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Development and characterisation of plant and dairy-based high protein Chinese steamed breads (mantou): Physico-chemical and textural characteristics

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ABSTRACT

High protein versions of popular, highly consumed food products such as Chinese steamed bread (CSB) can be useful to improve the health status of our populations. In the current study, high protein Chinese steamed breads (HPCSBs) were developed using plant (soy protein isolate, SPI) or dairy (rennet casein, RC and milk protein concentrate, MPC) proteins. These proteins were blended into wheat flour at two different levels (RC I, RC II; SM (soy protein isolate-SPI + milk protein concentrate-MPC) I, SM II) to prepare breads, which were then compared to a control (100% wheat flour-based) Chinese steamed bread for physico-chemical and textural characteristics. The addition of proteins darkened the colour of HPCSBs and decreased the specific bread volume with RC II showing the lowest. All the high protein formulations recorded an increase in RVA pasting temperature, whereas a decrease in the peak, final and breakdown viscosities of pastes was observed with the addition of both RC and SM at all levels. Similarly, the DSC onset transition temperatures were observed to increase when either RC or SM was added to the formulation. The textural characteristics of HPCSBs showed an increase in hardness, gumminess, and resistance for penetration along with tensile strength than the control CSB.

Introduction

Bread is one of the most important staple foods around the world (Cauvain, 2003). Over the centuries, conventional bread products have been developed. Chinese steamed bread (CSB) is prepared by fermenting wheat flour-based dough with yeast and then cooking it in a steamer (Huang, 2014). It is a staple food in parts of China for over two millennia and is now gaining popularity in other parts of the world (Hui and Evranuz, 2012; Zhu, 2014). It is well established that baking causes the Maillard reaction. This reaction decreases the nutritional value of the bread as it results in the loss of availability of soluble amino acids (mainly lysine). Liu *et al.* (2017) reported that lysine availability in steamed bread is higher than in baked bread. Thus, steamed bread is nutritionally richer than baked bread.

Bread is high in carbohydrates, and excessive intake can increase blood glucose and insulin levels in a short duration, which might lead to health issues. Therefore, there is a need for lowering the carbohydrate content of foods by the addition of non-starch or high protein ingredients (Guilherme, Virbasius, Puri, & Czech, 2008). Inadequate levels of dietary protein, particularly in Asian countries have prompted public demand for high-protein, low-carb foods.

While the protein content of normal wheat flours ranges from to 10-14%, such protein levels do not have enough nutritive value due to a lack of essential amino acids especially lysine (Huang, 2014). Protein enrichment complements this deficiency in cereals as it leads to an improvement in their amino acid content (Nogueira and Steel, 2018). One way to supplement the public diet with extra protein is to enrich food products that are already accepted and widely used, such as bread. Protein supplements, for example, soy protein and dairy protein are easily available for fortifying such baked products because of their proven health benefits. For a protein supplement to be commercially viable, the added proteins should not significantly change the quality of bread during the bread-making process.

Supplementation of protein in wheat flour at higher levels has not been successful so far because of its adverse effect on bread volume, texture, and organoleptic properties. (Du *et al.*, 2016; Liu *et al.*, 2016). Soy-fortified bread has been reported to exhibit undesirable characteristics such as diminished loaf volume and poor crumb grain. Also, soy protein has some antinutritional factors such as phytic acid, lectins, and hemagglutinins which may inhibit calcium and iron absorption and may

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cause diarrhoea (González-Pérez & Arellano, 2009). Thus, the blending of soy and dairy proteins in an appropriate combination may result in a new product containing both dairy and plant nutritional advantages.

Dairy ingredients can be used in bread for nutritional benefits, including increasing calcium content and protein efficiency ratio; and functional benefits including flavour, texture enhancement, and storage improvement. Dairy ingredients also enhance water absorption and can also improve dough-handling properties (Houben and Becker, 2012). Caseins are particularly rich in lysine and make excellent nutritional supplements for cereals, which are deficient in lysine (Rollema and Muir, 2009). Milk protein concentrate (MPC) contains significant quantities of calcium and phosphate. MPC can be used for its nutritional and functional properties. The high protein and low lactose ratio makes MPC suitable for protein-fortified beverages and low-carbohydrate foods (O'Kennedy, 2009).

Currently, little information is available in the literature on the fortification of CSB by adding dairy and plant proteins alone or in combination. Therefore, the main objective of this study was to study the effects of dairy (rennet casein; milk protein concentrate) and plant (soy protein isolate, SPI) proteins in the development of high protein versions of CSB. Physico-chemical, textural and thermal characteristics of the developed breads were investigated and compared with CSB. Microstructural characteristics and *in vitro* gastro-small intestinal digestion of the breads and microstructural properties of the digests obtained during gastro-small intestinal digestion are discussed in a companion paper.

2. Materials and Methods

2.1. Materials

Wheat flour (Pams, Auckland, New Zealand, protein 10%, carbohydrates 71%, fat 1.8% as mentioned on the label), sugar (Chelsea, Auckland, New Zealand), baking soda, yeast (Edmonds, Auckland, New Zealand), and soy protein isolate (SPI) (Davis Trading, Palmerston North, New Zealand; protein content- 89%) were purchased from a local market. RC (protein 95% as mentioned on the label) and MPC (protein 85% as mentioned on the label) were purchased from Fonterra Ltd. (Palmerston North, New Zealand). All the other chemicals and reagents used in this study were of analytical grade.

2.2. Formulations for making HPCSB

The production of HPCSBs was performed as per the method of Huang and Miskelly (2016). Experiments were conducted by replacing wheat flour with protein powders (rennet casein and a combination of SPI and MPC) at different levels. the combination of SPI and MPC has been observed to result in improved colour and flavour of bread during preliminary experiments. The different formulations studied in this research are provided in Table 1.

2.3. Bread Making - HPCSBs

The formulations for control and HPCSBs prepared in this study are shown in Table 1. The gluten level was calculated based on the amount of gluten in substituted wheat flour. Before mixing the dough, yeast and baking soda were dissolved in water (at 30 °C). For HPCSBs, different dry protein powders were mixed with wheat flour beforehand. To begin dough mixing, sugar and yeast solutions were poured into the dry ingredients. Mixing was then conducted at medium speed in a kitchen mixer (KMM021, Kenwood, China) as depicted in Table 2. The dough was then transferred into a pan and fermented at 35 °C for 30 min. After which, the dough was kneaded, and then sheeted by hand 5-10 times. The dough was then divided into eight pieces, hand-shaped, and proofed for 15 min followed by steaming for 20 min in a steamer (ST6650, Sunbeam, China). After steaming, the breads were cooled to room temperature (25 °C) for 20 min and then sealed in a plastic bag until further analysed.

Table 1
Formulations for control and high protein Chinese steamed breads.

