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# Influence of different hydrocolloids on dough thermo-mechanical properties and *in vitro* starch digestibility of gluten-free steamed bread based on potato flour

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ABSTRACT

The effects of hydrocolloids (hydroxypropylmethylcellulose (HPMC), Carboxymethylcellulose (CMC), xanthan gum (XG), and apple pectin (AP)) at different concentrations on dough thermo-mechanical properties and *in vitro* starch digestibility of gluten-free potato steamed bread were investigated. Results showed that hydrocolloids addition significantly increased the gelatinization temperature (from 52.0 to 64.2 °C) and water absorption (from 56.22 to 66.50%) of potato dough. Moreover, hydrocolloids may be interacted with protein and starch, the density of potato protein bands was decreased by hydrocolloids addition, the reason might be that higher molecular weight complexes might be formed between proteins-hydrocolloids or proteins-proteins, thus change the protein solubility. Furthermore, steamed breads with hydrocolloids presented higher specific volume and lower hardness, and the rapidly digestible starch and estimated glycemic index were significantly decreased from 45.51 to 20.64, from 69.54 to 55.17, respectively. In conclusion, HPMC and XG could be used as improvers in the gluten-free potato steamed bread.

## 1. Introduction

Steamed bread is a staple food of China, and has been consumed for at least 2000 years. Nowadays, steamed bread has been gaining considerable popularity in many countries, various studies have characterized this type of food because that the acrylamide content and loss of soluble amino acids of steamed bread are less than western baked bread (Sui, Zhang, & Zhou, 2016).

Gluten is essential to form the strong protein network required for the desired volume and structure of the steamed bread. However, the intake of gluten might lead coeliac disease (CD) or gluten sensitivity, the estimated prevalence of this disease is about 1% of the general population, and it affects persons of any age, race, and ethnic group (Wu et al., 2010). CD is not considered a condition that affects individuals of Chinese descent historically, largely because of a lack of data on the existence of CD in Chinese populations. However, Jiang, Zhang, and Liu (2009) examined 62 patients by capsule endoscopy for chronic diarrhea from June 2003 to March 2008. Four patients with chronic diarrhea and weight loss were diagnosed to have CD. Wu et al. (2010) demonstrated that the CD-predisposing human leucocyte antigen (HLA)-DQ alleles,

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accounting for ~30% of heritability in Caucasians, were not rare in Han inhabitants of this area. And the only effective treatment for patients with CD is a strict gluten-free (GF) diet through their lifespan. At present, GF products mainly contain starch, rice flour, corn flour, and are characterized by an excessive intake of carbohydrates and a reduced intake of protein, dietary fiber, vitamins and minerals, which worsens the nutritionally unbalanced diet of coeliac sufferers. To fulfil the expectations of CD sufferers, non-traditional flours such as pseudocereals flours (amaranth, quinoa and buckwheat), root and tuber flours (potato, cassava, sweet potato, taro and yams), and leguminous flours (chickpeas, lentils, dry beans, peas, and soybean) are gaining popularity in the production of GF food stuffs with major nutritional quality.

Potato is the fourth most important food crop in the world after rice, wheat, and maize. Potato protein has a balanced amino acid composition, which is superior to that of cereal proteins. Additionally, the contents of vitamins and minerals in potato flour are higher than in wheat flour. Furthermore, potato contains other phytochemicals such as phenolics, flavonoids, polyamines, and carotenoids, which are highly desirable in the diet due to their beneficial effects on human health. Therefore, potato flour addition into GF steamed bread would enhance the nutritional and functional qualities of GF products. However, the preparation of steamed bread without gluten may pose serious technological problem.

Hydrocolloids have a wide application as additives to improve the quality of GF breads. The functional effects of hydrocolloids stem from their ability to modify dough rheology and keep qualities of baked products (Nicolae, Radu, & Belc, 2016; Mezaize, Chevallier, Le Bail, & De Lamballerie, 2009; Mohammadi, Azizi, Neyestani, Hosseini, & Mortazavian, 2015). Mezaize et al. (2009) optimized GF formula for French style breads, based on rice and corn flour and potato starch using hydrocolloids such as guar (1.9%), CMC (1%), hydroxypropyl methylcellulose (HPMC) (2.3%) and XG (0.6%), and found HPMC and guar addition decreased the hardness and increased the specific volume of bread. Incorporation of guar gum and CMC also significantly increased bread specific volume based on rice flour or chestnut flour (Mohammadi et al., 2015; Nicolae et al., 2016). Moreover, CD is associated with a high incidence of type I diabetes, patients should maintain good glycemic control while adhering to a strict GF diet. Enzymatic digestion of starch can be affected by many factors such as granule structure, digestion conditions, particle size and physical structure. Bae, Jun, Lee, and Lee (2016) reported that apple dietary fiber content significantly influenced the *in vitro* starch digestibility in wheat flour gel. Glycemic response parameters slightly decreased in the maize starch with added guar gum (Dartois, Singh, Kaur, & Singh, 2010). Regarding GF bread, de la Hera, Rosell, and Gomez (2014) stated that the more compact the structure of GF rice bread, the lower the glycemic response. Wolter, Hager, Zannini, and Arendt (2013) observed that quinoa bread showed highest predicted GI (95). GIs of buckwheat (80), teff (74), sorghum (72) and oat (71) breads were significantly lower. In conclusion, although researchers have studied the effect of hydrocolloids on GF bread production, there is little information about GF steamed bread based on potato flour, and it is extremely difficult to predict the real effect of hydrocolloids on bread quality because of the differences in applied ingredients, structure of hydrocolloids, dough preparation and making procedures utilized by the researchers.

Therefore, the aim of this study was to assess the effects of different hydrocolloids (apple pectin (AP), XG, HPMC, CMC at the level of 0.5%, 1.0%, 2%, flour basis) on dough thermo-mechanical properties, *in vitro* starch digestibility and expected glycemic index of GF steamed bread based on potato flour.

## 2. Materials and methods

### 2.1. Materials

Fresh potato (cultivar: Shepody) was kindly provided by Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (Beijing, China). Potato tubers were peeled, washed, sliced, blanched, soaked in color-protecting liquid, and dried in a dryer where the temperature varied between 60 and 70 °C, then milled into flour by a hammer mill. Moisture, protein, ash, fat, dietary fiber, and starch content in potato flour were 10.23, 9.87, 1.86, 0.26, 6.28, 68.78 g/100 g, respectively. AP, XG, CMC and HPMC were purchased from Henan Zhongxing Chemical Co., Ltd. (Zhengzhou, Henan, P.R. of China). Potato flour with different hydrocolloids (0.5, 1.0, 2.0,% flour basis.) is formulated before being analyzed. Yeast was purchased from Angel Yeast Co., Ltd. (Hubei, China).

### 2.2. Thermo-mechanical properties

Thermo-mechanical properties were performed by Mixolab (Chopin, Tripetteet Renaud, Paris, France) which simultaneously determinates protein and starch characteristics during the process of mixing at a constant temperature, as well as during the period of heating and cooling. The Mixolab was set to follow the Chopin$^{+}$ program: Firstly, mixed for 8 min at 30 °C, secondly heated to 90 °C at a speed of 4 °C min$^{-1}$, then maintained at 90 °C for 7 min and lastly decreased the temperature to 30 °C at a speed of 4 °C min$^{-1}$. Additional details about the parameters of thermo-mechanical properties were previously reported (Moreira, Chenlo, & Torres, 2011).

