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Original article

Combination of resistant starches types II and IV with minimal amounts of oat bran yields good quality, low glycaemic index pasta

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Summary The objective of this work was to study the effects of the combination of resistant starch type II (RSII), resistant starch type IV (RSIV) and oat bran (OB) on technological and nutritional properties of pasta, applying response surface analysis. Cooking properties were improved by combining RSII and RSIV in pasta formulation, while OB addition negatively affected all technological attributes, and a negative synergistic effect was observed between this fibre and resistant starches in cooking losses. Considering nutritional properties, substitution of bread wheat flour with resistant starch type II and IV increased starch resistant to digestion and OB addition increased pasta starch hydrolysis. A positive synergistic effect was observed on glycaemic index by combining both types of resistant starches. Finally, we optimised the formulation considering three aspects separately: technological properties, nutritional attributes and these two features together. The combination of RSII 12.6, RSIV 3.1 and OB 0.6 g per 100 g of wheat flour will allow to obtain a pasta with low glycaemic index (GI = 69) and good technological characteristics.

Keywords Glycaemic index, oat bran, pasta quality, resistant starch, response surface analysis.

Introduction

Societies have recognised the important relationship between health, lifestyle and diet. As a result, consumers are more conscious of what they eat. Interest is not only focussed on obtaining an appropriate nutrient supply for healthy lifestyle, but also to regulate some diet-related diseases. In this sense, the significance of dietary fibre for the prevention of diet-related diseases makes us pay more attention to different types of foods rich in these components.

The development of healthier low glycaemic products with acceptable functional and sensorial quality, using different dietary fibres with diverse physicochemical properties, is of great importance to fulfil consumer expectations.

Potential health benefits of dietary fibre have been well documented in relation to bowel transit time (Green, 2001), prevention of constipation, reduced colorectal cancer risk (Sengupta *et al*., 2001), enhanced methanogenesis (Guarner & Malagelada, 2003), production of short-chain fatty acids (Gibson *et al*., 1996; Brouns *et al*., 2002) and promotion of colonic health (Gibson *et al*., 2004). Also, the contribution of dietary fibres to the development of viscosity in the gut appears to be related to the control of the metabolism of glucose and lipids (Jenkins *et al*., 2004; Horn *et al*., 2008; Kendall *et al*., 2010).

In our previous study (Bustos *et al*., 2011b), we reported an improvement in nutritional attributes of high-fibre pasta that was significant at 10% of flour replacement with resistant starches and at 5% of insoluble oat fibre, affecting textural and cooking properties of the resulting fortified pasta (Bustos *et al*., 2011a). Decreased functional quality of final pasta has been ascribed to a dilution of functional gluten proteins (Manthey & Shorno, 2002). In addition, insoluble fibre replacement of wheat flour could disrupt the starch–gluten matrix (Tudorica *et al*., 2002; Bustos *et al*., 2011a). In general, the poor quality of fibre-enriched pasta involves a decrease in firmness, an increase in adhesiveness as the amylose leached to cooking water increases, and a rough texture and mouth feel.

The effectiveness of response surface methodology (RSM) in the development and optimisation of cereal products has been highlighted by different authors (Malcolmson *et al*., 1993; Ribotta *et al*., 2010; Steffolani *et al*., 2011). The basic principle of RSM is

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to relate product properties of regression equations that describe interrelations between input parameters and product properties (Crowley *et al*., 2001).

By combining the benefits of pasta with the benefits of dietary fibres, novel functional food products associated with the prevention and treatment of diseases, such as coronary heart diseases and diabetes, may be developed (Tudorica *et al*., 2002; Sozer *et al*., 2007; Manno *et al*., 2009; Bustos *et al*., 2011a,b). More research has to be done in this area to investigate how different dietary fibre combinations affect pasta quality, cooking characteristics, pasta structure and starch digestibility.

The objective of this study was to determine the optimum levels of three dietary fibres (resistant starch type II, resistant starch type IV and oat bran) as independent variables leading to the lowest cooking losses and water absorption, and to the lowest *in vitro* digestibility and estimated glycaemic index of pasta, using response surface methodology (RSM). Pasta with these properties would be considered as an optimum product that can provide the desirable product, especially in terms of functional and technological properties. The variable responses were modelled through three independent variables to provide information regarding the optimum proportion of dietary fibres. It should be noted that the other critical parameters, such as optimum cooking time (OCT), swelling index, colour of cooked pasta, resistant starch content and kinetic parameters of pasta *in vitro* digestibility, have been investigated using the same optimisation procedure.

