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Berry fruits-enriched pasta: effect of processing and in vitro digestion on phenolics and its antioxidant activity, bioaccessibility and potential bioavailability

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ABSTRACT

Pasta samples were made by substituting wheat flour (2.5 and 7.5%) for lyophilized raspberry, boysenberry, and redcurrant and blackcurrant. Total polyphenol content showed minimum variation during processing while anthocyanins presented high degradation. In general, the first minutes of cooking showed the major detrimental effect on antioxidant activity. *In vitro* starch hydrolysis showed the lowest value with the addition of raspberries and boysenberries. During simulated digestion, polyphenols were released from pasta matrix reaching a 2.3 to 4.3-fold increase in bioaccessible polyphenols. Likewise, values observed for reducing power and free-radical scavenging activity ranged from a 0.7 to 2.0-fold and 1.6 to 6.8-fold increase in relation to cooked pasta, respectively. In addition, ≈40% of dialyzability was

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observed for scavenging activity. In conclusion, enrichment of pasta with fine fruits is an effective tool to obtain a product with enhanced antioxidant potential.

Keywords: pasta processing, *in vitro* digestion, berry fruits, antioxidant activity.

INTRODUCTION

Berry fruits represent a variety of small fruits characterized by red, purple, and blue colour. The most common include: blueberry, bilberry, cranberry, blackberry, raspberry, black, white or red currant, and strawberry. Berries are consumed as both fresh products as well as processed food (i.e., juices, beverages, jams, freeze-dried) (Seeram and Commission, 2008). These fruits are highly recognized for their high level of bioactive compounds with antioxidant activity like polyphenols including flavonoids (anthocyanins, flavanols, and flavanols), condensed tannins (proanthocyanins), hydrolysable tannins (ellagitannins and gallotannins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids, chlorogenic acid), stilbenes and lignans (Moyer *et al.*, 2002). It is believed that those compounds are largely responsible for the reduced risk of various lifestyle diseases associated with their consumption (Battino *et al.*, 2009; Vendrame *et al.*, 2016). Beyond the health benefits that could be provided by including fine fruit in the diet, such fruit has a very short shelf life (2-3 days at room temperature and 5-10 days under refrigeration) that conditions quantity and regularity of ingestion. This fact encourages the development of products like jams and nutritive concentrates for sports nutrition, having a considerable loss of bioactive compounds (Seeram and Commission, 2008).

Cereal-based food naturally rich or enriched with fibre and phytochemicals in the diet contribute to the health status of the consumer, dried pasta being one with long shelf life (>1 year) and thus a good option to achieve their efficient use (Bustos, Perez and Leon, 2015). Pasta is a popular very cheap food consumed by every social status. Inclusion of fruits in pasta could sound original and odd, however in a previous work we not only reported the good technological quality of the enriched pasta, but also the great acceptability found in the sensorial analysis (Bustos, Paesani and León, 2019). Berry-fruit are typically an acid fruit with low sweetness and mild flavor which results in an enriched pasta with a balanced acidity very pleasant for consumers. Therefore, the design of functional pasta could be a good alternative to reap the health benefits provided by berry fruits.

Since antioxidant compounds are quite sensitive to processing, their inclusion is not enough to guarantee good nutritional quality. That means that information on the stability of these health-promoting components during processing of enriched foods will be useful to food industry to assure presence of health-promoting compounds in the final product. In addition, changes in the microstructure and

hydrolysis of starch in cooked products during *in vitro* digestion determine the quantity of released compounds that may be available for absorption and use. As a consequence, the objective of the present research was to consider these two aspects which allow estimating the real contribution to health of berry-enriched pasta.

