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Changes in enzymatic activities and functionality of whole wheat flour due to steaming of wheat kernels

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Abstract
The effects of steaming wheat kernels on lipolytic degradation of resulting whole flour was studied by quantifying enzyme activities and lipid degradation products during storage. Lipase, lipoxygenase, polyphenol oxidase, and peroxidase activities were decreased by up to 81%, 63%, 22%, and 34%, respectively, as the time of steaming increased up to 90 s. Steaming had no effect on starch and gluten properties. Upon storage free fatty acids decreased with respect to time of steaming. Time of steaming did not affect lipid oxidation in flour; however, total carbonyls produced in dough made from stored flour were decreased with the increase in steaming duration. Thus, steaming wheat kernels prior to milling reduced lipase activity and consequently hydrolytic rancidity during storage without affecting starch and gluten fractions. Steam treatment did not affect oxidative rancidity in flour during storage, but did reduce oxidation once the flour was made into a dough.

Keywords: Lipase, Lipoxygenase, Flour storage, Free fatty acids, Rancidity

1. Introduction

Unlike refined wheat flour, which is essentially free of the bran and germ fractions, the functionality of whole wheat flour decreases rapidly upon storage due to the occurrence of enzymes present in the bran and germ

fractions that initiate lipolytic rancidity (O'Connor, Perry, & Harwood, 1992). Of the many enzymes in wheat bran and germ, lipase (triacylglycerol hydrolase, EC 3.1.1.3) and esterase (carboxyl ester hydrolases, EC 3.1.1.1), mainly present in the outer bran fraction of the wheat kernel, hydrolyze water insoluble and soluble esters yielding free fatty acids (FFA) that contribute to hydrolytic rancidity (Doblado-Maldonado, Pike, Sweley, & Rose, 2012; Goffman & Bergman, 2003). These FFA accumulate upon storage and are oxidized either non-enzymatically or enzymatically in hydrated flour by lipoxygenase (LOX, EC 1.13.11.12). Although, LOX is often added to bread formulations due to its ability to oxidize disulfide bonds (enhance loaf volume) and degrade carotenoid pigments (dough bleaching), it can have detrimental effects on flour quality by oxidizing polyunsaturated fatty acids arising from lipase activity in the presence of excess moisture during dough mixing (Delcros et al., 1998; Mann & Morrison, 1975). The hydroperoxide derivatives generated due to LOX activity undergo rearrangement and decomposition to yield secondary oxidation products including volatile compounds like hexanal and other ketones (Doblado-Maldonado et al., 2012). Products of hydrolytic and oxidative rancidity result in poor bread quality (Tait & Galliard, 1988; Zhang & Moore, 1999), production of bitter compounds (Bin & Peterson, 2016), and a decline in sensory properties (Hansen & Rose, 1996).

Besides lipase and LOX, the influence of polyphenol oxidase (PPO) and peroxidase (POD, EC 1.11.1.7) activities are important because they degrade hydrogen peroxide, which is an inhibitor of LOX, and they produce free radicals during oxidation that can attack unsaturated fatty acids and contribute to oxidative rancidity by promoting oxidation of FFA (Nicolas & Drapron, 1983). Like LOX, PPO and POD improve dough strength by crosslinking with gluten proteins or by cross linking gluten with polysaccharides, but can also contribute to oxidative rancidity (Takasaki, Kato, Murata, Homma, & Kawakishi, 2005; Matheis & Whitaker, 1984).

Due to the development of undesirable chemical changes in flour during storage, inactivation of enzyme activities may be an appropriate strategy to extend shelf life and maintain the functional properties of whole wheat flour. Previous studies have used different thermal processing methods including steaming, microwave heating, and passing through infrared and gamma radiation (De Almeida, Pareyt, Gerits, & Delcour, 2014; Rose, Ogden, Dunn, & Pike, 2008; Li et al., 2016; Jha, Kudachikar, & Kumar, 2013) to decrease lipolytic activities. Rose et al. (2008) reported a decrease in lipase activity of 93% and 96% when wheat bran was microwaved (1000 W) and steam treated for 60 s, respectively. Similarly, a reduction in lipase activity of 84% was observed when wheat grains were steam treated for 240 s (De Almeida et al., 2014). The residual lipase activity of wheat germ treated with

infrared (90 °C for 20 min) was found to be 18.02% (Li et al. 2016), whereas the lipase of wheat germ was inactivated by 31.2% when irradiated with 30 kGy gamma radiation (Jha et al., 2013). Although these studies have shown a decrease in lipase activity due to one or more thermal processing techniques, there is not much information on reaction products of lipolytic degradation on flours from steam treated grains during storage. Also, the steam treatment applied to grains may degrade starch (De Almeida et al., 2014) and denature gluten proteins (Prakash & Rao, 1999), which in turn may affect end use quality (Shin, Kim, & Kim, 2013) and dough properties of flour.

