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Development of a novel model dough based on mechanically activated cassava starch and gluten protein: Application in bread

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ABSTRACT

This study focused on the development of a novel model dough for leavened food production, which was obtained by blending gluten protein with damaged cassava starch (DCS) induced by mechanical activation (MA). The characteristics of model dough and the interaction between DCS and gluten were investigated, and the quality of bread made from the model dough was also evaluated. The results showed that both the addition of gluten and the increased damage of DCS could improve the strength of model dough. The damage of cassava starch prevented the formation of gluten network. The enhanced DCS-gluten interaction had an impact on the performance of dough, attributing to the interaction of hydrogen bonds between both of them. Moderate interaction was required to obtain the bread with desired quality, and MA for moderating structural damage to starch was an effective approach in promoting the interaction between starch and gluten protein.

1. Introduction

A wide variety of starch resources, including cassava starch, have long been considered to be the major calorie source for humans. However, reasonable and efficient utilization for developing them as staple foods is still a great challenge, partly because of the poor characteristics for food processing, such as the weak starch-water interaction with the low cold-water viscosity and poor solubility (Liu et al., 2019; Sun, Si, Xiong, & Chu, 2013). Cassava starch subjected to damage induced by mechanical activation (MA) to obtain damaged cassava starch (DCS) has been proven to improve the properties for food production by the increase in cold-water viscosity and solubility and changing the gelatinization property (Barrera et al., 2013; Zhang et al., 2013). Further study has found DCS to be able to provide suitable adhesion to form a starch-based model dough with desired viscoelasticity under appropriate mechanical damage level and water addition, which was successfully used to prepare crackers (Liu et al., 2019). Although the model dough has a viscoelastic quality close to that of cake flour, obvious differences exist in gluten-free formulation and chemical composition of starch. Compared with the wheat starch, cassava starch has relatively low amylose content, protein, and fat (Zhang, Mu, & Sun, 2018). Especially, amylose content can significantly influence the application performance. The starch-based model dough was not suitable to be utilized for the foods requiring higher dough strength, such as the leavened products and all kinds of noodles, which are traditionally made from the high-gluten or medium-gluten wheat flour. Especially for the leavened bread and steamed bread, they are in need of high elasticity and extensibility to expand big size and retain gas during the fermentation and baking.

Wheat gluten, a good quality and inexpensive vegetable protein, is an available by-product in the wheat starch industry (Day, Augustin, Batey, & Wrigley, 2006). It is generally recognized that gluten is mainly responsible for the unique viscoelastic properties in wheat flour dough by the formation of gluten network structure by interchain and intermolecular disulfide bonds (Day et al., 2006). Gluten protein is also believed to be safe for use as a dough strengthener, nutrient supplement, processing agent, stabilizing agent, and gelling agent (Asgar, Fazilah, Huda, Bhat, & Karim, 2010). Gluten is extensively used to fortify flours of lower protein content for the production of specialty breads or other bakery goods in North America and Europe (Day et al., 2006).

In the wheat flour, gluten protein and wheat starch coexist naturally, forming the integrity of starch-protein system. It has been reported that the proteins were adsorbed on the starch granules by non-

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ionic forces (Eliasson & Tjerneld, 1990). Reports on mixing the flour containing gluten and starch also confirmed that there was the interaction between both of them (Mohamed & Rayas-Duarte, 2003; Wang et al., 2017). Interaction between gluten and starch indicates a significant impact on the dough and its product quality (Ryan & Brewer, 2005). The integrity of the starch-protein system is nonexistent when exogenous gluten protein is added to the non-wheat starch for forming a composite model dough (Ryan & Brewer, 2005). In this system, gluten protein is separated from the mixed starch granules, so the interaction force of this blended flour is probably negligible (Eliasson & Tjerneld, 1990). Therefore, it is necessary to enhance the starch-protein interaction in the composite flour for modifying the dough quality and final product attributes.

Moderately damaged starch is reported to be beneficial for food production because of the effects to improve dough characteristic and promote fermentation activity in the wheat flour system (Liu et al., 2014; Ma et al., 2016). However, research involved the interaction between the dough components is scant. Damaged starch may form more reaction sites which are able to generate molecular force to change the structural characteristics of gluten (Ma et al., 2016). Compared to the raw starch, damaged starch generated partially pregelatinized starch along with incremental amylose and free hydroxyl groups in amorphous phase (Huang, Lu, Li, & Tong, 2007; Zhang et al., 2013). The gluten protein is rich in glutamine, and the hydrogen bonding would form between the amino groups of the glutamine and glucose molecules of the starch (Mohamed & Rayas-Duarte, 2003; Wang et al., 2017; Wang, Virgilio, Wood-Adams, & Heuzey, 2018). Gluten blended with damaged starch possibly facilitates hydrogen-bond interaction between them, which may influence the quality of their composite model dough and the resultant product. It may be desirable to add gluten protein to damaged starch for forming the composite model dough. It can be anticipated that the quality of dough can vary from different interaction between gluten protein and DCS, depending on the extent of damage.

Research on using a mixture of non-wheat starch with gluten protein for food processing is not reported to any significant extent. To the best of our knowledge, no study has been reported on the utilization of DCS-gluten protein composite flour for the development of foods. This study focused on development of a novel model dough composed of gluten protein with mechanically activated cassava starch for application in leavened foods, taking bread as an example. The objectives of this work were to investigate the effects of gluten protein and DCS with different damage levels on the qualities of the model dough and bread, and to evaluate the DCS-gluten interaction in the model dough.

2. Materials and methods

2.1. Materials

Cassava starch (10.9% moisture, 98.3% starch content in the dry basis) was supplied by Guangxi State Farms Mingyang Biochemical Group, INC (Nanning, China). Gluten protein (9.6% moisture, 85.8% protein, including 36.4% glutenin and 45.7% gliadin) was obtained from Henan Lotus Flour Co. Ltd. (Xiangcheng, China). Wheat flour (11.6% moisture, 12.8% protein, 2.96% damage level, 57.6% water absorption) for exclusive use of bread-making was procured from a local Wal-Mart supermarket. Starch Damage Assay Kit was purchased from Megazyme International Ireland Ltd. (Bray, Ireland). All other reagents and chemicals used were of analytical grade.