MATERIALS AND METHODS

Materials

Four species of berry fruits were selected: raspberry (*Rubus idaeus* var. Autumn Bliss) and boysenberry (*R. ursinus* × *R. idaeus* var. Black Satin), from San Pedro, Buenos Aires (Argentina); and redcurrants (*Ribes rubrum* sp.) and blackcurrants (*Ribes nigrum* sp.) from El Bolsón, Río Negro (Argentina). All berries were purchased from Dolphes Gourmet (Rosario, Argentina) as Individual Quick Frozen (IQF) fruits. Wheat flour (*T. aestivum*) without additives used for pasta production was provided by Industrias Alimenticias Tiranti S.R.L. (Argentina). Amylase from porcine pancreas (A3176), pepsin from porcine gastric mucosa (P7000), pancreatin from porcine pancreas (P7545), and bile salts (B8756) were purchased from Sigma-Aldrich (Buenos Aires, Argentina) and all other chemicals were of analytical grade unless otherwise stated.

Methods

Berry- enriched pasta making process

Freeze-dried berries were obtained by storage at -80 °C for 48 h and lyophilized (L-T8 RIFICOR, Argentina). Berry-enriched pasta was prepared according to Bustos et al., (2019) substituting 2.5 and 7.5 % of bread wheat flour for freeze-dried fruit powders. Pasta was dried in two steps: at 30 °C without controlling humidity in an air convection drier for 30 min and at 45 °C in a humidity-controlled (75%) drier for 17.5 hr, resulting in raspberry (RBP), boysenberry (BBP), redcurrant (RCP) and blackcurrant (BCP) enriched pasta. Samples were cooked in boiling water during 14 minutes for raspberry and boysenberry enriched pasta and 17 minutes for control and redcurrant and blackcurrant enriched pasta according to the Optimum Cooking Time (OCT). Samples were taken during elaboration to monitor bioactive compounds and antioxidant activity. Samples of 5 g were taken from ingredient mixture (IM), fresh pasta (FP), dried pasta (DP), pasta cooked until 50% of optimal cooking time (50%-CP) and cooked pasta (CP), and immediately freeze-dried to perform the analysis of polyphenols, anthocyanins and antioxidant activity.

Total polyphenol, anthocyanins and antioxidant activity

Sample extracts were made as reported in Bustos et al., (2018) by mixing two hundred milligrams of sample with 1 ml of acetone: water (70:30) with 0.1% HCl.

Total polyphenol content was determined using the Folin–Ciocalteu method, with gallic acid as a calibration standard (Prior, Wu and Schaich, 2005). Total polyphenol content was expressed as mg gallic acid per gram of fruit powder in dry basis.

Monomeric anthocyanin content was performed according to Giusti and Wrolstad (2001) using an extinction coefficient (B) of 26 900 l cm⁻¹ mg⁻¹ and a molecular weight of 449.2 g/mol of cyanidin 3-glucoside. Results were expressed as mg of cyanidin 3-glucoside/100 g of sample in dry basis.

ABTS^{•+} radical cation scavenging activity was measured according to Re et al., (1999) using Trolox as standard and results expressed as μmol of Trolox equivalent per gram of sample in dry basis.

Ferric reducing ability was determined by FRAP assay according to Pulido et al., (2000) using gallic acid as a standard.

In vitro digestion of berry-enriched pasta

In vitro digestion of berry-enriched pasta was performed according to Bustos *et al.*, (2017) based on the static method proposed by INFOGEST's scientists (Minekus *et al.*, 2014) and, with modifications to estimate dialyzability.

Briefly, the ratio used in the model was 50/50 w/v for: food/Simulated Salivary Fluid (SSF); oral content/Simulated Gastric Fluid (SGF) and gastric content/Simulated Intestinal Fluid (SDF). Five grams of cooked pasta were used to perform the digestion using α-amylase and pepsin from porcine pancreas, pancreatin and bile salts. Aliquots of 1 ml were withdrawn at time 0, after the salivary step, at 60 and 120 min of the gastric step and at 10, 30, 90 and 180 min of the intestinal step to monitor the hydrolysis degree of starch by analysing the reducing sugar content using the 3,5-dinitrosalicylic acid (DNS) method.

Non-linear models were applied to describe separately oral-gastric and intestinal digestion for starch hydrolysis using the SIGMA PLOT software (version 12) as reported in (Bustos *et al.*, 2017). The rate of starch digestion was expressed as the percentage of total starch hydrolysed at different times.