Materials and methods

Materials

Bread wheat flour without additives was provided by Industrias Alimenticias Tiranti S.R.L. (Argentina). RS type II (Hi maize 260, National Starch) and RS type IV (Novelose 480, National Starch) were supplied by Gelfix S.A. (Argentina). RSII is high-amylose maize starch (70% of amylose), and RSIV is defined as phosphated distarch phosphate, a cross-linked high-amylose maize starch. Oat bran (Canadian Harvest_ Oat Fibers 200/58 series, Sunopta, USA) was supplied by Saporiti S.A., Argentina.

Methods

Pasta making

Pasta was made using commercial bread wheat flour, water and three types of dietary fibre. Resistant starch type II (RSII) and type IV (RSIV) and oat bran (OB) were incorporated into recipes by replacing wheat flour according to experimental design (Table S1). An additional sample with no fibre included was also prepared as a control. Preparation of pasta was established according to Bustos *et al*. (2011a) using 50 g of flour, 500 mg of salt and distilled water necessary to produce a visually optimum dough prior lamination. After lamination, pasta was cut into strips approximately 2 mm wide and 15 cm long using cutting rolls. Finally, pasta was dried at low temperature in two steps: the first one was 30 min at 30 °C in an air convection drier; the second step was performed at 45 °C in a humidity-controlled (75%) drier for 17.5 h. The samples were wrapped in cling film and stored in airtight containers at room temperature until needed.

Technological quality parameters

The optimum cooking time (OCT) was determined according to method 16–50 (AACC International. American Association of Cereal Chemistry, 2000). After cooking and draining, the samples were analysed for water absorption (WA), swelling index (SI) and cooking losses (CL) according to Tudorica *et al*., 2002;. Water absorption of drained pasta was determined as follows: WA (%) = ((W1 − W2)/W2) × 100, where W1 and W2 were the weight of cooked and raw pasta, respectively. Swelling index of cooked pasta was evaluated by drying cooked pasta samples to constant weight at 105 °C, expressed as SI = (W1 − W3)/W3 where W3 was the weight after drying. Cooking loss of pasta was determined in water collected from each sample by evaporation to constant weight in an air oven at 105 °C. The residue was weighed and reported as percentage of the raw pasta sample.The colour of cooked pasta was determined with a Minolta 508d spectrophotometer (Ramsey, NJ, USA). Eight-millimetre measurement apertures, D65 illuminant, 10° angle of observer were set, according to approved methods 14–22 (AACC, 2000). At least eight readings were taken from the cooked pasta strand and recorded as CIE-LAB, L^* (lightness), a^* (redness-greenness) and b^* (yellowness-blueness) values (Joshi *et al*., 2002).

Starch analysis

Resistant, digestible and total starch were measured according to AACC 32–40, (2000). Both types of resistant starch fibres were analysed for resistant starch, resulting in (33.0 ± 0.8) g per 100 g for RSII and (21.7 ± 0.2) g per 100 g for RSIV.

In vitro digestion of pasta and estimated glycaemic index

In vitro digestion was performed using the multi-enzymatic method of Bustos *et al*. (2011b). Samples of pasta (4 g of cooked pasta) were mixed with 20 mL sodium potassium phosphate buffer (pH 6.9) (PBS). Thereafter, the pH was adjusted to 1.5 (using 8 M HCl), and 5 mL pepsin solution (115 U mL^{-1}) was added to the samples, followed by incubation at 37 °C for 30 min. Then, the pH was readjusted to 6.9 with

10% NaOH and the sample brought to 49 mL with PBS, and 1 mL of porcine pancreatic alpha amylase solution (110 U mL^{-1}) was added; each tube was incubated at 37 °C. Every 30 min for 3 h, aliquots of 1 mL from each tube were withdrawn for analysis of reducing sugar content using the 3,5-dinitrosalicylic acid (DNS) method. The maltose was converted into starch by multiplying by 0.9.

A nonlinear model was applied to describe the kinetics of starch hydrolysis. The first order equation has the following formula: $C = C_\infty\ (1 - e^{-Kt})$, where C corresponds to the percentage of starch hydrolysed at time t; C_∞ is the equilibrium percentage of starch hydrolysed after 180 min; K is the kinetic constant; and t is the time (min). Parameter estimation was carried out using ORIGIN PRO software, version 8 (OriginLab Corp., Northampton MA, USA). The rate of starch digestion was expressed as the percentage of total starch hydrolysed at different times (30, 60, 90, 120 and 180 min).