	Control (g)	RC I (g)	RC II (g)	SM I (g)	SM II (g)
Gluten	0	3.4	6.8	3.3	4.2
Wheat flour	250	219	187.9	220	212
RC	0	31	62.1	-	-
SPI	0	-	-	17.6	18
MPC	0	-	-	12.5	20
Sugar	8	8	8	8	8
Baking soda	0.5	0.5	0.5	0.5	0.5
Yeast	2.5	2.5	2.5	2.5	2.5
Water*	135	160	165	155	160

[1] RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.

* Water addition and kneading time were adjusted based on the performance of the dough during preliminary experiments.

Table 2
Production parameters for control and high protein Chinese steamed breads.

Time(min)	Control	RC I	RC II	SM I	SM II
Mixing*	7	5	5	4	2
Fermentation	30	30	30	30	30
Kneading*	3	1	1	0.5	0.5
Proofing	15	15	15	15	15
Steaming	15	15	15	15	15

RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively

* Mixing and kneading times were adjusted based on the performance of the dough during preliminary experiments.

2.4. Physico-chemical characterisation of HPCSBs

2.4.1. Colour characteristics

After cooling at room temperature for 30 min, the crust and crumb colour of steamed breads was determined using a Minolta Chroma Meter CR-200 (Chemiplas NA Ltd., AU), following the method of Kaack, Pedersen, Laerke, & Meyer (2006). Both crust and crumb colour of each sample were evaluated at three different points on four slices of breads.

Data were reported in the form of CIE L*, a*, and b* colour values. The white index of (WI) of HPCSBs and the control was calculated using the equation (Ballester, Gil, and Fernández, 2019):

$$WI = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{\frac{1}{2}}$$

$$\begin{aligned}\Delta L* &= (L * sample - L * standard)\\ &= difference\, in\, lightness\, and\, darkness\, (+ = lighter, -- = darker)\\ \Delta a* &= (a * sample - a * standard)\\ &= differenceinredandgreen(+ = redder,\ - - = greener)\\ \Delta b* &= (b * sample - b * standard)\\ &= differenceinyellowandblue(+ = yellower,\ - - = bluer)\end{aligned}$$

2.4.2. Specific bread volume

Bread samples were analysed for specific volume by the rapeseed displacement method as previously reported by Nwosu, Elochukwu, and Onwurah (2014).

2.4.3. Proximate analysis

The protein content of the HPCSBs was determined by the Kjeldahl method (AOAC 2000).

The moisture content of the bread samples was measured according to the standard AOAC method (AOAC, 1990). The oven temperature was set at 108 °C, the duration is 4 hours. The total starch content in HPCSBs was determined by using the method developed by Bordoloi, Singh, and Kaur (2012) and Goñi, García Diz, Mañas, and Saura Calixto (1996). HPCSBs and control bread samples were freeze-dried, ground and passed through a 0.5 mm mesh sieve and kept in a desiccator until analysed (Tamura, Singh, Kaur, and Ogawa, 2016). A total starch kit (K-TSTA, Megazyme International Ireland Ltd., Ireland) was used to determine the starch content.

2.5. Pasting properties of HPCSB formulations and freeze-dried HPCSBs

The pasting properties of different raw material formulations used for the preparation of HPCSBs along with freeze-dried HPCSB powders were analysed using a Rapid Visco Analyser (Perten RVA 4800, Australia). The data was analysed using Thermocline for Windows 10 (version 3.11).

The control sample (wheat flour-based) the raw material formulations with added protein (Table 1**)** (without yeast) and freeze-dried HPCSB powders were prepared as described earlier. Four (4 g) grams of each sample were transferred into an aluminium canister and 25 ± 0.1 mL distilled water was added (corrected to compensate for a 14 % moisture basis). An approved Method 76-21.01A (AACC International, 2000) was used for RVA analysis (4 g sample and 14 % moisture basis).

2.6. Thermal characteristics of HPCSBs and formulations

The gelatinization (thermal) characteristics of the HPCSB formulations, control (wheat flour), and fresh bread samples were determined using a differential scanning calorimeter (DSC) (TA Q100, TA Instruments, Newcastle, DE). An aluminium pan containing samples (approx. 5 mg, dry wt basis) was used, and distilled water was added to obtain a powder-water ratio of 1: 3 (w / w). The pan was then sealed and stored for at least 2 hours at room temperature before analysis. The sample pans were then heated from 20 to 100 °C at a rate of 10 °C / min. The temperature at the onset of gelatinization (T_o), peak temperature (T_p), conclusion temperature (T_c); enthalpy of gelatinization and (ΔH) temperature range (ΔT) were determined.

2.7. Texture measurements

2.7.1. Texture profile analysis

A double compression test was performed on bread samples using a TA-XT plus texture analyser (TA-XT plus, Stable Micro Systems, Surrey, United Kingdom), equipped with a 35 mm diameter cylindrical flat probe and a 5 kg load cell. The crust of the samples was removed, and the bread crumb was cut into a 3 × 3 × 2 cm cuboid. The pre and post-test speeds were set at 2 mm/s and the test speed was 0.2 mm/s. Hardness, gumminess, chewiness, springiness and cohesiveness values were obtained.

2.7.2. Tensile test

The purpose of carrying out pull-apart (tensile) analysis was to mimic hands pulling apart bread. The bread was griped on two sides by a metal fixator, and the position for gripping the sample was 3.5 cm from the centre. The analysis consisted of recording the force required to pull the sample apart (Dolores Romero de Ávila *et al.*, 2014; Farouk *et al.*, 2005). The texture analyzer was equipped with a tensile grip that was used to hold the bread for testing. The test speed was 0.5 mm/s with a rupture distance of 100 mm. Peak force, named resistance (g), the extensibility (mm) at peak force and the ratio of resistance and extensibility (R/E) were recorded.

Table 3

Colour characterisations of control and high protein versions of Chinese steamed breads.

Crust	*L**	*a**	*b**	White Index
Control	81.55±0.76^{a}	-1.86±0.06^{d}	17.36±0.62^{c}	74.58±0.39^{a}
RC I	79.08±0.33^{b}	-1.77±0.08^{c}	17.71±0.49^{c}	72.53±0.41^{b}
RC II	79.01±0.50^{b}	-1.42±0.10^{b}	20.24±0.57^{a}	70.80±0.49^{d}
SM I	78.20±0.55^{c}	-1.41±0.08^{b}	18.55±0.66^{b}	71.33±0.37^{c}
SM II	78.89±0.78^{b}	-1.33±0.06^{a}	20.08±0.49^{a}	70.82±0.40^{d}
Crumb	***L****	***a****	***b****	**White Index**
Control	77.24±0.77^{a}	-1.68±0.05^{c}	17.41±0.35^{c}	71.29±0.72^{a}
RC I	74.80±0.82^{b}	-1.63±0.12^{bc}	18.09±0.27^{b}	68.93±0.67^{b}
RC II	73.54±0.58^{c}	-1.58±0.08^{b}	18.41±0.32^{b}	67.72±0.51^{c}
SM I	72.80±0.72^{d}	-1.11±0.12^{a}	18.13±0.61^{b}	67.28±0.50^{c}
SM II	73.41±0.90^{cd}	-1.05±0.10^{a}	18.88±0.45^{a}	67.36±0.73^{c}

[1] Results are demonstrated as average (n=3) ± SD values in each column with the same superscript are not significantly different ($p < 0.05$). SM, SPI - MPC containing breads; RC, rennet casein containing breads

[2] RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.