2.2. Preparation of DCS

DCS was obtained by MA using a customized ball mill (Huang et al., 2007; Liu et al., 2019). Cassava starch (100 g) was added to a stainless steel chamber with approximately 300 mL of mill balls (5 mm diameter), and was ball-milled at a speed of 300 r/min and a fixed temperature of 30 °C by circulating thermostatic water in the jacket of the chamber. The milling was terminated at the designated time (0, 5, 10, 20, and 30 min) and the samples separated from the balls were sealed for storage and analysis. The damage level, amylose, and viscosity profile of DCS were measured by the methods reported by Liu et al. (2019).

2.3. Molecular weight determination of DCS

The chain length distribution of DCS was determined by the method of Gel Permeation Chromatography (PL-GPC50) with a refractive index (RI) detector. The samples were dissolved with dimethyl sulfoxide (DMSO) with concentration of 0.1 mg/mL with a bit of LiBr added as the hydrotropy agent, and then were filtered through a 0.45-μm membrane filter. Injection volume of the samples was 100 μL, and the eluent flow rate was 1 mL/min.

2.4. Determination of farinographical properties

The wheat flour for leavened products usually contains approximately 13% gluten protein, so based on the protein content in gluten (85.8%), 15.0% gluten protein content was chosen to be equivalent. Gluten protein was mixed with DCS of different damage levels for farinograph test. Farinographical properties of the mixture were measured in accordance with the standard AACC Method 54-21 on a farinograph (Brabender, Duisburg, Germany). Water absorption was defined as the amount of water (on 14% moisture flour basis) to reach a dough consistency of 500 FU (Farinograph units). The water absorption, development time, stability time, and softening degree of the model dough were recorded to evaluate the farinographical properties of DCS.

2.5. Preparation of the model dough

Gluten protein (15.0%) was added to different damage levels to prepare model doughs. Both of them were well-blended, and water addition was based on water absorption obtained from the farinograph test. Uniform model doughs (20 g in a batch) were prepared by mixing for 5 min. Meanwhile, wheat flour dough was prepared for a comparative study when necessary. The dough samples were covered with a protective film and allowed to rest for 30 min before further analysis. The fermentative model dough incorporated 1% of dried yeast power (Swallow Brand, France), fermenting at 30 °C (80% relative humidity) for 90 min.

2.6. Determination of free sulfhydryl

The free sulfhydryl content was determined according to a previous study (Liu, Wang, Hou, Huang, & Zhang, 2017) with some modification. Gluten protein was carefully washed out from the composite model dough. Next, gluten samples were frozen at −18 °C for 2 h and then dried in a vacuum freeze-dryer at −42 °C for 24 h. The dried gluten samples were pulverized and stored at −18 °C for further determination. To exclude the effect of the residue starch, purity of gluten protein was also determined using the Kjeldahl Method. Gluten samples (100 mg, based on the purity calculation) were suspended in 5 mL of Tris-Gly buffer (0.086 M Tris, 0.09 M glycine, and 0.04 M EDTA, pH 8.0) containing 8 M urea. The suspension was vortexed for 5 min and centrifuged at 8000*g* for 10 min. Subsequently, the clear supernatant was added to 150 μL of Tris-Gly/urea solution and 50 μL of DTNB (4 mg/mL, dissolved in 0.09 M Tris-Gly buffer). After incubating at 30 °C for 30 min, the absorbance of the supernatants was read at 412 nm against the reagent buffer as the blank. Results were calculated against the glutathione standard calibration curve, which is presented in Fig. S1 (Supplementary file). Determination of the free sulfhydryl content for wheat flour samples was also carried out with the same sample processing and testing method.

2.7. SEM analysis

The hydrated model doughs were frozen at −18 °C for 2 h and then dried in a vacuum freeze-dryer at −42 °C for 24 h. The dried samples were cut into small slices (approximately 0.5 cm) by a knife. The slices were placed on an aluminum specimen holder by double-sided Scotch tape and coated with a thin film of gold using a sputter coater for measurement. Micromorphology of the model dough was observed at 3000× magnification by an S-3400N scanning electron microscope (Hitachi, Japan).

2.8. Differential scanning calorimetry (DSC) analysis

Thermal properties of DCS and the model dough were measured using a differential scanning calorimeter (DSC200PC, Netzsch, Germany), following the method described by Liu et al. (2017) with some modification. The model dough after vacuum freeze-drying was ground into powder and sieved with particle size smaller than 150 μm. The samples (4 mg, dry basis) with deionized water (20 mg) were rested in a sealed aluminum pan for 2 h, and then scanned from 40 to 150 °C at a rate of 10 °C/min. The thermal enthalpy value was calculated using Proteus software (Netzsch, Germany).

2.9. Analysis of textural properties of the model dough

The texture evaluation of the composite model doughs before and after fermentation was performed by texture profile analysis (TPA) using a texture analyzer (TA-XT plus, Stable Micvo System, UK) equipped with a P/0.5 aluminum cylindrical probe. The samples ($1.2 \times 1.2 \times 1.2\ cm^3$) were compressed to 50% of their original height. The pre-test, test, and post-test speeds were 3, 1, 5 mm/s, respectively.

2.10. Preparation of bread

Bread formulation was prepared as follows: DCS (42.5 g) with different damage levels, gluten protein (7.5 g), sugar (3 g), blend oil (1.5 g, Arowana Brand, China), salt (0.5 g), dried yeast power (0.5 g, Swallow Brand, France), and water addition in accordance with the water absorption. A mixture of DCS, gluten, sugar, and salt was added with water including the dissolved yeast powder. All ingredients were mixed evenly for about 5 min to form the rough dough. Blending was continued until the dough was uniform and smooth when the oil was added. To observe the morphology of dough expansion, the dough samples were not put to the mold but retained the native shape during the fermentation and baking. After that, the dough was divided into three pieces of the same weight and hand-rounded. After fermenting at 30 °C (80% relative humidity) for 90 min, the dough was baked in an oven (SEC-3Y, Guangzhou Sain-Mate Machinery Co. Ltd, China) at 185 °C for 20 min. Breads were cooled at room temperature, and then sealed for storage at 4 °C before measurement. Meanwhile, both the DCS and gluten protein were substituted by wheat flour to prepare bread for the controlled trial with the same formulation and procedure.

