Heat damage and in vitro digestibility of infant biscuits are affected by the type of dairy ingredients present in their formulation

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Keywords:	Infant biscuit, digestibility, heat damage, structure

Title

Heat damage and *in vitro* digestibility of infant biscuits are affected by the type of dairy ingredients present in their formulation

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Abstract

The inclusion of dairy ingredients in the formulation of infant biscuits (IB) could translate in heat damage during baking and alteration of starch and protein digestibility. To study these phenomena, five experimental IB were prepared using milk protein concentrate (MPC), whey protein isolate (WPI) or skim milk powder (SMP). The highest heat damage measured as furosine and pyrraline contents characterized the IB prepared using SMP, which contained the highest amount of lactose. The IB including WPI and MPC and containing the highest amounts of whey protein showed the lowest *in vitro* protein digestibility, whereas the least starch digestibility was observed in the experimental IB containing the higher amount of milk protein. The ultrastructure of IB was not indicative of the diverse protein digestibility. The heat damage of six commercial IB was higher than that suffered by the experimental ones, whereas their starch and protein digestibility partially overlapped that revealed for experimental IB.

Keywords: infant biscuit, digestibility, heat damage, structure

Introduction

Biscuits for infants (IB) are targeted to meet the nutritional needs of infants (6-12 months) or toddlers (12-35 months) and they are commonly formulated using wheat flour, sucrose, vegetable fat, skimmed powdered dairy ingredients and supplemented with minerals and vitamins. Usually, oven baking of biscuits is carried out at temperature around 200 °C for less than 20 min with the goals of reducing the water content at values ≤ 10% and developing a superficial browning [1]. These time/temperature conditions promote starch gelatinization, protein denaturation, protein aggregation, and protein/sugar interaction via Maillard reaction (MR). In particular, the heat damage related to onset of MR in foods has been reported to impact on both protein and starch digestibility as well as bioavailability of indispensable amino acids and other nutrients [2]. In this regard, biscuits are among the cereal based products presenting the highest levels of chemical indices of the early (e.g. furosine, FUR) and the advanced stages (e.g. pyrraline, PYR; glycosyl and galactosyl isomaltol) of MR [1,3,4].

Dairy ingredients such as caseinates, skimmed milk powder (SMP), milk protein concentrate (MPC), whey protein concentrate (WPC) and whey protein isolate (WPI) are used to improve sensorial, rheological and nutritional properties of IB. The inclusion of such ingredients in the formulation of biscuits improves their flavor and texture besides hindering staling [5]. Focusing on IB administrated during weaning, the nutritional benefits have the major importance. Indeed, the presence of dairy proteins in IB help to modulate the transition from an exclusively milk based diet to a more diversified one. In addition, the presence of milk proteins brings benefits in terms of supplementation of indispensable amino acids among which lysine, methionine and tryptophan [6]. Dairy ingredients used in the formulation of IB mainly differ for concentration and type of milk protein and level of lactose they contain. Casein and whey protein are present in SMP and MPC in the same ratio as liquid milk whereas WPC and caseinate contain merely whey proteins and casein, respectively. The content of lactose is about 50% in SMP and varies from 5-10% to 40% in commercial WPC and MPC depending on the effectiveness of its removal during the ultrafiltration (UF) necessary for preparation of these milk derivatives [7]. The presence of dairy ingredients with different chemical or technological features in the formulation of IB could affect the extent of MR upon baking and the protein and starch digestibility of biscuit. Equally, denaturation and aggregation of protein themselves can vary the degree and kinetic of protein breakdown during gastrointestinal digestion (GID). In literature, the cited phenomena have been scarcely deepened in IB.

The aim of the present study was to assess the heat damage of experimental IB formulated with dairy ingredients with diverse protein composition and lactose content. The protein and starch digestibility of doughs and derived IB was assessed using an *in vitro* static protocol mimicking the gastrointestinal conditions of infants aged 6-12 months. Attempts to relate digestibility and the ultrastructural properties of IB was carried out by scanning electron microscopy (SEM). A further objective was to evaluate the heat damage and the digestibility of commercial IB of the main brand marketed in Italy and containing the same type of dairy ingredients.