Given that most enzyme activity, especially lipase activity, is concentrated in the outer layers of the wheat kernel while the gluten proteins and starch are located within the kernel and protected by the bran layer, we hypothesized that steaming wheat kernels prior to milling would inactivate lipase (and other enzymes) and thus stabilize lipids during storage without damaging flour end-use quality. The objectives of this study were to (1) quantify lipase, esterase, LOX, PPO, and POD activities in whole wheat flour obtained from steam-treated grains and determine the effects of steaming on the functionality of flour; and (2) determine the changes in hydrolytic and oxidative rancidity during storage of steam-treated whole wheat flour compared with non-steam-treated flour.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals and reagents used in this study were: 3,4-dihydroxybenzene (catechol) (120-80-9, ACROS), 3-(*N*-morpholino) propane sulfonic acid (MOPS) (1132-61-2, ACROS), potassium phosphate (7778-7-0, Fisher Bioreagents), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (30931-67-0, Sigma), hydrogen peroxide (7722-84-1, Fisher Bioreagents) *n*-propanol (71-23-8, Fisher Chemical), dithiothreitol (DTT) (3483-12-3, Fisher Bioreagent), lactic acid (50-21-5, Fisher Chemical), hexane (110–54-3, Fisher Chemical), chloroform (67-66-3, Fisher Chemical), methanol (67-56-1, Fisher Chemical), 3,3′-Bis [*N*,*N*-*bis*(*carboxymethyl*)*aminomethyl*]-*o*-cresolsulfonephthalein tetra sodium salt (xylenol orange) (3618-43-7, ACROS), barium chloride (10326-27-9, Fisher Bioreagent), iron (II) sulfate (7782-63-0, Sigma), iron (III) chloride (10025–77-1, Sigma), 2-propanol (67-63-0, Fisher Chemical), triphenylphosphine (603–35-0, Sigma), 2,4-dinitrophenylhydrazine (2,4-DNP) (119–26-6, Sigma), and potassium hydroxide (1310-58-3, Fisher Bioreagents).

2.2. Sample preparation and treatment

Two commercial blends from different lots of hard winter wheat were obtained from Bay State Milling (Winona, MN), and were steamed in the lab. Wheat kernels were steamed for 90 s in 15 s intervals. For each time interval, 25 g of kernels were spread in an even layer over a 40 mesh (0.42 mm) standard testing sieve. The sieve containing the kernels was then covered and placed over a boiling water bath. The distance between the grains on sieve and water was approximately 8 cm. After steaming, the grains were sealed in a plastic bag and kept at room temperature overnight and then milled with a cyclone mill equipped with a 1mm screen (UDY, Fort Collins, CO, U.S.A). The milled whole wheat flours were re-packaged in plastic bags until analysis. Milled whole grain flour thus obtained was used to quantify enzyme activity assays, functionality, and evaluate changes in lipid rancidity after storing for 6 months at ambient conditions (room temperature enclosed in a plastic sample bag) (**Fig. 1**). The moisture content of grains and flours were measured according to a standard method (approved method 44-19.01; AACC International, 2017). All measured response variables were reported on a dry weight basis.

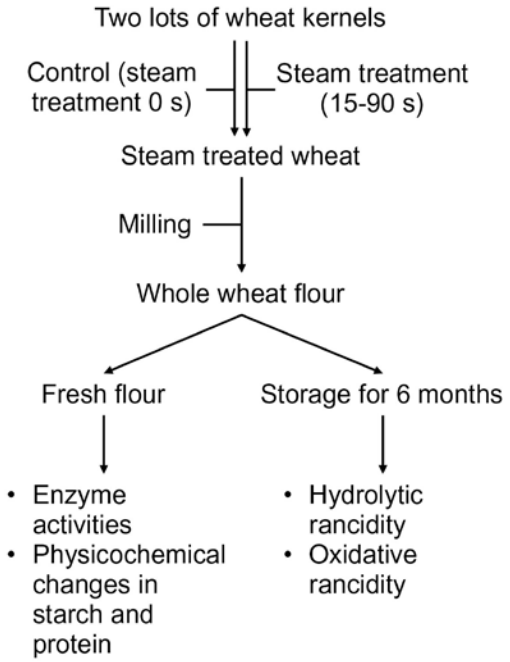

Fig. 1. Flow diagram of experimental design.

2.3. Enzymatic activities in fresh whole wheat flour

Lipase, esterase, and LOX activities were measured as described (Poudel, Bhatta, Regassa, & Rose, 2017).