2.7.3. Puncture test

The force required was recorded with the texture analyser to penetrate the bread crust by punching the bread surface at three different points. Experiments were carried out using a cylindrical probe, 2 mm in diameter. The test speed was 0.5 mm/s (Altamirano Fortoul, Hernando, and Rosell, 2013). Resistance (g) and extensibility (mm) were recorded.

2.8. Statistical analysis

The reported data are the average of at least three measurements. Minitab version 17.3.1 Statistical Software (Minitab Inc., State College, PA) was used for statistical analysis. The data were subjected to analysis of variance (ANOVA). Tukey's test at a 5% significance level was used for comparison. Standard deviation (SD) is shown in figures as error bars and is also shown in the tables. Pearson correlation coefficients for relationships between different physicochemical and textural properties were calculated. Principle component analysis (PCA) of HPCSB properties was carried out to provide a ready means of visualizing the difference and similarities among HPCSBs in terms of these properties.

3. Results and discussion

3.1. Physico-chemical characterisation of HPCSBs

3.1.1. Colour characteristics of HPCSBs

The physical appearance of control and HPCSBs are presented in Fig. S1. As given in Table 3, control bread had a significantly lighter colour ($p < 0.05$) as compared to other formulations. RC II showed the lowest white index (WI). A reduction in the average L^* and WI values for crust and crumb of RC were observed, whereas the * values and b^* values were higher when compared to the control, respectively. The higher amount of rennet casein led to lower L^* values and white index (WI) in RC I and RC II indicating that rennet casein incorporation darkened the appearance of CSB. An increase in b^* values was also observed for HPCSBs. Bread with a higher level of rennet casein was consequently more green and yellow than the control. These changes were attributed to the creamy yellow colour of rennet casein (Fox, Uniacke, McSweeney and O'Mahony, 2015). Su (2005) reported that for CSB the effect of the Maillard reaction on the colour of CSB was considered to be negligible as the steaming temperature is approximate 100°C and not > 150°C as the Maillard reaction might result in a darkened crust.

For SPI and MPC (SM) samples, as the protein levels increased, the L^* values (78.20) and white index (71.33) of the crust decreased compared

Table 4
The composition of control and high protein Chinese steamed breads.

	Moisture (%)	Protein (%)	Total starch (%)	Specific volume (mL/g)
Control	39.13 ± 0.46^{c}	8 ± 0.03^{c}	41.14 ± 1.58^{a}	2.22 ± 0.06^{a}
RC I	41.73 ± 0.25^{ab}	13 ± 0.11^{c}	34.22 ± 2.03^{b}	1.59 ± 1.59^{c}
RC II	42.90 ± 0.54^{a}	19 ± 0.13^{a}	27.89 ± 4.98^{c}	1.39 ± 1.39^{d}
SM I	41.05 ± 0.17^{b}	13 ± 0.14^{c}	32.32 ± 0.10^{bc}	1.96 ± 1.96^{b}
SM II	42.79 ± 0.82^{a}	15 ± 0.11^{b}	29.89 ± 0.67^{bc}	1.89 ± 1.89^{b}

[1] Results are demonstrated as average (n=3) ± SD values in each column
Values with the same superscript are not significantly different ($p < 0.05$). SM, SPI - MPC containing breads; RC, rennet casein containing breads
[2] RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.

to the control. A similar trend was observed for crumb colour characteristics. However, there was no significant difference in white index values, L^* values and a^* values among SM-containing samples, which may be attributed to the fact that SPI levels in both SM-containing samples were not much different. Du *et al.* (2016) reported similar findings.

3.1.2. Proximate analysis, total starch content and specific volume

In comparison to control, the moisture content of all the HPCSBs was observed to be higher (Table 4). RC II and SM II showed the highest moisture content whereas the control sample had the lowest. This might be attributed to the hydration properties of proteins which increase the moisture levels of the dough during processing. The added protein might have absorbed more moisture than the control sample during steaming (Kenny, Wehrle, Stanton, and Arendt, 2000).

As expected, the protein content of HPCSBs increased significantly ($p < 0.05$), whilst the starch content decreased. On the other hand, the specific volume decreased with protein addition, compared to the control bread (Table 4). The addition of proteins might have disrupted the well-defined natural protein (gluten)-starch complex of the dough, although the extra gluten was added, the effect was inconspicuous (Dhinda *et al.*, 2012). It is also confirmed through measurements of texture analysis (Section 3.4). Similar results were also observed by Dhinda *et al.* (2012) and Patel, Patel, and Singh, (2016).

A strong positive correlation between moisture and protein content has been revealed by Pearson correlation analysis (r = 0.93, $p < 0.05$, Table 9). A strong negative correlation between starch - protein content and starch - moisture content has also been observed (r =-0.958, -0.929, respectively, Table 9). The latter three components run in opposite directions on the PCA loading plot, suggesting a negative correlation among them (Fig. 2b).

3.2. Pasting properties

3.2.1. Pasting characteristics of bread formulations

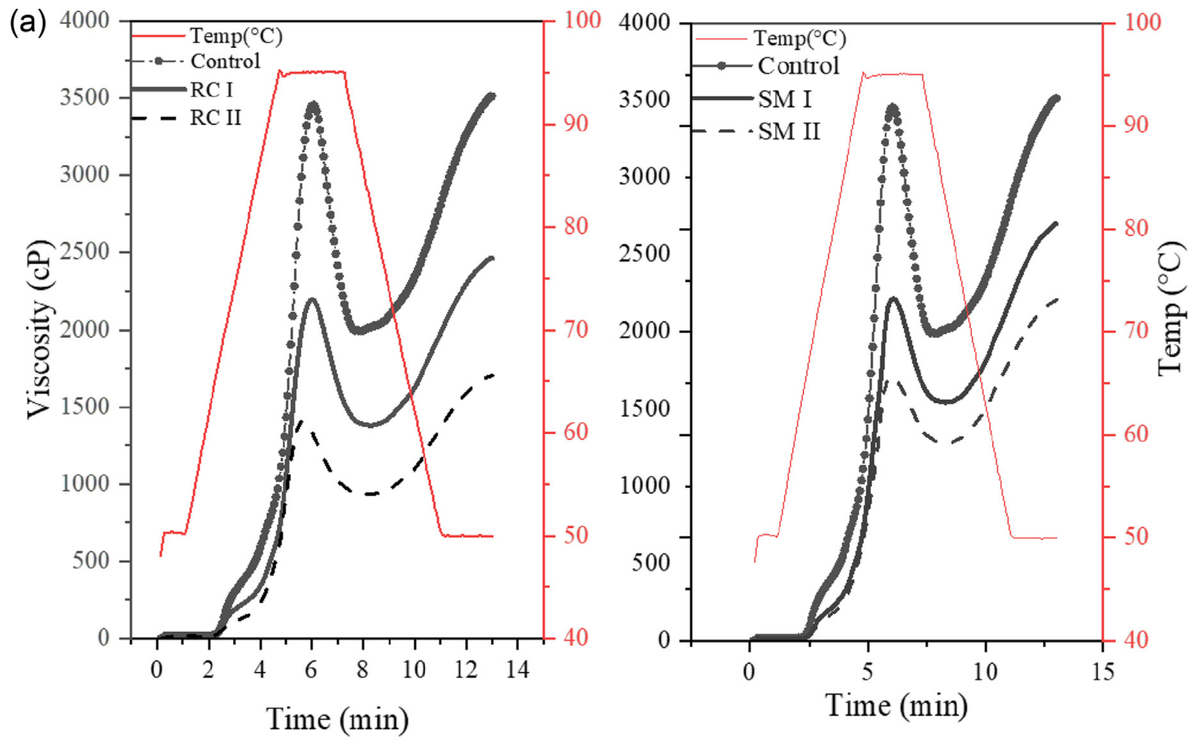
Significant differences in pasting properties were observed among all the bread formulations when compared to control (Fig. 1a, Table 5) with a more prominent effect observed at higher levels of protein addition.

