final report

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Processing of bovine plasma protein concentrate (PPC) for functional food ingredient application

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Abstract

This project planned in four stages aims to improve the sustainability of the Australian meat processing industry by developing value added products from effluent and by-product streams of meat processing. In the previous three stages of the project the following had been achieved: (i) development of processing methods for the manufacture of plasma protein powders (Milestone 1), (ii) powders reproducibly derived by these methods have shown to have excellent solubility, heat-induced gelation, foaming and emulsification properties (Milestone 2), and (iii) it had been shown that white bread, beef patties and beef sausages could be made with incorporation of plasma protein powders without detrimental effects on key quality attributes (Milestone 3). In the final project stage reported here, it has been shown that the plasma proteins can also be used to improve the protein content of low protein plant powders, such as wheat flour and gluten-free flour replacement blends, whilst improving the bread making properties of these low protein plant powders (Milestone 4).

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Executive summary

Cost-effective fractionation and isolation procedures for the manufacture of blood protein fractions are the subject of research worldwide. This research is driven by the need to both minimise and add value to effluent and by-products streams from meat processing with the overall aim to improve the long-term sustainability of the meat processing industry. The red cell fraction of animal blood is considered undesirable for application into most foods due to its strong colour and a metallic off-flavour. The red cell fraction can be readily separated from whole blood by centrifugation resulting in plasma as a source of nutritional and food-functional proteins. This project aims to develop tools for meat processors to establish isolation procedures for bovine blood protein fractions with composition and functionality desired by the food processing industry.

During the first phase of this project, three different protein ingredient powders had been manufactured from centrifuged bovine blood plasma in the CSIRO pilot plant. The processes to prepare these three ingredients involved i) spray-drying of the plasma, ii) membrane concentration and spray-drying of the plasma, or iii) membrane concentration, diafiltration and spray-drying of the plasma. In the second phase of the project, key food-functional properties of the plasma powders were benchmarked against several commercial food protein ingredients obtained from milk, egg and soybean sources. The results from the benchmarking of the functional properties of the plasma protein powders showed that (i) spray-dried plasma powders with high protein solubility over a wide pH range could be reproducibly produced using the processing regimes established at pilot scale, (ii) heat-induced gel formation at 90°C leads to strong elastic gels with good water-holding properties, (iii) foaming properties comparable with egg white could be achieved, and (iv) desirable emulsification properties could be achieved. In the third phase of the project, beef patties and sausages were enriched with plasma protein powders with the aim to replace some of the meat protein and/or binders (such as salts or starch) from the formulation. It was also shown that protein-enriched white bread could be made when plasma protein powders were incorporated. Key quality parameters of the foods were varied with plasma protein incorporation, but it was evident that acceptable foods could be prepared when plasma protein powders were incorporated. In addition, it had been found that it is likely that incorporation of plasma protein powders did not require significant modifications to processing regimes or equipment for all three products.

The final phase of the project described here investigates whether or not the plasma protein powders may be used to increase the protein content of low-protein plant-derived ingredients. Two different approaches were evaluated:

(i) Firstly, low protein wheat flour was enriched to similar protein content as wheat flour for bread making, and the potential of this blend in bread making was assessed. It was found that dough quality was affected by inclusion of the plasma powders. The dough colour was slightly more yellow and the dough was more sticky after plasma protein incorporation. However, it was found that after a short (15 min) resting time the dough could be processed according to the protocol used for the control. Baking loss was not changed with inclusion of plasma proteins. The incorporation of the plasma powders increased the loaf volumes of the breads. The crusts of all protein enriched breads were darker than those of control breads. The crumbs of breads with plasma protein fortification were found to have slightly increased yellowness and reduced colour lightness. Furthermore, the cell shape, porosity, cell wall thickness and number of cells in the crumb could be modified by the inclusion of plasma protein powders with subsequent changes in bread hardness and elasticity. Based on the results obtained here, it is suggested that it is possible to use the plasma protein powders to increase the protein content of low protein flour for bread making whilst matching key quality parameters of standard white bread. It also appears

that existing processing methods and equipment will be suitable in dough processing and bread baking. Further work establishing consumer acceptance and organoleptic properties of such

breads as well as optimisation of blend composition to match the desired bread characteristics and processing conditions is suggested before commercial implementation of these findings.

(ii) Secondly, it was found that plasma proteins could be used to improve the protein content of low protein gluten free bread mix comprising soy and rice flour, as well as maize and tapioca starch. Plasma protein powders were blended into gluten free bread mix to increase the protein content to 9.8% or 13.0% in the bread mix. Gluten free bread dough's with and without plasma protein fortification were more sticky and difficult to handle than traditional wheat flour based dough. Gluten free breads had a higher baking loss than traditional white breads, however, baking loss was not affected by plasma protein incorporation. Inclusion of plasma protein increased crust and crumb colour of gluten free breads. Crumb structure parameters (cell number, volume and shape, wall thickness) and textural properties of the crumb could be modified through the incorporation of plasma protein powders into the gluten free bread mix. The protein content of the baked bread could be increased from 3.9% of the control bread to 5.9% protein for the plasma fortified breads. The results suggested that the upper limit for protein fortification may result in bread with about 7.7% protein, but further evaluation of processing and quality parameter assessment needs to be carried out for commercial production of such breads.

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1 Background

The global increase in the human population combined with a rise in disposable income in developing and developed countries is promoting the demand for animal proteins including those derived from meat products. Blood is a by-product of the manufacture of meat products. Traditionally, the blood from slaughterhouses has been used as animal feed or been disposed of in ponds. Over the past decades there has been a mounting worldwide effort to add more value to this high-quality protein source and various methods aimed at the isolation of blood protein fractions have been developed. In order to make nutritionally valuable blood protein fractions available as food ingredients, both the manufacturing steps and functional properties of these proteins need to be evaluated and optimised.

Potential food applications for plasma protein fractions include bakery and processed meat products. Plasma protein fractions have been reported to have excellent heat-induced gelation properties and have also been reported to have excellent emulsifying, foaming and water holding capacity over a wide pH range (cf, Milestone Report 2 for this project). They may therefore find use as a food functional ingredient with the ability to increase the protein content of the processed food product.

2 Project Objectives

The agreed objectives of the project are:

1. To develop a cost-effective process for the production of a high quality plasma concentrate samples at pilot scale

2. To compare the food-functional properties of plasma protein concentrate samples and traditionally used protein ingredients

3. To incorporate plasma concentrate samples into model processed food products and evalute food product properties

4. To evaluate the potential of the plasma concentrate samples to increase the functionality of protein blends/formulations

5. To report details on all activities undertaken, results, conclusions and recommendations

In this report objective 4 is addressed. The protein content of low protein wheat flour and gluten-free bread mix were increased in order to establish if the bread making properties of these plant protein products could be improved.

3 Methodology

3.1 Materials

3.1.1 Protein powders

Three bovine plasma protein powders produced at pilot scale were selected to evaluate the possible improvement of the bread making properties of low quality plant proteins. The composition of the plasma protein powder is given in Table 3.1. Powder PPI 1 is a plasma protein isolate derived from bovine blood plasma by membrane concentration, diafiltration and

spray-drying. Powder PPC 2 was prepared by a process including membrane concentration and spray-drying. Powder SP was produced by a process involving spray-drying only. For further

information on the processing conditions of the powder refer to Milestone Report 1 (Production of PPC samples at pilot scale) of this project.

Table 3.1 Composition of plasma protein powders evaluated.

Plasma powder	Dry content (%)	Total protein on product basis (%)	Total protein on dry basis (%)	Fat (%)[1]	Ash (%)[1]
PPI1	93.91	89.99	95.82	0.14	0.9
PPC2	93.68	85.53	91.30	0.11	4.0
SP	90.50	72.43	80.04	0.07	11.7

[1]Analysis by Chemical Analysis (110 Merrindale Drive, Croydon, Vic 3136, Australia)

3.1.2 White bread baking procedure

The following ingredients were used for bread making:

- Biscuit flour (Manildra Group, Australia) with 9.4% protein content (here referred to as low protein flour)
- Wheat flour (Defiance , White Wings Food, Australia) with 12.5% protein content (high protein flour) (here referred to as “bread flour” and high protein flour)
- Instant dry yeast (Fermex, France)
- Table salt

3.1.3 Gluten-free (GF) bread baking procedure

The following ingredients were used for gluten-free bread making:

- Gluten free FG Roberts Cottage bread mix (Soy Products Pty Ltd, Australia) with 6.5% protein
- Instant dry yeast (Fermex, France)
- White vinegar
- Vegetable oil

3.2 Methods

3.2.1 White bread baking procedure

Twelve batches of bread including three control batches were prepared on three different days. Bread formulations are given in Table 3.1. Plasma protein powders were added to biscuit flour to adjust the protein content to 12.5% to match the protein content of high protein content wheat flour specifically marketed for bread making.

Table 3.1 Bread ingredient formulations (flour substitution levels in brackets).

Batch	Sample	Ingredients (g)						
		Biscuit flour	Plasma powder	Bread flour	Yeast	Salt	Water	Total
1 and 3	Biscuit flour control	600.0	-	-	7.5	12.0	336.0	955.5
	Biscuit flour (4% PPI1)	577.0	23.0	-	7.5	12.0	336.0	955.5
	Biscuit flour (4% PPC2)	576.0	24.0	-	7.5	12.0	336.0	955.5
	Biscuit flour (5% SP)	570.0	30.0	-	7.5	12.0	336.0	955.5
2	Bread flour control	-	-	600.0	7.5	12.0	336.0	955.5
	Biscuit flour control	600.0	-	-	7.5	12.0	336.0	955.5
	Biscuit flour (4% PPI1)	577.0	23.0	-	7.5	12.0	336.0	955.5
	Biscuit flour (5% SP)	576.0	24.0	-	7.5	12.0	336.0	955.5

Dry ingredients (except yeast) were mixed in a mixing bowl. Yeast and water (35^{o}C) were then added to the premixed dry ingredients. Mixing was then carried out with a dough hook for 1 min at low speed (setting 1) with a Hobart mixer. After 1 min the bowl was scraped down, and a further mixing for 10 min continued at a higher speed (setting 2). The formed dough was placed into a plastic tray, covered with a wet cloth and left to rest for 15 min. The dough was then partitioned into 104 g portions and placed into 8-mould Teflon-lined baking pans and proofed for 2 h at 30^{o}C in a high humidity cabinet. Bread was finally baked in 15 min at 230^{o}C in a top and bottom heated deck electrical oven (Willett, Australia) steamed with approximately 200 mL of water.

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Figure 3.1 Willett Space Saver deck oven (A), baking bread (B).

Bread loaves were removed from the pans, cooled to room temperature, stored in sealed plastic bags at ambient temperature and analysed on the following day.