The area under the hydrolysis curve (AUC) was calculated, and the hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (fresh white bread). Expected glycaemic index (GI) was estimated using the model GI = 39.21 + 0.803 (H_{90}).

Experimental design

The effect of combining dietary fibres on pasta quality attributes was studied by means of a response surface regression method. The following independent variables were selected: resistant starch type II, resistant starch type IV and oat bran at 0–10 g per 100 g of wheat flour. The fibre levels were selected according to previous results (Bustos *et al.*, 2011a,b). A rotatable central composite design was generated using Statgraphics plus 5.0 (Statpoint Technologies Inc., Warrenton, VA, USA); it was constituted by three factor combinations and five substitution levels of each factor. The experimental design is shown in Table S1. The experiment order was randomly selected to avoid the effect of a hidden variable. Four replicates at the central point made it possible to estimate the pure error of the analyses. Results were analysed by the multiple regression method. The fitness model quality was evaluated by ANOVA (Statgraphics plus 5.0), and the determination coefficient R^2 was obtained to fit each model of experimental data (Steffolani *et al.*, 2011).

Multiple regression equation was developed only with significant coefficients ($P < 0.05$). Tridimensional response surface plots were generated by each quality parameter. The optimal dietary fibres combination for pasta was determined using a multiple response method called 'desirability' (Ferreira *et al.*, 2007). This optimisation method incorporates desires and priorities for each of the variables.

The desirability function approach is one of the most frequently used multi-response optimisation techniques (Ribotta *et al.*, 2010; Steffolani *et al.*, 2011). The desirability lies between 0 and 1, and it represents the closeness of a response to its ideal value. If a response falls within the unacceptable intervals, the desirability is 0, and if a response falls within the ideal intervals or the response reaches its ideal value, the desirability is 1. The proposed desirability function transforms each response to a corresponding desirability value between 0 and 1. All the desirability can be combined to form a composite desirability function that converts a multi-response problem into a single-response one. The desirability function is a scale invariant index that enables quality characteristics to be compared to various units (Raissi & Eslami Farsani, 2009).

Results and discussion

Effect of combination of fibres on cooking properties of pasta

All combinations of fibre-enriched pasta showed a decrease in OCT compared with control pasta made with 100% wheat flour (10 min) (Table S6). Addition of RSIV and RSII into pasta formulation presented a negative quadratic effect in OCT; in the case of RSIV substitution, an additional positive linear effect was observed (Table S2). These results indicated that although the rise of RSIV content increased OCT, this increase was up to a level of fibre (Figure S1), because over these levels, the OCT decreased.

With regard to OB addition in pasta formulation, it showed only a linear positive effect in OCT (Table S2). No interactions between different types of fibre were observed. An explanation for this observation could be the disruption of the protein – starch matrix at high levels of oat bran addition in pasta formulation that we have discussed in a previous work (Bustos *et al.*, 2011a).

No significant coefficient was found in the regression equations of models ajusted for water absorption (SE = 1.8%) and swelling index in samples with resistant starch addition. On the other hand, OB addition presented a linear positive and negative quadratic effect for water absorption ($R^2 = 0.77$) and swelling index ($R^2 = 0.74$), indicating that this parameter increases with wheat flour substitution for OB until a certain value, above which a decrease was observed (Figure S1).

These observations agree with previous results (Bustos *et al.*, 2011a) which indicate that at low levels of OB incorporation, an evident improvement in pasta quality takes place, while at high levels, there is a disruption of protein matrix, increasing water absorption and swelling index as starch granules are more exposed (Fardet *et al*., 1998; Tudorica *et al*., 2002).

In the analysis of the swelling index (SE = 3.1%) of fibre-enriched pasta, we can establish a direct relationship between OCT and water absorption. The assays that presented high OCT (1, 2, 9, 14, 15, 17 and 18) showed high percentages of water absorption and swelling index, except for assay 8 – with 10 g per 100 g wheat flour of RSII, RSIV and OB – which had the lowest OCT and high levels of water absorption and swelling index, probably due to a disruption of the protein–starch matrix and the weakness of this one through the decrease in protein content.

Cooking loss (SE = 4.3%) is one of the most important parameters of pasta quality that affects consumers acceptability. All fibre combinations presented cooking losses below 8%, the value above which pasta quality is considered unacceptable (Dick & Youngs, 1988), even when all assays have lost <6.7 g per 100 g pasta, a value that corresponds to control pasta cooking losses.