### 2.3. Scanning electron microscopy (SEM)

SEM of dough prepared with different hydrocolloids was performed. Dough samples were fixed in 10% glutaraldehyde, submerged in acetone solutions and then in acetone 100% to obtain complete dehydration. Samples were dried at the critical point and coated with gold. A scanning electron microscope (JEOL 35 CF, Japan) was used to observe the samples.

### 2.4. Dough protein extraction and separation through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE under reducing conditions was performed according to the method described by Laemmli (1970) using an AE-6450 electrophoresis system (Atto Corporation, Tokyo, Japan). 0.2 g dough was transferred into a vial and mixed with 0.5 ml of sample buffer, vortex shaking for 30 min, and centrifuged for 20 min at 10,000 × g. The supernatant was directly dissolved (4:1, v/v) in the sample buffer. After boiling at 100 °C for 2 min, the samples were centrifuged at 10,000 × g for 20 min. The 15-μl samples and 10-μl marker solutions were placed into wells. Gel electrophoresis was run on 5% loading gels and 15% separating gels. The gels were stained using Coomassie Brilliant Blue R-250 method and distained with methanol wash solution. A marker kit containing bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase (35.0 kDa), REase Bsp98I (25.0 kDa), β-lactoglobulin (18.4 kDa), lysozyme (14.4 kDa) (Sigma) were used for evaluating the apparent molecular mass.

### 2.5. Preparation of steamed bread

The recipe consists of potato flour with or without HPMC, CMC, XG and AP, 70% water, 1% yeast w/w (based on flour basis).

Before mixing the dough, yeast was pre-dissolved in warm water (30–35 °C) and left standing for 10 min. Subsequently, the other dry ingredient was pre-mixing in a mixing bowl of KitchenAid® Mixer (Model 5KSM150PS, KitchenAid, USA). Mixing speed was fixed at level 2. Fresh yeast solution was then added in, and mixing was continued for 8 min. The dough was divided into 100 g pieces, and formed a round shape. The dough pieces were then put in a proofer maintained at 30 °C and 85% relative humidity for 60 min. After proofing, the dough was heated for 30 min in a steamer. The samples prepared were tested after cooling for 1 h.

### 2.6. Specific volume and porosity of steamed bread

The weight of steamed bread was measured to the nearest of 0.01 g. The volume of it was determined using rapeseed displacement method. Specific volume (ml/g) was calculated as the ratio of volume to weight of the loaf. The porosity was checked by modified method of Lazaridou, Duta, Papageorgiou, Belc, and Biliaderis (2007). The steamed bread was cut into 1.5 cm thick slices. Each slice was photographed with a digital camera (QImagining, Micro Publisher 5.0 RTV). The images were then analyzed with Image J (version 1.44; Wayne Rasband, National Institutes of Health, USA). The central image of the crumb was selected and the area was determined. To obtain a black and white threshold, the image was converted into 8-bit and binary segmentation was performed. The results were presented as a percentage of pore area in the total area.

### 2.7. Textural properties

Texture profile analysis (TPA) was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell and a 50 mm aluminium cylindrical probe. Parameter was set at a pre-test speed of 1.0 mm $s^{-1}$, a test speed of 4.0 mm $s^{-1}$, a post-test speed of 1.0 mm $s^{-1}$, the interval time between the first and second cycles was 1 s. Hardness, cohesiveness, chewiness, and springiness were counted through the graphic.

### 2.8. In vitro starch digestibility and expected glycemic index

GF steamed bread samples were frozen, freeze-dried and ground in a blender. A two-stage gastro-intestinal simulated *in vitro* digestion model (Dartois et al., 2010) was used in this study. According to the hydrolysis rate of starch, three different fractions were quantified as suggested Englyst, Vinoy, Englyst, and Lang (2003). Hydrolyzed glucose at 20 min (G20) and 120 min (G120) and total glucose (TG) were determined by the glucose oxidase/peroxidase colorimetric method. Free sugar glucose (FGS) was also determined by the method. Based on the results, rapidly digested starch (RDS) = 0.9 × (G20 − FGS), slowly digestible starch (SDS) = 0.9 × (G120 − G20), and resistant starch (RS) = 0.9 × (TG − G120).

Moreover, a non-linear model following the equation $[C = C_\infty (1 - e^{-kt})]$ was applied to describe the kinetics of enzymatic hydrolysis, where C was the concentration at t time, $C_\infty$ was the equilibrium concentration or maximum hydrolysis extent, k was the kinetic constant and t was the time chosen. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve (0–180 min) of the sample by the area of a standard material (glucose) over the same period of time. The estimated glycemic index (eGI) was calculated using the following equation,

$$eGI = 8.198 + 0.862HI$$

### 2.9. Statistical analysis

In order to establish the statistical differences between means, the data were treated by one-factor analysis of variance, and the least significant difference (LSD) at significance level 0.05 was calculated using Fisher post hoc test. Statistical analysis was performed using the Statistical Analysis System version 8.1 software (SAS Institute Inc., Cary, NC, USA).

## 3. Results and discussion

### 3.1. Thermo-mechanical properties

#### 3.1.1. Mixing period

Table 1 shows water absorption, development time and stability values obtained for PF doughs with and without hydrocolloids. Water absorption increased positively and significantly by hydrocolloids addition in comparison to the control, while low addition (0.5–1.0%) of XG and AP was not significantly different to control. Water absorption was increased with hydrocolloid content increasing. This behavior was broad agreement with the results found for chestnut flour when combinations of hydrocolloids and other components as chia (*Sativa hispanica L.*) were added (Moreira, Chenlo, & Torres, 2013). Similar result was previously reported for wheat dough with different types of HPMC (Correa, Añón, Pérez, & Ferrero, 2010). An increase of water absorption has also been stated by other researchers when various hydrocolloids such as XG, HPMC, agar, or agarose were added to wheat flour, whole wheat flour or GF formulations (Lazaridou et al., 2007; Sudha & Rao, 2009; Moreira et al., 2011). The reason could be attributed to the hydroxyl groups in the hydrocolloid structure, which allow more water interactions through hydrogen bonding (Rosell, Rojas, & De Barber, 2001). Higher water absorption with the addition of hydrocolloids suggests that the water binding capacity of hydrocolloids is higher than protein (Gharaie, Azizi, Barzegar, & Aghagholizade, 2015). Moreover, the influence degree on water absorption of hydrocolloids was based upon their structure and content, the present study stated that the order was HPMC > CMC > AP > XG.

Development time and stability values are indicators of the flour strength, with higher values suggesting stronger dough. The development time was affected in different ways by different hydrocolloids, the reason probably may be that the molecular structure of these polymers is determining the kinetics of the hydration process. HPMC remarkably increased the development time of PF dough (from 1.38 to 3.30 min at 2.0%), which suggested that a significant increase of the time needed for a complete hydration of the material. The results obtained in this study also agree with Rosell et al. (2001) and Sudha and Rao (2009), who reported that adding HPMC and XG increased water absorption and dough development time.

