

**Evaluation of different hydrocolloids to improve dough rheological properties and bread quality  
of potato-wheat flour**

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1           4    **Abstract**

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3           5    The aim of study was to investigate the effect of hydroxylpropylmethylcellulose (HPMC), arabic gum  
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6           6    (AG), konjac glucomannan (KG) and apple pectin (AP) at 2% (w/w, potato-wheat flour basis) on the  
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9           7    potato-wheat dough (the mass ratio was 1:1) rheological, fermentation properties and its bread quality.  
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11           8    The  $\tan \delta$  of potato-wheat dough was significantly increased by adding HPMC compared to those of  
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14           9    without adding hydrocolloids (from 0.337 to 0.425), which was close to wheat dough (0.531).  
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17           10   Moreover, the dough height during fermentation process was significantly improved by adding  
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20           11   hydrocolloids, with the order of HPMC (23.1mm) > AP (19.3mm) > AG (18.6mm) > KG (13.6mm). In  
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23           12   addition, the potato-wheat protein bands of potato-wheat dough turned pale by adding hydrocolloids,  
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26           13   suggesting higher molecular weight aggregation formed between proteins-hydrocolloids or  
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28           14   proteins-proteins after fermentation process. Furthermore, HPMC significantly increased specific  
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31           15   volume (from 1.45 to 2.22 ml/g), and hydrocolloids restrained the starch retrogradation of potato-wheat  
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34           16   breads.

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36           17   **Keywords:** Dough property; Hydrocolloid; Protein structure; Specific volume; Texture; Thermal  
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39           18   characteristics

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41           19   **Chemical compounds:** Hydroxylpropylmethylcellulose (PubChem CID: 57503849); Sodium dodecyl  
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44           20   sulfate (PubChem CID: 3423265); Polyacrylamide (PubChem CID: 3083321); Coomassie Brilliant  
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47           21   Blue G 250 (PubChem CID: 6333920); Mercaptoethanol (PubChem CID:1567); Sodium dihydrogen  
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50           22   phosphate (PubChem CID:1567); Sodium dihydrogen phosphate (PubChem CID:23672064); Disodium  
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53           23   hydrogen phosphate (PubChem CID:24203); Carbon dioxide (PubChem CID:280).

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1       25    **Introduction**

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3       26    Nowadays, the use of mixed and wheat-less flours for bread making is a very recent development  
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6       27    across the globe owing to some economic, social and health reasons. In some countries, research efforts  
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9       28    are devoted to partial substitution of wheat flour for bread baking purposes, in order to reduce the huge  
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12       29    expenditure on wheat importation and to increase the utilization of locally available food crops (Hager  
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14       30    and Arendt 2013).

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17       31    Potato is the fourth important food crop in the world after rice, wheat and maize (FAOSAT 2016).

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19       32    Potato protein is characterized by balanced amino acid composition, which repairs the defects of wheat  
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22       33    protein. Moreover, potato flour contains several phytochemicals such as phenolics, flavonoids, and  
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25       34    carotenoids, which play pivotal role in human health and are essential part of our routine diet (Ezekiel  
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28       35    et al. 2013). Due to its high nutritional value and low caloric content, potato is supposed to be a better  
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31       36    alternate of wheat flour in food industry. Moreover, potato flour addition may influence wheat dough  
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34       37    properties, which impact the physicochemical properties of bread. It has been proven that potato flour  
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37       38    up to 20% can be mixed with wheat flour to prepare acceptable bread (Ijah et al. 2014). However,  
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39       39    higher substitution of wheat flour has a negative impact on dough development during fermentation  
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42       40    process and the quality of the products, for example, dough height is reduced, specific volume is  
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45       41    decreased, and hardness is increased. Therefore, the addition of additives is a strategy to improve the  
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48       42    bread making performance in low gluten or gluten free breads.

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50       43    Hydrocolloids have a wide application as additives to improve the quality of wheat, low gluten or  
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53       44    gluten free breads. The functional effects of hydrocolloids stem from their ability to modify dough  
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56       45    rheology and keep qualities of baked products. Hydrocolloids consist of a number of water soluble  
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59       46    polysaccharides with different chemical structure, and provide various functional properties by  
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1 47 controlling the water molecule's mobility, thus affecting the dough rheology, dough development and  
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3 48 gas retention (Peressini et al. 2011; Mancebo et al. 2015; Nicolae et al. 2016). Various hydrocolloids  
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6 49 have been applied to improve dough rheology, the rheology of wheat dough can be improved with the  
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9 50 use of sodium alginate, k-carrageenan, xanthan gum (XG) and Hydroxylpropylmethylcellulose  
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12 51 (HPMC), alginate and HPMC showed an exceptional retardation of staling (Guarda et al. 2004). Shittu  
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14 52 et al. (2011) found that XG improved the quality of cassava-wheat bread (the mass ratio of cassava  
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17 53 flour and wheat flour was 1:9). Moreover, hydrocolloids have also been used extensively as improver  
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20 54 in gluten free bread, yielding higher specific volume and softer crumb (Moore et al. 2004; Korus et al.  
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23 55 2009; Nicolae et al. 2016). Peressini et al. (2011) found that propylene glycol alginate showed better  
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26 56 improvement than XG when they studied rice-buckwheat dough and bread quality. Conclusively, it's  
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29 57 still a challenge to predict the real effect of hydrocolloids on dough properties and bread quality due to  
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32 58 different applied ingredients, structure of hydrocolloids, dough preparation and baking procedures. Up  
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35 59 to date, a handful of information is available on the application of different hydrocolloids to improve  
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38 60 the dough property and quality of potato-wheat bread.

39 61 In the present study, the effects of hydroxylpropylmethylcellulose (HPMC), arabic gum (AG), konjac  
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42 62 glucomannan (KG) and apple pectin (AP) at the level of 2% (w/w, potato-wheat flour basis) on  
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45 63 rheological, fermentation properties and bread making performance of potato-wheat flour (the mass  
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48 64 ratio of potato flour and wheat flour was 1:1) were investigated. The effect of these hydrocolloids on  
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51 65 the protein structure and staling of the potato-wheat bread at 24 and 48 hours of storage were also  
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54 66 studied to improve the quality of bread.