For the formulations containing higher protein levels, a corresponding decrease in the peak viscosity was observed, which might be attributed to the dilution of total starch content (Bravo-Núñez, Garzón, Rosell, & Gómez, 2019). This possibly indicates that starch contributes mainly to network formation at lower protein levels (Kumar et al., 2018). This is in concordance with PCA findings where starch content is positively correlated with peak viscosity (r = 0.978). According to Ribotta et al. (2007), protein molecules can influence the starch gelatinisation process in many ways owing to their different properties, i.e they can aggregate and act as a filler, they can bind water to form a gel resulting in a weak gel whereas pure starch can form a more viscous gel (Noisuwan et al., 2008). For RC-containing bread formulations (RC I and RC II), the lower peak viscosity could be attributed to β-caseinates in RC which hinder starch granule swelling leading to a decreased peak viscosity due to their micelle forming capacity (Kenny *et al.*, 2000; Kumar, Brennan, Mason, Zheng, and Brennan, 2017). Similarly, MPC in SM-containing samples contains casein and whey protein, which might interact with amylose by non-covalent bonding. Furthermore, whey protein has poor water holding capacity than wheat protein (Sarabhai and Prabhasankar, 2015), which might be another reason for the decrease in peak viscosity. The less decrease in peak viscosity for SM II samples than RC II samples could be explained by the higher protein content of the latter. Also, soy protein contains various hydrophilic groups which can form crosslinks with starch, thus resulting in an increased paste viscosity (Ribotta *et al.*, 2007).

All the HPCSB formulations showed a significant decrease in final viscosity compared to the control (Table 5). Apart from the lower starch content of the former, the whey protein may have incapacitated the protein-starch network and acted as an inactive barrier during the process of amylose reordering. The higher amounts of the increased portion of lactose and lipid in the whey protein may also delay starch swelling and gelatinization (Zobel and Stephen, 2006). The drop in final viscosity of HPCSB formulations could also be ascribed to steric hindrance that might occur during the cooling process (Ktenioudaki *et al.*, 2013; Yang *et al.*, 2004; Sarabhai and Prabhasankar, 2015). Final viscosity was also found to have a positive correlation with specific bread volume (r = 0.904) and was negatively associated with bread firmness (r = -0.649).

The pasting temperature increased with the addition of protein ($p < 0.05$). The presence of proteins has been reported to minimize the availability of water to starch, resulting in increased pasting temperatures (Kaur, Singh, and Singh, 2005). Another possible explanation for RC-containing samples might be that casein is capable of forming micelles due to its self-association properties, and it might absorb onto the surface of starch granules and strengthen the granules, thereby restricting starch swelling and pasting temperatures (Noisuwan et al., 2007). The increased pasting temperature in SM-containing samples also indicates restrictions imposed by the protein on the swelling of starch granules (Kumar, Brennan, Zheng, and Brennan, 2018; Noisuwan *et al.*, 2008). These results also agree with DSC results (Table 6). The pasting temperature is positively correlated with the onset temperature (T_o) obtained from thermal properties (r = 0.748, data not shown). The onset temperatures for all samples were lower than the pasting temperature. This trend has been reported earlier (Noisuwan et al., 2008; Perez, Breene, and Bahnassey, 1998).

Setback and breakdown viscosities also decreased with the addition of proteins. As expected, RC II demonstrated the lowest setback value among all the samples probably because it had the lowest starch content. The setback viscosity also has been reported to be related to the hardness of bread texture (Lei, Tian, Sun, and Chun, 2008), which supported our Pearson correlation results (r = -0.715). Interestingly, SM II showed the