3.2.2 Gluten-free (GF) bread baking procedure

Nine batches of bread including two control batches were prepared on two different days. Bread formulations are given in Table 3.2. Plasma protein powders were added to the bread mix to adjust the protein content to 9.8% and 13.0%.

Table 3.2 Bread ingredient formulations (substitution levels in brackets).

Batch	Sample	Ingredients (g)						
		GF bread mix	Plasma powder	Yeast	Vinegar	Vegetable Oil	Water	Total
1	GF bread mix control	450.0	-	6.8	6.5	6.5	518.0	987.8
	GF bread mix (4% PPI1)	432.0	18.0	6.8	6.5	6.5	518.0	987.8
	GF bread mix (4% PPC2)	430.0	20.0	6.8	6.5	6.5	518.0	987.8
	GF bread mix (5% SP)	427.0	23.0	6.8	6.5	6.5	518.0	987.8
2	GF bread mix control	450.0	-	6.8	6.5	6.5	518.0	987.8
	GF bread mix (4% PPI1)	532.0	18.0	6.8	6.5	6.5	518.0	987.8
	GF bread mix (4% PPC2)	430.0	20.0	6.8	6.5	6.5	518.0	987.8
	GF bread mix (5% SP)	427.0	23.0	6.8	6.5	6.5	518.0	987.8
	GF bread mix (8% PPI1)	415.0	35.0	6.8	6.5	6.5	518.0	987.8

The warm water (~35°C) was placed into a mixing bowl. The yeast was sprinkled into the warm water, and oil and vinegar were added. The mixture was whisked until the yeast was dissolved (about 1 min). The dry ingredients were added to the liquid and subsequently mixed for 3 min at low speed (setting 1) using a dough hook fitted to a Hobart mixer. The resulting dough was partitioned into 104 g portions, placed into 8-mould Teflon-lined baking pans and proofed for 1 h at 30°C in a high humidity cabinet. Bread was baked for 35 min at 210°C in a top and bottom heated deck electrical oven (Willett, Australia) steamed with water.

3.2.3 Baking loss

The baking loss was determined by measuring the weight of 8 portions of dough for each batch

and 8 baked loaves and expressed as a percentage of the original weight of dough before baking (Equation 3.4).

$$Baking\ Loss\ (\%) = \frac{m_{dough} - m_{loaf}}{m_{dough}} \times 100 \quad \textbf{(3.1)}$$

3.2.4 Colour

The colour of the bread crusts and crumbs was measured by using Chroma Meter CR-300 (Minolta, Japan). Five loaves from each batch were used in colour evaluation and readings were taken at 2 different locations both on the crust surface and in the crumb area. Data from 10 measurements were averaged.

3.2.5 Crumb structure

The structure of bread crumb was characterised using a C-cell Imaging system (Calibre Control International, UK). Images of the bread slices (10 mm-thick) were captured (Figure 3.2) and analysed with the instrument software.

Figure 3.2 C-cell instrument (A) and the original, unprocessed image of the bread slice. Copied from http://www.c-cell.info/

Data derived from the crumb structure characterisation included:

- Slice area (mm^2): total area of the bread slice
- Number of cells: number of discrete cells detected within the slice
- Area of cells (%): the total area of cells as a percentage of the total slice area
- Cell elongation: the degree of overall elongation of the cell structure in a particular direction
- Wall thickness (mm): the average thickness of cell walls

Two slices from 5 different loaves were analysed from each batch. Data from 10 measurements were averaged.

3.2.6 Specific volume (SV)

Specific volume of bread is the reciprocal of bread density. It was determined by measuring the weight and exact thickness of 10 mm bread slices. The slice area was derived from crumb cell analysis using the C-cell imaging system. The specific volume was calculated using Equation 3.5.

$$Specific\ Volume\ (mL/g) = \frac{S_{slice} \times m_{slice}}{h_{slice}}$$

(3.2)

where S_{slice}: slice area (mm^2)
h_{slice}: slice height or thickness (mm)
m_{slice}: slice weight (g)

3.2.7 Textural properties

Five loaves from each batch were used to evaluate the texture of the crumb. Texture profile analysis (TPA) was performed using a TA-TX2 Texture Analyser (Stable Micro Systems, UK) equipped with the 5 kg load cell and a stainless steel probe with 12 cm length and 4 cm diameter. Two cubic samples (2.0×2.0×2.0cm) were taken from the centre of each loaf and subjected to a two-cycle compression test with 50% deformation at the test speed of 1 mm/s, post-test speed of 2 mm/s and with 5 s delay between the two “bites”. Texture parameters chosen to describe the crumb structure were calculated using the TA-TX2 software and included hardness, springiness and chewiness.

3.2.8 Statistical analysis

The experimental data were statistically analysed using one-way analysis of variance (ANOVA) and the means were compared by the Turkey test at a significance level of 0.05. These tests were carried out with MINITAB statistical software, release 14 (Minitab Inc, USA).

4 Results and discussion

4.1 Bovine plasma protein enrichment of low protein flour

The quality of flour for bread making is determined by its ability to produce a consistent finished product with a number of key characteristics such as high loaf volume, symmetrical loaf shape, attractive and even crust colour, fine and uniform crumb structure, smooth texture and light crumb colour.

The protein content of the flour is the key predictor for the quality of the bread dough and the finished bread. Generally, the higher the protein content of flour, the better its ability to produce a consistently strong dough. For bread making, flour protein levels between 11.0% and 13.0% are desirable, while 7.5 - 9.5% protein is preferred for flours for biscuit and cake preparation.

The objective of this work was to investigate the possibility of improving bread making properties of low protein wheat flour by increasing its protein content through the incorporation of plasma protein powders.

4.1.1 Dough quality

The preparation of dough with biscuit flour showed that several changes occurred with incorporation of plasma protein powders. Addition of 4.0% PPI1, 4.0% PPC2 and 5.0% SP resulted in an increase in water absorption and weakening of the dough (Figure 4.1).

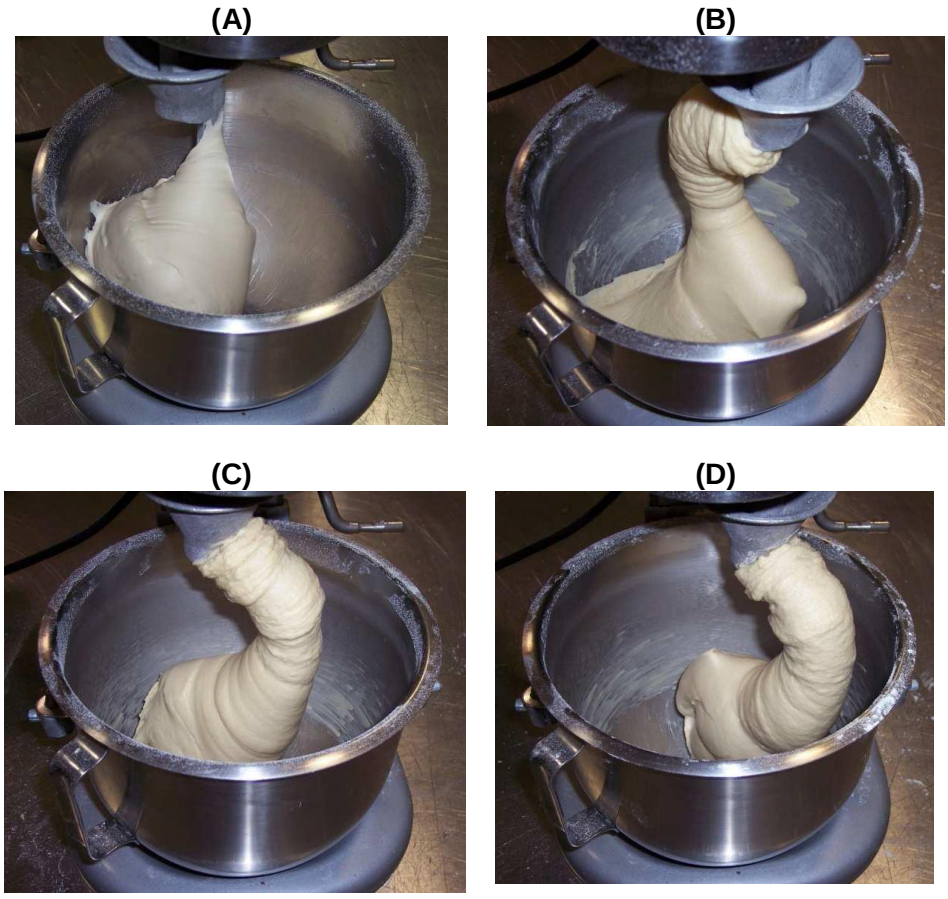

Figure 4.1 Images of dough produced from biscuit flour with no replacement (A), where 4.0 % was replaced by PPI1 (B), where 4.0% was replaced by PPC2 (C), and where 5.0% was replaced by SP (D).

The dough made without addition of plasma proteins was light in colour, semi-elastic, medium-stiff but non-sticky. Incorporation of plasma protein powders resulted in slightly yellow, wet and sticky dough. Resting the dough in bulk for 15 min at room temperature reduced its stickiness and the resulting dough could be moulded without problems.

4.1.2 Baking loss

The effect of flour enrichment with blood plasma proteins on baking loss is shown in Table 4.1. The results demonstrate that there was no significant difference (p >0.05) in baking loss between bread prepared with plasma protein powders and the controls. Baking losses varied from 11.4% to 12.2% (batch 1) and from 11.7% to 12.5% (batch 3). Moreover, it was found that baking loss in low protein control bread made with biscuit flower (12.2%) was similar (p>0.05) to baking loss estimated for bread prepared with high protein flour (11.4%).

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Table 4.1 Baking loss in bread prepared from biscuit flour with and without plasma proteins enrichment.