## 55 67 **Materials and methods**

### 58 68 **Materials**

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1 69 Wheat flour was purchased from Beijing Qijian Food Ltd. (Beijing, P.R. of China). Fresh potato  
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3 70 (Shepody) was provided by Institute of Vegetables and Flowers, Chinese Academy of Agricultural  
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6 71 Sciences (Beijing, China). Potato tubers were peeled, washed, then steamed for 30 min at 100 °C, and  
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9 72 dried between 170 and 200 °C, dried potatoes were milled into flour using hammer mill (Beijing  
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12 73 kaichuang tonghe Technology Development Co., Ltd. China) and sieved in 100 µm screen. The basic  
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14 74 compositions of wheat flour and potato flour were given in supplement Table 1. AP, AG, KG and  
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16 75 HPMC were obtained from Henan Zhongxin Chemical Co., Ltd. (Zhengzhou, Henan, P.R. of China).  
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19 76 Degree of esterification of pectin was 58.15. The viscosity of KG and AG were 36000 MPa/min (1%  
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21 77 aqueous solution at 25°C) and 5100 MPa/min (1% aqueous solution at 25°C), respectively. And the  
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23 78 degrees of methoxyl and hydroxypropyl substitution of HPMC were 28.2% and 7.8%, respectively.  
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26 79 Instant dry yeast was obtained from Angel Yeast Co. Ltd. (Yichang, Hubei, China). Tap water was used  
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29 80 to prepare dough.  
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### 32 33 34 81 **Dynamic rheological properties of dough**

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36 82 According to amino acid composition, the equipment adaptability of commercial process, the  
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39 83 acceptability of consumers, etc., the mass ratio of potato and wheat flour was 1:1, which was selected  
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42 84 throughout the study.  
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45 85 The water added for only wheat flour formulation was determined according to farinograph (water  
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47 86 absorption: 63±0.6%). And for the potato-wheat flour formulations (the mass ratio of potato flour and  
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50 87 wheat flour was 1:1) was 90%, and the level of hydrocolloids was 2% (w/w, potato-wheat flour basis)  
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53 88 (Supplement Table 2). The mixed flour and appropriate water (deprived of yeast) were mixed at the low  
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56 89 speed (80 rpm) for 10 min using a Hobart mixer A-120 (Tory, Ohio). Dynamic oscillatory tests were  
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59 90 performed in an Anton Par Physica MCR 301 equipped with a parallel plate geometry (25 mm diameter)  
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1 91 with a 1.0 mm gap between plates. Before measurements, samples were allowed to rest 10 min between  
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3 92 plates to relax. Deformation sweeps were performed at a constant frequency in order to determine the  
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6 93 linear viscoelastic range of each sample, frequency sweeps (from 1-100 rad/s) were performed at a  
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9 94 constant stress (0.1%) within the linear viscoelastic range. Experimental data were described by the  
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12 95 power law model (Korus et al. 2009):

$$14 \quad 96 \quad G'(\omega) = K' \cdot \omega^{n'}$$

$$17 \quad 97 \quad G''(\omega) = K'' \cdot \omega^{n''}$$

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20 98  $G'$  is a storage modulus (Pa),  $G''$ - loss modulus (Pa),  $\omega$ -angular frequency (rad s<sup>-1</sup>), and  $K'$ ,  $K''$ ,  $n'$ ,  $n''$   
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23 99 -experimental constants.

#### 25 100 **Rheofermentometer measurements**

28 101 Fermentation property was determined using a Rheofermentometer F3 (Chopin Technologies, France)  
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31 102 following the supplier specifications. The yeast content was 3% (flour basis). Dough was mixed  
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34 103 according to 2.2, placed (300 g) in the fermentation vat at the temperature of 30 °C for 3 h and a weight  
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37 104 constraint of 0.5 kg was applied. Fermentation parameters included:  $H_m$ , height under constraint of  
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39 105 dough at maximum development time (mm);  $V_T$ , total volume of CO<sub>2</sub> (ml) produced during  
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42 106 fermentation (Dahir et al. 2015).

#### 45 107 **Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)**

47 108 SDS-PAGE under reducing conditions was performed according to the method described by Laemmli  
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50 109 (1970) using an AE-6450 electrophoresis system (Atto Corporation, Tokyo, Japan). 0.2 g dough was  
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53 110 transferred into a vial and mixed with 0.5 ml of extraction solution (0.5 M Phosphate buffer at pH 7.4  
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56 111 with 2% SDS), vortex shaking for 30 min, and centrifuged for 20 min at 10,000 × g. The supernatant  
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59 112 was directly dissolved (4:1, v/v) in the sample buffer. After boiling at 100 °C for 2 min, the samples  
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1 113 were centrifuged at  $10,000 \times g$  for 20 min. The 15- $\mu$ l samples and 10- $\mu$ l marker solutions were placed  
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3 114 into wells. Gel electrophoresis was run on 5% loading gels and 15% separating gels. The gels were  
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6 115 stained using Coomassie Brilliant Blue R-250 method and destained with methanol wash solution. A  
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9 116 marker kit containing bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), lactate dehydrogenase  
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11 117 (35.0 kDa), REase Bsp98I (25.0 kDa),  $\beta$ -lactoglobulin (18.4 kDa), lysozyme (14.4 kDa) (Sigma) were  
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14 118 used for evaluating the apparent molecular mass.

### 17 119 **Bread making**

20 120 The yeast content was 3% (flour basis). Dough was mixed according to 2.2, then covered with a plastic  
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22 121 film, and was fermented 1h at 30 °C and 80 % relative humidity (RH). After first fermentation, dough  
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25 122 was divided into 50 g portions, rounded, and again kept at 30 °C and 80 % RH for 20 min. Baking was  
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28 123 performed in an electrical oven ACA model ATO-CF24B (Zhuhai, China) for 35 min at 160 °C. After  
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31 124 cooling, the bread samples were packed in bags and stored for 0, 24 and 48 hours at room condition  
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34 125 (25°C and 47% RH).

### 36 126 **Bread quality analysis**

39 127 Bread volume was assessed by rapeseed displacement. The height and diameter of breads were  
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42 128 measured using a vernier caliper and the shape was determined from the height/diameter ratio. Water  
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45 129 content, water activity ( $a_w$ ), and weight loss were determined according to the method described earlier  
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48 130 (Sahraiyen et al. 2013).

50 131 Additionally, analysis of crust color in CIE L\*a\*b\* system was performed by reflectance method using  
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53 132 Color i5 spectrometer (XRite, USA) set for the following parameters: measuring geometry d/8,  
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56 133 illuminant D65, observer 10°, slit width 25mm.