2.11. Bread quality evaluation

2.11.1. Texture analysis

Quality evaluation of the breads was carried out by measuring textural properties and specific volume. Textural properties of the breads were determined by a Texture Analyzer (TA-XT Plus, Stable Micvo System, UK) equipped with a P/100 aluminum cylindrical probe. Hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness were measured with pre-test speed of 3 mm/s, test speed of 1 mm/s, post-test speed of 5 mm/s, and the strain of 50%. The specific volume of the breads was determined by seed displacement according to the method reported by Wang, Rosell, and Benedito De Barber (2002).

2.11.2. Sensory analysis

The sensory quality of bread was evaluated using the method of quantitative descriptive analysis (QDA). The sensory parameters assessed were appearance, crust colour, crumb structure, crumb softness, crumb non-stickness, taste, and overall acceptance. Each parameter was assigned the score ranging from 0 to 10 on the basis of sensory quality. Sensory score criteria of the breads made from the model dough and wheat flour are presented in Table S1 (Supplementary file). A panel of twenty well trained judges was engaged in the scoring of bread.

2.12. Statistical analysis

All experiments were carried out at least in duplicate, and the significant differences among the samples were analyzed by One-way of Variance (One-way ANOVA) with Tukey's test ($p < 0.05$) using statistical of SPSS16 (SPSS Inc., USA).

3. Results and discussion

3.1. Physicochemical properties of DCS

Damage levels of the DCS samples with the MA time of 0, 5, 10, 20, and 30 min were determined to be 1.15%, 3.66%, 11.51%, 15.37%, and 18.65%, respectively. Corresponding to the increased damage level of the samples, the amylose contents of the DCS were 17.06%, 18.02%, 20.65%, 23.14%, and 25.72%, respectively. Besides, the viscosity profile and XRD analysis for the DCS with different damage levels are available in our previous study (Liu et al., 2019) with in-depth discussion.

Molecular weight is one of the important basic parameters for molecular structure of starch, which directly affects its physicochemical properties, such as the gelatinization temperature, viscosity characteristics, gel texture, and rheological properties. The data of the weight-average molecular weight (M_w) for DCS were determined to be 3.24×10^7, 3.18×10^7, 3.09×10^7, 2.87×10^7, and 2.45×10^7 g/mol, corresponding to the damage levels of 1.15%, 3.66%, 11.51%, 15.37%, and 18.65%, respectively. The M_w of DCS slowly decreased with the increasing damage level, indicating that MA destroyed the molecular chain of cassava starch and thus led to the decrease of its molecular weight.

3.2. Farinographical properties

Farinographical properties of the model dough with and without gluten protein are presented in Table 1, which were also compared with the dough made from wheat flour for evaluating dough characteristics. The samples without gluten protein at low damage levels of 1.15% and 3.66% were not measured because they did not form a suitable dough structure as other samples, which was also shown in a previous research (Liu et al., 2019). In wheat flour system, development time and stability time of farinographical properties reflected the resistance of mechanical force of the dough, both of which were closely associated with the strength of model dough (Liu et al., 2017). The softening degree, also called the mixing tolerance index, was an indicator for evaluating the degree of dough softening during a period of mixing (Li, Liu, Wu, Wang, & Zhang, 2016). Compared with the pure starch-based model dough, the addition of gluten protein significantly improved both the development time and stability time, but roughly decreased the softening degree. This suggests that gluten protein could enhance the strength of model dough, which was crucial for the fermented dough to inflate and retain gas.

Meanwhile, the damage level also had a great impact on farinographical properties of the model dough. With increasing damage level, water absorption of all the model dough significantly increased, which was mainly due to the formation of gel-forming materials in the outer layer of DCS rich in hydroxyl groups, allowing more water

Table 1
Farinographical properties of the starch-based doughs and their gluten-composite model doughs.

Samples	Water absorption (%)		Development time (min)		Stability time (min)		Softening degree (FU)	
	Without gluten	With gluten	Without gluten	With gluten	Without gluten	With gluten	Without gluten	With gluten
Control		57.6 ± 1.1[f]		3.70 ± 0.12[a]		4.51 ± 0.36[a]		89 ± 5[e]
DL 1.15%	–	64.1 ± 1.2[e]	–	1.60 ± 0.18[e]	–	2.54 ± 0.43[f]	–	239 ± 8[a]
DL 3.66%	–	69.6 ± 1.9[d]	–	1.63 ± 0.15[e]	–	2.65 ± 0.45[e]	–	162 ± 5[c]
DL 11.51%	72.1 ± 1.7[c]	75.2 ± 2.3[c]	0.66 ± 0.18[c]	1.75 ± 0.20[d]	0.72 ± 0.07[c]	2.76 ± 0.28[d]	261 ± 7[a]	154 ± 6[cd]
DL 15.37%	82.4 ± 1.5[b]	86.3 ± 1.8[b]	0.72 ± 0.08[b]	2.12 ± 0.11[c]	0.81 ± 0.10[b]	3.17 ± 0.51[c]	254 ± 12[b]	193 ± 9[b]
DL 18.65%	88.0 ± 2.2[a]	97.2 ± 1.6[a]	0.80 ± 0.12[a]	2.71 ± 0.16[b]	0.88 ± 0.13[a]	3.53 ± 0.30[b]	243 ± 10[c]	194 ± 8[b]

DL: damage level; "–": not detected; Control: wheat flour.
All values are the mean of triplicates. Values with the same letter in the same column do not differ significantly at $P < 0.05$.

interactions through hydrogen bonding (Liu et al., 2019). On the other hand, the damaged starch granules with the rough surface and destructive crystal structure allowed more water to penetrate into deep effortlessly. The development time and stability time significantly increased with the increasing damage level regardless of the addition gluten protein or not. Softening degree of the model dough was fluctuant, with higher values suggesting weaker strength of the dough. Besides the softening degree, the effect of damage level on farinographical properties of the model dough is essentially in agreement with the similar researches in the wheat flour (Barrera et al., 2013; Ma et al., 2016). However, comparing the model dough with wheat flour system, farinographical properties exhibited obvious differences. The development time and stability time of model dough were smaller, and the softening degree was larger. These results reflect that the gluten protein blended with exogenous cassava starch developed the relatively weak gluten strength. The variation of the farinographical parameters tended to be closer to that of the wheat dough, indicating that the damaged starch played a role in improving the dough strength. This is possibly attributed to the stronger interaction between damaged starch granules and gluten protein in the hydrated model dough. Farinographical properties indicate both the DCS and gluten protein made a contribution to improve the quality of the model dough.