Materials and methods

Model doughs, infant biscuits and commercial biscuits

Five model IB (named 0, 1, 2, 3, and 4) were prepared by oven baking doughs formulated as reported in Table 1. White wheat flour was produced from bread wheat cv. Bramante cropped in the fields of Consiglio per la ricerca e l'economia agraria (CREA), Sant'Angelo Lodigiano; olive oil and chemical leavening agents were purchased at the local market. Wheat starch was from Sigma-Aldrich (Milan, Italy). MPC, WPI and SMP were used for the formulation of doughs of IB 1 to 4. All these dairy ingredients were from Fonterra Co-operative Group (Auckland, NZ). Model IB were obtained

by oven cooking the doughs at 205 °C for 11 min in a Hobart HO 300E electric oven (National MFG CO, Lincoln, Nebraska, U.S.A.). Six commercial samples of IB of the main brands marketed in Italy were purchased at the local market. According to the information on the label, five of these IB contained dairy products among ingredients.

Determination of targeted heat-damage indices

Furosine was determined according to the Standard ISO 18329-2004 [8]. For the determination of PYR the method proposed by Resmini and Pellegrino [9].

Chemical determinations on dairy ingredients, doughs and biscuits

Protein and water contents were determined according to Standard ISO 8968:2014 [10] and method AACCI 44-15.02 [11], respectively. Soluble sugars were determined by HPLC as reported by Stuknyté et al. [12]. Lactose content in dairy ingredients was determined using the Standard ISO 22662:2007 [13]. In the same ingredients, soluble whey proteins were evaluated according to the Standard ISO 13875:2005 [14].

***In vitro* static gastrointestinal digestion**

Digestions of doughs and derived IB were carried out using the static *in vitro* protocol reported by Minekus et al. [15] modified according to McClean and Weaver [16] and Bourlieau et al. [17] in order to mimic the gastrointestinal conditions of infants aged from 6 to 12 months. In detail, samples (5 g) were mixed with 5 mL of simulated gastric fluid (SGF) supplemented with porcine pepsin (800 U/mL gastric digestate). The gastric phase of digestion was performed at 37 °C for 2 h at pH 3.0 (1 M HCl). Afterwards, 10 mL of simulated intestinal fluid (SIF) and bile salts (2 mM; Sigma-Aldrich, Milan, Italy) were added to the gastric digestate. Enzymes for intestinal digestion were porcine trypsin (100 U/mL intestinal digestate) and bovine chymotrypsin (35 U/mL intestinal digestate), pancreatic amylase (250 U/mL intestinal digestate), intestinal lipase (130 U/mL intestinal digestate) and co-lipase (molar ratio lipase/co-lipase 2:1). The intestinal phase of GID was performed at 37 °C for 2 h at pH 7.0. It was stopped by adding the protease inhibitor AESFB (Roche, Mannheim, Germany) to give a 1 mM final concentration. The intestinal digestates were immediately frozen at -40 °C and freeze-dried. All enzymes were purchased from Sigma-Aldrich (Milan, Italy). Each sample was submitted to three replicate digestions on the same day.

Evaluation of starch and protein degradation during *in vitro* GID of IB

The degree of starch hydrolysis following *in vitro* digestion of IB was assessed by determining the content of maltotriose, maltose and glucose as reported by Stuknyté et al. [12].

Aliquots of each *in vitro* digestate of IB and dough were submitted to UF (3 kDa) and the obtained permeates were submitted to the determination of nitrogen (N) content adopting the Standard ISO 8968:2014 [10]. Protein breakdown was calculated as follows:

Protein breakdown (%) = $[(N_{GID} - N_b) - N_{SB}]/ N_T \times 100$

with:

N_{GID}, the N content of the UF permeate of the IB after GID.

N_b, the N content of the UF permeate of the blank sample (enzymes and simulated digestive fluids) after GID.