58 134 Texture profile analysis was measured using a TA-XT2i Texture Analyser equipped with a 5 kg



1 135 load cell (Godalming, London). Pre-test speed 5.0 mm/s, test speed 1 mm/s, post-test speed 5.0 mm/s,  
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3 136 40% compression, 35 mm diameter cylinder probe. The waiting time between the first and second  
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6 137 compression cycle was 1 second. Hardness, springiness and chewiness were calculated from the  
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9 138 graphic.

### 10 11 139 **Thermal properties**

12 140 Thermal properties were characterized by differential scanning calorimeter (DSC) Q100 (New Castle,  
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14 141 Delaware, USA). 6-15 mg bread samples were placed into aluminum hermetic pans and analyzed at 0,  
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17 142 24 and 48 hours at room condition (25 °C and 47% RH ). An empty pan was used as reference. Sample  
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20 143 and reference were heated between 0 and 140 °C at a heating rate of 10 °C/min. The endothermic  
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23 144 transition temperature ( $T_p$ ), and enthalpy ( $\Delta H$ ) for starch gelatinization were computed from the  
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26 145 endothermic peaks. Enthalpies were expressed as J/g DW.

### 27 28 29 30 146 **Statistical analysis**

31 147 In order to establish the statistical differences between means, the data were treated by one-factor  
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34 148 analysis of variance, and the least significant difference (LSD) at significance level 0.05 was calculated  
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37 149 using Fisher post hoc test. Statistical analysis was performed using the Statistical Analysis System  
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40 150 version 8.1 software (SAS Institute Inc., Cary, NC, USA).

### 41 42 43 44 151 **Results and discussion**

#### 45 46 47 152 **Chemical composition of wheat and potato flour**

48 153 The composition of wheat and potato flour was shown in supplementary Table 1. Protein, ash, fat,  
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51 154 dietary fiber and starch content in wheat flour were 13.22±0.21%, 0.48±0.02%, 1.23±0.01%,  
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54 155 1.88±0.08%, 60.58±0.14%, respectively, and those of potato flour were 9.87±0.11%, 1.86±0.01%,  
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57 156 0.26±0.01%, 6.28±0.06%, 68.78±0.32%, respectively. Moreover, the dietary fiber content of potato  
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1 157 flour was 3.34- fold than those of wheat flour, K, P and vitamin C content were also significantly  
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3 158 higher than wheat flour, which are highly desirable in the diet due to their beneficial effects on human  
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6 159 health. Therefore, potato flour addition would enhance bread nutritional and functional qualities.  
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#### 9 160 **Dynamic rheological properties of dough**

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11 161 The storage modulus ( $G'$ ), loss modulus ( $G''$ ), and phase shift tangent ( $\tan \delta$ ) of the samples are  
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13 162 represented in supplementary Fig. 1. Table 1 shows the parameters of power law model. Storage  
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16 163 modulus were larger than those of loss modulus ( $G' > G''$ ) in all samples, which indicated that elastic  
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19 164 properties predominated viscous features, and solid like behavior of all tested samples. However,  
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22 165 concrete viscoelastic properties were different between samples. The  $G'$ ,  $G''$  and  $\tan \delta$  values of PW  
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25 166 dough were lower than wheat dough. Therefore, hydrocolloids with thickening effects in PW dough  
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28 167 might be more suitable for making bread. Compared to PW dough, the addition of AG, KG and AP  
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31 168 increased  $G'$  and  $G''$  values significantly (supplement Fig. 1), which was also confirmed by the values  
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34 169 of  $K'$  and  $K''$  (Table 1). However, HPMC addition decreased the  $G'$  accompanied with increasing  $G''$ .  
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37 170 Furthermore, AG and KG addition had an increase in  $K'$  and  $K''$  accompanied by the lower in  $n'$  and  $n''$   
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40 171 values, which suggested an increase in gel stability (Witczak et al. 2014). Studies also found that  
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43 172 addition of xanthan gum to rice-buckwheat dough increased  $G'$  and  $G''$  (Peressini et al. 2011). Because  
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46 173 of the tendency of AG and KG for aggregation or self-association, which may exhibit a wide range of  
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49 174 conformations in solution as the links along the polymeric chains, thus encourage extensive structuring  
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52 175 in the surrounding water, this hydrocolloid could contribute to batter strength with the formation of  
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55 176 weakly associated aggregates (Anton and Artfield 2008). Another contribution of AG and KG to dough  
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58 177 elasticity could be due to starch granule or protein-hydrocolloid interaction. Peressini et al. (2011)  
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61 178 found that some starch granules appeared to be glued together by the hydrocolloids and enveloped in a  
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1 179 coating suggesting a close association of hydrocolloids with starch granule.

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3 180 In order to compare the difference of samples,  $\tan \delta$  values at 1 Hz (within the linear region) was  
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6 181 conducted. Potato flour addition significantly reduced  $\tan \delta$  value compared to wheat dough (from  
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9 182 0.531 to 0.337).  $\tan \delta$  of HPMC-PW dough (0.425) was close to the wheat dough (0.531), and  
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11 183 significantly higher than PW dough (0.337) (Table 1). HPMC addition increased the  $\tan \delta$  and  
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14 184 decreased the storage modulus of the PW dough, which was similar to the influence of propylene  
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17 185 glycol alginate on the rice-buckwheat batters (Peressini et al. 2011). Hydrophobic groups of HPMC  
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20 186 introduced a bump on the chain that prevents close association of chains resulting in quite stable  
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23 187 polymer solution (Nammakuna et al. 2016). Although AP addition resulted in a significant growth of  $G'$   
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26 188 and  $G''$  values,  $\tan \delta$  only had a slight shift (0.348), which might be attributed to that AP enhanced the  
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29 189 elasticity and viscosity of dough in the similar degree. The reason could be explained that pectin is a  
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32 190 hetero-polysaccharide, which contains at least 65% (w/w) units of galacturonic acid, which is  
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35 191 significant from the other hydrocolloids. Our result agrees with the finding that the pectin had  
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38 192 contributed to the overall elasticity and viscosity of bread dough or potato starch paste (Witczak et al.  
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41 193 2014). AG and KG addition caused noticeable reduction of  $\tan \delta$  and loss of the relation between  
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44 194 moduli and oscillation frequency, which was illustrated by the lowest values of  $n'$  and  $n''$  (Table 1).  
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47 195 Therefore, AG and KG had a gelling effect than the thickening effect. Similar results found when agar,  
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50 196 HPMC and xanthan gum were added to chestnut dough (Moreira et al. 2011).