The three fractions of starch according to its digestibility as defined by Englyst (1996) were determined from adjusted curves from the intestinal phase.

Potentially bioaccessibility of polyphenols and antioxidant activity were evaluated after 180 minutes of digestion (last aliquot of intestinal phase). In order to analyse their dialyzability, a cellulose dialysis tube (molecular mass cut-off value 10,000-12,000 Da) filled with 25 ml of NaHCO₃ equivalent to the titrable acidity was included during the intestinal phase. After 3 h of incubation, aliquots were withdrawn from the inside (dialyzable) of the dialysis tube for analysis.

Statistical analysis

Four sets of each pasta sample were made, two were used to take samples during processing (each also analysed in duplicate) and the other two were used to perform *in vitro* digestion (twice in each set). All determinations in each aliquot from *in vitro* digestion were also performed in duplicate.

Results from the study of the effect of processing on bioactive compounds and its activity were analysed by adjustment to a model with fixed effects for a classification factor with nine levels (processing stage

and fruit type). The model included a variance function to consider the presence of an increasing variability pattern related to medium levels of response variable. The adjustment was carried out using an implementation in InfoStat Software of `gls` function from the `nlme` library of R. The variance function applied was a function of implementation of power variance `varPower()` from the `nlme` library. This type of statistical analysis allows comparing, simultaneously, the effect of processing stage and fruit type. All other results were analysed by analysis of variance (ANOVA). In both cases results from the analyses were evaluated by DGC test (Di Rienzo *et al.*, 2002) with a degree of significance of $P < 0.05$.

RESULTS AND DISCUSSION

In a previous research (Bustos, Paesani and León, 2019) we studied the effect of berry inclusion on technological and sensorial quality of pasta which was crucial to evaluate whether addition performed results in a final product with good quality. Now, in the present research, we evaluate polyphenols, anthocyanin and evolution of antioxidant activity during processing in order to identify the stage that produced the higher losses (Figure 1 and 2).

Total polyphenol content was slightly affected during berry-enriched pasta processing, although significant interactions were found between the stage of processing and the type of fruit and also with the level of addition used. Results showed that only the IM stage presented values significantly different from those of other processing stages (except for BCP) ($p < 0.05$). In addition, BBP followed by RBP showed the highest polyphenol content in all processing stages evaluated (except for IM) as can be seen in Figure 1A.

After kneading and sheeting, probably due to the release of bound polyphenols (Fares *et al.*, 2010), TPC increased significantly (except for BCP at 7.5%). It is worth noting that the initial differences on ingredient mixture generated by the different percentages of berry incorporation were minimized during pasta making process (Figure 1A). This observation could be due to the formation of indigestible complexes with gluten proteins and polyphenols reducing the proportion of extractable compounds as observed by Sun-Waterhouse *et al.*, (2013) and (Świeca *et al.*, 2013).

Anthocyanins are known by their low resistance to processing, alkaline pH, or temperature (Ioannou *et al.*, 2012), which agrees with the degradation observed during pasta processing (Figure 1B). As shown in polyphenols, the effect of the processing stage also depends on the type of fruit and level used in pasta samples (significant interactions, $P < 0.05$). The highest anthocyanin content was found in the ingredient mixture (IM), significantly different from that of all other stages, indicating the progressive degradation through processing (Figure 1B), being only detectable in BBP-7.5, RCP-7.5 and both BCP-2.5 and BCP-7.5 cooked pasta sample. It should be noted that during sheeting the major loss in anthocyanin was detected, probably due to the fact that this phase leads to the incorporation of water and oxygen in the dough, promoting their degradation. Anthocyanins from raspberry and redcurrant enriched pasta sample were

those mostly affected during processing, in agreement with our previous report on the effect of drying temperature during berry dehydration (Bustos *et al.*, 2018).