The tested hydrocolloids strikingly affected the stability of PF dough. PF dough containing CMC or 0.5% HPMC and AP exhibited lower stability than control. Conversely, the stability was increased by the addition of XG (1.0% and 2.0%) and HPMC (1.0%). Similar trends have been also reported for wheat flour and chestnut flour with XG and HPMC (Rosell et al., 2001; Moreira et al., 2011). Those results concluded that a unique trend was not existed and the optimum hydrocolloid concentrations needed to be further study, the reason can be attributed to that the effect of hydrocolloids on the mechanical properties greatly depends on the nature of the flour components, added polymer and the added amount (Moreira et al., 2011). Moreover, once the period of dough stability finished, the torque began to decrease because dough became stickier, less elastic, and losing gas holding properties in all studied systems,

**Table 1**
Thermo-mechanical properties of potato flour with and without hydrocolloids.

| | | Water absorption (%) | Development time(min) | Stability (min) | C2(Nm) | C3(Nm) | C4(Nm) | C5(Nm) | α(Nm/min) | β(Nm/min) | γ(Nm/min) | $T_0$–$T_1$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | | 56.22 ± 0.15d | 1.38 ± 0.01e | 0.73 ± 0.01cd | 0.16 ± 0.01fg | 3.14 ± 0.02g | 2.97 ± 0.03f | 3.84 ± 0.02e | 0.008 ± 0.001g | 0.014 ± 0.001k | −0.138 ± 0.001h | 52.0g–71.3d |
| HPMC | 0.5 | 58.96 ± 2.42bc | 1.23 ± 0.01g | 0.63 ± 0.01hg | 0.24 ± 0.01c | 3.12 ± 0.03g | 2.96 ± 0.02f | 3.76 ± 0.02f | 0.014 ± 0.001e | 0.066 ± 0.001h | −0.256 ± 0.004i | 59.3d–78.1a |
| | 1 | 59.56 ± 2.95bc | 3.03 ± 0.04a | 1.52 ± 0.03a | 0.42 ± 0.01a | 3.22 ± 0.01f | 3.46 ± 0.02a | 3.98 ± 0.02d | 0.014 ± 0.001e | 0.192 ± 0.001f | −0.27 ± 0.001j | 61.6b–77.0b |
| | 2 | 66.50 ± 0.19a | 3.30 ± 0.02a | 0.92 ± 0.01b | 0.34 ± 0.01b | 4.16 ± 0.01a | 3.47 ± 0.01a | 3.11 ± 0.03i | 0.010 ± 0.001f | 0.358 ± 0.003a | −0.044 ± 0.002b | 64.2a–77.8ab |
| CMC | 0.5 | 57.70 ± 2.42bcd | 1.33 ± 0.01ef | 0.60 ± 0.02h | 0.2 ± 0.01d | 2.94 ± 0.02h | 2.89 ± 0.01g | 3.57 ± 0.01h | 0.020 ± 0.001b | 0.102 ± 0.001g | −0.018 ± 0.001a | 59.2d–78.1a |
| | 1 | 59.23 ± 5.78bc | 1.31 ± 0.01f | 0.72 ± 0.02de | 0.24 ± 0.02c | 3.14 ± 0.01g | 2.83 ± 0.02h | 3.58 ± 0.02h | 0.016 ± 0.001c | 0.240 ± 0.001d | −0.02 ± 0.001a | 60.1c–78.5a |
| | 2 | 60.27 ± 6.88b | 1.31 ± 0.01f | 0.72 ± 0.01de | 0.25 ± 0.02c | 3.28 ± 0.02e | 2.91 ± 0.02g | 3.57 ± 0.02h | 0.014 ± 0.001e | 0.250 ± 0.001c | −0.018 ± 0.001a | 60.9c–78.6a |
| XG | 0.5 | 56.20 ± 2.04d | 1.12 ± 0.09h | 1.02 ± 0.02ab | 0.26 ± 0.01c | 3.12 ± 0.02g | 3.02 ± 0.03e | 3.68 ± 0.03g | 0.016 ± 0.001c | 0.338 ± 0.004b | −0.126 ± 0.003g | 59.5d–71.6d |
| | 1 | 56.27 ± 0.23d | 2.90 ± 0.03b | 1.53 ± 0.01a | 0.2 ± 0.01d | 3.91 ± 0.01b | 3.08 ± 0.01d | 4.39 ± 0.01a | 0.026 ± 0.002a | 0.320 ± 0.002b | −0.106 ± 0.001d | 63.9a–74.9c |
| | 2 | 57.10 ± 5.51cd | 2.20 ± 0.01d | 1.57 ± 0.05a | 0.17 ± 0.02ef | 3.46 ± 0.03d | 3.15 ± 0.02c | 4.31 ± 0.02b | 0.010 ± 0.001f | 0.340 ± 0.001b | −0.046 ± 0.001b | 64.1a–77.0b |
| AP | 0.5 | 56.15 ± 3.09d | 2.58 ± 0.06c | 0.68 ± 0.05ef | 0.2 ± 0.02d | 3.73 ± 0.02c | 3.30 ± 0.02b | 4.34 ± 0.01b | 0.008 ± 0.001g | 0.046 ± 0.001i | −0.12 ± 0.001f | 58.1e–74.8c |
| | 1 | 56.59 ± 1.25cd | 2.28 ± 0.01d | 0.77 ± 0.01c | 0.19 ± 0.01de | 3.48 ± 0.02d | 3.12 ± 0.02c | 4.09 ± 0.02c | 0.016 ± 0.001c | 0.038 ± 0.001j | −0.116 ± 0.001e | 56.4f–74.0c |
| | 2 | 60.47 ± 3.71b | 2.02 ± 0.01d | 0.63 ± 0.01hg | 0.16 ± 0.01fg | 3.13 ± 0.01g | 2.98 ± 0.01f | 3.58 ± 0.01h | 0.016 ± 0.001c | 0.230 ± 0.001e | −0.098 ± 0.001c | 63.0a–74.8c |

C2 (related to proteins weakening), C3 (related to starch gelatinization), C4 (cooking stability), C5 (related to retrogradation), α (proteins network weakening rate), β (gelatinization rate), γ (enzymatic activity rate), $T_0$–$T_1$ (gelatinization temperature range).

therefore, this stability value is an indicator of the capability to withstand mechanical shear stress during dough formation, and thus an increase in this parameter might positively affect the mechanical strength of the PF dough. The stability increasing of PF dough with assayed hydrocolloids is positive in order to improve the strength of PF doughs. However, the obtained values were low in comparison to those achieved for other flours usually employed in bakery industry such as wheat (5–8 min) or oat (4–5 min) flours, this parameter needed to be further improved through the other methods (Rosell et al., 2001).