### 51 197 **Fermentation properties of dough**

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53 198 Effect of hydrocolloids on dough development and total amount of gas ( $\text{CO}_2$ ) is shown in Table 2.  
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56 199 Dough development was characterized by dough height at maximum development time ( $H_m$ ), higher  
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59 200  $H_m$  suggested the condition of dough was more favorable to produce larger volume (Huang et al. 2008).  
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1 201  $H_m$  was significantly and negatively influenced by potato flour addition (from 58.7 mm to 10.8 mm).  
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3 202 This result was in agreement with the finding of Wang et al. (2002), who observed that bran addition  
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6 203 decreased the dough height by preventing the free expansion of wheat dough during fermentation.  
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9 204 Compared to PW dough, hydrocolloids addition increased the  $H_m$  significantly, especially HPMC (23.1  
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11 205 mm) and AP (19.3 mm), which agreed with that HPMC inclusion in the biscuit and whole meal flour  
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14 206 formulations improved dough development (Zannini et al. 2014). Mudgil, Barak, and Khatkar (2016)  
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17 207 also found that partially hydrolyzed guar gum addition increased the peak dough height and stability of  
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20 208 wheat dough. Similar results were also confirmed by Dahir et al. (2015), who reported that  
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23 209 carboxymethylcellulose improved the  $H_m$  of sorghum-wheat dough. The reason might be due to that  
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26 210 the hydrocolloids interact with protein and starch, thus influences dough stability during proofing and  
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29 211 confers additional strength to increase the gas retention leading to greater volume. The results of  
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32 212 Ribotta et al. (2005) and Correa et al. (2014) confirmed the explanation, they all found that  
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35 213 hydrocolloids could form hydrophilic complexes with protein or starch, which moreover changed the  
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38 214 viscoelasticity and influenced the fermentation character.  
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41 215 Regardless to the retarded dough development, compared to wheat dough, the gas behavior (total  
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44 216 volume of  $CO_2$ ,  $V_T$ ) was improved with potato flour addition (from 1713 ml to 2553 ml). The reason  
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47 217 might be the processing way of potato flour caused the gelatinization of starch and the break of  
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50 218 structure, an easier access of the enzyme to the active sites in starch and then yield sugars metabolized  
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53 219 by the commercial yeast addition. However, hydrocolloids addition did not have prominent effect on  
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56 220  $V_T$  of PW dough, which might be due to that hydrocolloids had no significant influence on the  
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59 221 enzymatic activity and sugar content pivotal for yeast growth.  
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## 222 **SDS-PAGE**

1 223 The typical protein electrophoretic profiles obtained by SDS-PAGE are shown in Fig. 1. Wheat dough  
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3 224 protein extract showed the presence of very faint bands at 61, 56 and 48.8 kDa that correspond to  
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5 225  $\omega$ -gliadins, followed by other polypeptides species of 41, 37, 35, 32 kDa which could be assigned to  $\alpha$ ,  
6  
7  
8 226  $\beta$ -and  $\gamma$  gliadins and low molecular weight glutenin subunits (LMW-GS), and bands at 26, 17 and 14  
9  
10 227 kDa that would correspond to albumin and globulins (Correa et al. 2014; Wang et al. 2016). The profile  
11  
12 228 of potato protein showed the presence of bands at 40 kDa and 18.4 kDa bands, which belongs to patatin  
13  
14 229 and protease inhibitor, respectively, which was accordance with the result of Koningsveld (2011).  
15  
16 230 Compared to the Wheat and PW dough, the protein bands of PW dough turned pale by adding  
17  
18 231 hydrocolloids, especially for patatin and protease inhibitor sites, which indicated a difficult extraction  
19  
20 232 of protein subunits. The possible explanation might be explained by that higher molecular weight  
21  
22 233 complexes might be formed between proteins-hydrocolloids or proteins-proteins after the fermentation  
23  
24 234 process, thus changed the protein solubility, and the capacity of complexation appears to be related to  
25  
26 235 the type of hydrocolloid. These results were demonstrated by Ribotta et al. (2005), who found the  
27  
28 236 carrageenan and pectin decreased the density of wheat protein bands because of forming hydrophilic  
29  
30 237 complexes with gluten proteins. However, the result was different with that high molecular weight  
31  
32 238 aggregates were observed at the top of the gel when hydrocolloids added into wheat dough (Correa et  
33  
34 239 al. 2014). We cannot rule out other types of interactions, such as electrostatic and hydrophobic  
35  
36 240 interactions in the aggregates present in the dough. The hydrocolloids addition also could affect the  
37  
38 241 protein properties through the change of secondary structure or forming non-covalent links, which were  
39  
40 242 confirmed by the results of Correa et al. (2014), who observed microcrystalline cellulose and HPMC  
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42 243 changed the  $\alpha$ -helix conformation percentage of wheat protein.  
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#### 57 244 **Bread quality analysis**

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1       245    **Specific volume and height/diameter ratio**