N_{SB}, the N content of UF permeates of the IB and doughs dissolved in 30 mL of water.

N_T, the total N content of the model IB.

Scanning electron microscopy (SEM)

IB were mounted on aluminum stubs and sputter-coated with gold. On each stub, cross-sectioned pieces (selected at random) of IB were mounted. Ultrastructures of IB were imaged using a LEO1430 SEM (Carl-Zeiss AG, Oberkochen, Germany) at an accelerating voltage of 7 kV.

Statistical analysis

Analysis of variance (ANOVA) was performed with Daniel's XL Toolbox adding for Excel, version 6.60, by Daniel Kraus, Würzburg, Germany (available at: http://xltoolbox.sourceforge.net/).

Results and discussion

Heat damage of model infant biscuits

The level of FUR and PYR was preliminarily determined in the dairy ingredients used for formulation of doughs. Among them, WPI showed the lowest content of FUR (18.8±0.5 mg/100 g protein). This finding is attributable to the low level of lactose (0.5±0.1 g/100 g), which was mostly removed by UF and diafiltration of whey protein retentate. The lack of lactose hindered FUR formation during concentration and drying applied to retentate for WPI manufacturing. On the contrary, the presence of lactose in MPC and SMP (5.9±0.1 g/100 g and 54.1±0.5 g/100 g, respectively) promoted a relevant protein glycation during processing as highlighted by their FUR content (172±3 and 273±5 mg/100 g protein, respectively). In this regard, the pre-heating conditions applied to liquid milk used for the production of the SMP sample proved to be particularly severe. Indeed, as evidenced by the Whey Protein Nitrogen Index value (1.4 mg/g) reported in the technical sheet, the SMP was assigned to the "High Heat" thermal class. All the studied dairy ingredients did not contain PYR.

The FUR levels of experimental IB 0, 1 and 2 (16.9±0.9, 38.3±1.6 and 25.1±1.5 mg /100 g protein, respectively) were low because of the absence or almost nil levels of lactose in their formulations as well as the low levels (<0.5 g /100 g) of reducing sugars from flour and starch hydrolysis during mixing step. Higher values of FUR were found for IB 3 (379±11 mg/100 g protein) and 4 (168±6 mg/100 g protein), which contained 2.95±0.28 and 0.78±0.11 g/100 g of lactose, respectively and only negligible amount of other reducing sugars. The FUR level of these experimental IB can be attributable to the enhancement of MR promoted by lactose during baking only in sample IB3, whereas the FUR content of sample IB4 was mainly attributable to the contribution (83%) of the FUR level (172.3 mg/100 g protein) of the MPC included in the formulation. To the authors knowledge the level of FUR in IB was not reported in literature previously. For this reason, our data can only be compared to those of similar baked products. For instance, the FUR levels of IB 0, 1 and 2 were slightly lower than those (32.6–46.5 mg/100 g protein) reported for experimental wheat water biscuits cooked at 200 °C for 25 minutes and formulated without the addition of dairy ingredients [3]. The relevant extent of the MR in IB 3, as revealed by the FUR index, is also supported by the formation of PYR (5.1±0.2 mg/kg), which was not detected in the remaining samples of IB. To date, no data concerning the presence of PYR in IB are reported in literature. Nonetheless, PYR values varying from 2.1 to 9.4 mg/kg were previously found in commercial cookies by Hellwig and Henle [4].

Breakdown of protein and starch during GID of doughs and model IB

The *in vitro* GID protocol adopted in this study is reported by Minekus et al. [15], with some changes to better mimic the physiological parameters of digestive tract of infant aged 6 to 12 months. The main modifications of the protocol consisted in the reduction of both the enzymes activity and the concentration of bile salts. Indeed, the gastrointestinal conditions of infant are far to be optimal for protein degradation, and large peptides can resist gastric and intestinal proteolysis thus remaining intact. Based on this, the degree of milk protein breakdown was evaluated by measuring the N content of UF permeates before and after *in vitro* GID of doughs and derived IB. A similar approach was previously adopted by Villemejane, et al. [18] for the evaluation of the protein breakdown upon *in vitro* digestion of short-dough biscuits and by Cattaneo et al. [19] to assess the *in vitro* digestibility of liquid model systems of infant formulas.