PPO was measured as described (Anderson & Morris, 2001) with a few modifications. To 0.2 g of whole wheat flour, 1.5 mL of catechol in 0.05M MOPS buffer (pH 6.5) was added and vortexed. This was followed by incubation at room temperature (22 °C) for 1 h with continuous horizontal shaking at 200 rpm in a reciprocal shaking water bath (model 2872, Thermo Scientific). Afterwards, the tubes were centrifuged and absorbance was recorded at 410 nm. The change in absorbance was compared against the substrate absorbance reading. PPO activity was expressed as U/g, where 1 U is defined as the increase in an absorbance per minute. POD activity was measured as described (De Almeida et al., 2014). To 0.125*g* of whole wheat flour, 2.5 mL of 0.1M potassium phosphate buffer (pH 5.0) was added which was followed by shaking (150 rpm) at room temperature for 30 min and centrifuging at 538*g* for 10 min. The supernatant was separated and considered as a crude enzyme extract. To a separate tube 2.9 mL of 9.1mM ABTS, 0.1 mL of 0.3% (w/w) hydrogen peroxide, and 0.05 mL of crude enzyme extract were added. For the blank reading, enzyme extract was replaced with buffer. The absorbance readings were taken at 405 nm. POD activity was expressed as U/g, where U was defined as the increase in absorbance at 405 nm per minute of the reaction.

2.4. Functional properties of fresh whole wheat flour

Changes in thermal and pasting properties of starch were assessed using a differential scanning calorimeter (DSC) (Pyris 1, Perkin-Elmer Co., Norwalk, Connecticut, USA) and a rapid visco analyzer (RVA) (RVA-4, Newport Scientific, Australia) respectively. For DSC, the method described by Ratnayake, Otani, and Jackson (2009) was used with minor modifications. Briefly, 10 mg of flour was weighed into an aluminum pan (B016-9321, Perkin-Elmer) and 0.03 mL of deionized water was added. The pan was hermetically sealed and kept at room temperature for 4 h and then analyzed. Each experimental replicate was analyzed twice. Thermal properties of starch were described by onset (To), peak (Tp), and conclusion (Tc) temperatures, and enthalpy of gelatinization. Pasting profile using RVA was analyzed according to a standard method (approved method 76-21.01; AACC International, 2017).

In addition to starch, changes in extractability of gluten proteins were assessed. Protein fractionation was performed as described in Suchy et al. (2007). Briefly, 10 mg of flour was extracted with 1.8 mL of 50% (v/v)

n-propanol for 30 min at 25 °C with occasional vortex mixing. After centrifuging (13500*g*) for 5 min, the absorbance of the supernatant was recorded at 280 nm. This absorbance reading, 50PS, contained mainly gliadin. For the total soluble protein (TSP), the extraction process was similar to the 50PS fraction, except the solvent was 50% (v/v) propan-1-ol containing 0.2% (w/v) DTT and the extraction temperature was 55 °C. The difference between TSP and 50PS gave a measure of the amount of high molecular weight glutenin. The gliadin and glutenin fractions were expressed as the percentage of total extractable protein. Besides protein fractionation, solvent retention capacity (SRC) using lactic acid, which is related to gluten protein functionality, was done according to a standard method (approved method 56–11.02; AACC International, 2017).

2.5. Products of lipolytic degradation in stored whole wheat flour

FFA, conjugated dienes, peroxide value, and hexanal were measured on stored flour. FFA and conjugated dienes were measured as described (Doblado-Maldonado, Arndt, & Rose, 2013). Peroxide value was measured as described (Wrolstad et al., 2005, chap. D2) with a few modifications. To 0.5 g of flour, 7 mL of hexane was added and the sample was vortexed for 5 min. Five mL of the supernatant was transferred to round bottom flask and the hexane was removed under partial vacuum at 37 °C on a rotary evaporator. The extract was dissolved in 9.9 mL of chloroform: methanol (7:3, v/v) and then 0.05 mL of 0.01M xylenol orange and 0.05 mL of iron (II) chloride. Iron (II) chloride solution was prepared by the addition of barium chloride solution (0.4 g $BaCl_2{\cdot}2H_20$ in 50 mL water) to iron (II) sulfate solution (0.5 g $FeSO_4{\cdot}7H_2O$ in 50mL water) with constant stirring followed by the addition of 2 mL concentrated HCl. The barium sulfate precipitate was filtered using Whatman no. 1 filter paper and the resulting solution was stored in an amber bottle. The sample was incubated at room temperature for 5 min and then the absorbance was measured at 560 nm against a reagent blank. Peroxide value was expressed as mEq active oxygen/g of flour. The standard curve was constructed using varying concentration of 0.05 mL iron (III) chloride (0.05 g iron (III) chloride, 5 mL concentrated HCl, and 0.1 mL of 30% hydrogen peroxide per 50 mL total solution) dissolved in 9.9 mL of chloroform: methanol (7:3, v/v) and 0.05 mL of xylenol orange. The reagent preparation and absorbance reading were taken in subdued light.