Cooking loss is one of the parameters more affected by fibre incorporation to pasta formulation because solid diffusion to cooking water is influenced by the integrity of the protein matrix (Fardet *et al*., 1998 and Fardet *et al*., 1999; Bruneel *et al*., 2010), which in turn is affected by protein content (Matsuo *et al*., 1972; Hennen & Brismar, 2003) and type of fibre incorporated (Tudorica *et al*., 2002; Sozer *et al*., 2007).

The three types of dietary fibre included in pasta formulation showed a linear positive and negative quadratic effect (R^2 = 0.97). Like many insoluble fibres, OB influenced the integrity of protein matrix negatively (Bustos *et al*., 2011a), so that it presented a negative interaction with both types of resistant starches (RSII and RSIV).

In colour analysis of cooked pasta (SE = 4.9%), assay 14 without OB addition presented the lowest b^* value (7.2) and the highest L^* value (66.9). On the other hand, assay 7 with high levels of OB substitution showed the highest b^* value (19.1) and the lowest L^* value (62.3). These results indicate that enrichment with OB strongly affects luminosity and yellowness of cooked pasta (Figure S2).

Wheat flour substitution with RSII, presented a negative quadratic effect in a^* value, while RSIV showed a positive linear effect (R^2 = 0.78). In this regard, a negative linear effect was determined for L^* parameter (R^2 = 0.90) and a positive linear effect for parameters a^* and b^* (R^2 = 0.97) when OB was included in pasta formulation. This analysis showed that the characteristics of oat bran presented the strongest influence in cooked pasta colour.

The highest value for R^2 was obtained for cooking loss and b^* parameter, which indicates that dietary fibre incorporation explains the variability observed in these parameters. It is important to point out that these variables represent the most important factors that affect consumers' acceptability (Sissons *et al*., 2005; Fu, 2008).

Resistant starch type II incorporation into pasta formulation presented significant positive linear effect only in the case of cooking losses, while a negative quadratic effect was observed in OCT, cooking losses and a^* parameter. RSIV enrichment of pasta had a positive linear effect and a negative quadratic effect on cooking losses.

On the other hand, the addition of OB to pasta lineally affects all parameters and produces a quadratic effect on OCT, water absorption, swelling index and cooking losses, which is associated with the high water retention capacity characteristic of this fibre (Manthey *et al*., 1999).

The addition of resistant starches did not present significant interactions in any of the cooking properties evaluated, while a separate negative interaction with OB in cooking losses was observed.

Effect of fibre combination on *in vitro* digestibility of pasta

It is widely accepted that the main quality of pasta nutritional attributes is a moderate glycaemic response and resistant starch content (SE = 4.6%), with a low kinetic constant of starch hydrolysis. Therefore, the objective was to minimise the response of glycaemic index (GI) (SE = 4.7%), kinetic constant of starch hydrolysis (K) (SE = 4.5%), hydrolysis index (HI) (SE = 4.1%), starch hydrolysed at equilibrium (C_∞) (SE = 4.6%) and digestible starch (SE = 4.6%); and to maximise the response of resistant starch content. For each group of response, a quadratic equation was generated with significant coefficients (P < 0.05) to obtain a R^2 value as high as possible. Based on these equations, the response behaviour can be predicted and presented as a response surface (Figs 1 and 2).

The experimental responses of nutritional attributes of fibre-enriched pasta are presented in Table S3. The highest R^2 values were determined for resistant and digestible starch content, 0.98 and 0.92, respectively; these indicate that the variability of all other parameters could be explained only in terms of dietary fibre addition.

Resistant starch type II and OB showed a negative linear effect for digestible starch content in cooked pasta (Fig. 1). These results agree with the fact that wheat flour substitution by fibre reduces starch content proportionally; in the case of resistant starch, these fibres presented a considerable digestible starch percentage, representing a minor impact in this parameter. Comparing observations with OB addition (minor coefficient in absolute value); in particular, RSII has