### 3.1.2. Heating-cooling period

The values of the main characteristic parameters (C2, C3, C4, C5) for studied doughs obtained using Mixolab complete tests are also shown in Table 1. The C2 value related to proteins weakening, which is the torque difference between the maximum torque at 30 °C and the torque at the end of the holding time at 30 °C. C2 value was significantly increased by tested hydrocolloids in exception of 2.0% AP. And different hydrocolloid addition induced significantly changes of C3 (related to starch gelatinization), C4 (cooking stability) and C5 (related to retrogradation) values. The value of C3 was increased significantly with the HPMC, CMC and XG concentration increasing, while the trend of adding AP was opposite. The reason could be explained that pectin is a heteropolysaccharide, which contains at least 65% (w/w) units of galacturonic acid, which is significant from the other hydrocolloids. Our result agrees with the finding that the pectin had contributed to the overall gelatinization characteristics of bread dough or potato starch paste (Witczak, Witczak, & Ziobro, 2014). Compared to control, the value of C4 was increased significantly with HPMC and XG addition in all tested concentration range and with AP addition below 1.0%, while the value for dough with CMC was no significant difference. Hydrocolloids affected the value of C5 in different ways, C5 was decreased with the CMC, 2.0% HPMC, and 2.0% AP, indicating these hydrocolloids addition reduced the effects of staling and crumbs firmness on bakery products made with PF. While, the C5 values was elevated with XG addition, which were in agreement with results previously obtained by Moreira et al. (2011) for chestnut flour dough with XG (from 0.5 to 2.0%). Comparing the values of C3, C4 and C5 at the same hydrocolloid concentration, HPMC exhibited the most obvious impact on these parameters. Particularly, for dough with HPMC addition at 2.0%, an increase in C4 accompanied by a significant reduction in C5 was found, which indicates higher starch stability and heat resistance to dough processing. However, the obtained values were also different in comparison to those achieved for wheat flour usually employed in bakery industry such as C2 (0.376), C3 (1.855), C4 (1.726) and C5 (2.470) (Banu, Măcelaru, & Aprodu, 2016). Recently, it has reported that wheat flours with high C3, C4 and C5 values (2.45, 2.46 and 3.38 Nm, respectively) can be considered as typical cookie flours with higher cookies diameter and spread ratio, while good cakes flours should present low C3, C4 and C5 values (1.43, 1.36 and 1.61 Nm, respectively) (Ozturk, Kahraman, Tiftik, & Koksel, 2008). Therefore, different thermal qualities were needed for different products.

Additional parameters like rate of protein weakening (−α), gelatinization rate (β), enzymatic degradation rate (γ) as well as initial and final gelatinization temperatures ($T_0$–$T_1$) derived from the analysis of heating-cooling cycle obtained using Mixolab complete test are also summarized in Table 2. Compared to control, the -α slope was decreased except for 2.0% HPMC, 2.0% XG, and 0.5% AP, the β slope was increased significantly with all tested hydrocolloids, and the γ slope value was improved except for 0.5% and 1.0% HPMC. Our results were opposite to the other researchers, who found the HPMC and agar reduced the γ slope of chestnut flour

**Table 2**
Effect of hydrocolloids on the texture parameters of gluten-free potato steamed bread.

| w/w (%, flour basis) | | Hardness (N) | Cohesiveness | Springiness | Chewiness (N) |
|---|---|---|---|---|---|
| Control | | 58.30 ± 0.15a | 0.42 ± 0.01g | 0.98 ± 0.01ab | 27.14 ± 1.35a |
| HPMC | 0.5 | 45.91 ± 1.42b | 0.46 ± 0.01f | 1.00 ± 0.00a | 8.66 ± 0.37g |
| | 1.0 | 34.74 ± 1.95def | 0.47 ± 0.04ef | 1.00 ± 0.01a | 9.97 ± 0.92g |
| | 2.0 | 28.89 ± 0.19h | 0.47 ± 0.02ef | 1.00 ± 0.00a | 10.02 ± 0.82g |
| CMC | 0.5 | 45.91 ± 1.23b | 0.49 ± 0.01de | 1.00 ± 0.00a | 22.60 ± 1.15b |
| | 1.0 | 32.74 ± 1.78g | 0.49 ± 0.01de | 1.00 ± 0.01a | 16.11 ± 2.83ef |
| | 2.0 | 33.89 ± 0.88efg | 0.50 ± 0.01cd | 1.00 ± 0.01a | 16.98 ± 3.24de |
| XG | 0.5 | 35.85 ± 0.23d | 0.52 ± 0.09bc | 1.00 ± 0.00a | 18.76 ± 1.34cd |
| | 1.0 | 29.55 ± 1.04h | 0.54 ± 0.03b | 1.00 ± 0.01a | 16.04 ± 1.08ef |
| | 2.0 | 24.11 ± 0.51i | 0.58 ± 0.01a | 1.00 ± 0.01a | 13.72 ± 0.94f |
| AP | 0.5 | 35.44 ± 1.09de | 0.50 ± 0.06cd | 0.98 ± 0.00b | 17.69 ± 0.71cde |
| | 1.0 | 33.56 ± 0.25fg | 0.50 ± 0.01cd | 1.00 ± 0.01a | 16.84 ± 0.37de |
| | 2.0 | 41.91 ± 0.71c | 0.48 ± 0.01def | 1.00 ± 0.01a | 19.96 ± 1.22c |

(Moreira et al., 2011), the reason can be attributed to the different materials.

$T_0$ ranged from 52.0 to 64.2 °C, and $T_1$ ranged from 71.3 to 78.6 °C. The presence of hydrocolloids caused a significant rise in the initial temperature, being more pronounced when adding HPMC at the same concentration. The effect of hydrocolloids in delaying the starch gelatinization has been previously identified (Moreira et al., 2011). This delay in starch gelatinization is crucial for improving the texture and other qualities of starch based products (Sharma, Oberoi, Sogi, & Gill, 2009). The temperatures of $T_1$ appeared similar trends with $T_0$. However, this result was different from the finding that the final gelatinization temperature decreased for whole wheat flours with 1.0% HPMC addition (Sudha & Rao, 2009). Moreover, the range of gelatinization temperatures (the range of initial temperature and final gelatinization temperature) was from 11 to 19.3, which was in accordance with the result that the difference between the initial and final gelatinization temperatures was usually of 10–15 °C. Compared to the gelatinization temperatures range of control (19.3), the hydrocolloids addition decreased the value (from 18.9 at 0.5% CMC addition to11.8 at 2% AP addition). It seems possible that changes in gelatinization character are the consequence of changes in macromolecular organization due to the interactions between components of the system. The aforementioned authors pointed out that the change in pasting temperature seemed to be due to some kind of interaction between starch and the hydroxyl groups of the hydrocolloids (Sharma et al., 2009).

### 3.2. SEM analysis

Starch is the main component of gluten-free dough and, therefore, the characteristics of starch significantly influence the quality of bread. Fig. 1 shows representative SEM micrographs of the dough with and without hydrocolloids. The structure of all gluten-free PF dough was not like that of wheat dough, protein matrix formed a smooth, enveloping, veil-like network stretched over the starch granules. The starch granules of control sample seemed to be scattered and exposed. Chaisawang and Suphantharika (2006) reported that in SEM micrographs of tapioca, XG totally wrapped the native starch granules. In our SEM study, hydrocolloids influenced the starch structure in different level due to the different structure and added amount of hydrocolloids, the potato starch granules seemed to be wrapped by a tight adhering hydrocolloids layer (arrows show). This causes restricted swelling at high temperatures, limitation of the increase in viscosity and subsequently retardation of gelatinization of starch granules (Table 1). These results were consistent with Smitha, Rajiv, Begum, and Indrani (2008), who reported that the large and small starch granules seem to be coated prominently with gum in the micrograph of parotta dough containing HPMC. Xia et al. (2014) also informed that the surface of starch was all wrapped tightly by adhering lignin, hemicellulose or cellulose. Moreover, due to the association of hydrocolloids with starch, they could retard starch gel aging in the storage process. It was suggested that hydrocolloids, such as xanthan gum, was capable of enwrapping the starch granules and physically stabilizing the particle by acting as a physical barrier to prevent amylopectin chain linkages during storage (Heyman, Depypere, Meeren, & Dewettinck, 2013).