The production of carbonyl compounds in dough made from stored flours were quantified as described (Endo, Li, Tagiri-Endo, & Fujimoto, 2001) with a few modifications. The dough was prepared by adding water (flour: water, 1:1). After thoroughly mixing the flour and water to facilitate the production of carbonyl compounds from the action of LOX on polyunsaturated

fatty acids released by lipase, the dough was rested for 2.5 h at room temperature. To the dough, 7 mL of 2-propanol was then added and vortexed for 5 min followed by centrifuging at 4713*g* for 7 min. Five mL supernatant and 5 mL of 2-propanol containing triphenylphosphine (0.4 mg/mL) were added in a separate tube and vortexed for 2 min which was referred as the sample solution. In a separate 15 mL glass tube, 1 mL of sample solution and 1 mL of 2,4-DNP were added to start the reaction. 2,4-DNP solution was prepared by dissolving 0.05 g 2,4-DNP in 100 mL 2-propanol containing 3.5 mL of concentrated HCl. The glass tubes were capped and incubated in water bath for 20 min at 40 °C. After cooling under running water, 8 mL of 2% potassium hydroxide dissolved in 2-propanol was added. The test tube was centrifuged for 5 min at room temperature and the absorbance reading of the upper layer was measured at 430 nm. The carbonyl values were expressed as U/g, where 1 U is defined as μ mole of carbonyl released per minute.

2.6. Data analysis

Data were analyzed using SAS software (version 9.4, SAS Institute, Cary, NC, USA). Mean Comparisons were done using Tukey adjustment at $P \leq 0.05$. Percentage reduction in enzymatic activities with respect to time of steaming were reported. Pearson's correlations were calculated to quantify the relationships among response variables.

3. Results and discussion

3.1. Moisture content in grains and flour

An increase in moisture content of steamed grains (measured 12 h after steaming) with respect to time of steaming was observed (Supplementary Table 1). In particular there was an increase of 5% moisture, from 8.8% to 13.7%, in wheat kernels steamed for 90 s compared with the control that was steamed for 0 s (i.e., not steamed). Since steamed grain samples were sealed immediately in a plastic bag to prevent moisture loss, difference in flour moisture content was expected in our study. Since the moisture content of even the samples steamed for the longest time (90 s) was not excessive, it would be interesting in the future to study how steaming could be used not only as a means of stabilizing whole wheat flour, but also as a means of delivering moisture to wheat kernels for tempering in preparation for milling.

An increase in moisture content up to 17% (90 s, control = 12.8%) was reported by Rose et al. (2008) following steaming of wheat bran; however,

they did not find any difference when moisture was measured after 24 h. The increase in moisture content of wheat grains with the extended time of steaming was expected and had been reported by De Almeida et al. (2014) previously. However, they found the time of steaming had no effect on flour moisture content after milling, which contrasted with our findings. In both studies, they have stored grains either on paper bag (Rose et al., 2008) or plastic trays (De Almeida et al., 2014) for a certain duration before milling, which may have caused loss of moisture from grains and hence no change was reflected in the flour.

3.2. Enzymatic activities in fresh whole wheat flour

Lipase (LA-O and LA-P), lipoxygenase, and esterase activities were decreased with an increase in time of steaming of grains (**Fig. 2**a and b). A decrease in lipase activity by 81% (LA-O), 97% (LA-P), esterase activity by 60%, and LOX activity by 64% were observed. The different reduction percentage of lipase activity using different substrate was expected, since we previously reported that these substrates were found to give different results for the same wheat samples (Poudel et al., 2017). Our results suggested that LA-O measured the lipase activity that was more stable to steam treatment than that measured with LA-P. The other possible reason for difference may be the isozymes measured using LA-P were right on the surface of kernel thus had more exposure to heat during steaming than the isozymes measured using LA-O.

PPO and POD activities were decreased slightly with extended steaming time up to 22% and 34%, respectively (Fig. 2c and d). De Almeida et al. (2014) found a decrease in lipase and POD activities of 75.3% and 90.2% respectively when the grains were steamed for 120 s. As POD enzyme is more heat stable than lipase enzyme (Cenkowski, Ames, & Muir, 2006; Bookwalter, Lyle, & Nelsen, 1991) the 90 s of steam time in our study appears enough to inactivate lipolytic enzymes with only slight effect on POD activity. Initially, an increase in PPO activity was observed due to steam treatment, which may be due to inactivation of a PPO inhibitor. Extended steaming then decreased PPO activity, presumably due to denaturation of the enzyme.