3.3. Free sulfhydryl content

Changes in free sulfhydryl content are considered to be a persuasive indicator of the variation in disulfide bonds, which is directly related to the gluten network structure in gluten protein important for wheat flour dough to maintain appropriate dough characteristics (Liu et al., 2017; Shewry & Tatham, 1997). The free sulfhydryl contents of the gluten from the model dough were 27.8, 31.5, 35.9, 41.6, and 43.1 $\mu mol \cdot g^{-1}$, corresponding to the DCS with the damage level of 1.15%, 3.66%, 11.51%, 15.37%, and 18.65%, respectively, and the free sulfhydryl in wheat flour was 56.1 $\mu mol \cdot g^{-1}$. All the model doughs were added with the same content of gluten protein, and the total sulfhydryl content of the gluten from the model dough was 63.7 ± 2.6 $\mu mol \cdot g^{-1}$. Generally, the lower the content of free sulfhydryl, higher is the extent of formation of disulfide bonds. There were different contents of disulfide bonds, indicating the influence of damage level of DCS on the formation of gluten network. Therefore, this indicates that the damaged starch in the model dough prevented the gluten protein from forming disulfide bonds, relevant to the damage level. Some dietary fibers, such as barley β-glucan and some kind of hydrocolloids, are known to interfere with the formation of the gluten network. This effect has been attributed to competing with protein for water absorption because of the formation of gluten network requiring lots of water. Similarly, damaged starch in the wheat flour with high water absorption tended to compete with gluten protein for water (Barrera, Pérez, Ribotta, & León, 2007), which may also explain the phenomenon in this study (Nawrocka, Szymańska-Chargot, Miś, Wilczewska, & Markiewicz, 2017a, 2017b) verified that dietary fibre polysaccharides not only competed for water with gluten proteins but also interacted with them based on the formation of gluten-pectin mixtures by the thermal analysis. According to the farinographical properties of the model dough, the addition of gluten protein increased the strength of the model dough in direct ratio to the damage level of DCS, while the formation of gluten network based on disulfide bond content was inversely proportional. This discrepancy possibly implies that DCS exhibited an important influence on the characteristics of the model dough, and there might be the interaction between DCS and gluten protein.

3.4. SEM analysis

The morphologies of the DCS with different damage levels and the structures of their corresponding dough without the addition of gluten protein were investigated in a previous research (Liu et al., 2019). The granules of DCS were able to cohere together to form a starch-based dough structure when damage level was not less than 11.51% in the presence of a certain amount of water. In this study, gluten protein was blended with the DCS with different damage levels to obtain composite model doughs, and their morphologies were observed by SEM, as shown in Fig. 1. With increasing damage level of the DCS, the model doughs exhibited various microstructures. For the model dough with raw starch (Fig. 1a), the gluten network structure could be clearly observed. The components of starch granules and gluten protein did not glue together tightly as well as the starch granules in this model dough. The isolated components allowed gluten protein to relatively freely aggregate to form the gluten network structure. For the DCS with higher damage level in the model dough (Fig. 1b and c), gluten protein became more closely connected to the surface of starch granules, and the starch granules also mutually adhered. Although no continuous gluten network was observed, an interpenetrating network structure appeared, which was a net synergistic effect based on the relatively weak gluten network and strong network structure of the starch-based dough from adhesive damaged starch granules. This structure resulted in the more compact dough than the model dough of raw starch and stronger interaction among the dough fractions. When the damage levels of DCS reached 15.37% and 18.65%, gluten protein almost integrated with the outer of DCS, tending to form very tight composite doughs. The close-contact dough fractions probably generated some forces of interaction, and apart from this, space steric hindrance from the bonding of starch granules might also have an effect on the gluten protein crosslinking.

The study on farinographical properties showed that the adhesion between the dough fractions was very much related to the characteristics of dough, depending heavily on the damage level of the DCS. However, appropriate characteristics of dough were required for the production of leavened products. The dough with too loose structure (Fig. 1a) may be unfavorable for gas retention during the fermentation and baking. However, the dough with too compact structure (Fig. 1e) may hinder the gas expansion, leading to a lower volume of bread.