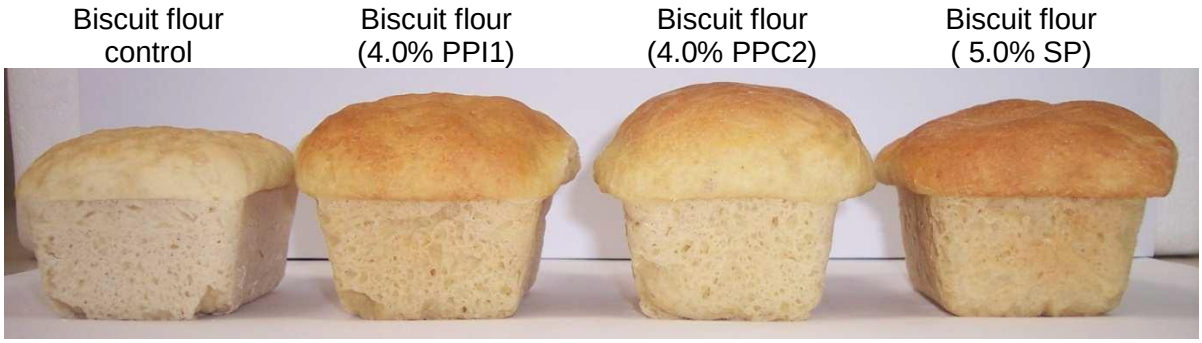
Batch	Sample	Baking loss (%)*
1	Biscuit flour control	11.57 ± 0.58 (a)
	Biscuit flour (4% PPI1)	12.22 ± 0.90 (a)
	Biscuit flour (4% PPC2)	12.13 ± 0.79 (a)
	Biscuit flour (5% SP)	11.42 ± 0.74 (a)
2	Bread flour control	11.37 ± 0.40 (b)
	Biscuit flour control	12.16 ± 0.54 (ab)
	Biscuit flour (4% PPI1)	12.56 ± 0.60 (a)
	Biscuit flour (5% SP)	11.83 ± 0.97 (ab)
3	Biscuit flour control	12.37 ± 0.46 (a)
	Biscuit flour (4% PPI1)	12.27 ± 0.66 (a)
	Biscuit flour (4% PPC2)	12.45 ± 0.45 (a)
	Biscuit flour (5% SP)	11.67 ± 0.83 (a)

* - Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of eight determinations for each sample.

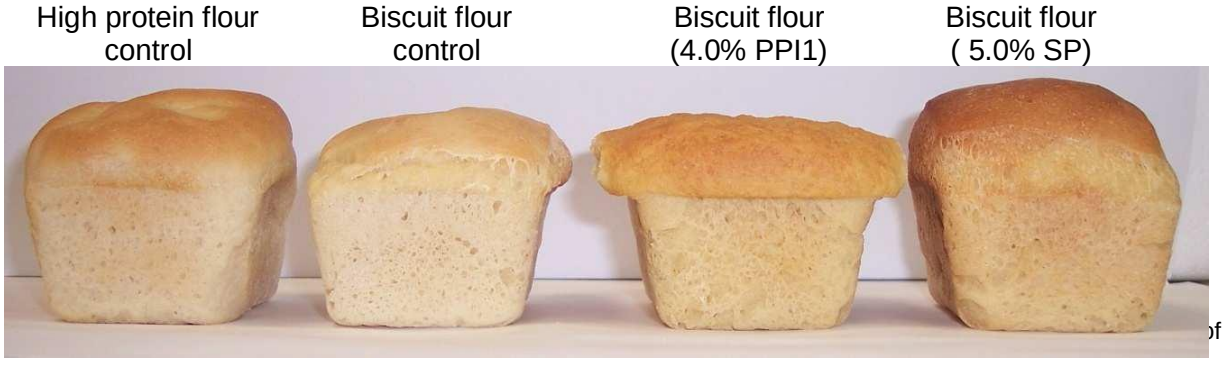
The results indicated that under current experimental conditions baking loss was not influenced by the type of flour used for bread making and also was not affected by incorporation of plasma protein powders into low protein flour.

4.1.3 Bread appearance and colour of crust and crumb

Visual appearance of bakery products strongly influences consumer preferences. The breads produced from high protein flour and from low protein flour with and without plasma protein fortification are shown in Figure 4.2.



(A)



(B)

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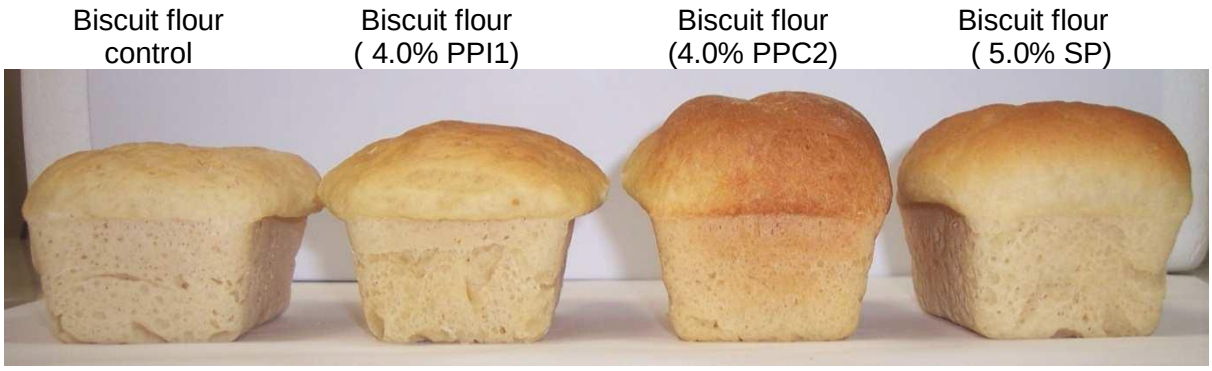

(C)

Figure 4.2 Images of bread loaves produced from high protein wheat flour, low protein flour and low protein flour enriched with plasma protein powders at 4.0% and 5.0%. (A) batch 1, (B) batch 2, (C) batch 3.

The loaf volume of bread prepared from biscuit flour control was smaller compared to the high protein control (batch 2). The incorporation of plasma protein powders to biscuit flour resulted in increased loaf volume which was more visible in bread prepared with 4.0% PPC2. Breads fortified with SP powder were bigger than breads enriched with PPI1 powder (batch 2 and 3), but they had similar sizes when made in batch 1. This finding indicates day-to-day variations in loaf volume and loaf shape due to unknown factors.

All protein enriched breads were more coloured on sides compared to the control breads. The visual appearance of the bread crusts is shown in Figure 4.3.

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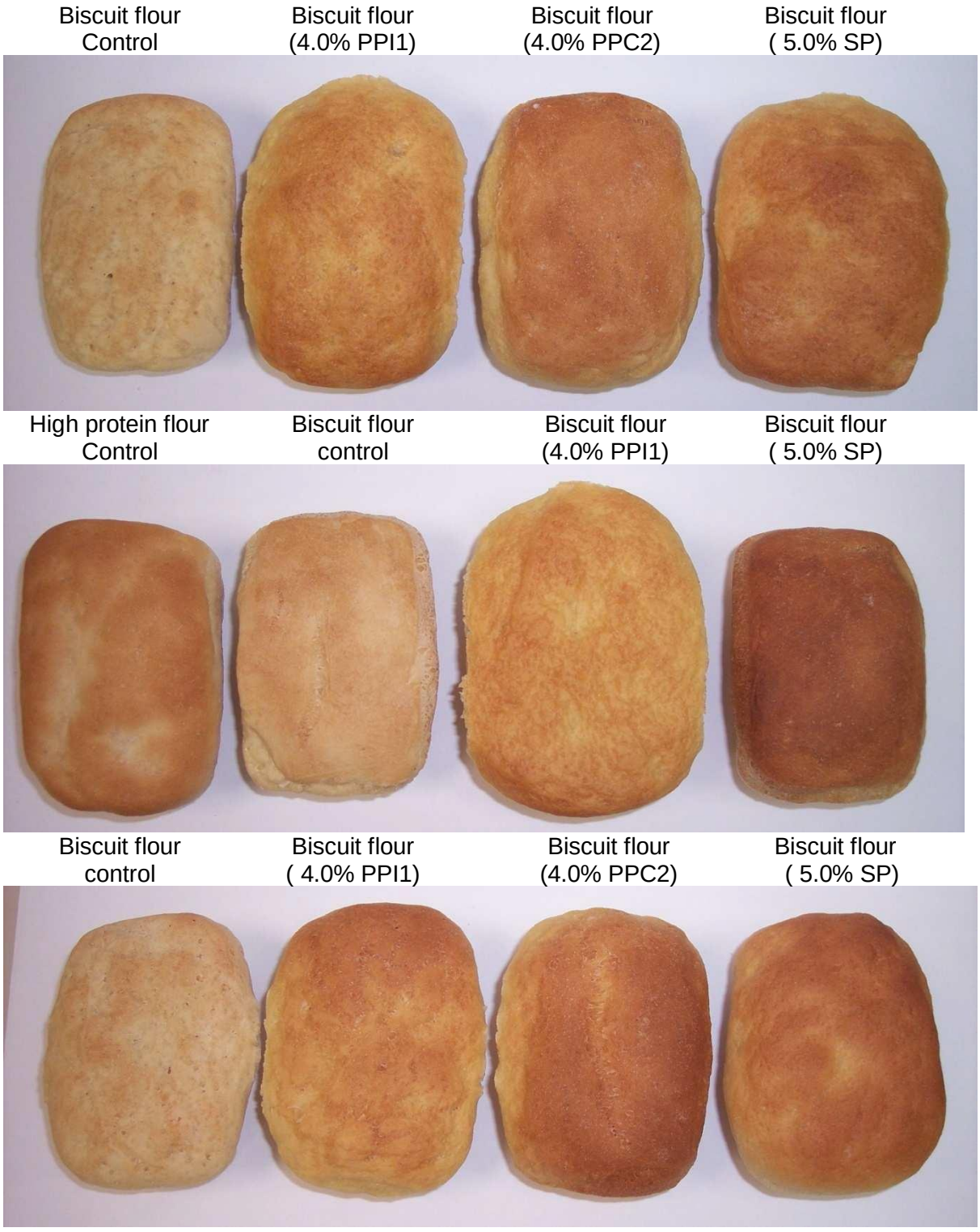

Figure 4.3 Images of bread crusts produced from high protein flour, low protein flour and biscuit flour enriched with plasma protein powders at 4.0% and 5.0%. (A) batch 1, (B) batch 2, (C) batch 3.

The crust colour of the low protein control breads was lighter compared to the high protein control. The crusts of all protein enriched breads were darker than the control breads. The surface of crusts in control samples and bread with 4.0% PPI1 powder was rough and non-

uniform. The smoothest and most appealing crusts were obtained in breads produced with 4.0% PPC2 powder.

The colour of bread crusts was analytically evaluated using L*, a* and b* parameters. Results from the crusts colour measurements are presented in Table 4.2.

Table 4.2 Colour of bread crusts produced from high and low protein flours and fours enriched by plasma protein powders.