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3       246    The photographs of breads are shown in supplement Fig.2. The bread prepared with 100% wheat flour  
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6       247    had the biggest specific volume (3.08 ml/g, Table 3), accompanied by a good distribution of pores on  
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9       248    the crumb surface. The smallest specific volume of bread (1.45 ml/g, Table 3) was found in PW. It was  
10  
11       249    well known that gluten (gliadins and glutenins) was responsible for the protein starch interaction which  
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13       250    is related to gas cell formation, including stabilization and retention of the gas cells during the proofing  
14  
15       251    and baking process (Khatkar, Barak, and Mudgil 2013), and potato flour addition destructed the gluten  
16  
17       252    structure. Therefore, the lowest specific volume of PW bread might be due to the “dilution effect” of  
18  
19       253    the gluten proteins and different starch granule, amylose/amylopectin, particle size between potato and  
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21       254    wheat flour. Hydrocolloids addition significantly increased the specific volume of bread, which was in  
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23       255    agreement with the result that 0.5 and 1.0 g/100 g guar and all concentrations of locust bean gum-flour  
24  
25       256    blends gave higher loaf volume compared to control (Hammed, Ozsisli and Simsek 2016), 1% sodium  
26  
27       257    carboxymethyl cellulose increased volume of rice bread (Nicolae, Radu and Belc 2016), xanthan gum,  
28  
29       258    guar gum, and locust bean gum increased the specific volume of gluten-free bread based on small  
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31       259    broken rice berry flour (Numfon 2017). The reason could be attributed to that hydrocolloids improved  
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33       260    dough rheological properties (Table 1) and fermentation capability (Table 2), and the increased extent  
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35       261    appeared to be related to the type of hydrocolloid. This might be because too high or low viscosity and  
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37       262    elasticity caused a limited gas cell expansion during fermentation, which was in agreement with the  
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39       263    results of Lazaridou et al. (2007), who observed that viscosity and elasticity up to a certain level have  
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41       264    the advantage to allow for a larger increase in volume. HPMC-PW bread had the biggest specific  
42  
43       265    volume (2.22 ml/g), which was 1.53-folds of PW bread, this could be confirmed by the result that  
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45       266    HPMC increased the  $\tan \delta$  (near to wheat dough) (Table 1) and maximum dough height ( $H_m$ ) of PW  
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1 267 dough (Table 2). HPMC contains hydrophobic groups, which increase the interfacial activity within the  
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3 268 dough system during fermentation, and form gel networks on heating during bread making process  
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5  
6 269 (Mancebo et al. 2015). Such network structures serve to further strengthen the boundaries of the  
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8  
9 270 expanding cells in the dough, thus increase gas retention and lead to a better bread specific volume  
10  
11 271 consequently (Lazaridou et al. 2007). AP addition also increased the specific volume of bread from  
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13  
14 272 1.45 to 2.04 ml/g, coinciding with the results of Correa et al. (2012) and Lazaridou et al. (2007), who  
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16  
17 273 found that pectin increased the specific volume of gluten free rice bread. These height/diameters of all  
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19  
20 274 the samples were higher than 0.5, suggesting a spherical shape was formed. AG had the highest value  
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22 275 (0.74), even more than the sample with only wheat flour (0.67). On the other hand, PW showed the  
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24  
25 276 lowest height/diameter (0.58), which showed that the shape was close to round.

#### 277 **Crust colour**

278 The Hunter parameters of the crust are provided in Table 3. Compared to wheat bread, the addition of  
279 potato flour and hydrocolloids decreased  $L^*$ , the one reason might be that  $L^*$  of potato flour was lower  
280 than wheat flour, and the other reason might be that the  $L^*$  was affected by the making process of bread.  
281 The parameter  $a^*$  denotes the balance between green (negative values) and red (positive values). The  
282  $a^*$  of PW bread was significantly increased by AP and HPMC addition, suggesting an increase in the  
283 red colour of these samples, while the opposite trend was found for breads with AG and KG addition.  
284 In the case of parameter  $b^*$ , all tested sample showed evidence of the dominance of yellow ( $b^* > 0$ ).  
285 The lowest value of  $b^*$  was observed in the wheat bread (35.1). Hydrocolloids addition significantly  
286 increased the value of the parameter  $b^*$ , especially AP (41.2) and HPMC (42.0), which was different to  
287 the result of Lazaridou et al. (2007), who obtained bluer gluten free breads supplemented with different  
288 hydrocolloids compared to control, the reason might be different material composition and making

1 289 process of the researchers.

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3 290 **Water activity, weight loss and water content**

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6 291 Water properties of bread are important for bread quality, especially for the retrogradation during  
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9 292 storage. The water activity ( $a_w$ ) values of the bread of all potato-wheat flour recipes are presented in  
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11  
12 293 Table 3. The results demonstrated that the values of  $a_w$  varied within the ranges of 0.9645  
13  
14 294 (Wheat)-0.9652 (AG-PW). The  $a_w$  value was not affected by hydrocolloids addition, which was in  
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16  
17 295 accordance with the result of Sahraiyen et al. (2013), who found the lepidium sativum seed and guar  
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20 296 gum had no significant influence on the  $a_w$  of rice-wheat bread. In contrast, Rosell et al. (2001) reported  
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23 297 an increase of  $a_w$  as well of moisture retention due to the higher water holding capacity of the gum.  
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26 298 Correa et al. (2014) reported that significantly higher relaxation times were observed when modified  
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29 299 celluloses were added. Moreover, hydrocolloids addition significantly decreased the weight loss during  
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32 300 the baking process because of the higher water holding capacity, the better dough stability, or the gas  
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35 301 cells with a more continuous surface and a thicker appearance with respect to the control (Fadda 2014).  
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38 302 The weight loss of all the PW bread were lower than the wheat bread, the reason could be attributed to  
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41 303 that water holding capacity of potato flour was higher than wheat flour because of the different  
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44 304 composition, such as protein, starch, dietary fiber and phosphorus content. Besides, hydrocolloids  
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47 305 addition significantly affected the water content of PW bread during storage. After the 24 and 48 h of  
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50 306 storage, a trend of decreasing water content of all the bread was found, while the degree of reduction  
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53 307 was inhibited by hydrocolloids with the exception of AP (Table 3).