### 3.3. SDS-PAGE

Fig. 2 exhibits the typical protein electrophoretic profiles obtained by SDS-PAGE. At the reducing condition, previous study found the profile of potato protein showed the presence of bands at 40, and 5–25 kDa that corresponded to patatin, and protease inhibitor, respectively, and the bands above 40 kDa might be the aggregates of potato protein (Koningsveld, 2001). Our result was similar to this discovery. Compared to control, hydrocolloids addition decreased the density of potato protein bands, especially the protein extracts of HPMC and XG added dough. There were also qualitative differences between the profiles of different hydrocolloids contents. This indicated that the protein content of dough supernatant was decreased, the reason might be that higher molecular weight complexes might be formed between proteins-hydrocolloids or proteins-proteins, thus change the protein solubility. Rosell and Foegeding (2007) studied lower protein solubility in HPMC-gluten systems and suggested that HPMC could have an interfering role in protein network formation. Borreani, Llorca, Larrea, and Hernando (2016) reported that the aggregates formed in the dairy protein-konjac glucomannan mixtures. These results were also demonstrated by Ribotta, Ausar, Beltramo, and Leon (2005), who found the carrageenan and pectin decreased the density of wheat protein bands because of forming hydrophilic complexes with gluten proteins. The other possible reason was the changing of secondary structure or forming non-covalent links. Correa, Ferrer, Añón, and Ferrero (2014) found GG, XG, HPMC and CMC (1%–1.5% f.b.) decreased a-helix conformation, increasing more unfolded, disordered structures.

### 3.4. Specific volume

Among various studied external characteristics of steamed bread, specific volume and texture were considered most important by the consumers. Specific volume is one of the most important visual characteristics of steamed bread, strongly influencing consumer's choice. Hence, it is a key parameter evaluating steamed bread quality. Fig. 3a displays the specific volume of gluten-free potato steamed bread without or with different hydrocolloids. There were significant differences in specific volume between gluten-free potato steamed bread without hydrocolloids

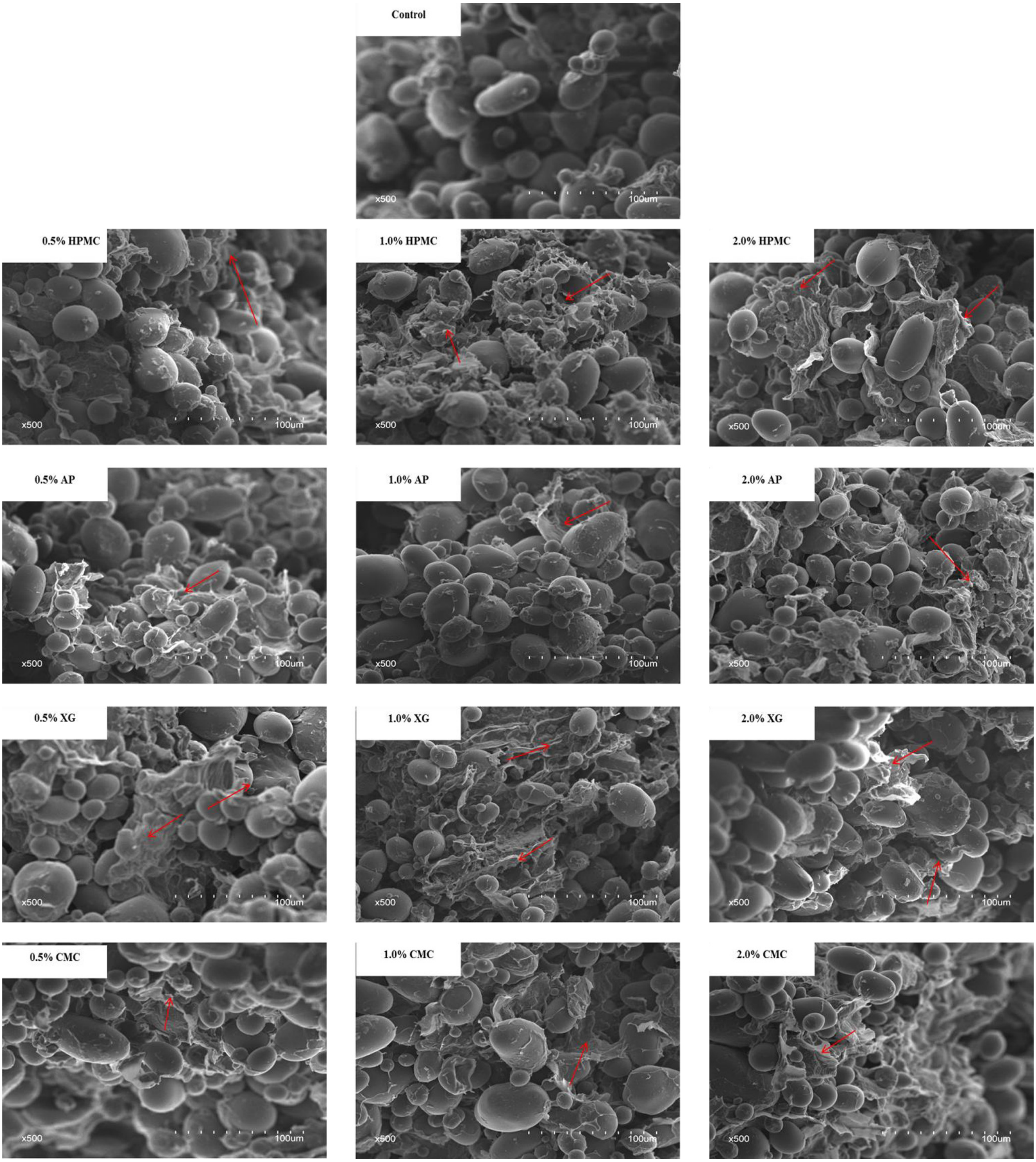

**Fig. 1.** SEM micrographs of the gluten-free potato flour dough samples obtained using different hydrocolloids, magnification:500×.

(1.23 ml/g) and its samples with hydrocolloids (from 1.45 to 2.03 ml/g). Compared to control, the specific volume was increased significantly with hydrocolloids addition. HPMC had a prominent positive linear effect on specific volume, followed by XG, CMC, and AP. The trend was similar with the result that CMC and high-methylated-pectin increased the specific volume of bread in a linear effect (Eduardo, Svanberg, & Ahrné, 2014). The larger specific volume of HPMC added steamed bread (2.03 ml/g) compared with the other hydrocolloids may be explained by a higher ability of HPMC to interact with the potato protein or starch, resulting in more stable dough. Similar effects on specific volume have been reported with additions of β-glucan to gluten-free rice bread (Moreira et al., 2013), of HPMC to gluten-free maize-teff bread (Hager & Arendt, 2013), and of pectin and CMC to gluten-free formulations (Lazaridou et al., 2007). These findings might be a result of the formation of a gel network during heating that strengthens the expanding cells of the dough and, as a result, improves gas retention and specific volume (Moreira et al., 2013). However, Xanthan addition had a negative linear effect on specific volume of rice, maize, teff, and buckwheat breads (Hager & Arendt, 2013),