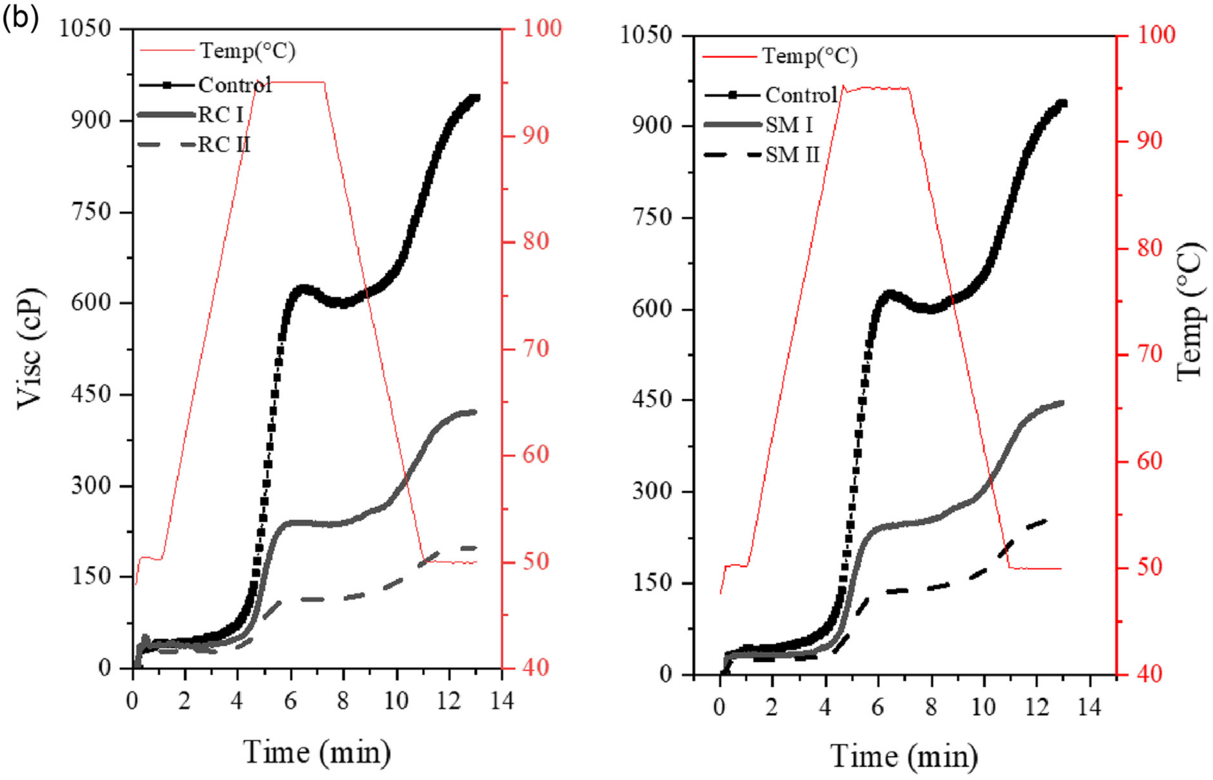


Fig. 1. a.Pasting curves for control and high protein Chinese steamed bread formulations.
RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.

Fig. 1b. RVA profiles of freeze-dried control and high protein Chinese steamed breads
RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.

Table 5
Pasting characteristics of control and high protein Chinese steamed bread formulations and freeze-dried bread samples.

Formulations	Peak viscosity (cP)	Holding strength (cP)	Break down (cP)	Final viscosity (cP)	Setback viscosity (cP)	Pasting Temp (°C)
Control	3452 ± 120.86^{a}	1991.33 ± 65.01^{a}	1460.67 ± 82.81^{a}	3514.33 ± 61.34^{a}	1523.00 ± 31.19^{a}	68.52 ± 0.10^{b}
RC I	2193 ± 9.54^{b}	1381.33 ± 10.50^{b}	811.67 ± 11.06^{b}	2464.67 ± 25.17^{c}	1083.33 ± 20.98^{b}	68.80 ± 0.43^{b}
RC II	1419 ± 27.62^{d}	933.33 ± 31.09^{c}	485.67 ± 16.07^{d}	1704.67 ± 53.46^{d}	771.33 ± 24.11^{b}	86.97 ± 0.89^{a}
SM I	2215 ± 72.63^{b}	1546.33 ± 39.46^{b}	668.67 ± 41.86^{c}	2701.33 ± 75.80^{b}	1155.00 ± 39.34^{ab}	70.97 ± 0.08^{b}
SM II	1774 ± 123.72^{c}	1354.67 ± 82.34^{b}	419.33 ± 44.81^{d}	2307.67 ± 152.58^{c}	953.00 ± 70.55^{b}	83.20 ± 8.40^{a}
Freeze-dried Sample						
Control	624.67 ± 20.79^{a}	597.33 ± 19.76^{a}	27.33 ± 4.04^{a}	938.00 ± 11.36^{a}	340.67 ± 9.07^{a}	95.28
RC I	240.67 ± 1.15^{b}	236.67 ± 3.79^{b}	4.00 ± 2.65^{b}	422.00 ± 4.58^{b}	185.33 ± 2.08^{bc}	ND
RC II	114.67 ± 13.32^{c}	111.67 ± 13.43^{c}	3.00 ± 3.00^{b}	198.33 ± 21.39^{c}	86.67 ± 8.62^{d}	ND
SM I	248.33 ± 16.74^{b}	241.00 ± 15.57^{b}	7.33 ± 3.21^{b}	447.00 ± 21.70^{b}	206.00 ± 6.24^{b}	ND
SM II	184.67 ± 18.58^{d}	179.33 ± 16.07^{d}	5.33 ± 3.21^{b}	343.00 ± 29.87^{d}	163.67 ± 13.80^{c}	ND

[1] Results are demonstrated as average (n=3) ± SD values in each column with the same superscript are not significantly different ($p < 0.05$). SM, SPI - MPC containing breads; RC, rennet casein containing breads. Control: pure wheat flour

[2] RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.

[3] ND: not detected.

Table 6
Thermal properties of control and high protein Chinese steamed bread formulations and bread samples.

Formulations	Onset temp. (°C)	Peak temp. (°C)	Conclusion temp. (°C)	Enthalpy ΔH (J/g)	R (°C)
Wheat Flour	57.06 ± 0.41^{b}	62.95 ± 0.30^{ab}	76.61 ± 2.37^{a}	6.81 ± 0.71^{a}	19.55 ± 2.67^{a}
RC I	56.65 ± 0.55^{b}	62.17 ± 0.37^{b}	77.95 ± 2.03^{a}	6.43 ± 1.37^{a}	21.30 ± 2.55^{a}
RC II	57.71 ± 0.11^{ab}	62.42 ± 0.82^{ab}	76.73 ± 1.51^{a}	3.97 ± 0.59^{a}	19.02 ± 1.62^{a}
SM I	57.62 ± 0.35^{ab}	62.21 ± 0.45^{ab}	80.63 ± 0.94^{a}	6.44 ± 0.34^{a}	23.01 ± 1.19^{a}
SM II	58.48 ± 0.80^{a}	63.63 ± 0.69^{a}	79.45 ± 2.47^{a}	5.15 ± 1.25^{a}	20.96 ± 2.97^{a}
Fresh bread samples					
Control	41.51 ± 0.99^{a}	58.53 ± 0.45^{a}	ND	0.69 ± 0.59^{a}	-
RC I	43.05 ± 0.53^{a}	59.12 ± 0.47^{a}	ND	0.07 ± 0.03^{a}	-
RC II	39.08 ± 2.27^{a}	48.99 ± 0.35^{b}	ND	0.20 ± 0.21^{a}	-
SM I	40.54 ± 1.40^{a}	59.78 ± 1.89^{a}	ND	0.52 ± 0.09^{a}	-
SM II	39.10 ± 1.31^{a}	56.65 ± 2.32^{a}	ND	0.37 ± 0.10^{a}	-

[1] Results are demonstrated as average (n=3) ± SD average mean in each column with the same superscript are not significantly different ($p < 0.05$). SM, SPI - MPC containing breads; RC, rennet casein containing breads. Control: pure wheat flour

[2] RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively. [3] ND: not detected.

lowest breakdown viscosity among the five samples. The reason for this could be the higher amount of whey protein in SM II compared to SM I, which may have contributed to plasticity that prevented the starch granule from swelling and maintained gelation during the cooling cycle (Carvalho, Onwulata, and Tomasula, 2007).