Batch	Sample	Colour parameters (*)		
		*a**	*b**	L*
1	Biscuit flour control	4.04 ± 1.14 (c)	26.60 ± 1.38 (c)	66.81 ± 1.25 (a)
	Biscuit flour (4.0% PPI1)	10.40 ± 1.30 (b)	30.16 ± 0.93 (a)	56.85 ± 1.64 (b)
	Biscuit flour (4.0% PPC2)	13.67 ± 1.10 (a)	29.08 ± 1.12 (ab)	53.77 ± 1.64 (c)
	Biscuit flour (5.0% SP)	11.72 ± 1.25 (b)	28.57 ± 0.84 (b)	54.42 ± 1.36 (c)
2	Bread flour control	10.29 ± 1.14 (b)	29.80 ± 0.71 (a)	62.29 ± 1.49 (a)
	Biscuit flour control	9.17 ± 1.20 (b)	27.47 ± 0.85 (b)	63.62 ± 2.46 (a)
	Biscuit flour (4.0% PPI1)	9.78 ± 0.92 (b)	28.97 ± 0.53 (a)	57.77 ± 0.84 (b)
	Biscuit flour (5.0% SP)	15.07 ± 0.53 (a)	24.57 ± 1.19 (c)	49.70 ± 1.43 (c)
3	Biscuit flour control	5.91 ± 1.37 (c)	27.80 ± 1.52 (bc)	65.48 ± 0.85 (a)
	Biscuit flour (4.0% PPI1)	11.90 ± 1.13 (b)	30.01 ± 0.82 (a)	56.39 ± 1.27 (b)
	Biscuit flour (4.0% PPC2)	14.45 ± 0.45 (a)	26.62 ± 1.57 (c)	50.57 ± 1.92 (b)
	Biscuit flour (5.0% SP)	14.65 ± 0.81 (a)	28.33 ± 1.35 (b)	51.93 ± 2.01 (b)

(*) - Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of six determinations for each sample.

The *a** value characterises the redness of bread crusts. The highest *a** values (13.7 – 15.1) of the crusts were found in bread made with 4.0% PPC2 and 5.0% SP powders. The crust yellowness (*b** parameter) was greater in bread prepared with 4.0% PPI1 powder (29.0 – 30.2) compared to the biscuit flour controls (26.6 – 27.8). However, the high protein flour control from batch 2 also had a high crust *b** value (29.8). The crusts of breads containing plasma proteins were darker with lower L* values (49.7 – 57.8) when compared to the biscuit flour control (63.62 – 66.81) and high protein flour control (62.29). The baked breads with 4.0% PPC2 and 5.0% SP powders had the darkest crusts, with L values from 49.7 to 54.4. The results from the objective colour measurement were in good agreement with the visual observations reported above.

The crumb structure of breads produced in the current study was visually examined. Images of bread slices showing the crumb structure and colour are presented in Figure 4.4.

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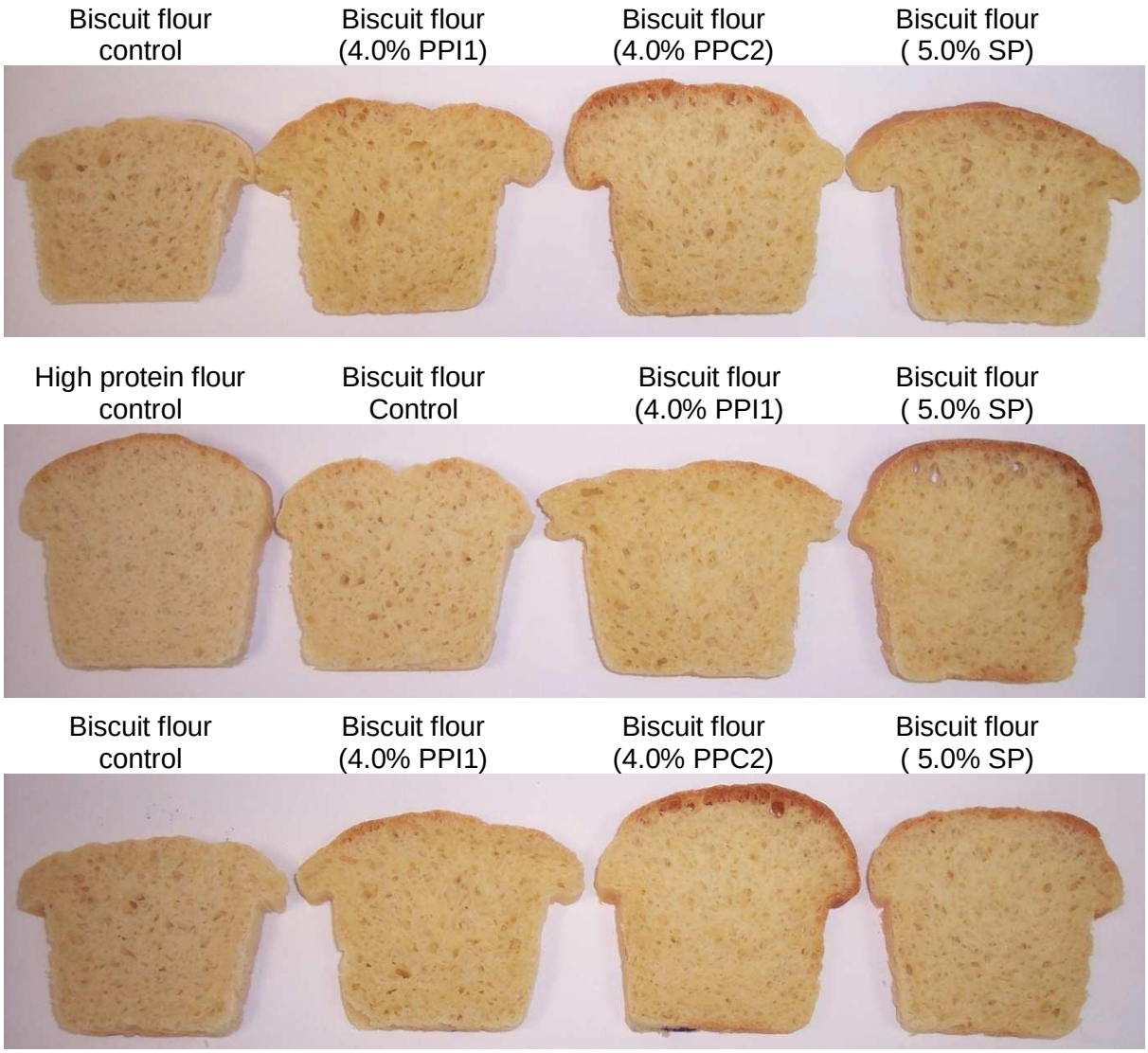

Figure 4.4 Images of bread slices produced from high protein flour, biscuit flour and biscuit flour enriched with plasma protein powders. (A) batch 1, (B) batch 2, (C) batch 3.

The bread baked from high protein control flour (batch 2) demonstrated the most homogeneous distribution of small, relatively round cells in the crumb. The crumb of the controls prepared from low protein control flour was markedly less uniform with a number of bigger cells. The addition of plasma powders to biscuit flour resulted in the formation of more irregular and larger cells in bread crumb. The crumb of plasma enriched breads was more yellow compared to the control samples.

The visual assessment of bread crumb colour was supported by colour measurements using the Minolta ChromaMeter. Results are shown in Table 4.3.

Table 4.3 Colour characteristics of crumbs of plasma protein enriched breads.

Batch	Sample	Colour parameters (*)		
		*a**	*b**	L*
1	Biscuit flour control	-0.91 ± 0.12 (a)	16.16 ± 0.72 (b)	67.73 ± 1.49 (a)
	Biscuit flour (4% PPI1)	-1.25 ± 0.22 (b)	18.28 ± 1.50 (a)	64.25 ± 1.39 (b)
	Biscuit flour (4% PPC2)	-1.34 ± 0.20 (b)	19.02 ± 0.46 (a)	65.59 ± 1.70 (b)
	Biscuit flour (5% SP)	-0.99 ± 0.16 (a)	18.62 ± 0.65 (a)	64.99 ± 1.29 (b)
2	Bread flour control	-0.88 ± 0.09 (a)	14.57 ± 0.60 (d)	72.70 ± 1.08 (a)
	Biscuit flour control	-1.11 ± 0.12 (b)	16.19 ± 0.61 (c)	68.99 ± 1.24 (b)
	Biscuit flour (4% PPI1)	-1.23 ± 0.17 (b)	19.55 ± 0.66 (a)	67.00 ± 1.31 (c)
	Biscuit flour (5% SP)	-1.19 ± 0.13 (b)	18.65 ± 0.66 (b)	67.08 ± 1.32 (c)
3	Biscuit flour control	-0.98 ± 0.12 (a)	15.45 ± 1.08 (c)	68.91 ± 1.07 (a)
	Biscuit flour (4% PPI1)	-1.36 ± 0.16 (b)	19.28 ± 1.13 (a)	67.36 ± 1.15 (ab)
	Biscuit flour (4% PPC2)	-1.53 ± 0.21 (b)	18.13 ± 0.94 (a)	66.30 ± 1.05 (b)
	Biscuit flour (5% SP)	-1.39 ± 0.18 (b)	16.86 ± 1.18 (b)	65.97 ± 1.82 (b)

(*) Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of six determinations for each sample.

All breads including controls (high and low protein flour) demonstrated negative *a** values indicating diminished redness and the presence of green component in the colour of all crumbs. The *a** varied from -1.53 to -0.88 among the samples and controls.

As expected, with the addition of plasma powders the most affected colour characteristic of bread crumb was the yellowness parameter b*. All plasma fortified breads had significantly ($p < 0.05$) higher *b** values compared to the controls. Breads with 10% PPI1 had the greatest *b** values 19.95 (batch 1) and 20.95 (batch 3). The bread crumb became darker with addition of plasma powders. Significant difference ($p < 0.05$) was found between the L values of the control breads and those supplemented with 10% SP or 10% PPI1 (batch 1), 5% PPI1 (batch 2), 5% PPC2 (batch 3) and 10% PPI1 (batch 3). The results were in good agreement with visual observations.

4.1.4 Specific volume (SV)

Another important bread characteristic which influences consumer perception of bread is the loaf specific volume. The results for SV of breads prepared with and without plasma protein enrichment are summarised in Table 4.4.

Table 4.4 Specific loaf volume of bread produced from low and high protein flour and biscuit flour enriched with plasma protein powders at 4% and 5%.

Batch	Sample	Specific Volume (*) (mL/g)
1	Biscuit flour control	2.35 ± 0.10 (b)
	Biscuit flour (4% PPI1)	2.76 ± 0.29 (a)
	Biscuit flour (4% PPC2)	2.84 ± 0.14 (a)

	Biscuit flour (5% SP)	2.57 ± 0.19 (b)
2	Bread flour control	2.82 ± 0.16 (a)

Batch	Sample	Specific Volume (*) (mL/g)
	Biscuit flour control	2.63 ± 0.08 (b)
	Biscuit flour (4% PPI1)	2.73 ± 0.14 (ab)
	Biscuit flour (5% SP)	2.84 ± 0.11 (a)
3	Biscuit flour control	2.57 ± 0.14 (c)
	Biscuit flour (4% PPI1)	2.95 ± 0.28 (a)
	Biscuit flour (4% PPC2)	3.26 ± 0.14 (a)
	Biscuit flour (5% SP)	2.90 ± 0.19 (b)

(*) Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of 10 determinations for each sample.