54 308 **Texture properties analysis**

55  
56 309 Table 4 shows selected texture parameters of bread. The interactions between starch and other  
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59 310 macromolecular constituents are especially important for structural changes occurring in bread. In  
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1 311 traditional wheat based bakery products, the primary role is played by gluten and starch.  
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3 312 Polysaccharide constituents are generally more important in establishing bread structure than protein in  
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6 313 the case of wheat-less products. Moreover, the presence of polysaccharide significantly influence on  
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9 314 bread staling, which occurs due to the changes in water binding and starch retrogradation. Initial  
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11 315 hardness of wheat bread was 19.23 N and increased to 31.02 N and 46.58 N after the 24 and 48 h of  
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13  
14 316 storage, respectively. As for the PW, the hardness was 19.24, 30.19, and 38.56 N on the 0, 24, and 48 h,  
15  
16  
17 317 respectively. These hydrocolloids addition did not affect the hardness of the fresh bread (Table 4).  
18  
19  
20 318 There was a report on correlation between loaf volume and hardness (Moore et al. 2004). However, this  
21  
22  
23 319 result was not certified by the study, as the Wheat and PW had the different specific volume, and the  
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26 320 hardness of them was almost similar at the same time.  
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28 321 HPMC addition inhibited a significant reduction of hardness during storage, which was in agreement  
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30  
31 322 with the results observed earlier by Hager and Arendt (2013). Similar result was also reported by  
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33  
34 323 Burešová et al. (2016), who found that the hardness of rice-buckwheat crumb without hydrocolloids  
35  
36  
37 324 (12.2 N) was decreased by calcium caseinate (4.3 N), xanthan gum (9.1 N) and carboxymethyl  
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40 325 cellulose (9.3 N). The hardness of PW bread with AG and AP addition were less than PW bread after  
41  
42  
43 326 24 h of storage. Similarly, the lower hardness was measured for bread supplemented with AG (30.08  
44  
45  
46 327 N) and AP (33.48 N) after 48 h of storage. Hydrocolloids significantly limited the hardening of the PW  
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48  
49 328 bread. These results could be confirmed by the results that  $\Delta H$  of starch retrogradation was restrained  
50  
51  
52 329 by hydrocolloids addition (Fig.2b). Hydrocolloids addition delayed the hardening of the bread probably  
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54  
55 330 by preventing water migration, which was confirmed by the higher water content after the 24 and 48 h  
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58 331 of storage (Table 3). The other possible reason might be that hydrocolloids addition affected the  
59  
60  
61 332 viscosity of the host product interfering with the water diffusion phenomena (Guarda et al. 2004).  
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1 333 Conversely, the addition of KG increased the hardness of the bread that could be the consequence of  
2  
3 334 the thickening effect on the bread walls surrounding air spaces as proposed by Rosell et al. (2001). This  
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6 335 finding was in agreement with Sim et al. (2011), who reported that the hardness of steamed bread  
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8  
9 336 added with KG became firmer when compared with the control counterpart.

10  
11 337 Springiness didn't show any notable difference between samples. Chewiness is the energy required to  
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13  
14 338 masticate a solid food to a state ready for swallowing and significantly related to hardness, there was  
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16  
17 339 no significant difference of fresh samples. The most pronounced reduction of chewiness was caused by  
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20 340 the addition of AG at the same storage time, the reason might be due to that the AG had the highest  
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22  
23 341 influence on storage modulus ( $G'$ ) and loss modulus ( $G''$ ) (Table 1), this viscoelastic character might  
24  
25  
26 342 inhibition of the retrogradation. Similar data was found by Shalini and Laxmi (2007), who observed  
27  
28  
29 343 that the addition of guar gum might affect the amylose network avoiding the formation of spongy  
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31 344 matrix.

### 34 345 **Thermal properties analysis**

35  
36 346 Thermal properties of breads using different hydrocolloids are shown in Fig.2. All fresh breads did not  
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39 347 show any endothermic transition, which suggested that the starch was completely gelatinized after  
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41  
42 348 baking (data not shown). Kim et al. (2003) postulated that gluten absorbs 30% of the water in dough,  
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44  
45 349 but gluten denatures and transfers the water to the starch granules for gelatinization during baking. The  
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48 350 hydrophilic hydrocolloids used in this work probably have a similar mechanism of water transfer. After  
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51 351 storage of 24 h or 48 h, supplementation of bread with potato flour resulted in an increase in peak  
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54 352 temperature ( $T_p$ ) of about 1°C compared to wheat bread, and the addition of hydrocolloids increased  
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57 353 the peak temperature in different extents (Fig.2a). The increase in  $T_p$  could be caused by the interaction  
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60 354 between added hydrocolloids and starch polymers, which in turn might limit the retrogradation and  
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1 355 aging of bread. It is in a general accordance with earlier observation on significant decrease in hardness  
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3 356 with hydrocolloids addition (Table 4). The result of Rosell and Santos (2010) showed that dietary fiber  
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6 357 at medium-high levels (6%–34%) increased the higher initial and peak gelatinization temperatures,  
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8  
9 358 lower gelatinization enthalpy ( $\Delta H$ ), the reason can be attributed to that fibers would restrict starch  
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11 359 swelling by restricting the availability of water for the remaining ungelatinized granules.

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14 360 As expected,  $\Delta H$  increased with the storage time increasing due to starch retrogradation and the loss of  
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17 361 water, but was significantly lower compared to the PW when addition of hydrocolloids with the  
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20 362 exception of AP (Fig.2b), which attributed to controlling and maintaining the moisture content,  
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22 363 stabilizing the dough, and influencing the crust structure (Rosell and Gomez 2007). Yeh et al. (2009)  
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25 364 observed that hydrocolloids increased water retention of bread. Therefore, the retained water could be  
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28 365 utilized to starch gelatinization. The results of both hardness changes and enthalpy of melting of  
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31 366 retrograded amylopectin clearly showed that the applied hydrocolloids except AP could act as an  
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33  
34 367 antistaling factor. The  $\Delta H$  was lower in cassava-maize-wheat bread with hydrocolloids and emulsifiers,  
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36 368 6.7-11.0 J/g compared to 20.0 J/g for the reference bread (Eduardo, Svanberg, and Ahrné 2015).

### 39 **Conclusion**

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42 370 The results of the present study showed that the functionality of potato-wheat flour in terms of dough  
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45 371 rheology and bread making performance can be successfully improved by the addition of hydrocolloids.  
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47 372 SDS-PAGE result indicated that hydrocolloids might form higher molecular weight aggregation  
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50 373 between proteins-hydrocolloids or proteins-proteins after the fermentation process, and which were  
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53 374 closely related to the type of hydrocolloids. Specific volume and crumb structure results suggested that  
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56 375 HPMC addition provided higher quality potato-wheat bread than AP, AG and KG addition.  
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58 376 Furthermore, all the tested hydrocolloids with the exception of AP showed antistaling effect on  
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1 377 potato-wheat bread according to the results of thermal properties and water properties. Considering the  
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3 378 dough rheology, specific volume, texture, and thermal properties comprehensively, HPMC addition  
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6 379 gave promising results for the production of high quality potato-wheat bread, which provides a  
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8  
9 380 potential possibility for replacing wheat flour with potato flour for making bread.