3.2.2. *Pasting properties of freeze-dried HPCSB powders*

The pasting profiles of freeze-dried Chinese steamed breads were different from those of their formulations, without showing a very little breakdown in viscosity following peak viscosity, and much higher final viscosities (Fig. 1b). The pasting values were lower than that of raw their counterpart formulations (Table 5). Moreover, pasting temperatures of freeze-dried samples were not detected except for control wheat bread, indicating that the starch in freeze-dried bread samples was fully gelatinized due to steaming (Krupa, Rosell, and Sadowska, 2010). This could be corroborated through SEM images and DSC values (Table 4). As expected, RC II showed the lowest RVA values of parameters. Low peak and final viscosities also indicate higher heat damage to starch granules due to the plasticization of the starch-protein structure (Shittu, Raji, & Sanni, 2007). Becker, Hill, and Mitchell (2001) indicated that the presence of added ingredients can noticeably vary the starches' properties in water. Another reason for the decrease in the peak viscosity of freeze-dried samples might be the freeze-drying process (Krystyjan et al., 2017), which damages hydrogen bonds that stabilise the structure. Juszczak, Fortuna, Witczak, and Dymel (2004) also suggested that wheat starch is especially susceptible to this process since amylose dendrites formed during the retrogradation process are not easily hydrated, therefore the structure of the continuous phase of the paste, formed by amylose, is more susceptible to shearing.

All pairwise pasting properties (except peak time) were positively correlated, whereas pasting temperature was negatively associated with all other traits (Table 7). Moreover, except for breakdown viscosity, all pasting parameters had a positive correlation with bread texture parameters, springiness and adhesiveness and extensibility, whereas they had a negative correlation with hardness, gumminess, chewiness, and resistance (Table 7**).**

3.3. *Thermal properties*

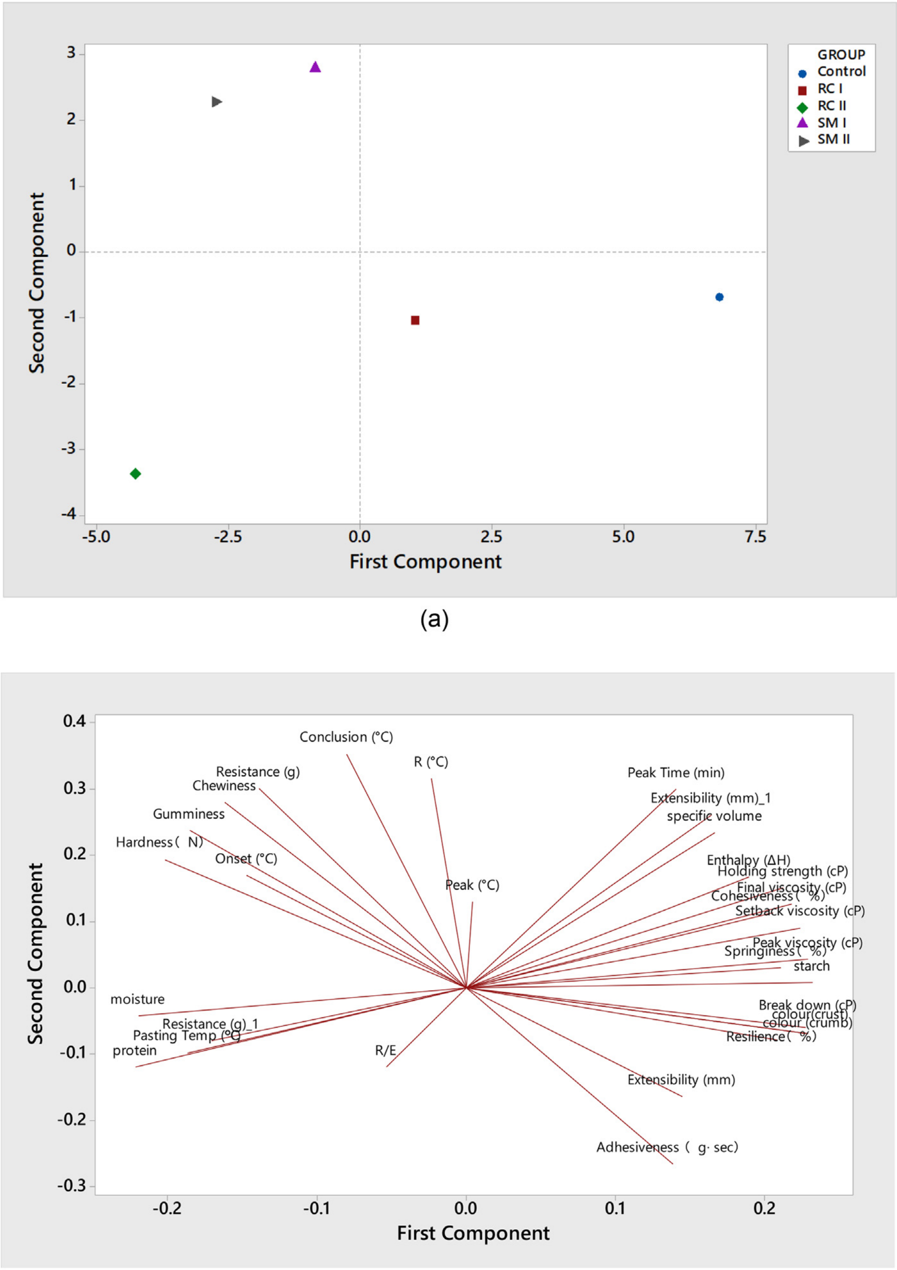
3.3.1. *Thermal properties of raw HPCSB bread formulations*

As observed in Figs. S2-a and b, a single endothermic transition was observed for the bread formulations, which correspond to the gelatinization of starch (Fig. 2a and b). Yu, Liu, Li, and Jiang (2016) reported that the DSC thermograms of mixture samples (soybean 11S globulin with nonwaxy maize starch) exhibited two peak temperatures in the range of (1) 60–80°C and (2) 80–110°C (II). However, this was not observed in the current study (Fig. 2b). The probable reason for this might be that the commercial SPI used in the study is already denatured during processing and therefore did not show any desaturations in the current experiment. The addition of SPI resulted in differences in the thermal characteristics of the bread formulations.

Table 7
Texture profile analysis (TPA) of control and high protein Chinese steamed breads.

Sample	Hardness (N)	Springiness (%)	Cohesiveness (%)	Gumminess	Chewiness
Control	15.55±0.42^{d}	0.90±0.01^{a}	0.76±0.00^{a}	10.97±0.28^{e}	9.86±0.39^{c}
RC I	19.32±0.91^{c}	0.89±0.03^{ab}	0.74±0.01^{b}	13.27±0.53^{d}	11.76±0.46^{b}
RC II	22.25±1.23^{b}	0.82±0.02^{c}	0.69±0.01^{d}	14.29±0.50^{c}	11.71±0.53^{b}
SM I	24.21±0.49^{a}	0.85±0.01^{abc}	0.72±0.00^{c}	16.16±0.32^{b}	13.79±0.08^{a}
SM II	25.08±0.48^{a}	0.85±0.04^{bc}	0.73±0.01^{bc}	16.92±0.25^{a}	14.32±0.52^{a}

[1]Results are demonstrated as average (n=3) ± SD average mean in each column with the same superscript are not significantly different ($p < 0.05$). SM, SPI - MPC containing breads; RC, rennet casein containing breads. Control: pure wheat CSB. [2] RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.