A comparable degree of protein degradation upon GID characterised the doughs containing milk proteins, irrespective of the dairy ingredient added to the formulation, whereas the lowest protein degradation was observed in IB 0 containing gluten proteins as the sole protein source (Table 2). The heat treatment applied during baking translated in a decrease of protein digestibility in all the studied samples with the exception of the dairy-protein free IB (sample 0). The protein degradation upon *in vitro* digestion of this sample kept substantially unmodified between dough and baked sample. Several authors studied the effect of heat treatment on gluten digestibility by comparing those of flour and doughs with that of the corresponding baked product. Smith et al. [20] observed a reduction of digestibility of purified gluten proteins from wheat flour and wheat flour after heat treatment. The greater surface area of flour with respect to baked products was reported to provide better enzymes accessibility to protein and starch. An opposite effect of baking on protein degradation during digestion was reported by Abdel-Aal and Rabalski [21] who observed an increase of digestibility of wheat biscuits upon baking. The mechanisms involved in this phenomenon were not highlighted by the author. It can be hypothesized that the lower digestibility of the wheat doughs is determined by its high viscosity, which hinders the protein degradation during GID [22]. Pasini et al. [23] reported the protein digestibility to be largely dependent on the heat load during baking. In this regard, the digestibility of bread dough was found to be similar to that of bread crumb, but markedly higher than crust digestibility. The same authors justified this evidence with the different heat load suffered by crust and crumb during baking that led to a different degree of formation of Maillard type interactions and/or interpeptides cross-linking. As evidenced by the low level of FUR, it is likely that the baking conditions applied for cooking of the studied IB were too mild to affect protein digestibility of sample IB 0.

The decrease of protein digestibility was substantial in all model IB formulated with dairy ingredients (Table 2). The highest decrease was revealed in samples IB 2 and IB 4, which contained the highest amount of whey protein. Despite the dairy ingredients added to the formulation of IB contained different amounts of soluble whey protein (Table 2), it is likely that baking conditions led to a complete denaturation and aggregation of these proteins. According to De Wit [24] α-lactalbumin denaturation occurs above 64 °C, whereas β-lactoglobulin begins to loss the native structure above 78 °C and the formation of whey protein aggregates occurs when temperature is higher than 85 °C. It was also reported [25] that baking of bread containing WPC at 230 °C promotes the denaturation of the whey protein added to the formulation and the subsequent formation of denatured whey proteins aggregates scarcely susceptible to enzyme degradation [2]. In addition, the occurrence of polymerization between gluten proteins and whey protein *via* disulphide bonds was reported by Lambrecht et al. [26]. The formation of protein aggregates hardly susceptible to gastrointestinal degradation could be a factor contributing to the decrease in digestibility of the whole protein fraction of IB. Finally, the reduction of protein digestibility in IB 1, 3 and 4 could also be ascribed to a hindered degradation of casein by gastrointestinal enzymes due to the interactions occurring upon heating between denatured whey protein and casein [27].

The decrease of protein digestibility upon baking paralleled the amount of whey protein added in the formulation via dairy ingredients, with the exception of IB 3. In this case, beside whey protein denaturation, the relevant extent of MR

that led to PYR formation represents a factor contributing to the decrease in protein digestibility [2]. Overall, data emerging from our study demonstrated that protein digestibility of IB is lowered when dairy ingredients containing whey protein, regardless of their chemical status before baking, are included in the initial formulation of dough.

The starch *in vitro* digestibility was assessed by measuring the release of maltotriose, maltose and glucose following *in vitro* GID of doughs and IB (Table 3).