Specific volume of control bread prepared from the biscuit flour (2.63 mL/g) was significantly lower than the specific volume of bread flour control bread (2.82 mL/g). The incorporation of plasma proteins into biscuit flour increased specific volume of baked loaves. Bread prepared with 4% PPC2 and 4% PPI1 (batch 3) demonstrated the highest SV 3.26 mL/g and 2.95 mL/g, compared to SV 2.57 mL/g in the control sample. These findings support the subjective observations of loaf volume described in Section 4.1.3 above.

4.1.5 Crumb structure

Images of crumb structure are presented in Figure 4.4 and Table 4.5 summarises the crumb structure parameters determined by digital image analysis.

Table 4.5 Crumb structure of breads produced from high and low protein flour controls and biscuit flour enriched with plasma protein powders at 4% and 5%.

Batch	Crumb cell characteristics (*)					
	Sample	Slice Area (mm^2)	Number of cells	Area of cells (%)	Average cell elongation	Wall thickness (mm)
1	Biscuit flour control	2743.4 ± 67.8 (c)	2012 ± 146 (a)	49.29 ± 0.84 (c)	1.47 ± 0.02 (b)	0.44 ± 0.01 (b)
	Biscuit flour (4% PPI1)	3190.9 ± 191.3 (b)	1880 ± 105 (a)	51.92 ± 0.85 (ab)	1.51 ± 0.02 (a)	0.47 ± 0.01 (a)
	Biscuit flour (4% PPC2)	3402.2 ± 123.8 (a)	1956 ± 105 (a)	52.36 ± 0.44 (a)	1.52 ± 0.02 (a)	0.48 ± 0.01 (a)
	Biscuit flour (5% SP)	3042.5 ± 157.6 (b)	1886 ± 74 (a)	51.23 ± 1.01 (b)	1.51 ± 0.03 (a)	0.47 ± 0.01 (a)
2	Bread flour control	3130.9 ± 119.2 (b)	2471 ± 83 (a)	48.23 ± 0.82 (c)	1.49 ± 0.01 (b)	0.42 ± 0.01 (c)
	Biscuit flour control	3008.6 ± 160.5 (b)	2064 ± 82 (bc)	49.92 ± 0.67 (b)	1.48 ± 0.02 (b)	0.45 ± 0.01 (b)
	Biscuit flour (4% PPI1)	3032.7 ± 80.9 (b)	1986 ± 84 (c)	50.36 ± 0.74 (b)	1.53 ± 0.03 (a)	0.45 ± 0.01 (b)
	Biscuit flour (5% SP)	3286.5 ± 90.3 (a)	2086 ± 36 (b)	51.37 ± 0.41 (a)	1.53 ± 0.02 (a)	0.46 ± 0.01 (a)
3	Biscuit flour control	2804.5 ± 98.3 (c)	2008 ± 84 (a)	49.28 ± 0.34 (c)	1.47 ± 0.02 (b)	0.44 ± 0.01 (b)
	Biscuit flour (4% PPI1)	3275.6 ± 116.5 (b)	1981 ± 117 (a)	51.74 ± 0.79 (ab)	1.51 ± 0.03 (a)	0.47 ± 0.01 (a)
	Biscuit flour (4% PPC2)	3574.8 ± 101.9 (a)	2043 ± 119 (a)	52.39 ± 0.58 (a)	1.51 ± 0.02 (a)	0.48 ± 0.01 (a)

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	Biscuit flour (5% SP)	3345.5 ± 128.2 (b)	2039 ± 95 (a)	51.29 ± 0.65 (b)	1.51 ± 0.03 (a)	0.47 ± 0.01 (a)

(*) Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of 10 determinations for each sample.

There was no significant difference in slice area between the high and low protein flour control breads (batch 2). In most cases bread baked from biscuit flour fortified with plasma protein powders had significantly ($p < 0.05$) larger bread slice area compared to the controls. The biggest slice area was observed in bread produced with 4% PPC2: 3402.2 mm^2 (batch 1) and 3574.8 mm^2 (batch 3).

In contrast to the slice area, there was a significant difference in a number of cells measured in high and low protein control breads. Bread produced from high protein flour had a higher number of cells (2471) than bread baked from the low protein flour (2064). Increasing protein content by incorporation of plasma powders into the low protein flour did not have an effect on a number of cells formed in baked bread.

The porosity of bread determined through the total area of cells was affected by the type of flour used for bread making. The high protein flour control had the smallest area of cells (48.23%) indicating a denser structure of bread crumb. Bread produced from low protein flour similarly demonstrated a dense crumb with a total cell area of 49.92%. Higher values for area of cells (> 50.0%) was found in plasma enriched breads, indicated a more open texture of the crumbs. The most porous breads were produced with 4% PPC2 having cell areas of 52.36% (batch1) and 52.39% (batch 2).

Cell elongation describes of how far the pore shape differs from a circle, with values close to 1 indicating a circle. The more rounded cells (1.47 to 1.49) were formed in both types of control breads, whereas pores in plasma fortified bread crumb were more elongated (1.51 to 1.53). While statistically significant, the importance of this relatively small elongation of pores in the fortified breads to consumer preference is not known.

Cells formed in high protein control bread had the smallest wall thickness of 0.42 mm. Bread prepared from the biscuit flour showed higher cell wall thickness (0.44 to 0.45 mm). When plasma powders were added to biscuit flour, baked bread had wall thickness higher (>0.45 mm) than the control sample, with one exemption for bread made with 4% PPI1 (batch 2) with wall thickness similar to the control bread (0.45).

4.1.7 Textural characteristics

Texture of bread crumb was characterised by hardness, springiness and chewiness derived from TPA (texture profile analysis). Results are presented in Table 4.6.

Table 4.6 Textural characteristics of bread produced from high and low protein flour and biscuit flour enriched with plasma protein powders at 4% and 5%.

Batch	Sample	Textural parameters (*)		
		Hardness (N)	Springiness	Chewiness (N)
1	Biscuit flour control	5.41 ± 0.52 (a)	0.87 ± 0.02 (b)	2.84 ± 0.25 (b)
	Biscuit flour (4% PPI1)	3.37 ± 0.22 (c)	0.93 ± 0.02 (a)	2.64 ± 0.16 (c)
	Biscuit flour (4% PPC2)	3.12 ± 0.34 (c)	0.92 ± 0.02 (a)	2.47 ± 0.28 (c)
	Biscuit flour (5% SP)	4.64 ± 0.59 (b)	0.93 ± 0.02 (a)	3.61 ± 0.43 (a)
2	Bread flour control	3.82 ± 0.43 (c)	0.91 ± 0.02 (a)	2.55 ± 0.39 (c)
	Biscuit flour control	5.47 ± 0.60 (a)	0.89 ± 0.01 (b)	3.00 ± 0.34 (ab)

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Biscuit flour (4% PPI1)	3.98 ± 0.34 (c)	0.91 ± 0.02 (a)	2.82 ± 0.27 (bc)

	Biscuit flour (5% SP)	4.81 ± 0.53 (b)	0.93 ± 0.01 (a)	3.35 ± 0.34 (a)
3	Biscuit flour control	5.55 ± 0.55 (a)	0.89 ± 0.02 (b)	3.09 ± 0.33 (a)
	Biscuit flour (4% PPI1)	3.98 ± 0.36 (c)	0.92 ± 0.02 (a)	2.72 ± 0.20 (b)
	Biscuit flour (4% PPC2)	3.23 ± 0.39 (d)	0.91 ± 0.04 (ab)	2.22 ± 0.29 (c)
	Biscuit flour (5% SP)	4.71 ± 0.43 (b)	0.93 ± 0.01 (a)	3.15 ± 0.31 (a)

(*) Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of 10 determinations for each sample.

The hardness of control samples made from biscuit flour varied from 5.41 N to 5.55 N which was significantly higher ($p < 0.05$) than the hardness of high protein control bread (3.82 N). The incorporation of plasma proteins into biscuit flour significantly decreased bread hardness. The softest crumb was formed in bread prepared with 4% PPC2 (batch 1 and 3) with corresponding hardness values of 3.12 N and 3.23 N. Bread prepared with 4% PPI1 powder had crumb hardness of 3.98 N (batches 2, 3) which was similar to the bread control sample (3.82 N). The hardness of biscuit bread with 5% SP varied from 4.64 to 4.81 N.

The springiness of biscuit flour control bread (0.89) was lower than the springiness of bread flour control sample (0.91). Fortification of biscuit flour with plasma proteins resulted in more “elastic” and resilient crumb with springiness higher than or equal to 0.91.

Chewiness of bread crumb was dependent on the type of flour used for bread making. Bread prepared from bread flour was less “chewy” than biscuit flour control bread with corresponding chewiness values of 2.55 N and 3.00 N. Addition of 4% PPI1 and 4% PPC2 powders to biscuit flour reduced the chewiness of biscuit control bread on average by 8% and 20%, respectively. In contrast, incorporation of 5% SP powder did not have an effect on bread crumb chewiness (batches 2 and 3) or increased chewiness from 2.84 N to 3.61 N (batch 1).

The findings indicate that softer, springier and less chewy bread crumb could be produced if low protein biscuit flour was fortified with 4% PPI1 and 4% PPC2 powders. At this fortification level, the protein content of the baked bread would be about 9.2 % and equal to that of the high protein control bread compared with the protein level of 6.6% of the low protein control bread.

4.2 Bovine plasma protein enrichment of gluten-free bread

The market for gluten-free products is increasing due to the increasing occurrence of gluten-triggered adverse reactions in individuals with compromised immune systems. Production of bakery products without gluten is a big challenge for bakers and cereal researches: In such products the role of gluten to form a three dimensional protein network during dough preparation is replaced by other ingredients, including proteins from milk, egg or soy (Houben *et al*. 2012).

Commercially available gluten-free breads are typically lacking in protein content as the flour sources have lower protein content than wheat flour. The objective of this work was to assess the opportunity for developing high protein gluten-free breads.

4.2.1 Dough quality

The absence of gluten in dough can markedly affect dough quality. Gluten free (GF) dough is typically less cohesive and elastic than wheat dough (Houben *et al*. 2012). The effect of plasma protein incorporation on dough appearance is shown in Figure 4.5.