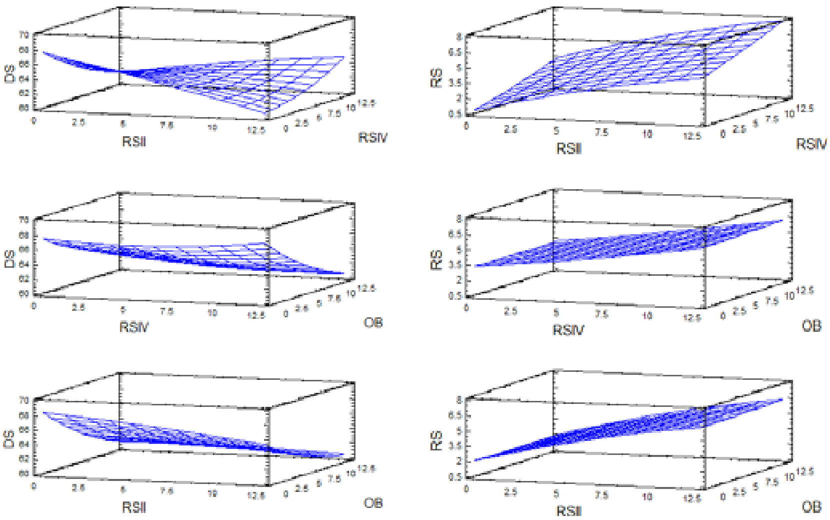

Figure 1 Response surface plots of digestible (DS) and resistant (RS) starch content (g per 100 g pasta) in cooked pasta. Resistant starch starch type II (RSII) and IV (RSIV), oat bran (OB).

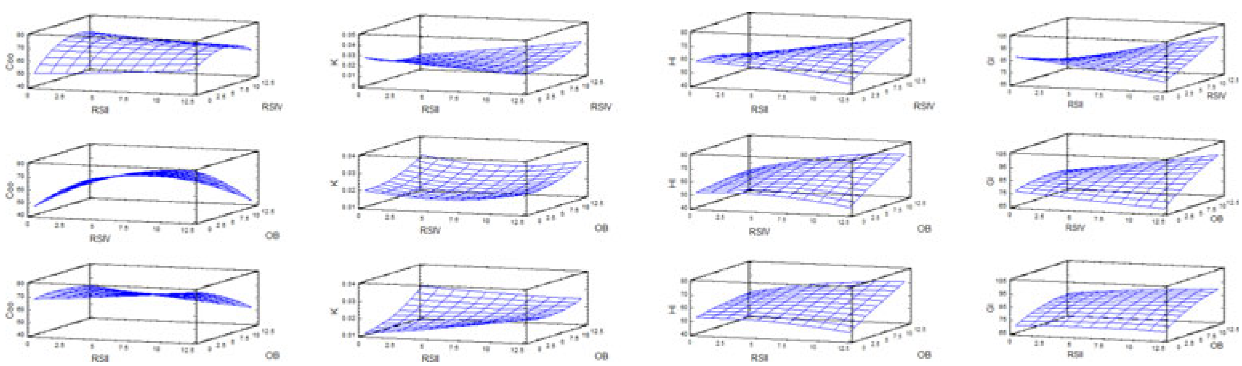

Figure 2 Response surface plots of pasta *in vitro* digestibility parameters. Starch hydrolyzed at equilibrium (C_∞), kinetic constant (K), hydrolysis index (HI) and estimated glycaemic index (GI). Resistant starch starch type II (RSII) and IV (RSIV), oat bran (OB).

the highest resistant starch content (33 g per 100 g), leading to a more important decrease in digestible starch of cooked pasta.

In this regard, according to previous observations (Bustos *et al.*, 2011b), pasta with a high level of RSII incorporation in formulation leads to a resistant starch generation. This fibre presented a positive linear effect for resistant starch content; the same result was obtained for RSIV with a minor coefficient.

Resistant starch type II addition in pasta formulation only presented linear significant coefficients for digestible and resistant starch contents, being negative and positive, respectively (Table S4). Pasta enrichment with RSIV presented a positive linear effect for resistant starch content and C_∞, and in the case of this last parameter, a quadratic negative effect was observed (Fig. 1).

Wheat flour substitution with OB presented a negative linear effect for digestible starch and a linear positive effect for C_∞, K, HI and GI, which is related to the negative effect observed in cooking properties with substitution levels above 5 g per 100 g of wheat flour (Bustos *et al.*, 2011b). We observed that the incorporation of OB affects the protein matrix formation, as it threatens its integrity and makes starch more accessible to enzymatic attack (Colonna *et al.*, 1990; Fardet *et al.*, 1998).

Resistant starches presented a positive interaction for digestible starch and GI and a negative interaction between OB and resistant starch IV for C_∞ (Table S4).