3.3. Functional properties of fresh whole wheat flour

From the DSC thermograms, a difference in onset temperature (To) of starch gelatinization was observed, however, there were no differences in conclusion temperature (Tc), peak temperature (Tp), and enthalpy of starch due to steaming of grains (**Table 1**). This implies that the initial stages of gelatinization were affected by the steam treatments, but after continued heating

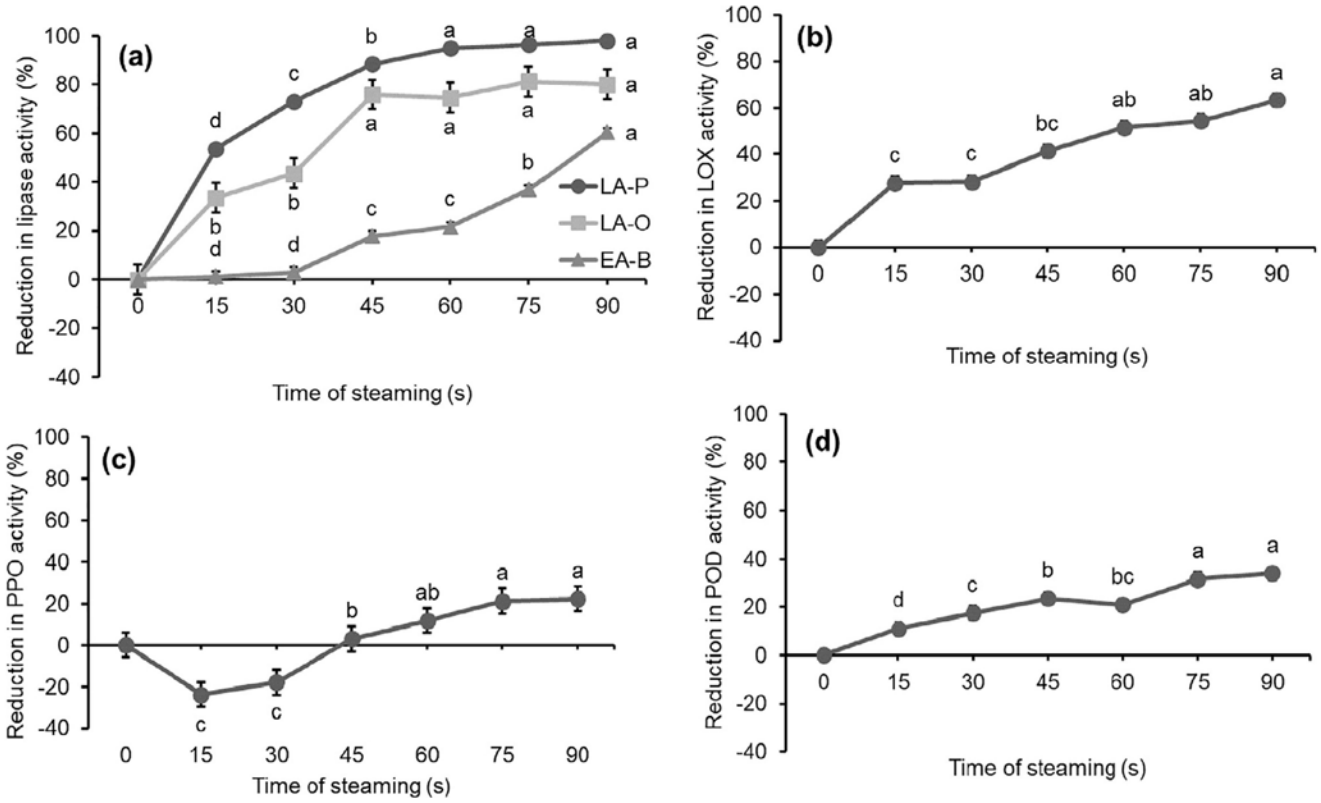

Fig. 2. Reduction in enzyme activities as a function of time of steaming: (a) lipase using p-nitrophenyl palmitate (LA-P), olive oil (LA-O), and esterase using p-nitrophenyl butyrate (EA-B) as substrates; (b) lipoxygenase (LOX); (c) polyphenol oxidase (PPO); and (d) peroxidase (POD); within each subfigure points labeled with different lower-case letters are significantly different at α = 0.05.

Table 1. Mean values of differential scanning calorimeter (DSC) gelatinization endotherms and rapid visco analyzer (RVA) pasting properties of untreated and steam-treated whole wheat flours.