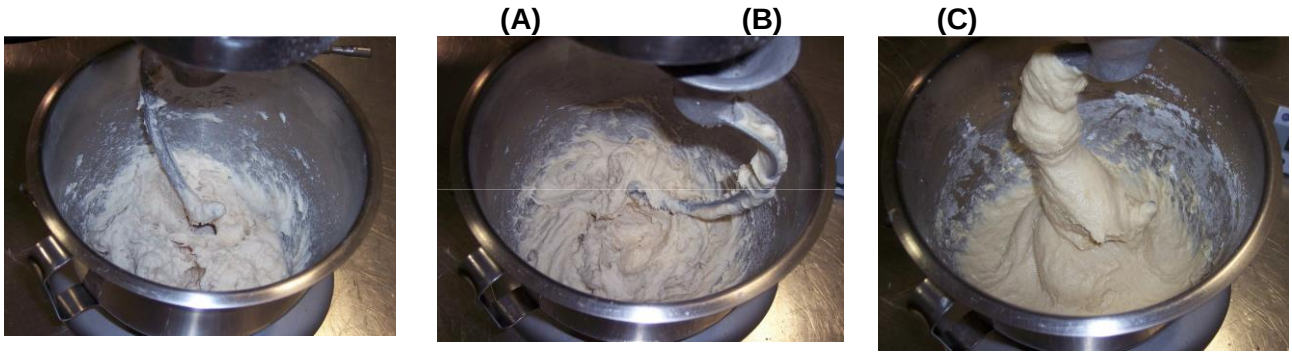


Figure 4.5 Images of dough produced from GF bread mix control (A), GF bread mix replaced by 4 % PPI1 (B) and 8 % PPI1 (C).

The dough made from GF bread mix without protein fortification was light in colour, non-elastic and sticky. It was more like the batter of a cake than a typical bread dough. Incorporation of 4.0 % PPI1 powder resulted in formation of slightly yellow and stickier dough. The dough produced with 8.0% PPI1 powder had intense yellow colour, was very moist and excessively sticky and was therefore difficult to handle.

4.2.2 Baking loss

The effect of incorporation of plasma proteins into GF bread mix on bread baking loss is shown in Table 4.7.

The results demonstrate that there was no significant difference (p >0.05) in baking loss between bread samples prepared with plasma protein powders and the controls. Baking losses varied from 19.0% to 20.6% (batch 1) and from 19.2% to 19.6% (batch 2). The baking loss of GF bread in this study was on average 1.7 times higher than the baking loss of bread samples prepared from wheat and biscuit flours (Table 4.1).

Table 4.7 Baking loss in bread produced from GF bread mix with and without plasma protein enrichment.

Batch	Sample	Baking loss (%)*
1	GF bread mix control	19.95 ± 1.08 (a)
	GF bread mix (4% PPI1)	20.56 ± 1.04 (a)
	GF bread mix (4% PPC2)	20.12 ± 0.79 (a)
	GF bread mix (5% SP)	20.30 ± 0.69 (a)
2	GF bread mix control	19.30 ± 1.02 (a)
	GF bread mix (4% PPI1)	19.23 ± 1.04 (a)
	GF bread mix (4% PPC2)	19.45 ± 1.05 (a)
	GF bread mix (5% SP)	19.58 ± 0.90 (a)
	GF bread mix (8% PPI1)	19.23 ± 0.90 (a)

* Sample means for each batch with different letters in brackets are significantly different (p < 0.05). Variation of the mean represents standard deviation of eight determinations for each sample.

4.2.3 Bread appearance and colour of crust and crumb

The breads produced from GF bread mix with and without plasma protein incorporation are shown in Figure 4.6.

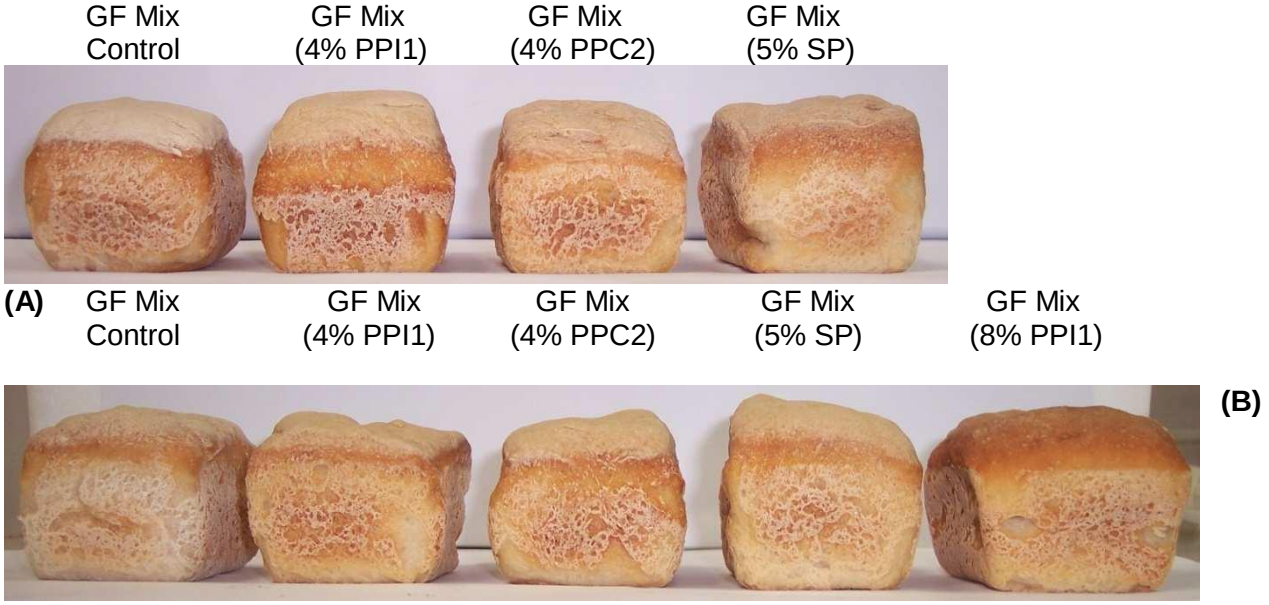
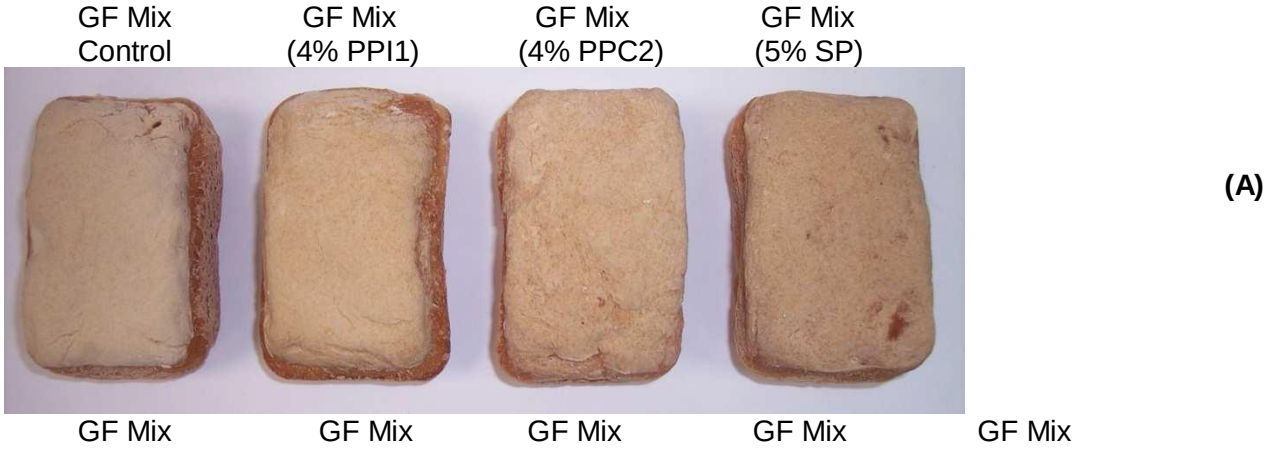


Figure 4.6 Images of bread loaves produced from gluten-free bread mix control and the bread mix fortified with plasma protein powders at 4, 5 and 8%. (A) batch 1, (B) batch 2.

The shape of baked loaves was irregular and it was very difficult to compare loaf volume of the control bread samples with breads containing 4% and 5% plasma powders. Bread loaves with 8% PPI1 powder, however, were visibly larger in size than the control bread.

All GF breads were unevenly baked and coloured on sides and bottom. The shade of the sides and bottom did not conform to that of the crust in control samples and breads with 4 and 5% plasma proteins as the top of these breads had a lighter beige colour. The bread crusts of all breads are shown in Figure 4.7.



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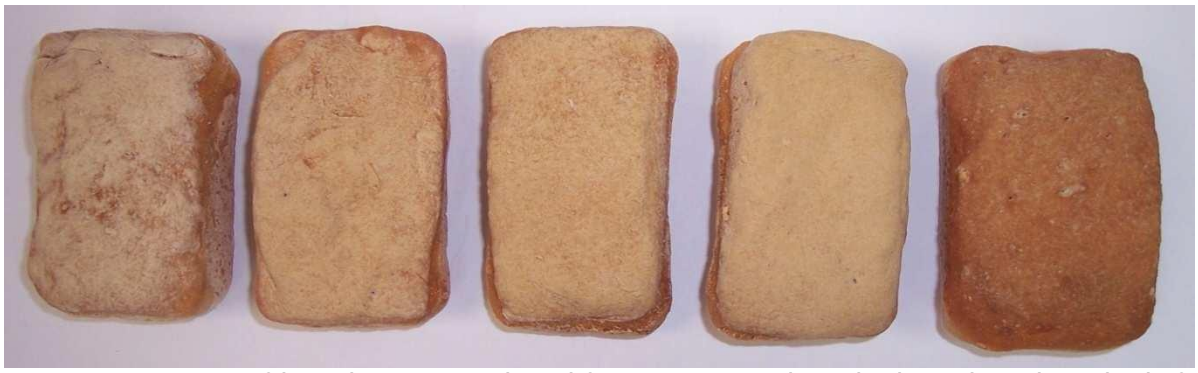
Control	(4% PPI1)	(4% PPC2)	(5% SP)	(8% PPI1)



(B)

Figure 4.7 Images of bread crusts produced from 100% GF bread mix and GF bread mix fortified with plasma protein powders at 4, 5 and 8%. (A) batch 1, (B) batch 2.

The bread loaves produced with the addition of 8% PPI1 powder had darker crusts than all the other loaves. Overall, these loaves had a better external appearance and resembled typical wheat-bread loaves more closely than the gluten-free control.

The colour of bread crusts was analytically evaluated using L*, a* and b* parameters. Results from the crust colour assessment are presented in Table 4.2.

Table 4.8 Colour characteristics of bread crust produced from gluten-free bread mix and the bread mix fortified with plasma protein powders at 4, 5 and 8%.