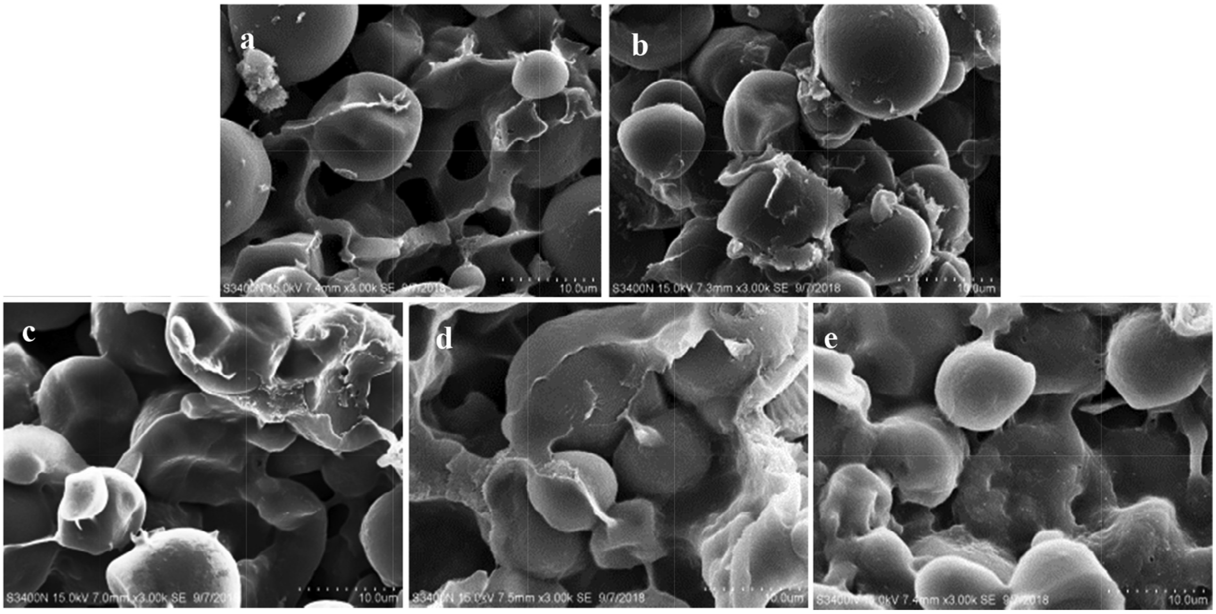

Fig. 1. SEM micrographs (3000 ×) of the model doughs obtained with gluten and different damage levels of DCS: (a) 1.15%, (b) 3.66%, (c) 11.51%, (d) 15.37%, and (e) 18.65%.

3.5. DSC analysis

Thermal properties of DCS with different damage levels and their corresponding gluten composite model doughs are shown in Table 2, including onset temperature (T_0), peak temperature (T_p), conclusion temperature (T_c) and thermodynamic enthalpy (ΔH). For all the samples, two endothermic peaks were detected, labeled as Peak 1 and Peak 2, respectively. Peak 1 is the melting of double-helical crystallite of amylopectin and Peak 2 is the vaporization of water (Huang et al., 2007). For the Peak 1 of DCS without gluten, all the values of temperature and ΔH showed a decreasing tendency with the increasing of damage level, which is consistent with a previous study (Barrera, León, & Ribotta, 2012; Morrison, Tester, & Gidley, 1994). This is attributed to the increasing destruction of crystalline structure induced by MA. The similar study on wheat starch by Barrera et al. (2012), Morrison et al. (1994) reported the same decrease for ΔH, but showed first increased and then decreased on T_0 at the high moisture addition with the increasing damage level. For the Peak 2 of DCS without gluten, the values of temperature and ΔH showed slight increase with the increasing damage level. Water evaporation and migration may contribute this trend, based on the increased interaction between the damaged starch and water (Liu et al., 2019).

Compared with the DCS samples, almost all of thermodynamic onset temperature and enthalpy of the composite model doughs increased with the increase of damage levels for both Peak 1 and Peak 2. The ΔH values for the composite model doughs were higher than those of the DCS that presented constant decrease, which indicated the existence of the interaction force between DCS and gluten. This result was also probably because that DCS facilitated the formation of hydrogen bonding in the outer layer of hydrous starch granules, which interacted with amino acid groups from gluten protein.

3.6. Textural properties of the model dough before and after fermentation

Textural properties of the composite model dough are usually connected with the final product quality, and the results are shown in Table 3. Due to focusing on leavened food production, the model doughs before and after fermentation were investigated. According to Table 3, there were significant differences on texture properties among different damage levels. The hardness and adhesiveness of the model doughs with or without fermentation were much higher than those of the control (wheat flour dough). However, the corresponding data on springiness, cohesiveness, and resilience of the control were within the range of those of the model dough. On the whole, the results of the model doughs with cassava starch at damage level of 11.51% were similar to the control. Based on Table 3, it can be concluded that the

Table 2
Thermal properties of the DCS and their gluten-composite model doughs.

Samples	Peak 1				Peak 2			
	T_0 (°C)	*Tp* (°C)	*Tc* (°C)	ΔH (J/g)	T_0 (°C)	*Tp* (°C)	*Tc* (°C)	ΔH(J/g)
DL 1.15%	55.4 ± 0.8^{d}	65.3 ± 0.5^{c}	77.7 ± 0.4^{c}	13.6 ± 1.5^{e}	100.1 ± 0.4^{d}	110.5 ± 0.4^{f}	116.9 ± 0.7^{f}	450.1 ± 5.6^{j}
DL 3.66%	54.6 ± 0.6^{d}	64.5 ± 0.8^{c}	75.3 ± 0.6^{d}	11.9 ± 1.0^{f}	100.4 ± 0.3^{d}	112.7 ± 1.1^{e}	120.6 ± 0.8^{e}	454.3 ± 9.0^{i}
DL 11.51%	52.3 ± 0.5^{e}	61.8 ± 0.6^{d}	73.1 ± 0.8^{e}	8.2 ± 1.2^{g}	100.9 ± 0.4^{d}	117.5 ± 0.5^{d}	127.1 ± 1.0^{d}	462.8 ± 5.6^{h}
DL 15.37%	50.8 ± 0.7^{f}	61.0 ± 0.5^{d}	72.5 ± 0.7^{e}	6.1 ± 0.9^{h}	101.5 ± 0.9^{cd}	119.4 ± 0.6^{c}	135.8 ± 0.9^{b}	476.7 ± 8.1^{g}
DL 18.65%	47.5 ± 1.0^{g}	59.1 ± 0.7^{d}	70.4 ± 0.5^{f}	4.3 ± 0.6^{i}	102.1 ± 0.8^{c}	120.1 ± 0.8^{c}	136.5 ± 1.1^{b}	483.2 ± 6.0^{f}
DL 1.15%-G	67.1 ± 0.7^{c}	76.3 ± 0.6^{b}	86.4 ± 0.7^{b}	18.4 ± 1.1^{d}	100.6 ± 0.3^{d}	120.8 ± 1.0^{c}	138.3 ± 1.3^{a}	523.6 ± 6.4^{e}
DL 3.66%-G	68.6 ± 0.8^{b}	75.7 ± 1.0^{b}	87.8 ± 1.2^{b}	19.1 ± 1.6^{d}	101.4 ± 0.7^{cd}	121.0 ± 0.9^{c}	130.6 ± 0.9^{c}	548.1 ± 5.8^{c}
DL 11.51%-G	69.8 ± 1.1^{b}	76.5 ± 1.0^{b}	88.3 ± 0.8^{a}	22.5 ± 2.0^{b}	102.0 ± 0.6^{c}	121.7 ± 1.0^{c}	128.1 ± 0.8^{d}	582.2 ± 10.1^{c}
DL 15.37%-G	71.2 ± 1.0^{ab}	77.2 ± 0.8^{a}	88.7 ± 0.9^{a}	28.6 ± 1.8^{b}	103.7 ± 0.8^{b}	124.2 ± 1.1^{b}	128.7 ± 1.2^{d}	638.5 ± 7.6^{b}
DL 18.65%-G	72.5 ± 0.9^{a}	77.6 ± 1.1^{a}	88.1 ± 1.3^{a}	30.6 ± 1.5^{a}	105.8 ± 0.9^{a}	125.9 ± 0.8^{a}	129.5 ± 1.0^{c}	670.1 ± 4.7^{a}