	DSC				RVA						
Time of steaming (s)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	Peak viscosity (cP)	Trough viscosity (cP)	Break-down (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (min)	Pasting temperature (°C)
0 (control)	62.0bc	65.4	68.0	6.0	895	647	248	1452	806	5.3	89.2
15	62.2bc	65.2	68.1	5.9	888	628	259	1445	817	5.2	89.2
30	61.7c	65.1	68.0	6.3	887	650	238	1431	782	5.4	88.8
45	61.9bc	66.4	68.3	6.4	888	649	239	1424	775	5.3	89.2
60	62.9ab	66.0	68.9	5.9	892	633	260	1409	776	5.3	89.2
75	61.7c	65.2	68.3	6.6	888	620	268	1412	792	5.3	89.2
90	63.9a	66.9	69.8	5.9	884	608	276	1433	825	5.3	89.5
p-value	0.03	0.13	0.18	0.25	0.37	0.75	0.8	0.35	0.71	0.49	0.84

a. Means followed by different lower-case letters within a column are significantly different at α = 0.05.

all samples achieved similar properties. Although there were subtle differences in onset temperature, the differences appeared unrelated to the time of steaming. This indicated that the steaming treatment used in this study had minimal effects on starch gelatinization properties. This is in agreement with De Almeida et al. (2014), who also reported minimal effects of steaming wheat kernels on starch gelatinization. However, using a steam treatment of 170 °C for 4 min, gelatinization of wheat starch was observed (Hu, Wang, Zhu, & Li, 2017).

The pasting profile from RVA showed no differences for peak viscosity, breakdown, final viscosity, setback, peak time, and pasting temperature due to steaming (Table 1). Previous studies have shown a decrease in the peak, trough, and final viscosities due to steaming (Hidalgo, Brandolini, & Gazza, 2008; Hu et al., 2017). However, the steaming conditions applied in those studies were different ours. Hidalgo et al. (2008) used hulled kernels for steam treatment at varying temperature, time and pressure combination from 115 °C, 5 min, 1.8 bar up to 120 °C, 15 min, and 2.1 bar, whereas Hu et al. (2017) applied the steam treatment in soft wheat flours.

There were no differences in lactic acid SRC values and protein extractability due to steaming of grains (**Fig. 3**). Both results suggested no change in functional contribution of gluten protein with respect to non-steamed samples. In contrast to our results, denaturation of gluten and increase in dough stiffness were reported due to steam treatment of wheat flour (Prakash & Rao, 1999). However, the steam treatment was different in their study compared to ours. Prakash and Rao (1999) applied steam treatment on wheat flour up to 30 min, whereas wheat kernels were steamed only for 90 s in our study.

3.4. Products of lipolytic degradation in stored whole wheat flour

The accumulation of FFA in the stored flour was inversely related to the time of steaming with 2.2 and 8.8 μmol/g FFA liberated in flour made from wheat kernels steamed for 90 s and 0 s, respectively (**Fig. 4**). This was expected because the lipase activity was greatly reduced due to steaming of grains and evolution of FFA was a function of lipase activity over time (Rose & Pike, 2006). FFA in stored flour were highly correlated with the lipase activity of fresh flour (LA-O: $r = 0.95$, $p < 0.001$; LA-P: $r = 0.91$, $p = 0.002$).

Unlike FFA, steaming of grains did not affect the primary oxidation products, conjugated dienes and peroxide value, or the secondary oxidation product, hexanal (Fig. 4). When bran fractions were given steam treatment for 60 s, no effect of treatment was reported for conjugated dienes (Rose et al., 2008). The whole wheat flour was stored in low-density polyethene