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1	<b>Table captions</b>
2	<b>Table 1</b>
3	Parameters of the power-law functions describing dependence of storage and loss moduli on angular
4	frequency
5	<b>Table 2</b>
6	Fermentation properties of potato-wheat dough obtained using different hydrocolloids
7	<b>Table 3</b>
8	Technological parameter of potato-wheat breads obtained using different hydrocolloids
9	<b>Table 4</b>
10	Texture of potato-wheat breads obtained using different hydrocolloids

11 **Table 1**

Samples	$G' = K' \cdot \omega^{n'}$		$G'' = K'' \cdot \omega^{n''}$		tan $\delta$ At 1Hz
	$K' \times 10^{-3} (\text{Pa s}^{n'})$	$n'$	$K'' \times 10^{-3} (\text{Pa s}^{n''})$	$n''$	
Wheat	11.42±0.52d	0.191±0.002b	6.86±0.21c	0.192±0.006b	0.531±0.001a
PW	8.74±0.23e	0.148±0.003d	3.17±0.19e	0.188±0.004c	0.337±0.006c
HPMC-PW	8.04±0.12f	0.218±0.006a	3.81±0.08d	0.252±0.005a	0.425±0.005b
AG-PW	34.59±1.59a	0.114±0.001f	8.16±0.27a	0.152±0.008d	0.222±0.001e
KG-PW	24.46±0.67b	0.132±0.004e	7.28±0.19b	0.160±0.005d	0.283±0.004d
AP-PW	20.43±0.42c	0.172±0.006c	7.52±0.09b	0.196±0.009c	0.348±0.002c

12 PW, potato flour-wheat flour; HPMC, hydroxypropylmethylcellulose; AP, apple pectin; AG, arabic gum; KG,

13 konjacglucomannan

14

15 **Table 2**

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Samples	Dough development	Gas behavior
	H <sub>m</sub> (mm)	V <sub>T</sub> (mL)
Wheat	58.7 ± 0.2a	1713 ± 20e
PW	10.8 ± 0.1f	2553 ± 25c
HPMC-PW	23.1 ± 0.1b	2368 ± 38d
AP-PW	19.3 ± 0.2c	2614 ± 12b
AG-PW	18.6 ± 0.1d	2671 ± 31a
KG-PW	13.6 ± 0.1e	2714 ± 29a

17 PW, potato flour-wheat flour; HPMC, hydroxypropylmethylcellulose; AP, apple pectin; AG, arabic gum; KG,  
18 konjacglucomannan; H<sub>m</sub>, dough height at maximum development time; V<sub>T</sub>, total volume of CO<sub>2</sub> (mL).

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21 **Table 3**

Samples	Weight loss (%)	Height/diameter	Water activity	Specific volume(ml/g)	Water content (%)			L*	a*	b*
					0 (h)	24 (h)	48 (h)			
Wheat	22.65 ± 0.21a	0.67 ± 0.04bc	0.9645 ± 0.001a	3.08 ± 0.01a	42.17 ± 0.12l	37.49 ± 0.11m	36.85 ± 0.02n	67.9 ± 0.2a	6.9 ± 0.1e	35.1 ± 0.3e
PW	19.69 ± 0.14b	0.58 ± 0.02d	0.9646 ± 0.002a	1.45 ± 0.02f	49.07 ± 0.17c	46.28 ± 0.07h	45.24 ± 0.06i	64.9 ± 0.4c	8.2 ± 0.2c	38.7 ± 0.2d
HPMC-PW	18.30 ± 0.13c	0.63 ± 0.03cd	0.9650 ± 0.008a	2.22 ± 0.03b	49.74 ± 0.21b	47.44 ± 0.02e	45.02 ± 0.12j	60.9 ± 0.5d	10.5 ± 0.1b	42.0 ± 0.4a
AP-PW	18.30 ± 0.20c	0.61 ± 0.03cd	0.9650 ± 0.007a	2.04 ± 0.01c	49.15 ± 0.08c	46.77 ± 0.15g	43.65 ± 0.11k	58.6 ± 0.1e	13.4 ± 0.2a	41.2 ± 0.1b
AG-PW	18.20 ± 0.08c	0.74 ± 0.06a	0.9652 ± 0.004a	1.70 ± 0.02e	49.15 ± 0.08c	48.66 ± 0.21d	47.08 ± 0.02f	66.6 ± 0.1b	5.8 ± 0.1f	38.7 ± 0.1d
KG-PW	16.94 ± 0.11c	0.69 ± 0.02ab	0.9649 ± 0.006a	1.82 ± 0.01d	51.31 ± 0.09a	48.67 ± 0.03d	47.51 ± 0.08e	64.4 ± 0.2c	7.7 ± 0.1d	39.8 ± 0.2c

22 PW, potato flour-wheat flour; HPMC, hydroxypropylmethylcellulose; AP, apple pectin; AG, arabic gum; KG, konjac glucomannan

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24

25 **Table 4**

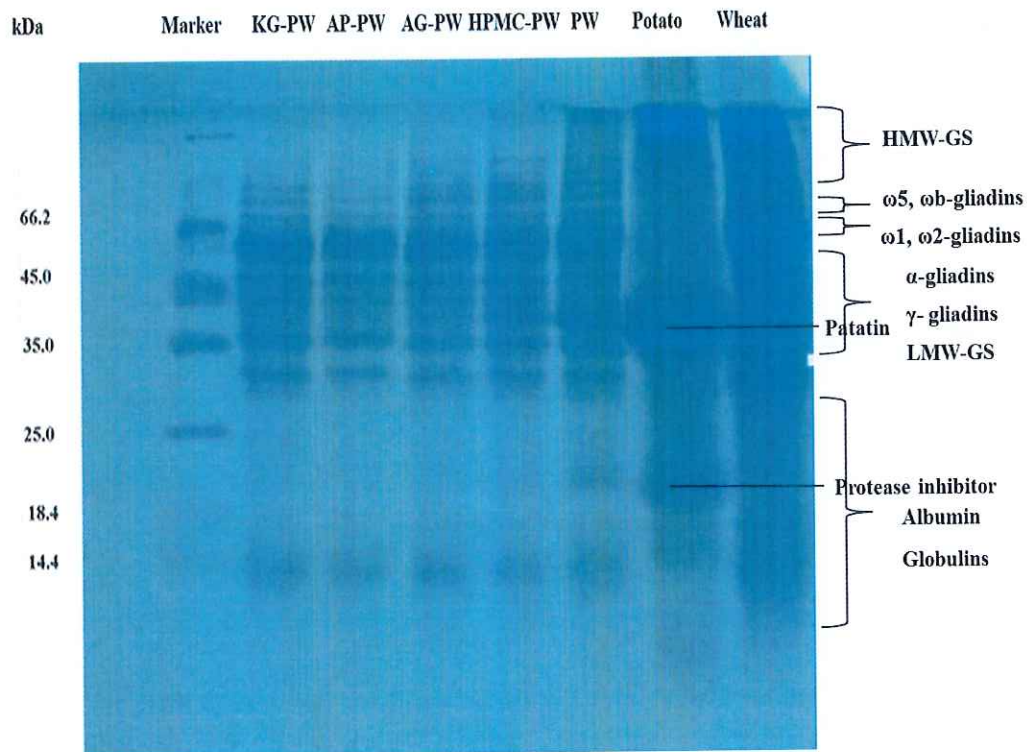
Samples		Hardeness(N)	Springiness	Chewinness(N)
Wheat	0h	19.23±1.25de	0.90±0.01a	7.84±1.34f
	24h	31.02±1.44c	0.93±0.01a	13.71±0.73d
	48h	46.58±3.04a	0.94±0.01a	19.44±2.36abc
PW	0h	19.24±2.20de	0.90±0.01a	8.82±0.80ef
	24h	30.19±3.05c	0.94±0.01a	14.75±0.90d
	48h	38.56±2.35b	0.93±0.01a	17.43±1.14c
HPMC-PW	0h	16.76±1.40e	0.90±0.01a	8.61±0.43ef
	24h	31.02±1.44c	0.91±0.01a	13.99±0.54d
	48h	46.58±3.04a	0.91±0.01a	19.84±1.10ab
AP-PW	0h	17.65±1.70de	0.92±0.01a	8.89±0.76ef
	24h	20.73±1.72d	0.91±0.02a	9.56±1.20ef
	48h	33.48±1.35c	0.93±0.01a	14.75±0.48d
AG-PW	0h	16.56±0.92e	0.91±0.02a	9.09±2.28ef
	24h	20.54±0.64d	0.93±0.01a	10.02±0.21e
	48h	30.08±0.39c	0.94±0.01a	13.42±0.23d
KG-PW	0h	20.33±3.78d	0.91±0.02a	9.77±2.37ef
	24h	40.54±1.39b	0.93±0.01a	17.95±0.65bc
	48h	50.06±2.44a	0.91±0.01a	21.00±1.20a