Not much research has focused on the degradation of total polyphenols during processing. Instead, some studies have analysed the evolution of tocopherols and carotenoids during bread, biscuit or pasta making, which determined that major loss takes place during the kneading step, where water and oxygen levels facilitate the action of lipoxygenase, accelerating the rate of their decomposition (Icard-Vernière and Feillet, 1999; Hidalgo and Brandolini, 2010). In our study, we decided to analyse total polyphenols as a first approach to evaluate effect of processing. Accordingly, it is necessary to link the changes in TPC results with the evolution of antioxidant activity in order to establish whether the probable depolymerization of polyphenols leads to an increase in activity.

Figure 2 shows the evolution of ABTS^{•+} radical cation scavenging activity and ferric reducing ability in each stage of the pasta making process. As observed in polyphenols and anthocyanins, significant interactions were found between the stage of processing and fruit type and the level of addition, in both mechanisms of antioxidant activity.

Boysenberry-enriched pasta showed the highest values of ABTS^{•+} radical cation scavenging activity during processing at both levels of addition ($P < 0.05$). On the other hand, red currant enriched pasta presented the lowest values within the same percentage of addition ($P < 0.05$), in agreement with that discussed above.

From ingredient mixture to cooked pasta the scavenging activity decreased between 11.4% for BB 2.5 and 63.5% for RC7.5. The greatest loss was observed during cooking, since part of the bioactive compounds was released by the pasta matrix; yet, such loss was particularly low, suggesting that, despite the heat, the antioxidant properties measured *in vitro* were conserved. The ingredient mixture of pasta with the same level of fruit enrichment had a very similar radical cation ABTS^{•+} scavenging activity. However, after processing and cooking, BBP and BCP were the samples that best kept their antioxidant activity. This observation is in agreement with our previous report where we observed that boysenberry and blackcurrant better maintained their antioxidant properties during berry drying (Bustos *et al.*, 2018).

Ferric reducing ability was very low and showed the same tendency as that of scavenging activity (main mechanism), with a decrease in activity between 0.3% for RB7.5 and 66.8% for RC2.5 throughout pasta making process.

In general, our results confirm that the kneading and sheeting phases led to greatest loss, particularly in the case of anthocyanins, while the drying step caused no significant changes. During cooking, boiling water could enhance the extraction of some bound polyphenols from pasta matrices (increasing the TPC measured), while some of the sensitive ones could reduce their activity (decreasing the ABTS^{•+} scavenging

activity values) (Fares *et al.*, 2008; Sun-Waterhouse, Jin and Waterhouse, 2013), although a considerable proportion of bioactive compounds remains in pasta structure and could provide health benefits. Moreover, pasta with addition of fruits from *Rubus* genus showed a better conservation of polyphenols, while boysenberry and blackcurrant enrichment revealed the slightest loss of antioxidant activity.

Research into the effect of incorporation of functional ingredients in food matrix requires knowing how much of that active compound could survive the digestion process and be bioavailable. As a result, we compared the effect of berry fruits incorporated into pasta with that on starch *in vitro* digestion and antioxidant activity potentially available.

Table 1 shows the rate and extent of starch hydrolysis during oral, gastric and intestinal phases. Inclusion of dried berry in pasta structure resulted in a detriment of its integrity due to gluten network disruption (Bustos, Paesani and León, 2019), developing a more open structure where digestive enzymes may penetrate easily. That explains the higher hydrolysis of starch observed during the oral phase in berry-enriched pasta, as compared to control ($P<0.05$), and also the higher starch degradation in all pasta samples with 7.5% of fruit addition, compared to the corresponding 2.5% counterpart ($P<0.05$), especially for RBP-7.5 that showed a higher starch hydrolysis than control at the end of *in vitro* digestion. It is noticeable that fruits from *Ribes* genus showed the highest starch hydrolysis according to the observed detriment in pasta quality, already reported (Bustos, Paesani and León, 2019). At gastric phase conditions, the berry-enriched pasta samples showed a decrease of around 50% in the extent of starch hydrolysis compared to control sample (Table 1). Once again, red and black currant enrichment leads to a greater starch hydrolysis than other enriched pasta samples ($P<0.05$). These results could be attributed to the fact that at the beginning of digestion the integrity of the pasta structure governs starch hydrolysis facilitating the penetration of enzymes in the matrix. However, when the food is degraded, polyphenols are released from matrix and may inhibit amylolysis of starch as reported by many authors (McDougall *et al.*, 2005; McDougall, Kulkarni and Stewart, 2008). In this regard, the lowest starch hydrolysis values were found in raspberry-enriched pasta, the fruit that has already been associated with inhibition of the porcine pancreatic amylase (Zhang *et al.*, 2010; Grussu, Stewart and McDougall, 2011). Since *Ribes* fruit caused major disruption of the gluten matrix with higher cooking loss than produced by *Rubus* fruit enrichment, hence, a lower starch proportion remained in cooked pasta, reaching a higher percentage of hydrolysis after *in vitro* digestion (Table 1).