Undigested samples showed similar levels of soluble sugars. No significant differences in starch degradation were found after *in vitro* GID of doughs containing wheat flour (samples IB 0 to IB 3). Contrarily, the sample IB 4 formulated without wheat proteins showed the higher content of released sugars upon digestion. In this sample the susceptibility of starch to enzymatic hydrolysis was enhanced by the absence of the gluten network. In this regard, it has been widely demonstrated that the presence of a gluten protein network surrounding starch granules impairs their breakdown by amylases [28-30].

After baking, a marked reduction (32.4-55.3%) in the total amount of released soluble sugars was highlighted for all the model IB samples submitted to *in vitro* GID (Table 3). Our data seem to contrast with some evidences reported in literature [31] according to which an increase of starch digestibility was observed after baking of biscuit containing wheat flour. These authors stated that the enhancement of the rate of amylolysis upon heat treatment lies in different starch modifications such as chemical breakdown, gelatinization and retrogradation (occurring in presence of water), that make it more susceptible to amylases. However, according to Singh et al. [29] baking is less effective in enhancing starch digestibility than other processing, such as puffing, roasting, pressure cooking and autoclaving. Contrary to what reported by Sterbova et al. [31] and according to the evidences of the present experimentation, Roopa and Premavalli [32] reported that baking of finger millet promotes a decrease in starch digestibility as a consequence of the limited starch gelatinization occurring during dry thermal processing. The ultrastructural observations confirmed that this phenomenon characterized the baking process of IBs, as the native structure of the starch granules seemed to be conserved in all IB samples. The evaluation of starch digestibility in cereal products cannot be examined separately from the effect of the presence of ingredients other than starch on amylolysis. Targeted studies regarding the effect of milk protein on starch degradation during digestion are lacking, but it is worth noting that sample IB 4 presented both the lowest amount (29.36±0.88 g/100 g d.m.) of sugars released upon *in vitro* GID and the highest reduction (55.3%) in released sugars after baking. Differently from the other IB, the protein matrix of this sample was solely constituted by casein and whey protein, which were present in MPC at the same ratio (80:20) of liquid milk. The interactions between casein and starch formed upon heating seems to preserve starch from hydrolysis in a more performing way than gluten alone or gluten/whey protein aggregates. In this regard, a reinforcement of the starch granule structure due to the formation, *via* lipid-protein layer, of aggregates deriving from the interaction of starch with β-casein and α_s-casein has been reported by Kett et al. [33] in maize starch/casein/oil model systems submitted to heat treatment (70 °C) and containing an amount (10%) of water similar to that of IB model systems. The same authors report the inability of whey protein to both interact with the lipid fraction and consequently to reinforce starch granule structure. These evidences confirm the relationship between the different degree of starch digestibility and the presence of diverse protein matrices.

Besides the interaction with lipid-protein layer, the starch digestibility can be affected by the formation of starch-lipid interactions as well [29]. Since a similar amount (5.7-5.9%) of olive oil was added in the IB samples here considered, differences in starch digestibility cannot be directly ascribed to the presence of lipids in the formulation of IB.

Ultrastructure of baked model IB

The ultrastructure of different model IB was investigated by SEM (Figure 1). After baking, the protein matrix of IB 0 looked continuous with starch granules distributed inside the protein network. In sample IB 1 the presence of MPC seemed

to render the protein matrix more uniform. Moreover, the addition (14.0 g/100 g) of wheat starch into the initial dough's formulation reflected in more visible granules regularly distributed within the protein matrix. At greater magnification (x 300, not shown), these granules appeared only partially deformed and poorly gelatinized. When WPI was present (sample IB 2), the protein matrix seemed more aggregated, but in the same time a little more porous than in IB 1. Also in IB 2, the granules of added starch (14.0%) were evenly distributed and only negligible gelatinization was observed (not shown). The SEM image of IB 3 showed the presence of SMP to determine a similar physical protein and starch organization in the final product. Finally, in sample IB 4, the absence of gluten and the addition of greater amount (45.6%) of starch resulted in an irregularly shaped protein matrix in which the starch granules were not easily recognizable. In general, the SEM of model IB revealed a certain uniformity among ultrastructure of samples containing dairy ingredients. In the absence of gluten, SEM showed a less regular structure likely due to the large incorporation of starch and poor presence of protein. Overall, the SEM images of IB 0 to 3 showed the baking process to induce similar changes in the characteristics of starch so partially explaining the comparable starch digestibility observed for these IB samples. On the contrary, the ultrastructure of the protein network could not be related to the diverse protein digestibility observed for the studied experimental IB.