DL: damage level; G: gluten.
All values are the mean of triplicates. Values with the same letter in the same column do not differ significantly at $P < 0.05$.

Table 3
Textural properties the composite model doughs before and after fermentation.

Samples	Hardness		Adhesiveness	Springiness	Cohesiveness						
	BF	AF		BF	AF	BF	AF	BF	AF	AF	
Control	108.30 ± 8.2[e]	95.63 ± 7.30[f]	4.66 ± 0.8[f]	8.75 ± 1.2[e]	0.44 ± 0.1[d]	0.41 ± 0.0[d]	0.51 ± 0.1[b]	0.48 ± 0.0[b]	0.10 ± 0.00[c]	0.09 ± 0.00[c]	
DL 1.15%-G	260.01 ± 9.7[a]	220.08 ± 10.2[a]	35.67 ± 4.7[c]	51.84 ± 5.3[b]	0.97 ± 0.2[a]	0.86 ± 0.1[a]	0.60 ± 0.1[a]	0.53 ± 0.1[a]	0.06 ± 0.00[e]	0.06 ± 0.00[d]	
DL 3.66%-G	162.52 ± 8.6[c]	141.65 ± 9.1[d]	23.63 ± 2.4[d]	36.03 ± 2.8[c]	0.96 ± 0.1[a]	0.83 ± 0.2[a]	0.53 ± 0.0[c]	0.46 ± 0.2[b]	0.07 ± 0.00[e]	0.07 ± 0.00[d]	
DL 11.51%-G	142.37 ± 5.3[d]	130.21 ± 7.4[e]	10.97 ± 3.3[e]	28.61 ± 3.6[d]	0.41 ± 0.0[d]	0.46 ± 0.1[d]	0.41 ± 0.1[d]	0.40 ± 0.0[c]	0.09 ± 0.01[d]	0.08 ± 0.01[cd]	
DL 15.37%-G	161.49 ± 7.2[c]	155.82 ± 6.8[c]	68.86 ± 6.3[b]	52.47 ± 4.2[b]	0.54 ± 0.0[c]	0.63 ± 0.0[c]	0.49 ± 0.1[b]	0.46 ± 0.1[b]	0.13 ± 0.02[b]	0.12 ± 0.01[b]	
DL 18.65%-G	176.35 ± 8.1[b]	172.4 ± 5.6[b]		87.27 ± 8.2[a]	68.35 ± 3.1[a]	0.67 ± 0.1[b]	0.68 ± 0.1[b]	0.61 ± 0.0[a]	0.57 ± 0.1[a]	0.17 ± 0.01[a]	0.16 ± 0.02[a]

DL: Damage level; G: gluten; Control: wheat flour; BF: Before fermentation; AF: After fermentation.
All values are the mean of quintuplicates. Values with the same letter in the same row do not differ significantly at $P < 0.05$.

composite model doughs were no match for the wheat flour dough. To reach the level of conventional wheat flour, modification of dough properties could be indispensable. Reports have shown that some hydrocolloids, such as guar gum and xanthan gum, revealed an improvement in the network strength and elasticity of the gluten-free dough (Cai et al., 2016; Motta Romero, Santra, Rose, & Zhang, 2017; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). Sourdough fermentation with lactic acid bacteria was proven to be an effective measure to make the dough included maize flour more cohesive, soft, and less elastic (Falade, Emmambux, Buys, & Taylor, 2014) These methods could be also suitable for the improvement of these composite model doughs.

3.7. Bread quality evaluation

The quality of bread prepared by the model dough and wheat flour dough was investigated by textural properties, the specific volume, sensory quality and the morphology, as shown in Fig. 2 and Table 4. Obviously, damage level of starch negatively correlated with the specific volume of the bread, which was consistent with the similar study in the wheat flour system (Barrera et al., 2007). The bread made from the raw cassava starch model dough (Fig. 2A) was macroporous with relatively higher specific volume, and its shape was collapsed and scraggly. This morphology of bread was due to overexpansion of the model dough but poor capacity for gas retention, which was related to the network structure of gluten protein and the relatively incompact dough structure. The breads made from the model dough with damage levels of 3.66% (Fig. 2B) and 11.51% (Fig. 2C) had relatively suitable expansion ability and appearance, with the specific volume and shape close to the control sample (wheat flour bread). A lower specific volume for the model dough with higher damage levels of starch was observed, perhaps because the poor expansion resulted from the weak gluten network and the strong interaction between the gluten protein and the DCS (Fig. 2D and E).