Finally, during the intestinal phase, the extent of starch hydrolysis in berry-enriched pasta was higher than that in control sample, particularly in the case of *Ribes* genus fruit ($P<0.05$). That means that, at the end of the intestinal phase, the structure of pasta sample was so much disrupted and degraded, that the starch became easily available for enzymes and the extent of hydrolysis increased. Considering the entire *in vitro*

digestion, the enrichment of pasta with 2.5% of raspberry or boysenberry led to a decreased digestibility, despite the fact that, when a higher amount of fruit was added, the weakening of the gluten network allowed contact between enzymes and starch and the effect of inhibition turned less meaningful. Kinetic constant in berry-enriched pasta remained very similar during the entire *in vitro* digestion.

For nutritional purposes, starch can be classified into three classes depending on their rate and extent of digestion as established by Englyst (1996). These include rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS), as shown in Table 2. It is clear that the effect of fruit components on starch hydrolysis takes place at the beginning of *in vitro* digestion by significantly reducing the RDS fraction (except for RCP-7.5), as compared to control pasta. Then, the SDS fraction was significantly higher in all enriched pasta samples than that in control (except for BBP-2.5) ($p < 0.05$). It should be noted that the incorporation of fruit from *Ribes* genus significantly increases the SDS fraction, which indicates that, in addition to the highest amount of total starch hydrolysed observed ($p < 0.05$), it takes place more slowly, decreasing the amount of RDS associated to poor nutritional quality (Hasjim *et al.*, 2010). Even so, a resistant starch fraction higher than that found in the control sample was observed in enriched pasta with those fruits ($p < 0.05$). These results indicate that considering the total amount of hydrolysed starch at the end of digestion should not be enough, since the rate at it takes place could increase SDS, involved in health claims (Vinoy *et al.*, 2015).

In order to exert a health benefit, polyphenols have first to be bioaccessible, i.e., they have to be released from the pasta matrix and solubilized (Halliwell, Rafter and Jenner, 2005; Seeram and Commission, 2008). As a result, the potentially bioaccessible and dialyzable fractions of polyphenols and antioxidant activity were determined (Figure 3).

Berry-enriched pasta samples showed an increase in potentially bioaccessible polyphenols and scavenging activity compared to cooked pasta, suggesting that phenolics probably underwent depolymerization during simulated GI digestion. As a result, the average content of polyphenols and scavenging activity on cooked pasta was 251 mg GA/100 g sample and 1232 μmol Trolox/100g sample, respectively, while the corresponding values after digestion were: 729.8 mg GA/100 g sample and 4207 μmol Trolox/100g sample, respectively.

In general, the potentially bioaccessible polyphenols were higher in pasta enriched with *Rubus* genus fruits than with *Ribes* ones. Yet, in relation to the results shown by cooked pasta, raspberry- and boysenberry-enriched pasta showed an increase of around 260%, while red and black currant enrichment presented around 360% increase in polyphenols. Thus, fruits from *Ribes* genus better conserved the bioactive compounds during digestion, as compared to addition of *Rubus* fruits, around 26% and 34% of

bioaccessible polyphenols being dialyzable, respectively. However, the total polyphenol content in the potentially bioaccessible fraction was very similar between all the berry-enriched pasta.