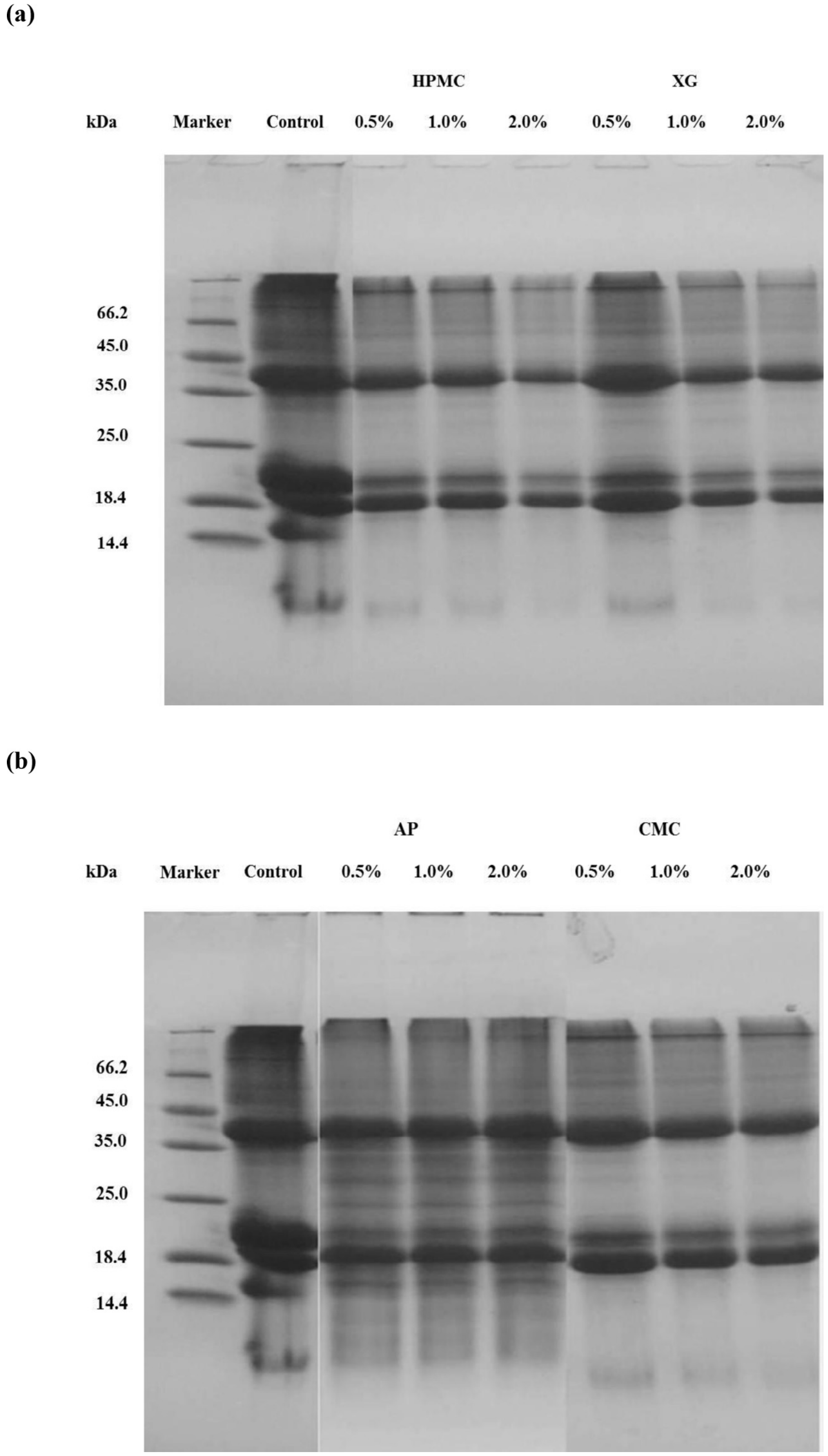

**Fig. 2.** SDS-PAGE micrographs of the gluten-free potato flour dough samples obtained using different hydrocolloids under reducing condition, (a) HPMC and XG, (b) CMC and AP.

which was not consistent with our results, the reason could be attributed to different materials, water addition, and making process between these researches.

### 3.5. Porosity of the crumb

Other important physicochemical parameter, as determinants of steamed bread quality, is the porosity of the crumb (Fig. 3b). Compared to control, the porosity increased in the case of hydrocolloids addition, illustrating that the area occupied by the cells was higher in hydrocolloids-added crumb (from 36.53 to 46.20%) than in control crumb (31.84%), which was in agreement with findings of Lazaridou et al. (2007) and Mezaize et al. (2009), who found that the CMC and β-glucan addition increased the porosity of GF rice bread, the reason might be due to these hydrocolloids stabilized air cells in the steamed bread dough and prevented coalescence of the cells. Porosity was improved by increasing the hydrocolloids level, followed the order of