(a)

(b)

Fig. 2. a.Score plot of physico-chemical properties of control and high Chinese protein steamed breads.
RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.
Fig. 2b. Loading plot of physico-chemical properties of control and high protein Chinese steamed bread.
RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.

No significant differences were observed in the transition temperatures, enthalpy, and transition range (R) among different levels of added proteins (Table 6). When compared to the control formulation, only the SM II sample exhibited significantly higher onset temperature (To) of starch gelatinisation. All the other samples did not differ in any of the thermal properties.

A plausible reason for this might be that when SM addition was done at level II, more molecular interactions between starch and protein might have taken place resulting in competition for water which hindered starch gelatinization thus increasing the transition temperatures. Furthermore, SPI also absorbs more water compared to milk protein, thus resulting in less amount of water available for starch which may have delayed the gelatinisation resulting in a higher transition temperature (Zayas, 1997). Similar results were reported for soybean protein-starch mixtures (Colombo, León, and Ribotta, 2011; Li *et al.*, 2014; Yu *et al.*, 2015). The difference in the thermal properties of samples containing RC and SM formulations could be attributed to the high water-retention capacity and swelling characteristics of soy protein, which reduces the available water for starch and shifts the endotherm peaks to higher temperatures (Ribotta *et al.*, 2007; Zayas, 1997).

3.3.2. Thermal properties of fresh HPCSBs

Fresh HPCSB samples showed dramatically lower onset, peak and conclusion temperatures than their raw flour-protein formulations (Table 6). As expected, the control wheat bread has the highest To and Tp while the SM II presented the lowest ones ($p > 0.05$). Since most starch granules were already gelatinized, this leads to the formation of crystalline structures that are less stable and less organized compared to those of native starches. Consequently, less energy is required to melt the restructured crystals, and therefore, the enthalpy for retrogradation of gelatinized starch samples is also lower than that of the raw formulations (Diamantino et al., 2019).

3.4. Texture measurements

An informal sensory analysis of the breads showed that all the breads had acceptable standards. As the session was conducted informally, the details and data are not included in the manuscript. Full sensory and consumer acceptability studies would be done in future.

3.4.1. Texture profile analysis (TPA)

The profile analysis curve of control and HPCSBs are present in Figs. S3a and b. The hardness, gumminess, and chewiness of the breads increased with the protein addition compared to control whereas springiness and cohesiveness, decreased (Table 7). Soy and milk protein (SM) containing samples presented higher hardness, chewiness and gumminess than RC-containing samples. It was reported that the addition of soy protein and whey proteins increased the hardness, gumminess and chewiness of bread along with a slight decrease in springiness and cohesiveness (Ivanovski, Seetharaman, & Duizer, 2012; Yang, Liu, Ashton, Gorczyca and Kasapis, 2013). Rennet casein (RC I) containing bread was closest to the texture of control bread in terms of the studied textural attributes.

The increment in hardness, gumminess and chewiness may also be attributed to the less amount of gluten as compared to the control sample (wheat flour only). Although extra gluten was added, it was noticed that it did not lead to the formation of the gluten-starch network in the same way as natural gluten forms wheat flour dough. Dairy proteins especially caseins have strong water absorption properties, which may lead to finer, denser crumb structures in bread (Gallagher, Kunkel, Gormley and Arendt, 2003). Therefore, the dilution of gluten matrix in wheat flour and the addition of casein would likely lead to an increase in the movement of the water from the bread crumb to the crust, resulting in an increased crumb hardness (Roach and Hoseney, 1995).

The addition of soy and milk protein at the higher level (SM II) obtained the highest values for the texture parameters tested among the five samples, although its protein content (15%) was lower than RC II (19%). The possible explanation for this might be due to the interchange of disulphide bonds between soy and gluten proteins and also because of increased water absorption by SPI causing an increase in dough viscosity. This may have resulted in lower specific volume and increased density (Du et al., 2016; Shin, Kim and Kim, 2013). Pearson correlation findings have also reported a negative correlation between protein content and loaf volume of the breads (-0.854). There was also a moderate positive correlation between protein content and hardness ($r = 0.679$).

3.4.2. Tensile test

Control CSB was soft as observed from its low resistance values, and also possessed less extension before rupture (extensibility). The R/E ratio was observed in an order of control >SM II >SM I; control>RC I > RC II. The extensibility of control CSB increased (absolute value) in RC I and SM I, whereas it dropped slightly for level II samples. The incorporation of both RC and SM significantly increased the resistance to tear (Table 8). Additionally, the resistance in SM-containing samples was higher than those in RC, which might be attributed to the properties of SPI with its rigid nature of protein hydration in SM doughs, resulting in bread with a rubberier texture and hard to be torn (Nishinari et al., 2018; Zhou et al., 2018; Tang and Liu, 2017). Furthermore, protein incorporation and dilution of the gluten content lead to a decrease in the free water in the dough, leading to the sticky dough and hard-to-sheet texture, thus, lowering its processing performance (Shalini and Laxmi, 2007).

3.4.3. Puncture test

No significant difference was found in crust resistance (Table 8). The extensibility values for RC I, RC II and SM II were observed to be lower than the control sample while there was no significant difference ($p > 0.05$) between SM-containing samples. This might be attributed to the addition of rennet casein which leads to a weaker gluten network, resulting in less extensibility (Gallagher, Kunkel, Gormley and Arendt, 2003). On the other hand, the interaction between SPI and MPC might lessen the negative effect of protein towards starch, which maintained the extensibility of SM steamed bread (Augustin et al., 2011; Hui and Evranuz, 2012). A similar finding was reported by Sołowiej et al. (2016).

The variation in textural properties of HPCSBs is mainly influenced by variations in the protein and starch content. Statistically positive correlation of springiness, cohesiveness with starch content were observed ($r = 0.93, 0.909, p < 0.05$, respectively), whereas gumminess was negatively correlated ($r = -0.782, p < 0.05$). In terms of extensibility from tensile analysis, it was negatively correlated with protein content ($r = -0.871, p < 0.05$) (Table 8).

3.5. Principal component analysis (PCA) and Pearson correlation analysis

The PCA plots provide an overview of the similarities and differences among HPCSBs and the control, and the interrelationships between the measured properties (Figs. 2a and 2b). The properties whose curves lie close to each other on the plot are positively correlated while those whose curves run in opposite directions are negatively correlated.