Commercial IB

Among commercial IB, the samples A, D, E and F included in their formulation both dairy and cereal ingredients. Sample B contained protein exclusively deriving from dairy ingredients, whereas sample C contained exclusively cereal proteins. Commercial IB showed a content of FUR ranging from 268±5 to 2376±12 mg/100 g protein (Table 4). This range partially overlaps the level of FUR (25.0-982 mg/100 g protein) reported by García-Baños et al. [34] in commercial cookies containing different amounts of lactose. The extent of MR during baking led to a content of PYR from 10.2 to 29.8 mg/100 g protein (Table 4). The content of PYR (10.2±0.4 – 29.8±0.8 mg/100 g protein) in commercial IB was slightly higher than that reported for cookies (2.1-9.4 mg/kg) and roughly lies in the range of wheat bread (6.3-56.7 mg/kg) [4]. The highest level of heat damage *via* MR was observed for sample B, which contained SMP and MPC to replace gluten. Besides different baking conditions, the remarkably more relevant heat damage in terms of MR suffered by commercial samples of IB compared to that of the experimental ones could also be ascribed to the presence of diastatic malt flour containing partially hydrolysed starch added to the formulation.

Protein breakdown of commercial IB ranged from 68.9±2.2 to 86.0±0.9% (mean 75.6±2.0%) (Table 4). The heat damage related to MR has been reported to impact both protein digestibility in foods [2]. In particular, an effect of glycation *via* MR occurring during baking on protein digestibility can be observed since sample B showed the highest values of both FUR and PYR, and the lowest digestibility (Table 4). On the contrary, sample D was characterized by a limited heat damage, which translated in the highest observed protein digestibility.

The amounts of sugars released during *in vitro* digestion of commercial IB ranged from 27.4±1.1 and 32.2±1.7 g/100 g d.m. and partially overlapped those arising from starch hydrolysis in experimental IB. Due to the presence of unknown amounts of diastatic flour in the formulation of commercial IB, it is difficult to relate the degree of starch digestibility to the heat damage and/or to the protein digestibility of these samples.

Conclusions

The modifications of starch and protein digestibility in baked cereal products are generally evaluated in relation to the heat treatment conditions applied during processing. This approach could be limiting when dairy protein based ingredients are included in the formulation of IB. Data obtained in the present research highlighted that type and amount of milk

protein together with the presence of lactose can impact on the digestibility of these components. In particular, the presence of whey protein greatly hindered the protein digestibility due to denaturation and aggregation of protein upon baking. Modifications arising from the MR seemed to affect digestibility in presence of high levels of heat damage, as emerged from the data obtained for commercial IB. Based on the above results, manufacturers of IB, besides considering the positive role in terms of nutritional and rheological properties of dairy ingredients, should consider that the selection of dairy derivatives with proper characteristics in terms of protein composition and lactose content may be crucial for modulating the digestibility of IB.

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Caption to figure

Fig. 1 Scanning electron microscopy images of model infant biscuits prepared with different formulations (0-4; see Table 1). (S: starch; P: protein matrix)