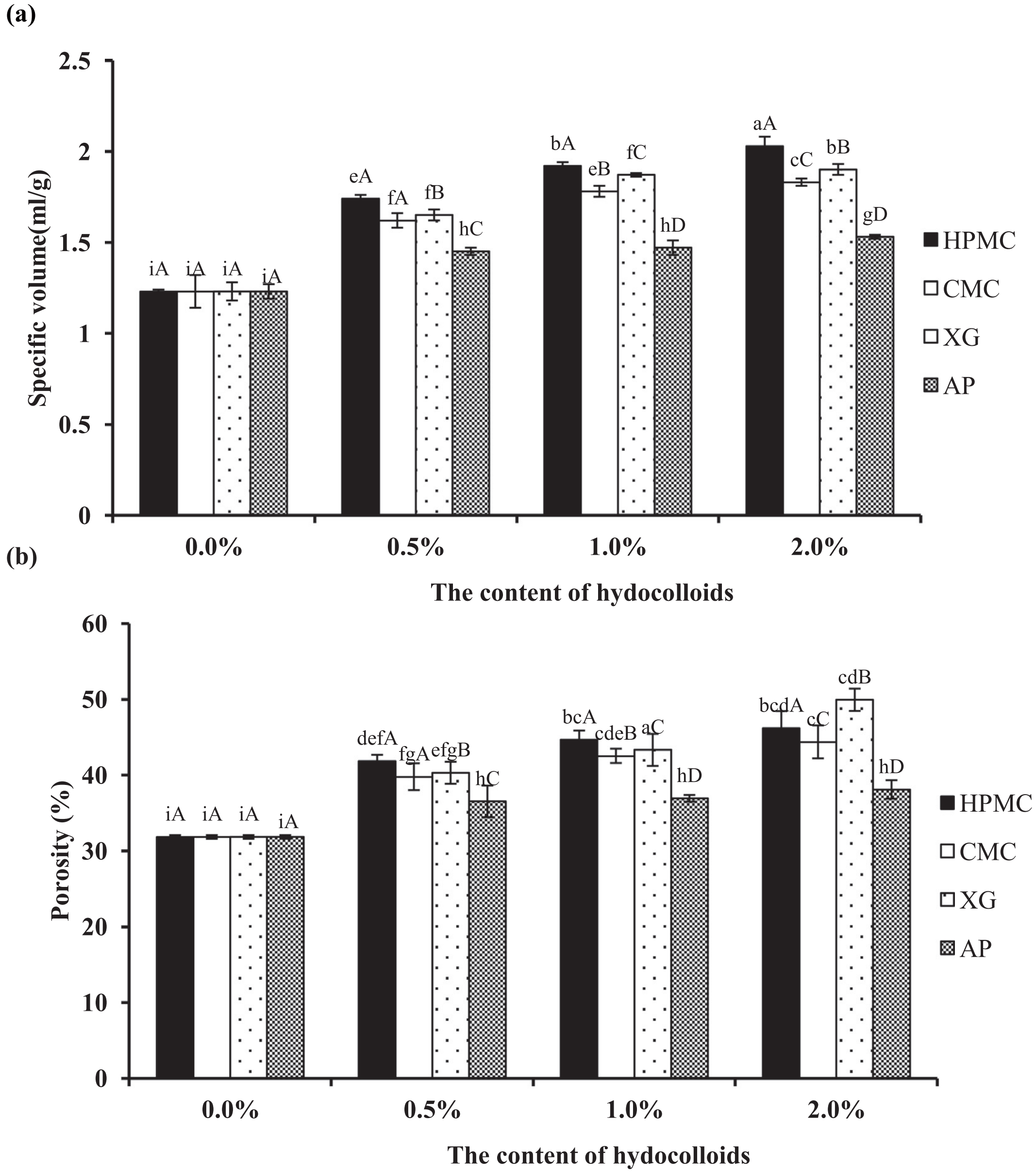

**Fig. 3.** The specific volume and porosity of gluten-free potato steamed bread using different hydrocolooids. *Note:* $^{a-e}$ Values labelled with a different letter are significantly different ($P < 0.05$).$^{A-C}$ Values labelled with a different letter in the same concentration of hydrocolloids are significantly different ($P < 0.05$).

HPMC > XG > CMC > AP. Porosity will influence the sensorial assessment, higher scores were obtained for higher porosity compared to lower porosity (Mohammadi et al., 2015). Ozkoc, Sumnu, and Sahin (2009) also observed that the highest pore area fraction was obtained for breads formulated with xanthan-guar. It is also worthy to note that the porosity result corroborates the volume result. However, Mezaize et al. (2009) found HPMC decreased the porosity while increased the specific volume of GF rice bread.

### *3.6. Texture analysis*

The interactions between starch and other macromolecular constituents are of particular importance for structural changes occurring in steamed bread. In traditional wheat based products the primary role is played by gluten. Polysaccharide constituents are generally more important in establishing steamed bread structure than protein in the case of gluten-free products. Table 2 demonstrates selected texture parameters of steamed bread. The hydrocolloids addition significantly affected the texture behavior of gluten-free potato steamed bread. The hardness of steamed bread without the hydrocolloid (58.30 N) was decreased by hydrocolloids addition, especially XG (24.11 N) and HPMC (28.89 N) at the level of 2%. Our results showed that hydrocolloids had a softening effect on gluten-free steamed bread, which was in agreement with Correa et al. (2010), who reported that CMC, HPMC and pectin decreased the bread crumb hardness. However, our results differed from those obtained by Lazaridou et al. (2007), who observed that pectin and CMC did not affect the crumb firmness of gluten-free breads. The contrasting results could probably be explained by the different bread formulations (rice flour, corn starch and potato flour) and making process (baking and steaming). Interestingly, a negative correlation was obtained in the current study between hardness and specific volume. The smaller the specific volume, the denser and more tightly packed crumb structure, and subsequently the higher hardness values were observed in the present study. A negative relationship between bread volume and hardness has been previously reported for gluten-free bread by Mezaize et al. (2009), who reported that a significant decrease in the crumb hardness resulting from the addition of HPMC could be attributed to the higher specific volume and less dense crumb structure of these breads.

In the case of cohesiveness, samples with hydrocolloids were more cohesive (ranged from 0.46 to 0.58) than control (0.42), suggesting a more integrated matrix. Low cohesiveness negatively influences consumer's acceptance of steamed bread, because it results in high crumbling. From this point, the hydrocolloids addition could decrease the crumbling, therefore improve the sensory quality.

The differences between samples were small in the case of springiness. In comparison to control sample, slightly lower values were measured only in the case of steamed bread with 0.5% AP, the differences were not statistically significant in all the other cases.

Chewiness is calculated as the product of springiness, cohesiveness and hardness, although cohesiveness increased, the decrease in hardness was relatively higher, so chewiness was significantly lower for hydrocolloids-added samples, particularly in the case of HPMC. The results of two-factor analysis of variance showed a significant influence of both factors i.e. type of hydrocolloids and its level as well as the interaction between them on the cohesiveness and chewiness of gluten-free steamed bread.

### 3.7. In vitro starch digestibility of potato GF steamed bread

*In vitro* starch digestibility of GF potato steamed breads using different hydrocolloids is shown in Table 3. In a previous study, RDS was correlated with *in vitro* glycemic response and could be a proxy indicator of GI value, which was influenced by many factors, such as starch granules, bread structure, viscoelasticity, etc. (Englyst et al., 2003). Compared to control, the hydrocolloids addition significantly decreased the RDS content, which was consistent with the result that the Malva nut gum addition reduced the *in vitro* starch digestibility of wheat bread (Srichamroen, 2014). The results also agreed with a study indicated that hydrocolloid might form a layer around starch granules, which could then shield the starch granules from enzyme attack. Moreover, with increasing the content of hydrocolloids, the RDS content decreased significantly with AP added concentration increasing, the reason could be attributed to that higher hydrocolloids addition changed the viscosity significantly, which coated the surface of the starch granules, thus acting as a physical barrier to either the enzyme attack or to the release of hydrolysis products (Dartois et al., 2010). The other possible reason might be that the hydrocolloids addition changed the starch gelatinization qualities, thus affecting the starch digestibility (Rosell et al., 2001). However, the trend was opposite in the term of XG, it seems that the enzymatic hydrolysis of the starch in steamed breads made with XG may be mostly affected by the volume, resulting in increased accessibility of amylases to starch granules because of the higher specific volume, rendering starch more susceptible to hydrolysis. The reason may be that the backbone of XG is made up of five sugar units (two units of glucose, two units of mannose and one unit of glucuronic acid), and the molecular weight is so different among hydrocolloids. Perez-Quirce, Lazaridou, Biliaderis, and Ronda (2017) found that the effect of β-glucan on *in vitro* starch digestibility based on rice bread was dependent on its molecular weight and concentration.

SDS is slowly digested in the small intestine and induces a gradual increase of postprandial plasma glucose and insulin levels. SDS ranged from 34.85% (0.5% CMC) to 46.04% (0.5% HPMC). In this study, the digestible starch content was lower as compared that reported by de la Hera et al. (2014), who found that the digestible starch content of GF rice bread is higher than 90%. Differences in the values could be due to the different food ingredients and formulation used to prepare the steamed bread samples.

RS in steamed bread samples significantly increased in the presence of hydrocolloids, especially HPMC and XG. The result was in consistent with that NaCMC and XG increased the RS content of banana pseudo-stem flour-wheat composite bread (Ho, Tan, Aziz, & Bhat, 2015). According to Dartois et al. (2010), dietary fiber performs the role of a sponge and is capable of absorbing water in human intestine. Additionally, it helps in mixing with the foodstuff to form an entangled network, and thereby can slow down the rate of digestion and absorption.