In the analysis of parameters obtained by *in vitro* digestibility of fibre-enriched pasta, for the total starch hydrolysed in equilibrium (C_∞), RSIV showed positive linear and negative quadratic effects. These results agree with previous observations that RSIV presented an important effect at low levels of substitution in pasta digestibility (Fig. 2). Besides, a positive linear effect was observed for OB, which agrees with the disruption associated with fibre incorporation, which is an important factor affecting starch digestibility and C_∞ (Colonna *et al*., 1990; Fardet *et al*., 1999; Aravind *et al*., 2011).

The kinetic constant for *in vitro* digestibility of pasta was only affected by a low coefficient for OB substitution, with a positive linear effect. Only OB affects HI with a negative linear effect, according to the negative impact of inclusion of this fibre in pasta formulation.

The glycaemic index (GI) is one of the most important parameters for the nutritional evaluation of the final product and the suitability of pasta for diabetic people. This parameter was affected by OB incorporation with a positive linear effect and a positive interaction between both resistant starches (Fig. 2).

Optimisation

Three optimisation procedures of pasta formulation with different types of fibres were performed. In the first optimisation, we tried to minimise water absorption and cooking losses and to maximise L^* and b^* parameters of cooked pasta, simultaneously. Results indicate that a combination of RSII 12.5, RSIV 0.08 and OB 2.19 g per 100 g of wheat flour will allow to obtain a type of pasta with a 24% decrease in cooking losses, compared with control sample (without fibre inclusion), and with a lower water absorption, L^* and b^* parameter (Table S5). This formulation almost excludes RSIV, which indicates that RSII principally contributes to cooked pasta properties, while OB influences water absorption and yellowness.

In a second optimisation, we focused on nutritional attributes, so we tried to minimise the swelling and the glycaemic indexes and to maximise resistant starch content, simultaneously. Results indicate that a combination of RSII 3.96, RSIV 12.6 and OB 0.03 g per 100 g of wheat will result in a type of pasta with a low GI and moderate resistant starch content (Table S5). This formulation includes a high level of substitution of RSII and almost excludes OB from pasta formulation, indicating that OB affects pasta structure negatively, as it alters nutritional attributes of cooked pasta, in agreement with previous results (Bustos *et al*., 2011b).

In the third optimisation, we considered technological and nutritional characteristics of cooked pasta; therefore, we tried to minimise the glycaemic index, cooking losses and water absorption and to maximise resistant starch content, simultaneously. Results indicates that a combination of RSII 12.6, RSIV 3.1 and OB 0.6 g per 100 g of wheat flour will result in pasta with low GI, a 8.2% decrease in cooking losses and a 57.1% increase in resistant starch content (Table S5).

This optimised formulation allows to obtain pasta with good technological quality, improved nutritional attributes through the incorporation of a high percentage of starch high in amylose (RSII) – considered an important improvement in quality in a previous work (Bustos *et al*., 2011a,b) – and a minimal quantity of chemically modified starch (RSIV) showing an improvement in digestibility at low percentages of incorporation. On the contrary, OB generates a disruption of protein matrix so it affects pasta quality negatively.

Conclusion

The effects of combining resistant starch type II and type IV and oat bran on technological and nutritional attributes of pasta were studied using response surface methodology. The prediction models developed had high R^2, indicating that insoluble fibre inclusion in pasta formulation explains the variability of dependent variables analysed.

Resistant starch type IV contributed mainly to the reduction in cooking loss, while RSII incorporation improved all other parameters evaluated, except colour attributes that were affected by OB addition.

Resistant starch type II produced the most important improvement in technological and nutritional properties of cooked pasta, while OB was the variable that most strongly affects parameters associated with protein–matrix integrity. In conclusion, the optimisation of pasta formulation indicated that high levels of RSII, moderate levels of RSIV and a minimal addition of OB yield cooked pasta where cooking loss is below 6%. This enriched pasta could be classified as low glycaemic index pasta (GI $\geq$ 79) with moderate resistant starch content. The consumer acceptance test will be necessary to confirm its desirable attributes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Response surface plots of pasta technological quality parameters.

Figure S2. Response surface plots of pasta luminosity (L), redness (a) and yellowness (b).

Table S1. Rotatable central composite design for the optimization of RSII, RSIV and OB addition.

Table S2. Significant coefficients of the regression design fitting model for cooking properties of fiber enriched pasta.

Table S3. Experimental responses of nutritional attributes of fiber enriched pasta.

Table S4. Significant coefficients of the regression design fitting model for nutritional attributes of fiber enriched pasta.

Table S5. Expected response values for each optimization.

Table S6. Experimental responses of cooking properties of fiber enriched pasta

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