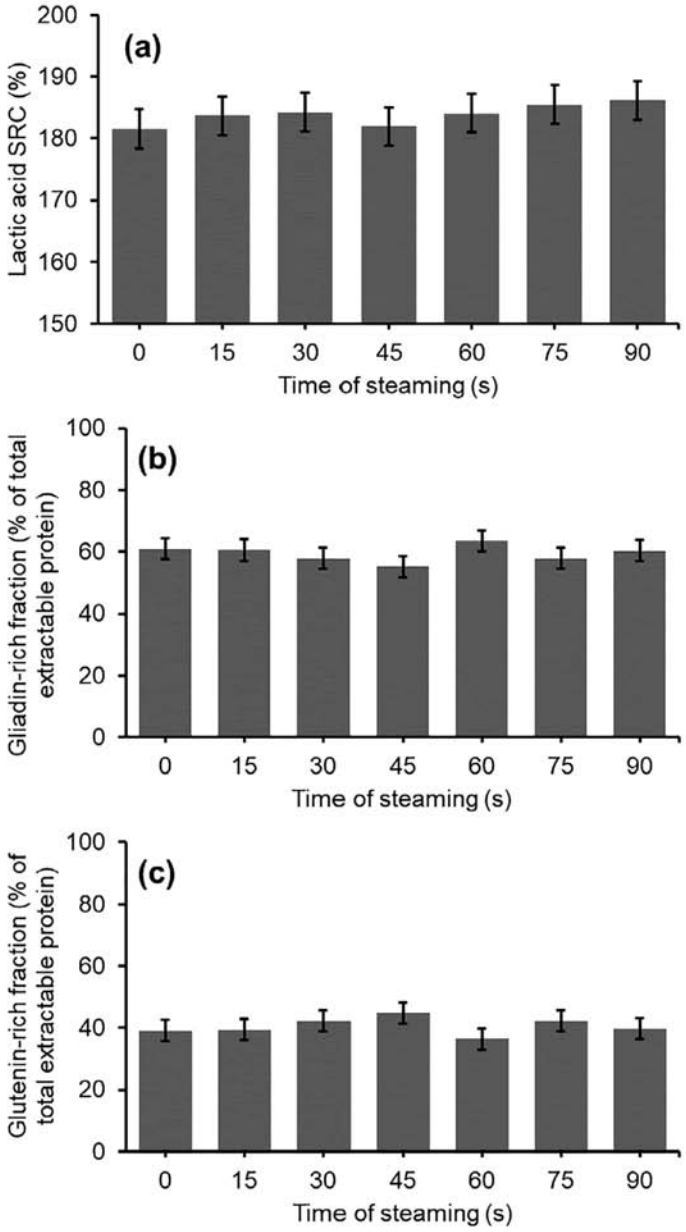

Fig. 3. Influence of steaming on gluten protein properties: (a) lactic acid solvent retention capacity (SRC); (b) extractability of gliadin-rich fraction; and (c) extractability of glutenin-rich fraction; within each subfigure no bars are significantly different at $\alpha = 0.05$.

(LDPE) plastic bags. These bags were not impermeable to oxygen (Evans & Quinton, 1978). Over a period of 6 months, the cumulative permeation of oxygen into the storage bag would have been significant. Furthermore, no effort was made to expel oxygen when sealing the bags for storage. Therefore,