Figure 1

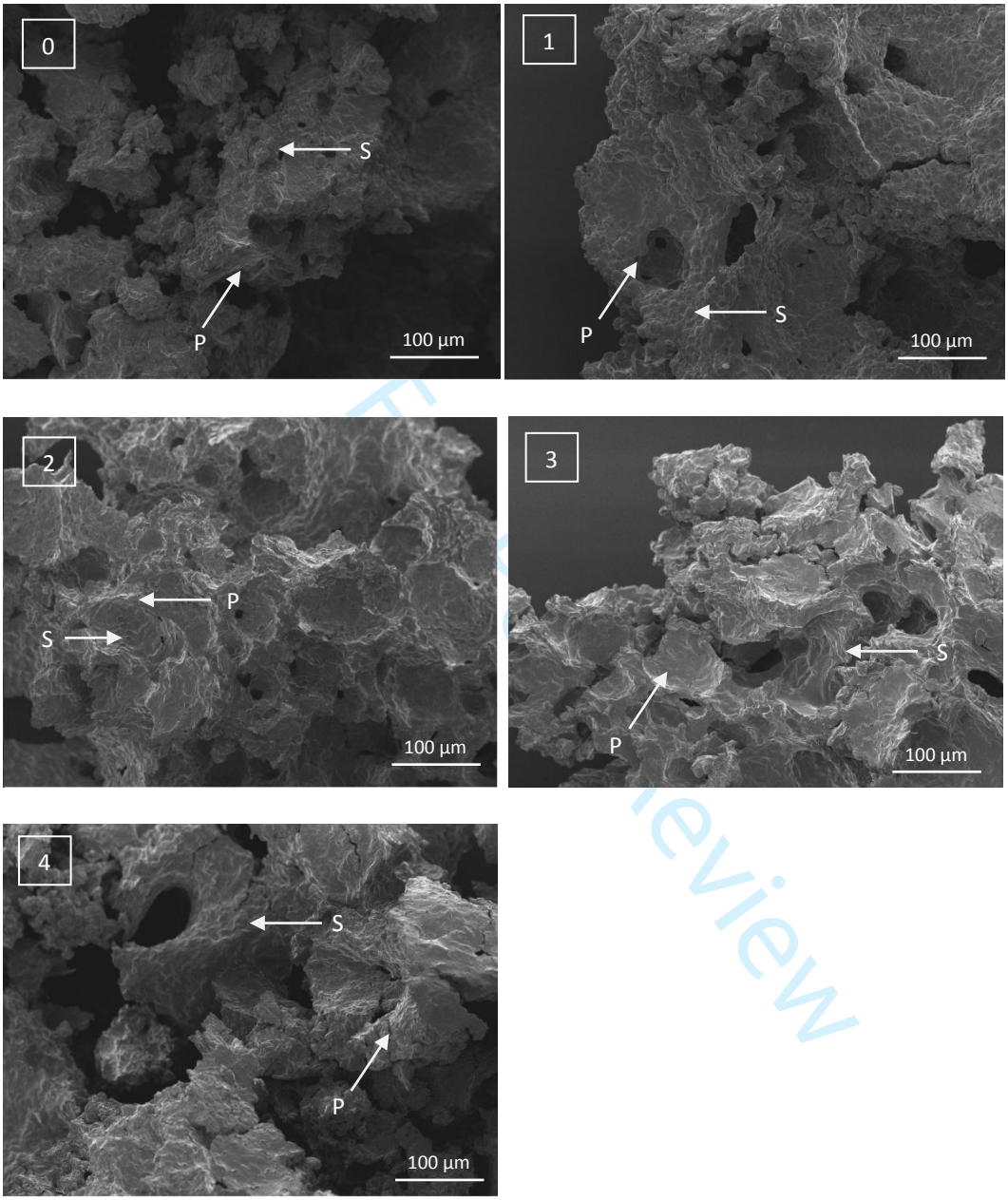

Table 1 Ingredients and formulation of experimental infant biscuits

Ingredients (g/100 g)	Doughs				
	0	1	2	3	4
Wheat flour	58.9	43.0	43.0	43.0	n.p.
Sucrose	18.2	18.9	18.9	16.3	18.2
Olive Oil	5.7	5.9	5.9	5.9	5.7
MPC[1]	n.p.	1.9	n.p.	n.p.	7.4
WPI[2]±	n.p.	n.p.	1.7	n.p.	n.p.
SMP[3]	n.p.	n.p.	n.p.	4.7	n.p.
Wheat starch	n.p.	14.0	14.0	14.0	45.6
Leavening agents[4]	0.4	0.4	0.4	0.4	0.4
Water	16.8	15.8	15.8	15.8	22.9