As the proportion of fruit added to pasta increased, the potentially bioaccessible scavenging activity increased significantly (except for BBP) ($P < 0.05$). Hence, adding berry fruit to pasta formulation allowed release of polyphenols from pasta matrix into the gastrointestinal tract, with an increase of around 400% of activity, compared to cooked pasta; 40% of potentially bioaccessible activity was detectable in dialyzable fraction. Reducing ability was very similar to that observed in cooked pasta with around 20% being dialyzable.

In addition to the low values of dialyzability index, even if bioactive compounds remained unabsorbed, the bioaccessibility of antioxidants at the gastrointestinal tract produced a wide variety of reactive species such as reactive oxygen species (ROS), which is of significant interest. As a result, antioxidants may play an important role in the GI tract by maintaining redox equilibrium against harmful oxidants, preventing GI tract diseases linked to ROS generation during the digestion process (Halliwell, Rafter and Jenner, 2005).

As a consequence, adding raspberry or boysenberry to pasta may produce a considerable release of polyphenols during digestion which remain active and could provide health benefits as well as decrease in the extent and rate of starch hydrolysis. Furthermore, we cannot disregard the fact that the limited time of interaction between digesta and the dialysis tube, which is markedly different from that *in vivo*, also reduced the fraction of dialysable phenolics.

CONCLUSION

Our results have demonstrated that the kneading and cooking steps play a crucial role in the preservation of bioactive compounds. Berry fruits are a rich source of potentially bioaccessible polyphenols, and the current study shows the potential of raspberry and boysenberry to reduce the glycaemic response to pasta products through *in vitro* digestion, which retards starch hydrolysis (probably due to inhibition of digestive enzymes), promoting an increase in slowly digested starch and reducing the total starch hydrolysed. In addition, after *in vitro* digestion the polyphenols greatly increased compared to those found in cooked pasta, particularly red and blackcurrant, probably due to depolymerization, increasing the scavenging activity. In addition, free soluble antioxidants that could cross a cellulose membrane and thus be potentially available for uptake, account for only 20% of polyphenols in *Rubus* enriched pasta and almost 40% of scavenging activity, compared to potentially bioaccessible fraction in the intestinal digesta. It should be noted that the data obtained with a static *in vitro* model of simulated GI digestion cannot be directly extrapolated to human *in vivo* conditions. Our results represent a preliminary and necessary step for studying berry enrichment of pasta as an effective tool that allows obtaining functional food with a

significantly enhanced nutraceutical potential. Together with awareness raising, the use of dehydrated berry fruit in pasta may contribute to consumption of more sustainable food.

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DATA AVAILABILITY, ETHICAL AND CONFLICT OF INTEREST STATEMENTS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval was not required for this research. The authors declare that they have no conflicts of interest.

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FIGURE CAPTIONS

Figure 1.- Evolution of total polyphenol (A) and monomeric anthocyanin (B) content during pasta making process.

IM: ingredients mixture, FP: fresh pasta, DP: dried pasta, 50%-CP: pasta cooked until 50% of cooking time, CP: cooked pasta. RBP: raspberry pasta, BBP: Blackberry pasta, RCP: redcurrant pasta, BCP: blackcurrant pasta. 2.5 and 7.5 indicate substitution level. Stripped columns: 2.5% and filled columns: 7.5% of substitution. *: not detectable.

Figure 2.- Evolution of scavenging activity of radical cation ABTS^{•+} (A) and ferric reducing ability (B) during pasta making process.

IM: ingredients mixture, FP: fresh pasta, DP: dried pasta, 50%-CP: pasta cooked until 50% of cooking time, CP: cooked pasta. RBP: raspberry pasta, BBP: Blackberry pasta, RCP: redcurrant pasta, BCP: blackcurrant pasta. 2.5 and 7.5 indicate substitution level. Stripped columns indicate 2.5% of substitution and filled columns 7.5%.

Figure 3.- Potentially bioaccessible (striped bars) and dialyzability (solid bars) fractions of polyphenols and antioxidant activity after *in vitro* berry-enriched pasta*

* C: control pasta, RBP: raspberry pasta, BBP: Blackberry pasta, RCP: redcurrant pasta, BCP: blackcurrant pasta. 2.5 and 7.5 indicate substitution level.