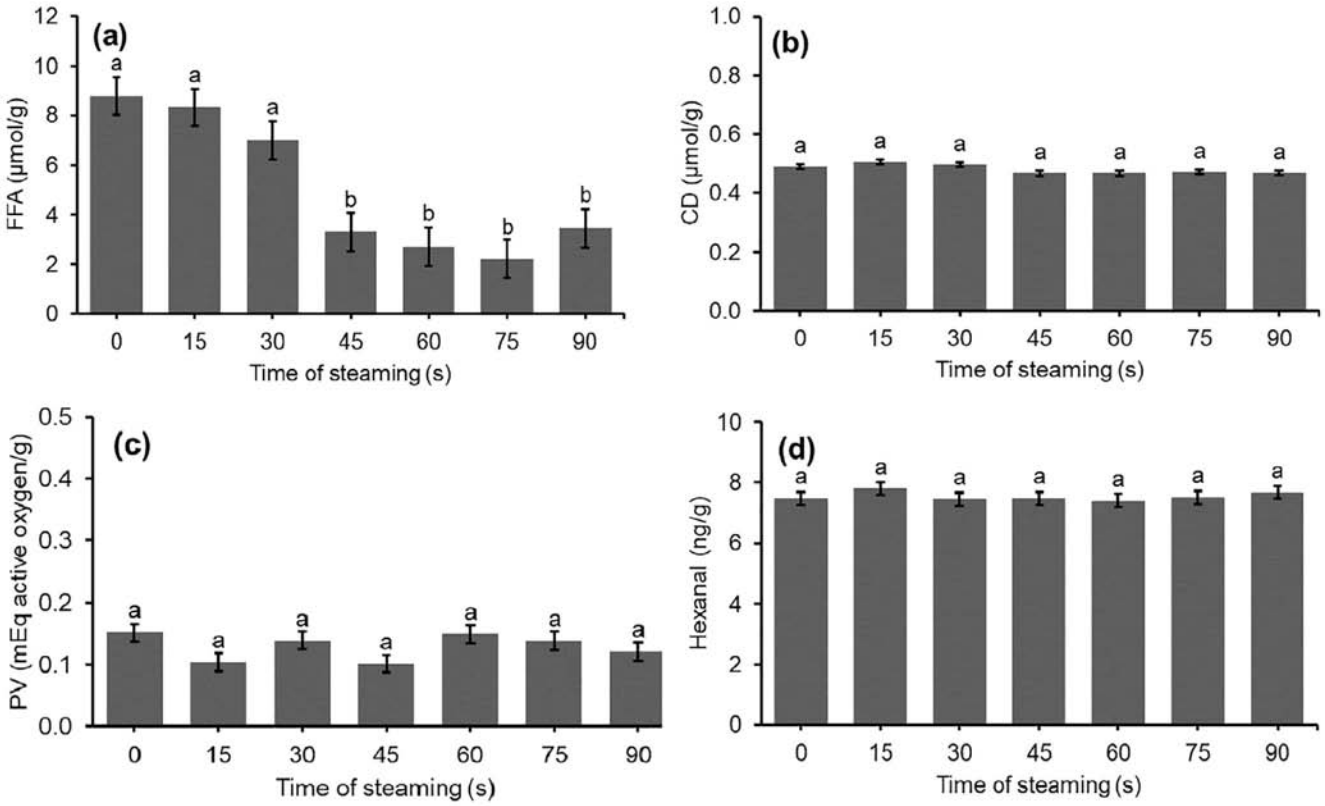

Fig. 4. Products of lipid degradation measured in stored whole wheat flours that were milled from untreated or steam-treated wheat: (a) free fatty acids (FFA); (b) conjugated dienes (CD); (c) peroxide value (PV); and (d) hexanal; within each subfigure bars labeled with different lower-case letters are significantly different at $\alpha = 0.05$.

we expect that low availability of oxygen was not the reason that we did not observe lipid oxidation during storage. The reason for no effect of steaming on oxidation products in our study may be due to short storage time, which did not provide enough time for oxidation in flour.

Although the stored whole grain flour did not show signs of lipid oxidation, we hypothesized that the stored flours that had higher accumulation of FFA would be more prone to oxidation once the flour was mixed with water (i.e., made into a dough). Therefore, we measured the change in carbonyl compounds, which are one of the major end products of lipid hydroperoxide breakdown, upon making the flour into a dough.

Dough made from the control flour had more carbonyls than the dough made from steam treated flours (**Fig. 5**). The production of carbonyls in the dough was correlated with the FFA concentration in the stored flour ($r = 0.81$, $p = 0.01$) and LOX activity in fresh flour ($r = 0.88$, $p = 0.001$). Therefore,

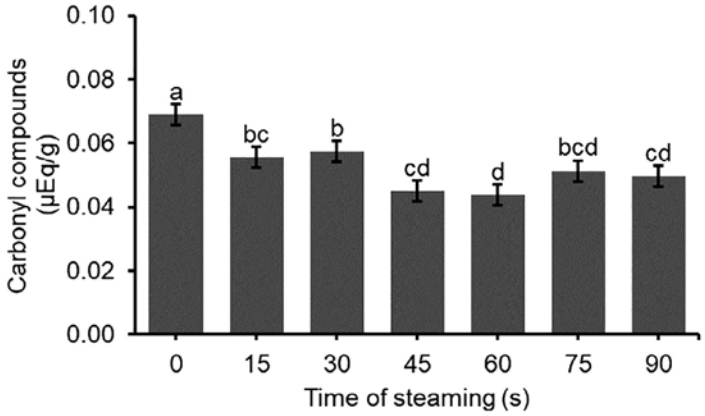

Fig. 5. Carbonyl compounds produced in dough made from stored whole wheat flours that were milled from untreated or steam-treated wheat; bars labeled with different lower-case letters are significantly different at $\alpha = 0.05$.

it appeared that the steam treatment was able to prevent lipid oxidation once flour was made into a dough by limiting the substrates (FFA) available for oxidation and by partially inactivating LOX. This would be expected to produce a better product from a sensory point of view, since lipid oxidation products arising from stored whole grain products have been described as undesirable (Doblado-Maldonado et al., 2012; Bin & Peterson, 2016) Future studies targeting the decrease in LOX activity and FFA accumulation, along with the addition of antioxidants at various concentrations, will be interesting and may have an advantage to the industry to prevent lipid oxidation during dough mixing and handling.

4. Conclusions

Results from our study suggested that lipolytic activities of whole wheat flour can be reduced by steaming of wheat kernels without deteriorating starch and gluten properties. Reduction in lipolytic activity reduced the evolution of FFA during storage and prevented the generation of lipid oxidation products in dough made from stored whole wheat flour. In addition, the correlation of FFA and LOX activity with total carbonyl compounds in the dough provides an opportunity to understand and prevent lipid oxidation in prepared doughs. Furthermore, it would be interesting to study sensory properties of flour from steamed grains and if steaming can be applied as a measure to reduce the microbial loads on wheat grains.

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