.46 ^a	4.22±0.31 ^a	3.35±0.33 ^ª	4.42±0.42 ^a	4.52 ±0.48 ^a	6.42±0.24 ^a

Table 6. Sensory evaluation and overall acceptability of gluten-free pasta.

¹5-point scale, ²9-point hedonic scale (n = 55).

^{a+}Means with the same superscript within a column are not significantly different (p > 0.05).

The best scores were recorded for appearance and stickiness of the optimum pasta, as confirmed by the texture measurements; it can be attributed to the enhancing effect of the hydrothermal treatment of rice flour added to the pasta recipe in our study. Moreover, the selected optimum pasta gathered superior scores (values above 5) in the overall acceptability in comparison with the control sample without the addition of PGRF.

3.5. Microstructure of dry pasta

The microscopic pictures can be helpful in analyzing the appearance, texture or integrity of food products (GORINSTEIN *et al.*, 2004). The surface of traditional laminated GF pasta is presented in Fig. 4.



Figure 4. SEM surface of laminated pasta from control and optimized recipe at different magnifications; control pasta: A) x200, B) x600, optimum pasta: C) x200, D) x600.

The analysis of the micrographs of pasta manufactured from the RFBS recipe without PGRF (Fig. 4A) exhibits the presence of small cracks with a small amount of empty spaces, which suggests some lack of continuity due to the absence of gluten. Also, the graphs showed the structure of poorly agglomerated starch granules and separate granules visible on the pasta surface (Fig. 4B). The microstructure images, presented in Fig. 4C, highlighted the differences in starch organization and collocation observed on the surface of traditional pasta laminated from the optimized recipe with the addition of PGRF. It was

characterized by a compact and homogeneous matrix. The agglomerates of starch and proteins are surrounded by pre-gelatinized starch, which forms a continuous phase responsible for the better results of the CL and a low quantity of starchy components passing into the cooking water. Pre-gelatinized rice flour forms aggregates combine all pasta components, which is clearly visible at high magnification (Fig. 4D). Similar morphological features could be observed and confirmed by analyzing the cross-sectional microstructure of pasta (Fig. 5).



Figure 5. Cross-section of control RFBS pasta: A) x200, B) x600, C) x1500, and optimum pasta with the addition of PGRF: D) x200, E) x600, F) x1500 observed with SEM.

Fig. 5A and 5B show the irregular and discontinuous internal structure of RFBS pasta with a small amount of swollen starch granules and visible higher dimensions, and only few places are linked by a protein fibrous fraction that comes from field bean flour (Fig. 5C). A more compact and uniform structure can be observed if pre-gelatinized rice flour was used as a pasta improver (Fig. 5D). The incorporation of PGRF caused the ultra-structural reorganization of matrix and influenced the excellent microstructure. Comparing the size and amount of visible starch granules, pasta with the addition of treated rice flour showed the lower amount of free starch and a more uniform distribution of components inside the tread (Fig. 5E). Starch granules are surrounded by a continuous phase of gelatinized starch responsible for the limited leaching of components during cooking and the enhanced consistency of PGRF-improved pasta (Fig. 5F).

4. CONCLUSIONS

Laminated pasta based on rice and enriched with field bean semolina provides an important quantity of dietary fiber and protein with a positive amino acids balance. The supplementation of cereals with legumes is likely enhance the nutritional value of glutenfree pasta products. Optimization made by an experimental design showed that the amount of water and improver are the important factors having an influence on the quality of gluten-free pasta. The optimum formulation of rice and field bean containing 5.845 g of pre-gelatinized rice flour and 59.266 mL of water was selected based on the desirability function approach with the value of 0.775, which showed the optimum pasta properties. The hydrothermal treatment of rice flour resulted in the changes to the starch structure and the physical properties of PGRF pasta, such as the cooking and texture parameters, pasting and hydrations properties, thermal features, microstructure, and sensory profile. The obtained results also showed that the addition of pre-gelatinized flour induced significant differences (p<0.05) in all parameters in comparison with the control pasta. The pasta processed with the optimized formula was characterized by the improved cooking quality and texture. As regards the sensory evaluation, the optimum recipe showed acceptable scores for all the sensory attributes and the overall quality of glutenfree pasta. Optimized GF rice and field bean pasta obtained using hydrothermally treated rice flour as the improver allow the manufacture of gluten-free laminated pasta with improved nutritional characteristics and appropriate quality suitable for celiac people. Hydrothermal treatment, as an environmentally friendly technique of modifying starch functionality at low cost, fits the public demand for natural products and is highly promising in the long perspective.

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