Batch	Sample	Colour parameters (*)		
		a*	b*	L*
1	GF bread mix control	4.75 ± 0.25 (d)	21.10 ± 0.59 (b)	75.41 ± 1.02 (a)
	GF bread mix (4% PPI1)	7.20 ± 0.51 (c)	24.89 ± 1.61 (a)	69.73 ± 0.82 (b)
	GF bread mix (4% PPC2)	8.08 ± 0.36 (b)	24.85 ± 0.41 (a)	67.21 ± 1.13 (c)
	GF bread mix (5% SP)	8.59 ± 0.46 (a)	23.95 ± 0.96 (a)	64.49 ± 1.72 (d)
2	GF bread mix control	6.01 ± 0.42 (d)	20.67 ± 0.96 (d)	72.16 ± 1.13 (a)
	GF bread mix (4% PPI1)	8.57 ± 0.49 (bc)	24.06 ± 0.73 (c)	66.20 ± 1.26 (b)
	GF bread mix (4% PPC2)	8.83 ± 0.43 (b)	26.01 ± 0.96 (ab)	65.57 ± 0.76 (b)
	GF bread mix (5% SP)	8.20 ± 0.24 (c)	25.63 ± 0.52 (b)	66.76 ± 0.78 (b)
	GF bread mix (8% PPI1)	13.66 ± 0.74 (a)	26.94 ± 0.49 (a)	54.46 ± 1.33 (c)

* - Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of ten determinations for each sample.

The *a** value represents the redness of bread crusts. The lowest *a** values 4.75 (batch 1) and 6.01 (batch 2) were found in control samples indicating that the redness component in crust colour increased with addition of plasma protein powders. The bread produced with 8.0% PPI1 powder displayed the highest crust *a** value (13.7).

The crust yellowness, described by the *b** parameter, was greater in protein-enriched breads (24.1 – 26.9) compared to GF controls with *b** values 21.1 (batch 1) and 20.7 (batch 2).

The lightness of bread crust tended to decrease with addition of plasma proteins. The darkest crust with the lowest L* value (54.5) was formed in bread fortified with 8.0% PPI1 powder (batch 2). The GF controls had the highest L* values 75.4 (batch 1) and 72.2 (batch 2) indicating the greatest lightness.

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The crumb structure and colour of GF breads were visually examined. The representative images of bread slices are shown in Figure 4.8.

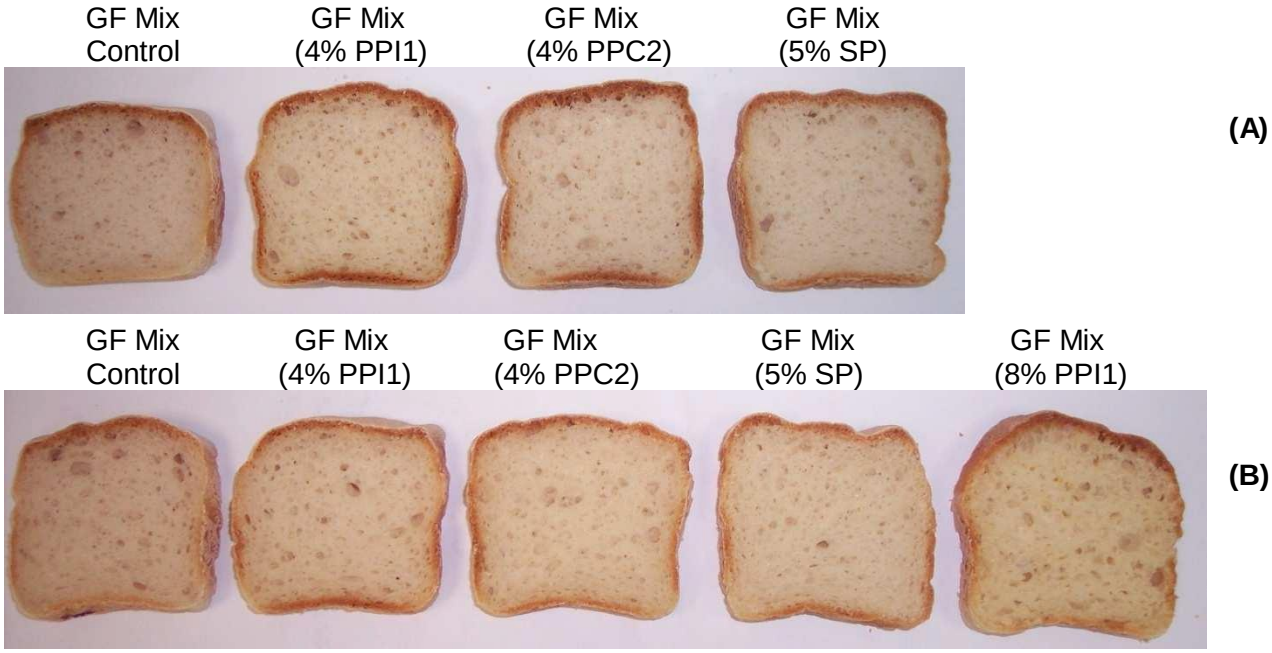

Figure 4.8 Images of bread slices produced from gluten-free bread mix and the bread mix fortified with plasma protein powders at 4, 5 and 8%. (A) batch 1, (B) batch 2.

The crumb of GF controls demonstrated the non-uniform distribution of small and big cells. The crumb was moist and rubbery. Breads prepared with incorporation of 4% PPI1 and 4% PPC2 and 5% SP powders presented a crumb structure similar to that of the control. The crumb of the bread produced with 8% PPI1 powder displayed larger cells and was spongier than other plasma protein containing breads.

The colour of the bread crumb became more yellow with addition of plasma protein powders. Colour characteristics obtained by instrumental analysis are presented in Table 4.9.

Table 4.9 Colour characteristics of bread crumb produced from gluten-free bread mix and the bread mix fortified with plasma protein powders at 4, 5 and 8%.

Batch	Sample	Colour parameters (*)		
		a*	b*	L*
1	GF bread mix control	1.59 ± 0.13 (a)	9.84 ± 0.51 (b)	69.20 ± 0.70 (c)
	GF bread mix (4% PPI1)	0.22 ± 0.16 (c)	12.57 ± 0.67 (a)	70.78 ± 0.77 (b)
	GF bread mix (4% PPC2)	0.43 ± 0.15 (b)	12.28 ± 0.65 (a)	71.29 ± 0.62 (ab)
	GF bread mix (5% SP)	0.41 ± 0++.06 (b)	12.03 ± 0.52 (a)	72.00 ± 0.73 (a)
3	GF bread mix control	1.58 ± 0.15 (a)	9.63 ± 0.33 (d)	70.19 ± 0.52 (c)
	GF bread mix (4% PPI1)	0.77 ± 0.21 (b)	11.93 ± 0.42 (c)	70.90 ± 0.55 (bc)
	GF bread mix (4% PPC2)	0.30 ± 0.21 (b)	12.12 ± 0.47 (c)	70.47 ± 1.04 (bc)
	GF bread mix (5% SP)	0.32 ± 0.15 (b)	12.83 ± 0.80 (b)	72.57 ± 0.95 (a)
	Bread Mix (8% PPI1)	-0.30 ± 0.17 (c)	15.39 ± 0.61 (a)	71.39 ± 0.35 (b)

* - Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of ten determinations for each sample.

The bread produced with 8% PPI1 powder demonstrated a very small negative *a** value indicating the presence of green component in crumb colour. Other breads including controls had positive *a** values showing increasing crumb redness. The *b** parameter, representing yellowness, in all plasma containing bread samples had significantly higher values ranging from

11.9 to 15.4 than the GF controls with *b** values 9.8 (batch 1) and 9.6 (batch 2). The crumb lightness (L* value) significantly increased with the addition of plasma protein powders (batch 1). A similar effect on crumb lightness was observed with incorporation of 5.0% SP and 8% PPI1 powders (batch 2). However, the crumb lightness was not significantly influenced by 4 % PPI1 and 4 % PPC powder addition (batch 2).

4.2.4 Specific volume (SV)

The loaf specific volume measured in GF control bread was 2.04 mL/g (day 1) and 2.10 mL/g (day 2). That was lower than the loaf specific volume of traditional white bread (2.82 mL/g) as shown in Table 4.4. The reduced loaf specific volume of gluten-free bread could be due to the lack of a cohesive protein matrix, elasticity and extensibility of the gluten-free dough as suggested by Hager *et al.* (2012).

The incorporation of plasma protein powders generally resulted in an increase in loaf volume as shown in Table 4.10 compared with the control. For instance, addition of 4% PPI1 and 5% SP powders into bread mix increased loaf specific volume by 7.6% and 9.0%, respectively (batch 1). An increase in loaf specific volume by 12.1% and 12.6% was observed when GF bread mix was replaced by 5% SP and 8% PPI1 (batch 2). The other formulations produced breads with loaf specific volume not significantly different from the control. The results suggest that the bread loaf volume can be increased by fortification of gluten-free bread mix with plasma proteins.

Table 4.10 Specific loaf volume of bread produced from gluten-free bread mix and bread mix fortified with plasma protein powders at 4, 5 and 8%.

Batch	Sample	SV (mL/g) (*)
1	GF bread mix control	2.04 ± 0.9 (b)
	GF bread mix (4% PPI1)	2.20 ± 0.10 (a)
	GF bread mix (4% PPC2)	2.13 ± 0.08 (ab)
	GF bread mix (5% SP)	2.22 ± 0.11 (a)
2	GF bread mix control	2.10 ± 0.07 (bc)
	GF bread mix (4% PPI1)	2.10 ± 0.08 (c)
	GF bread mix (4% PPC2)	2.20 ± 0.09 (b)
	GF bread mix (5% SP)	2.37 ± 0.11 (a)
	GF bread mix (8% PPI1)	2.36 ± 0.04 (a)

- Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of ten determinations for each sample.

4.2.5 Crumb structure

Images of the crumb structure of the gluten-free breads are shown in Figure 4.8 and the results from crumb structure analysis are summarised in Table 4.11.

Table 4.11 Crumb structure characteristics of bread produced from gluten-free bread mix and the bread mix fortified with plasma protein powders at 4, 5 and 8%.