The first and the second principal components (PC1 and PC2) explained 61.7% and 21.5%, respectively, of the overall variation (data not shown). The distance between the locations of any two samples on the score plot is directly proportional to the degree of the difference/similarity between them (Fig. 2a) The RC II is located at the far left of the score plot with a large negative score in PC1, and the control had a large positive score while RC I was intermediate between these two (Fig. 2a). However, they differ only slightly in terms of PC2. In Fig. 2a RC I are located close to zero in PC1 while SM I and SM II showed positive scores in PC2, indicating that they differed mainly in terms of properties such as chewiness and resistance whose curves in Fig. 2b lie relatively close to the PC2 axis. Overall, RC II and the control exhibited the greatest difference in their properties, especially those

Table 8

Tensile and puncture profiles of control and high protein Chinese steamed breads.

Tensile	Resistance (g)	Extensibility (mm)	Resistance/Extensibility (R/E)
Control	2007.00±104.25^{c}	-6.85±1.13^{a}	-293.21±37.64^{c}
RC I	2128.04±191.55^{bc}	-12.36±0.29^{c}	-172.21±15.15^{a}
RC II	2431.45±91.18^{b}	-10.04±0.03^{b}	-242.25±10.83^{b}
SM I	3218.96±98.87^{a}	-12.61±0.02^{c}	-255.27±7.80^{bc}
SM II	3086.67±119.40^{a}	-11.36±0.57^{bc}	-271.71±9.77^{bc}
Puncture	Resistance (g)	Extensibility (mm)	
Control	122.93±2.80^{a}	11.94±0.82^{a}	
RC I	134.21±15.39^{a}	8.91±0.55^{bc}	
RC II	138.97±9.91^{a}	7.02±0.22^{c}	
SM I	137.15±4.81^{a}	11.38±0.78^{a}	
SM II	128.36±1.82^{a}	9.84±1.93^{ab}	

[1] Results are demonstrated as average (n=3) ± SD average mean in each column with the same superscript are not significantly different ($p < 0.05$). SM, SPI - MPC containing breads; RC, rennet casein containing breads. Control: pure wheat flour

[2] RC I and RC II, samples contain rennet casein at 14% and 33% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively; SM I and SM II, samples contain soy protein isolate and milk protein concentrate at 7%, 5% and 7%, 8% (dry wt. of added protein (excluding gluten)/dry wt. of wheat flour), respectively.

Table 9

Pearson correlation analysis for different properties of control and Chinese steamed breads.

	C-crust	C-crm	SV	Protein	Moisture	Starch	PV	Trough	BD	FV	Ptemp	R	Hd	Gmm	Chw
C-crm	1														
SV	0.602	0.602													
Protein	-0.885	-0.885	-0.854												
Moisture	-0.903	-0.903	-0.787	0.93											
Starch	0.98*	0.98*	0.714*	-0.958	-0.929										
PV	0.948*	0.948*	0.813*	-0.969*	-0.976*	0.978									
Trough	0.831	0.831	0.93	-0.984*	-0.93	0.914	0.959*								
BD	0.987*	0.987*	0.648	-0.885*	-0.948*	0.967*	0.966*	0.852							
FV	0.866	0.866	0.904	-0.988	-0.954	0.936	0.977	0.997	0.888						
Setback	0.904	0.904	0.859	-0.985	-0.977*	0.958*	0.991*	0.983*	0.927	0.994*					
ΔH	0.725	0.725	0.709	-0.916	-0.814	0.831	0.82	0.87	0.715	0.874	-0.972*				
Hd	-0.941*	-0.941*	-0.313	0.679	0.753	-0.86	-0.794	-0.597	-0.917	-0.649	0.59	0.471			
Spr	0.887	0.887	0.572	-0.896	-0.757	0.93	0.847	0.808	0.822	0.823	-0.852	0.062	-0.774		
Coh	0.831	0.831	0.755	-0.933	-0.753	0.909	0.864	0.89	0.778	0.884	-0.761	0.1	-0.644		
Gmm	-0.889	-0.889	-0.21	0.578	0.697	-0.782	-0.72	-0.496	-0.874	-0.556	0.505	0.55	0.989*		
Chw	-0.806	-0.806	-0.107	0.446	0.612	-0.674	-0.622	-0.375	-0.803	-0.439	0.362	0.659	0.948*	0.984*	
Rsl	0.922	0.922	0.401	-0.802	-0.723	0.912	0.807	0.692	0.854	0.725	-0.779	-0.151	-0.898	-0.83	-0.721
Adh	0.69	0.69	0.062	-0.362	-0.625	0.558	0.553	0.312	0.734	0.382	-0.376	-0.509	-0.829	-0.892	-0.929
P-rss	-0.727	-0.727	0.088	0.342	0.415	-0.595	-0.474	-0.225	-0.669	-0.285	0.329	0.647	0.91	0.934	0.934
T-ext	0.577	0.577	0.967*	-0.871	-0.808	0.707	0.802	0.93	0.626	0.908	-0.692	0.429	-0.274	-0.168	-0.047

Colour(crust), C-crs; Colour (crumb), C-crm; Specific volume, SV; Protein content, Protein; Moisture content, Moisture; Starch content, Starch; Peak viscosity, PV; Holding strength, Trough; Break down, BD; Final viscosity, FV; Setback viscosity, Setback; Pasting temp, Ptemp; Enthalpy, ΔH; Hardness, Hd; Springiness, Spr; Cohesiveness, Coh; Gumminess, Gmm; Chewiness, Chw; Resilience, Rsl; Adhesiveness, Adh; Puncture-resistance, P-rss; Puncture-extensibility, Tensile-extensibility, T-ext

[*] $P \leq 0.0$

properties such as protein and moisture whose curves in Fig. 2b lie relatively far from each other.

The Pearson correlation coefficients for the relationships between different starch properties are presented in Table 9. A positive correlation was exhibited between pasting properties (peak viscosity, breakdown viscosity), colour (crumb and crust) and starch content (r = 0.978, r = 0.967, r = 0.98, r = 0.98, respectively), whereas hardness, protein and moisture content presented a negative correlation with starch (r = -0.86, -0.958, -0.929, respectively). The principal components analysis and Pearson correlation analysis discriminated between samples mainly based on starch, protein and moisture contents.

4. Conclusion

The fortification of proteins especially RC has increased the protein content of CSB (up to 19% compared to 8% control for wheat CSB). There were no dramatic differences observed among the four HPCSBs in terms of the colour of the breads compared to the control CSB. As the level of protein (RC/ SM) supplementation increased, the specific bread volume decreased while the moisture content increased compared to the control CSB. The protein incorporation had a significant effect on both crust and crumb colour, with the high protein breads showing darker colours with an increase in the level of proteins. Crumb hardness, gumminess and chewiness increased whereas the cohesiveness and springiness with an increase in protein level, demonstrating that the texture became firmer. Among all the high protein samples, the physio-chemical, textural attributes of rennet casein (RC I) containing breads were found to be closest to the control CSB.

The *in vitro* digestion characterisations of these bread samples are reported in another companion paper.

Ethical statement

No animals or humans experimentation was involved in this study

Declaration of Competing Interest

Authors declare no conflict of interest

Data Availability

Data will be made available on request.

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Supplementary materials

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