As seen from Table 4, the breads made from the model dough with increasing damage level of DCS showed the ever-decreasing hardness, gumminess, and chewiness, but overall increased cohesiveness and almost unvaried springiness. The textural properties of the breads were closely related to dough viscosity, starch amylose/amylopectin content, and gluten protein aggregation (Alvarez-Jubete, Auty, Arendt, & Gallagher, 2010). Based on previous research (Liu et al., 2019), DCS with the higher damage level had the bigger water absorption, dough viscosity, and amylose content. The decreasing bread hardness, gumminess, and chewiness might be attributed to the increasing water absorption and dough viscosity in the model dough by retaining more moisture in the bread. The changes in the cohesiveness of bread were possibly associated with both the increasing water absorption and amylose content. Springiness of breads was the comprehensive result of the protein aggregation, interaction between starch and gluten, and water retention. Sensory analysis (Table 4) of the breads further indicated the difference of bread quality from different composite model doughs. Breads with DCS of moderate damage levels (3.66% and 11.51%) exhibited the favorable overall acceptance, in spite of being slightly inferior to the control. Bread with original cassava starch (damage level 1.15%) showed poor appearance, crumb structure, and softness, which were consistent with the photographs of the morphology and hardness. Bread with DCS of high damage levels (15.37% and 18.65%) showed inferior crumb structure, big stickiness, and taste, resulting in inferior quality. It was worth noting that the adhesiveness of the breads with damage levels of 15.37% and 18.65% was detected by texture analysis, which was in agreement with the sensory analysis with big crumb stickiness. This result led to undesired mouthfeel unsticky to teeth, owing to the DCS with too large water absorption and excessive retention ability. Although there were slight differences between the breads made from the model dough and wheat flour dough, it was obvious that DCS improved the quality of bread on some textural

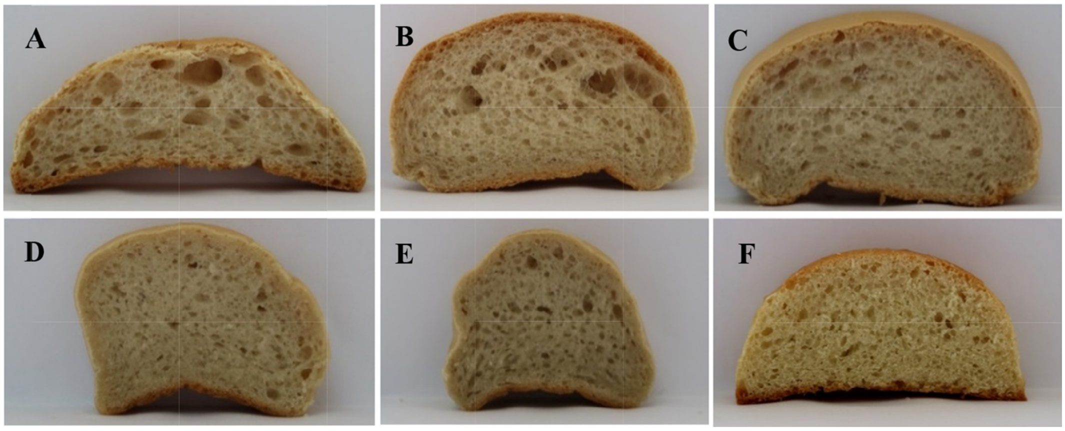

Fig. 2. Photographs of the morphology of the breads made from the model doughs with different damage levels of DCS: (A) 1.15%, (B) 3.66%, (C) 11.51%, (D) 15.37%, and (E) 18.65%, and from (F) the wheat flour dough.

Table 4
Textural properties, specific volume and sensory analysis of the bread prepared by the composite model doughs.

	Control	DL1.15%-G	DL3.66%-G	DL 11.51%-G	DL15.37%-G	DL 18.65%-G
Specific volume	2.18 ± 0.12^{b}	2.34 ± 0.23^{a}	2.23 ± 0.16^{b}	2.20 ± 0.10^{b}	1.25 ± 0.13^{c}	1.17 ± 0.08^{c}
Texture						
Adhesiveness	–	–	–	–	2.12 ± 0.42^{b}	3.28 ± 0.86^{a}
Hardness	9455 ± 38^{e}	26331 ± 26^{a}	20302 ± 31^{b}	16009 ± 43^{c}	12086 ± 29^{d}	8900 ± 34^{f}
Springiness	0.91 ± 0.07^{c}	0.95 ± 0.10^{a}	0.92 ± 0.08^{bc}	0.93 ± 0.05^{b}	0.94 ± 0.07^{a}	0.95 ± 0.06^{a}
Cohesiveness	0.58 ± 0.04^{c}	0.60 ± 0.03^{c}	0.66 ± 0.04^{b}	0.70 ± 0.02^{a}	0.72 ± 0.03^{a}	0.73 ± 0.02^{a}
Gumminess	5507 ± 12^{f}	15661 ± 25^{a}	13602 ± 23^{b}	11606 ± 18^{c}	8489 ± 15^{d}	6478 ± 17^{e}
Chewiness	5030 ± 8^{f}	14801 ± 12^{a}	12459 ± 15^{b}	11049 ± 21^{c}	8065 ± 18^{d}	6047 ± 10^{e}
Sensory scores						
Appearance	9.1 ± 0.3^{a}	5.4 ± 0.7^{d}	8.3 ± 0.2^{b}	9.5 ± 0.2^{a}	7.6 ± 0.3^{bc}	7.0 ± 0.2^{c}
Crust colour	8.6 ± 0.1^{a}	8.1 ± 0.2^{b}	8.7 ± 0.3^{a}	8.5 ± 0.3^{a}	7.1 ± 0.4^{c}	6.8 ± 0.4^{c}
Crumb structure	8.9 ± 0.2^{a}	5.0 ± 0.4^{e}	7.6 ± 0.2^{c}	8.4 ± 0.3^{ab}	7.3 ± 0.2^{c}	6.7 ± 0.3^{d}
Crumb softness	8.2 ± 0.2^{b}	4.5 ± 0.3^{e}	6.8 ± 0.4^{d}	7.5 ± 0.4^{c}	8.8 ± 0.3^{a}	9.2 ± 0.2^{a}
Crumb non-stickiness	9.8 ± 0.1^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.1^{a}	6.2 ± 0.2^{b}	5.8 ± 0.3^{b}
Taste	8.8 ± 0.4^{a}	8.1 ± 0.7^{b}	8.3 ± 0.5^{b}	8.6 ± 0.3^{a}	7.1 ± 0.5^{c}	6.7 ± 0.2^{c}
Overall acceptance	9.0 ± 0.3^{a}	6.6 ± 0.4^{d}	8.2 ± 0.3^{b}	8.4 ± 0.2^{b}	7.5 ± 0.3^{c}	6.8 ± 0.3^{d}

DL: Damage level; G: gluten; SV: specific volume ($mL·g^{-1}$); Control: wheat flour; "–": not detected.
All values are the mean of quintuplicates. Values with the same letter in the same row do not differ significantly at $P < 0.05$.

properties and sensory quality.