Table 2 Whey protein content in IB samples and protein breakdown upon *in vitro* gastrointestinal digestion of experimental doughs and biscuits. ([1]MPC: milk protein concentrate; [2]WPI: whey protein isolate; [3]SMP: skim milk powder)

Sample	Dairy ingredient	Whey protein		Protein breakdown	
		Total	Soluble	Doughs	Biscuits
		(g/100 g)		(%)	
0	-	-		69.3±1.1[b]	70.6±0.9[b]
1	MPC[1]	0.32	0.17	77.9±2.2[a]	69.0±1.2[b]
2	WPI[2]	1.59	1.49	80.3±2.5[a]	61.5±1.8[d]
3	SMP[3]	0.26	0.00	78.8±3.1[a]	65.4±1.1[c]
4	MPC[1]	1.10	0.75	76.7±2.8[a]	60.2±2.2[d]

Values of protein breakdown are presented as means ± SD (n=3). Data with different superscript differ ($p<0.05$)

Table 3 Content (g/100 g dry matter) of sugars in experimental biscuits and doughs before and after *in vitro* gastrointestinal digestion

		Biscuits				Doughs
	Sample	Maltotriose	Maltose	Glucose	(Total)	(Total)
0	*Undigested*	0.14±0.02^{e}	0.36±0.05^{e}	0.06±0.01^{d}	0.56±0.08^{c}	1.00±0.11
	Digested	10.58±0.88^{c}	19.94±0.27^{c}	2.86±0.10^{c}	33.38±1.25^{b}	59.53±1.12^{a}
1	*Undigested*	0.12±0.02^{e}	0.32±0.01^{e}	0.06±0.02^{d}	0.53±0.05^{c}	0.78±0.08
	Digested	12.60±0.12^{a}	22.28±0.15^{a}	3.77±0.11^{a}	38.95±0.38^{a}	57.63±1.55^{ab}
2	*Undigested*	0.110±.01^{e}	0.35±0.02^{e}	0.09±0.01^{d}	0.55±0.04^{c}	0.88±0.09
	Digested	11.45±0.44^{b}	21.74±0.23^{a}	3.09±0.11^{b}	36.28±0.78^{a}	57.99±1.62^{ab}
3	*Undigested*	0.12±0.01^{e}	0.32±0.04^{e}	0.07±0.01^{d}	0.52±0.06^{c}	0.89±0.04
	Digested	9.33±0.54^{d}	20.36±0.44^{b}	2.80±0.04^{c}	32.19±1.02^{b}	55.12±0.91^{b}
4	*Undigested*	0.12±0.01^{e}	0.29±0.05^{e}	0.06±0.00^{d}	0.51±0.06^{c}	1.14±0.06
	Digested	8.95±0.28^{d}	17.46±0.57^{d}	2.95±0.03^{c}	29.36±0.88^{c}	65.72±1.15^{c}

Values are presented as means ± SD (n=3). Data with different superscript differ ($p<0.05$)

Table 4 Contents of furosine (FUR) and pyrraline (PYR), and protein breakdown upon *in vitro* gastrointestinal digestion of commercial infant biscuits

Sample	FUR (mg/100 g protein)	PYR (mg/100 g protein)	Protein breakdown (%)
A	777.2±7.1^{b}	10.6±0.2^{e}	73.5±1.5^{c}
B	2376.1±12.3^{a}	29.8±0.8^{a}	68.9±2.2^{d}
C	268.0±5.2^{f}	18.2±0.6^{c}	77.7±1.6^{b}
D	410.3±3.2.^{e}	13.2±0.2^{d}	86.0±0.9^{a}
E	691.8±5.6^{d}	26.1±1.1^{b}	72.2±2.8^{c}
F	790.5±8.3^{b}	10.2±0.4^{e}	75.2±3.1^{b}

Values are presented as means ± SD (n=3). Data with different superscript differ ($p<0.05$)