Accepted Article

Table 1.- Adjusted parameters obtained with kinetic equations for starch hydrolysis during *in vitro* oral-gastric and intestinal phases*

Sample	Starch hydrolysed at oral-gastric phase (%) ($C_{g\infty}$ * ¹)		Kinetic constant at oral-gastric phase (min^{-1}) (K_g * ¹)	Initial starch concentration at intestinal phase (%) (C_0 * ²)	Starch hydrolysed at intestinal phase (%) ($C_{i\infty}$ * ²)	Kinetic constant at intestinal phase (min^{-1}) (K_i * ²)	Total starch hydrolysed (%)
	Oral phase	Gastric phase					
CP	1.4 ^a	32.5 ^g	0.018 ^a	29.6	37.1 ^a	0.026 ^d	70.9
RBP- 2.5	2.5 ^b	12.4 ^b	0.029 ^b	14.9	45.2 ^c	0.021 ^c	60.2
RBP- 7.5	3.7 ^c	16.3 ^d	0.020 ^a	19.4	52.1 ^d	0.029 ^e	72.0
BBP- 2.5	3.7 ^c	9.7 ^a	0.016 ^a	15.8	39.3 ^b	0.040 ^e	52.6
BBP- 7.5	5.1 ^e	11.4 ^c	0.019 ^a	18.1	54.8 ^e	0.029 ^e	71.2
RCP- 2.5	4.1 ^c	15.5 ^d	0.018 ^a	17.8	66.0 ^g	0.013 ^a	85.7
RCP- 7.5	5.7 ^f	15.1 ^f	0.024 ^b	23.5	51.1 ^d	0.026 ^d	77.6
BCP- 2.5	4.1 ^c	16.3 ^d	0.019 ^a	18.5	59.0 ^f	0.016 ^b	79.4
BCP- 7.5	4.7 ^d	18.3 ^e	0.018 ^a	22.3	58.4 ^f	0.017 ^b	81.3

Note: *¹Parameters of the kinetic equation $C = C_{\infty} (1 - e^{-Kt})$. *²Parameters of the kinetic equation $C = C_0 + C_{\infty} (1 - e^{-Kt})$.

Parameter C_0 has no statistical analyses since it is equivalent to $C_{g\infty}$. Values are presented to demonstrate the accurate adjustment of both equations. CP: control pasta, RBP: raspberry pasta, BBP: Blackberry pasta, RCP: redcurrant pasta, BCP: blackcurrant pasta. 2.5 and 7.5 indicate substitution level. *Different letters in the columns indicate significant difference $p < 0.05$.

Table 2.- Rapidly (RDS), slowly (SDS) and resistant digestible starch (RS) fractions in berry-enriched pasta samples*

Sample	RDS	SDS	RS
	(g/100 g starch)		
CP	44.8 ^f	20.3 ^a	1.3 ^a
RBP- 2.5	30.6 ^a	26.0 ^b	2.5 ^c
RBP- 7.5	42.6 ^e	27.3 ^c	1.3 ^a
BBP- 2.5	32.7 ^b	21.1 ^a	1.1 ^a
BBP- 7.5	42.1 ^e	29.1 ^d	1.4 ^a
RCP- 2.5	33.2 ^b	37.2 ^f	7.3 ^e
RCP- 7.5	44.0 ^f	28.2 ^d	1.9 ^b
BCP- 2.5	34.4 ^c	34.1 ^e	5.5 ^d
BCP- 7.5	38.7 ^d	33.9 ^e	5.1 ^d

*Fractions calculated as proposed by Englyst and Hudson (1996) from starch digestion curves obtained from in vitro digestion. CP: control pasta, RBP: raspberry pasta, BBP: Blackberry pasta, RCP: redcurrant pasta, BCP: blackcurrant pasta. 2.5 and 7.5 indicate substitution level. Different letters in the columns indicate significant difference $p < 0.05$.