Batch	Sample	Crumb cell characteristics (*)				
		Slice Area (mm²)	Number of cells	Area of cells (%)	Average cell elongation	Wall thickness (mm)
1	GF bread mix Control	2364.9 ± 39.6 (b)	2031 ± 81 (a)	47.76 ± 0.85 (b)	1.55 ± 0.03 (bc)	0.414 ± 0.008 (c)

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GF bread mix (4% PPI1)	2522.8 ± 76.5 (a)	1819 ± 49 (b)	49.42 ± 0.61 (a)	1.58 ± 0.02 (a)	0.443 ± 0.006 (a)
GF bread mix (4% PPC2)	2466.3 ± 56.5 (a)	1939 ± 49 (ab)	48.67 ± 0.86 (ab)	1.56 ± 0.02 (ab)	0.426 ± 0.008 (b)

Batch	Sample	Crumb cell characteristics (*)				
		Slice Area (mm²)	Number of cells	Area of cells (%)	Average cell elongation	Wall thickness (mm)
	GF bread mix (5% SP)	2522.9 ± 86.0 (a)	2059 ± 163 (a)	48.97 ± 0.90 (a)	1.52 ± 0.01 (c)	0.415 ± 0.014 (bc)
2	GF bread mix Control	2321.7 ± 48.2 (bc)	2080 ± 75 (a)	46.49 ± 0.43 (d)	1.54 ± 0.02 (a)	0.411 ± 0.005 (c)
	GF bread mix (4% PPI1)	2282.2 ± 45.5 (c)	1949 ± 86 (b)	46.49 ± 1.15 (d)	1.55 ± 0.03 (a)	0.423 ± 0.008 (c)
	GF bread mix (4% PPC2)	2367.1 ± 61.0 (b)	1785 ± 111 (c)	48.53 ± 0.71 (c)	1.52 ± 0.02 (a)	0.442 ± 0.014 (b)
	GF bread mix (5% SP)	2468.3 ± 75.5 (a)	1760 ± 136 (c)	50.54 ± 0.89 (b)	1.46 ± 0.03 (b)	0.442 ± 0.013 (b)
	GF bread mix (8% PPI1)	2532.2 ± 57.5 (a)	1355 ± 87 (d)	53.15 ± 0.54 (a)	1.44 ± 0.54 (b)	0.512 ± 0.013 (a)

* Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of ten determinations for each sample.

The control bread had on average 2343.3 mm^2 slice area. The slice area increased by 4.4% when 4% PPC2 powder was added to the bread mix, and by 6.7% when 4% PPI1 or 5% SP powders were added to the bread mix (batch 1). Bread prepared with 8% PPI1 demonstrated the greatest slice area 2532.2 mm^2, which was 9.1% higher compared to the control (batch 2).

The addition of plasma powders into GF bread mix resulted in a significant reduction of the number of cells in bread produced on batch 2 and in bread made with 4% PPI1 (batch 1). The smallest number of cells (1355) was observed in bread prepared with 8% PPI1 powder. The reduction was 34.9% compared to the control bread which had the highest number of cells (2080).

The most porous bread was produced with 5% SP and 8% PPI1 having an area of cells of 50.54% and 53.15%, respectively (batch 2).

The most rounded cells with an average cell elongation 1.44 and 1.46 were found in GF bread samples fortified with 8% PPI1 and 5% SP (batch 2), whereas voids in other plasma enriched breads and control breads were a little more elongated (1.52 – 1.58).

Cells formed in bread baked with 8% PPI1 powder had the highest wall thickness 0.51 mm (batch 2). The wall thickness values for other protein enriched bread ranged from 0.42 to 0.44 mm compared to the controls with wall thickness of 0.41 mm.

4.2.6 Textural characteristics

Textural characteristics of gluten-free bread with and without plasma protein inclusion are presented in Table 4.12.

Table 4.12 Textural characteristics of bread produced from GF bread mix fortified with plasma protein powders at 4, 5 and 8%.

	Sample	Textural parameters (*)		
		Hardness (N)	Springiness	Chewiness (N)
1	GF bread mix control	1.992 ± 0.238 (a)	0.874 ± 0.025 (a)	1.247 ± 0.137 (b)
	GF bread mix (4% PPI1)	1.845 ± 0.224 (b)	0.887 ± 0.058 (a)	1.245 ± 0.188 (b)
	GF bread mix (4% PPC2)	2.463 ± 0.227 (a)	0.906 ± 0.034 (a)	1.645 ± 0.181 (a)

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	GF bread mix (5% SP)	1.997 ± 0.150 (a)	0.882 ± 0.032 (a)	1.352 ± 0.094 (b)
2	GF bread mix control	2.014 ± 0.266 (b)	0.847 ± 0.032 (bc)	1.256 ± 0.150 (c)
	GF bread mix (4% PPI1)	2.310 ± 0.406 (b)	0.840 ± 0.046 (c)	1.447 ± 0.220 (bc)

	Sample	Textural parameters (*)		
		Hardness (N)	Springiness	Chewiness (N)
	GF bread mix (4% PPC2)	2.347 ± 0.279 (b)	0.902 ± 0.042 (a)	1.632 ± 0.316 (b)
	GF bread mix (5% SP)	1.875 ± 0.333 (b)	0.837 ± 0.053 (c)	1.202 ± 0.217 (c)
	GF bread mix (8% PPI1)	2.897 ± 0.295 (a)	0.894 ± 0.030 (ab)	1.969 ± 0.194 (a)

* Sample means for each batch with different letters in brackets are significantly different ($p < 0.05$). Variation of the mean represents standard deviation of ten determinations for each sample.

The crumb hardness of GF bread was significantly reduced from 2.0 N to 1.8 N by addition of 4% PPI1 powder into bread mix (batch 1). Increasing the level of substitution from 4 to 8% resulted in more firm crumb with hardness 2.9 N. The crumb hardness of other plasma protein enriched bread was not significantly different from the corresponding controls.

Fortification of GF bread mix with plasma proteins did not affect the “elasticity” of bread crumb measured by springiness which varied from 0.84 to 0.90. The one exception was the bread produced with 4% PPC2 powder which had crumb springiness significantly higher than the control sample (batch 2).

Chewiness of bread crumb was dependent on the type of plasma powders used for protein enrichment. Bread prepared with 4% PPI or 5% SP powders (batch 1 and batch 2) had chewiness similar to the corresponding controls. The addition of 4% PPC2 (batch1 and batch 2) or 8% PPI1 (batch 2) into bread mix increased the chewiness of the bread crumb by 31 and 56%, respectively.

5 Conclusions and recommendations

Low protein wheat flour was enriched to similar protein content as wheat flour for bread making, and the potential of this blend in bread making was assessed. It was found that dough quality was affected by inclusion of the plasma protein powders. The dough colour was slightly more yellow and the dough was more sticky after plasma protein incorporation. However, it was found that after a short (15 min) resting time the dough could be processed according to the protocol used for the control. Baking loss was not changed with inclusion of plasma proteins. The incorporation of the plasma powders increased the loaf volumes of the breads. The crusts of all protein enriched breads were darker than those of control breads. The crumbs of breads with plasma protein fortification were found to have slightly increased yellowness and reduced colour lightness. Furthermore, the cell shape, porosity, cell wall thickness and number of cells in the crumb could be modified by the inclusion of plasma protein powders with subsequent changes in bread hardness and elasticity. Based on the results obtained here, it appears to be possible to use the plasma protein powders to increase the protein content of low protein flour for bread making without significant loss of textural quality. It also appears that existing processing methods and equipment will be suitable in dough processing and bread baking. Further work establishing consumer acceptance and liking of such breads as well as optimisation of formulation and processing conditions is required before commercial transfer of this application.

Plasma proteins could also be used to improve the protein content of low protein gluten-free bread mix comprising soy and rice flour, as well as maize and tapioca starch. Plasma protein powders were blended into gluten free bread mix to increase the protein content to 9.8% or 13.0% in the bread mix. Gluten-free bread dough’s with and without plasma protein fortification

were more sticky and difficult to handle than traditional wheat flour based dough. Gluten free breads had a higher baking loss than traditional white breads but baking loss was not affected by plasma protein incorporation. Inclusion of plasma protein increased crust and crumb colour of

gluten free breads. Crumb structure parameters (cell number, volume and shape, wall thickness) and textural properties of the crumb could be modified through the incorporation of plasma protein powders into the gluten free bread mix. The protein content of the baked bread could be increased from 3.9% protein for the control bread, to 5.9% protein for the plasma fortified breads. The results suggested that the apparently upper limit for protein fortification results in bread with about 7.7% protein, but further optimisation of effects of formulation, processing and quality needs to be carried out before commercial application.

The overall project results have successfully demonstrated:

- Bovine blood plasma proteins could be concentrated, diafiltered and spray-dried in a cost effective manner (Milestone 1).
- The resulting plasma protein ingredients exhibited comparable functionality to other food protein ingredients such as dairy and soy proteins. Plasma proteins displayed good solubility over a wide pH range, formed strong elastic gels with good water-holding capacity, foamed and emulsified oils well (Milestone 2).
- The plasma protein ingredients could be incorporated into food products such as sausages, meat patties and bread and with acceptable functional properties. The foods fortified with plasma proteins had improved nutritional and/or functional properties and no major changes in processing protocols or equipment setup was considered to be necessary (Milestone 3).
- Plasma proteins could be incorporated into low protein wheat flour to produce white bread with improved nutritional properties and acceptable quality attributes. High protein gluten free breads could also be prepared with addition of plasma proteins without the loss of bread quality (Milestone 4).

Based on these preliminary results and with further formulation design and optimisation, plasma proteins could be further developed as an ingredient to improve the nutritional and functional properties of food. In order to achieve these outcomes, it is recommended:

- The specific food formulations used by industry are more complex and may include additional ingredients to enhance desired properties. Therefore, further work should be conducted to understand the interaction of plasma proteins with such other ingredients and establish the impact on the desired product functionality. The information gained through these studies will enable the definition and optimisation of food formulations using plasma proteins.
- Sensory properties are of primary importance in consumer acceptance, hence understanding the interaction of the plasma ingredients and its impact on sensory properties needs to be further evaluated.
- Incorporation of plasma protein ingredients into food products such as bread is an ideal vehicle to provide enhanced nutrition. The importance of generating a new source of proteins to provide nutrition will become more significant with the forecasted deficit in the global supply of protein for human consumption. Therefore, the nutritional properties of plasma protein after incorporation into food products should be evaluated in comparison with other sources.
- The use of blood in sausages and other meat products is acceptable in certain communities, but it is still not widely used in others. Therefore, the plasma protein powders may be initially introduced to processed meat products to gain consumer acceptance and confidence in plasma protein sources. The incorporation of plasma protein into non-meat products would likely generate consumer resistance, but in an

environment of low protein supply and high protein prices the extent of such resistance is not known. Therefore, understanding the acceptability of plasma protein in processed food, including non-meat products in targeted consumer groups needs further study.

- Further development and optimisation of a plasma protein isolation procedure need to be conducted. This work will need to focus on meeting food grade standards in plasma handling and processing whilst delivering economic and sustainable processing solutions.
- Regulatory requirements (locally and globally) for incorporating plasma proteins into non-meat processed food need to be understood, and where appropriate they need to influence development of these ingredients.
- Further information on labelling requirements for processed foods incorporating plasma proteins is required.
- An industry strategy promoting plasma proteins as functional and nutritional ingredients should be developed, especially with the intention of incorporating the ingredients into non-meat food products in the medium to long term. In formulating the strategy, information gained from the above recommendations should be considered.

6 References

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