For the wheat flour dough system, the gluten network with relatively continuous and compact structure is responsible for the stability of prepared doughs in the processing of products (Li et al., 2016). However, gluten protein in these model doughs did not form similar network structure like that of the wheat flour dough based on the SEM analysis. The DCS played an important role in improving the whole dough characteristics, due to the interaction between the DCS granules as well as between DCS granules and gluten protein.

3.8. Interaction between DCS and gluten protein

According to the above research results, there was a certain interaction among the components of the model dough that contained gluten protein, DCS, and water. Generally, interactions between proteins and polysaccharides may occur non-covalently and covalently (Wijaya, Patel, Setiowati, & Van der Meeren, 2017). In this study, since both the components were physically mixed, it is almost impossible to form covalently interaction. However, it was quite possible that the gluten-DCS interaction by non-covalently bonds did enhance the dough strength. A schematic diagram of DCS and its corresponding model dough based on different damage levels of starch and gluten protein is presented in Fig. S2 (Supplementary file). Starch is one of the crystalline solid materials, and the hydrogen bonding in the chain structure of raw starch is relatively strong. The crystalline structure of cassava starch was significantly destroyed by MA, resulting in breaking the intramolecular and intermolecular hydrogen bonds in the chain structure of starch. The hydrogen bonding in starch chains was increasingly and severely damaged with the increasing damage level. So the DCS induced by MA generated plenty of the free hydroxyl groups in the damaged portion (Huang et al., 2007).

Gluten protein is mainly composed of gliadins and glutenins, containing different polypeptides connected by peptide bonds, as well as intermolecular and intramolecular disulfide bonds (Asgar et al., 2010). According to the previous studies (Mohamed & Rayas-Duarte, 2003; Wang et al., 2017), gluten is rich in glutamine with amino groups, and hydrogen bonding could occur between the amino groups of the glutamine in gluten and the second or third free hydroxyl of the glucose, as shown in Fig. S2B. The sulphydryl groups of cysteine residues in the polypeptides of gluten protein were responsible for the formation of intramolecular and intermolecular disulfide bonds. The content of disulfide bonds decreased with the increasing damage level of DCS in the model dough, possibly because some cysteine residues formed hydrogen bonding with the glucose units. It is also possible that some gluten protein entered into the damaged starch granule through small cracks during the dough-making, causing the decreased in disulfide bonds. Meanwhile, some other protein residue subunits may occur through the similar non-covalently bound with hydroxyl groups in the DCS.

The gluten-DCS interaction depended on the damage level, which had a direct correlation with the qualities of both the model dough and its final product. Compared with the conventional wheat flour, this composite model dough required the starch with higher damage level to remedy the qualities of dough and final product. The schematic diagram (Fig. S2) of gluten-DCS interaction could explain how the model dough affected the final product quality. For the model dough with raw cassava starch that hardly possessed free hydroxyl, the gluten-starch interaction was relatively weak, and both of them separately existed with big void (Fig. S2A), leading to poor gas preservation and shape retention during the fermentation and baking. The model dough with slightly high damage level (between 3.66% and 11.51%) of DCS could form the relatively compact network structure based on both the adherence among DCS granules as well as with gluten protein (Fig. S2B) generating moderate interaction among dough fractions with the appropriate characteristics of the dough. The characteristics of this type of model dough were close to the wheat flour dough, which was verified by texture analysis, and the corresponding bread showed consistent result. For the model dough with the damage level of DCS at 15.37 and 18.65% (Fig. S2C) the most compact dough structure was related to the stronger interaction by hydrogen bonding, as well as the very weak gluten network structure, resulting in the poor expansion capacity during the fermentation. The model dough with more disulfide bonds possessed weaker strength, indicating that DCS played a crucial role in improving the characteristics of dough. Moderate interaction between gluten protein and DCS was required to obtain desired qualities of dough and the fermented product. MA for moderate structural damage of starch was an effective approach in improving the starch characteristic for bread production.

When the model doughs and the corresponding product made from DCS were compared with those from the conventional wheat flour, there are some drawbacks to be overcome, such as unsuited hardness, cohesiveness, and their related gumminess and chewiness for the dough and bread. Only the indispensable ingredients used for bread preparation in this work, formulation may need further optimisation to meet full consumer acceptability (Zhu, 2014). A variety of hydrocolloids (Mir, Shah, Naik, & Zargar, 2016; Bárcenas, O-Keller, & Rosell, 2009), including guar gum, hydroxy propyl methyl cellulose (HPMC), xantham gum, pectin, carboxymethylcellulose (CMC), food protein (Crockett, Ie, & Vodovotz, 2011), inulin (Rodriguez Furlán, Pérez Padilla, & Campderrós, 2015), etc., were proven to be effective in improving the bread texture characteristics. Further works will be required to investigate the food processability and application of DCS in more complex food matrices.

4. Conclusions

This study provides a new insight into the utilization of non-wheat flour to exploit leavened foods. The composite model dough prepared by blending DCS with gluten protein had unique dough characteristic and microstructure. The addition of gluten protein into the model dough could significantly improve the dough strength. Compared with the cassava raw starch, the damage of cassava starch exerted an important influence on the farinographical and texture properties of the model dough. DCS prevented the formation of gluten network depending on the damage level. The DSC results indicated the enhanced interaction between DCS and gluten protein with increasing damage levels of the DCS. Bread prepared with the moderate damage level of DCS had relatively acceptable texture characteristics, sensory quality, specific volume, and morphology. The DCS-gluten interaction was associated with the formation of hydrogen bonds between them. MA for moderating structural damage to starch was an effective approach in developing appropriate model dough for production of leavened foods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Notes

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125196.

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