### 3.8. Kinetics of the in vitro starch hydrolysis and estimated glycemic index

The parameters derived from the *in vitro* digestion of the GF steamed breads are listed in Table 3. There is a lack of information about starch digestibility and glycemic response of GF food, although some authors have reported that the GI of GF bread is significantly higher than that of traditional bread (Segura & Rosell, 2011). The maximum hydrolysis ($C_\infty$), or the hydrolysis degree showed significantly influence by the hydrocolloids used in the recipe, especially HPMC. The $C_\infty$ value of control was similar to the result of de la Hera et al. (2014), who observed that the $C_\infty$ value of GF rice bread (70% water content) was 96.5. The kinetic constant (k) values, indicative of the hydrolysis rate in the early stage, were between 0.0112 and 0.0281 $\text{min}^{-1}$, which was reduced by the hydrocolloids addition. Segura and Rosell (2011) observed the values between 0.0527 and 0.1458 ($\text{min}^{-1}$) in different GF breads based on starch. Therefore, a lower constant was obtained for GF potato steamed breads than those determined for pasta and baked bread, showing the low susceptibility of these starchy products to enzymatic hydrolysis. The values of the estimated

**Table 3**

*In vitro* starch digestibility and its kinetic parameters of gluten-free steamed bread.

| w/w (%, f.b.) | | RDS (%) | SDS (%) | RS (%) | $C_\infty$ (g/100 g) | K ($\text{min}^{-1}$) | HI | eGI |
|---|---|---|---|---|---|---|---|---|
| Control | | 45.67 ± 1.21a | 44.02 ± 0.73bc | 10.31 ± 0.09m | 94.46 ± 1.01a | 0.0281 ± 0.0011a | 75.58 ± 0.76a | 73.35 ± 1.22a |
| HPMC | 0.5 | 36.83 ± 0.96d | 46.04 ± 0.44a | 17.13 ± 0.11k | 90.22 ± 0.83c | 0.0213 ± 0.0002b | 65.92 ± 0.43e | 65.02 ± 0.72d |
| | 1.0 | 32.24 ± 1.62f | 44.68 ± 0.25b | 23.08 ± 0.07h | 87.85 ± 1.21d | 0.0183 ± 0.0009de | 60.69 ± 0.92g | 60.52 ± 1.07f |
| | 2.0 | 32.89 ± 0.84f | 42.92 ± 0.78d | 24.19 ± 0.23f | 81.63 ± 0.72f | 0.0212 ± 0.0004b | 58.81 ± 1.32h | 58.89 ± 0.49g |
| CMC | 0.5 | 40.05 ± 0.43c | 43.38 ± 1.01cd | 16.57 ± 0.06l | 92.41 ± 0.22b | 0.0210 ± 0.0006b | 67.35 ± 0.11cd | 66.25 ± 1.00cd |
| | 1.0 | 43.67 ± 1.09b | 38.92 ± 0.24f | 17.41 ± 0.04j | 93.76 ± 1.25ab | 0.0211 ± 0.0004b | 69.82 ± 1.00b | 68.38 ± 1.46b |
| | 2.0 | 40.08 ± 0.77c | 37.89 ± 0.18g | 22.03 ± 0.12i | 90.51 ± 0.26c | 0.0180 ± 0.0009e | 67.72 ± 0.74c | 66.57 ± 1.51c |
| XG | 0.5 | 32.08 ± 1.23f | 40.02 ± 0.52e | 27.90 ± 0.03c | 92.92 ± 1.99ab | 0.0112 ± 0.0011h | 63.23 ± 0.42f | 62.71 ± 0.85e |
| | 1.0 | 32.71 ± 0.49f | 38.82 ± 0.08f | 28.47 ± 0.08b | 88.41 ± 0.74d | 0.0154 ± 0.0004f | 63.22 ± 0.53f | 62.70 ± 0.28e |
| | 2.0 | 35.24 ± 0.25e | 35.12 ± 0.13h | 29.64 ± 0.05a | 76.98 ± 1.11g | 0.0196 ± 0.0001c | 63.90 ± 1.00f | 63.28 ± 0.09e |
| AP | 0.5 | 38.23 ± 0.19d | 34.85 ± 0.64h | 26.92 ± 0.11d | 84.58 ± 0.86e | 0.0185 ± 0.0003de | 66.00 ± 0.91e | 65.09 ± 0.64cd |
| | 1.0 | 32.74 ± 0.24f | 43.45 ± 0.23cd | 23.81 ± 0.06g | 93.61 ± 0.27ab | 0.0140 ± 0.0002g | 65.69 ± 0.83e | 64.83 ± 0.25d |
| | 2.0 | 30.62 ± 0.16g | 44.83 ± 0.15b | 24.55 ± 0.04e | 83.28 ± 0.45e | 0.0193 ± 0.0001cd | 66.04 ± 0.24de | 65.12 ± 0.16cd |

*Note:* RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; $C_\infty$, equilibrium concentration; k, kinetic constant; HI, hydrolysis index; eGI, estimated glycaemic index. Values followed by different letters in each column and each parameter indicate significant differences ($P < 0.05$).

glycemic index (eGI) ranged from 58.89 to 73.35, which was lower than the GF bread, this could be explained by that water content of steamed bread was commonly less than baked bread, and the amorphous starch regions that remain as part of the starch granular structure and the limited gelatinization of the starch granules, which are less prone to be attacked by alpha amylase. The other possible reason was that some composition (dietary fiber, polyphenols, alkaloids, etc.) in GF potato steamed bread reduced the rate of starch hydrolysis, the incorporation of the components into products restricted the hydration and gelatinization of starch, resulting in the retardation of starch hydrolysis by the enzyme (Sui et al., 2016). Jun, Bae, Lee, and Lee (2014) showed that the cake samples prepared with fiber-enriched materials exhibited a lower predicted glycaemic index. Sui et al. (2016) observed that anthocyanin addition reduced the digestion rate of wheat bread. Furthermore, the hydrocolloids addition reduced estimated glycemic response, which was testified by the lower DS and higher RS. Our result was agreement with that the estimated glycemic index (eGI) values were noticeably reduced when wheat flour was replaced with an equal blend of soluble and insoluble dietary fibers at 20% by weight (Bae et al., 2016). The risk of developing cardiovascular diseases is associated with an elevated blood glucose levels in healthy individuals. Moreover, low to moderate GI (<70) are considered favorable to health. This study demonstrated that GF steamed bread with hydrocolloids addition emerged as a “healthier” alternative to control. The number and variety of ingredients of GF steamed bread can be considered important factors that will determine the starch digestibility.

## 4. Conclusions

In conclusion, hydrocolloids (HPMC, CMC, XG and AP) greatly improved the mixing and thermal behavior of gluten-free potato dough, the hydrocolloids addition increased the $T_0$ and $T_1$, the reason could be explained by that hydrocolloids covered starch granules and delayed water absorption. And the result of SDS-PAGE indicated that hydrocolloids might form higher molecular weight aggregation between proteins-hydrocolloids or proteins-proteins, and which were closely related to the type and content of hydrocolloids. Moreover, the specific volume, hardness and porosity were improved by the hydrocolloids addition. Furthermore, the hydrocolloids addition reduced *in vitro* starch digestibility and estimated glycemic index. Therefore, it is possible to obtain improved GF steamed bread by incorporating hydrocolloids, HPMC was the optimal hydrocolloids for potato steamed bread making, followed by XG, CMC, and AP. These formulations also can be rendered suitable to be explored commercially as dietary supplement in people requiring low glycemic index food.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.07.047.

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