26 PW, potato flour-wheat flour; HPMC, hydroxypropylmethylcellulose; AP, apple pectin; AG, arabic gum; KG,  
 27 konjacglucomannan

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## Figure

- 1 **Figure captions**
- 2 **Figure 1**
- 3 SDS-PAGE micrographs of the dough obtained using different hydrocolloids
- 4 **Figure 2**
- 5 Thermal characteristics of breads obtained using different hydrocolloids, (a)  $T_p$ , (b)  $\Delta H$ .
- 6



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8 PW, potato flour-wheat flour; HPMC, hydroxypropylmethylcellulose; AP, apple pectin; AG, arabic

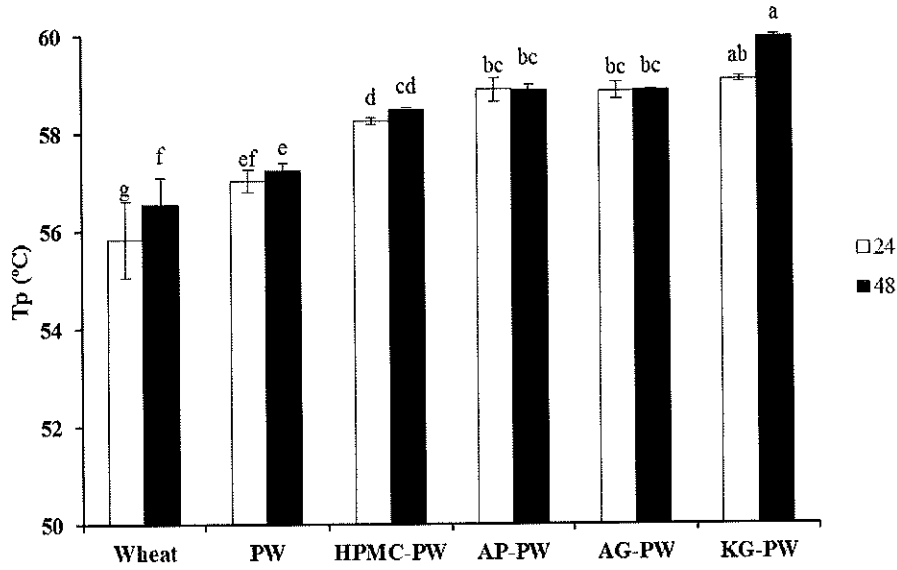
9 gum; KG, konjacglucomannan

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Figure 1

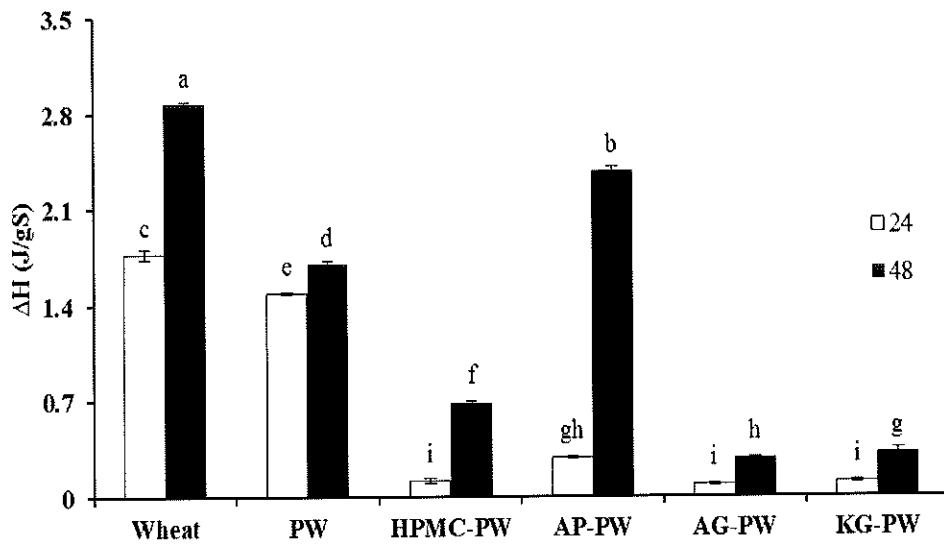
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12 (a)



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14 (b)



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16 Tp stands for peak temperature; ΔH stands for retrogradation enthalpy; PW, potato flour-wheat flour;

17 HPMC, hydroxypropylmethylcellulose; AP, apple pectin; AG, arabic gum; KG, konjacglucomannan

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Figure 2