Inhibitory effects of ultrasound combined with ascorbic acid or glutathione on enzymatic darkening of whole-wheat raw noodles

Meng Niu, Gary G. Hou, Xiaodan Li, Li Wang, Zhengxing Chen

Keywords: Ultrasound, Ascorbic acid, Glutathione, Enzymatic darkening, Whole-wheat raw noodle

1. Introduction

Whole-wheat foods, which are rich in dietary fiber and phytochemicals, have become increasingly popular in many parts of the world (Jiang, Martin, Okot-Kotber, & Seib, 2011). Asian noodles account for approximately 20–50 percent of the total wheat consumed in Asia (Hou, 2010a); thus, noodles made from whole-wheat flour (WWF) should be a major subject of scientific research.

Color is a key quality trait of noodles because of the visual impact at the point of sale. It also provides some indication of the raw material quality and shelf life of the product (Mares & Campbell, 2001). Polyphenol oxidase (PPO) has been implicated as a leading cause of discoloration in raw Asian noodles and other wheat products (Fuerst, Anderson, & Morris, 2006). PPO catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones. The quinone products of PPO reaction with a number of functional groups, such as amines, thiois, and phenolics, and form complex colored products (Whitaker & Lee, 1995). For noodles made from WWF, the issue concerning discoloration is much more important than that of regular noodle, because the PPO and phenolic compounds are primarily located in the bran layer of wheat, and the whole-wheat noodle (WWN) contains all of these components (Hatcher & Kruger, 1997). Therefore, the inactivation of PPO is of vital importance in preventing the enzymatic browning of WWN.

Enzyme deactivation is a requisite for stabilization of some food materials. Although enzymes can be easily deactivated by heat treatment, heat can negatively modify some food properties such as flavor, color, or nutritional value. This is the primary driving force for increased interest in alternative methods of enzyme inactivation (Ercan & Soysal, 2011). One such alternative method is ultrasound. Ultrasound causes enzyme inactivation by cell lysis using vibration energy, which produces cavitation bubbles and temporarily generates spots of extremely high pressure and temperature when imploded (Morris, Brody, & Wicker, 2007). When combined with other processes, such as high pressure and heat, ultrasound can be more effective (Mason & Panivnyk, 2003). On the other hand, several studies have focused on the inhibition of enzymatic browning by antioxidants. Ascorbic acid, a highly effective inhibitor of enzymatic browning, can reduce o-quinones produced by PPO-catalyzed oxidation of polyphenols back to dihydroxy polyphenols.
However, the effect of ascorbic acid is temporary since once it is completely oxidized, o-quinones will accumulate and form browning pigments (Rojas-Graü, Sobrino-López, Tapia, & Martín-Belloso, 2006). Moreover, glutathione (GSH, reduced form), a potent cellular reducing agent, has been reported as an effective inhibitor of enzymatic browning. Nevertheless, it loses its ability to inhibit enzymatic darkening when fully oxidized.

Several studies on the inhibition of enzymatic darkening by ultrasound, ascorbic acid, or glutathione have been conducted (Arias, Gonzalez, Peiro, Orià, & Lopez-Buesa, 2007; Ercan & Soysal, 2011), however, the combined treatments of ultrasound and antioxidants have seldom been studied. More specifically, the effect of combined treatment of ultrasound and antioxidants on the inactivation of PPO in WWF has never been reported. The objective of this research was to investigate the effects of ultrasound combined with ascorbic acid or GSH on the inactivation of PPO in WWF and prevention of enzymatic darkening of WWN. The residual activity of PPO, thermal properties, and some functional contents were measured after treatments to verify whether the changes of physicochemical properties and nutritional values of WWF had occurred. In addition, the discoloration (ΔL*) and textural characteristics of WWN were investigated to determine whether these treatments affected the quality of the finished product.

2. Materials and methods

2.1. Materials

Wheat cultivar Jimai 22, a good quality flour for noodles, was obtained from the seed station of food ministry in Shandong province, China. The moisture, protein, fiber, and ash contents of the wheat seeds were 12.4 g/100 g, 12.5 g/100 g (14 g/100 g mb), 2.25 g/100 g (14 g/100 g mb), and 1.8 g/100 g (14 g/100 g mb), respectively. WWF was produced by first milling the seeds in a disc-mill and then grinding the coarse whole meal in a MZF-4L impact superfine grinder (Huantai Dongqi Powder Manufacturing Co., Ltd, Shandong, China) for 15 min. The mean particle size of the resultant WWF was 75 μm, as determined by the laser diffraction method using the Microtrac S3500 Particle Analyzer (Montgomeryville, Pennsylvania, USA). The WWF samples were packed in airtight plastic bags and stored at room temperature for further use. Ascorbic acid, glutathione were obtained from Shanghai Yuanye Biotech Corporation (Shanghai, China), and other chemicals used in the study were purchased from Sigma–Aldrich Co. (Shanghai, China).

2.2. Ultrasound treatment combined with antioxidants

Different combinations of ultrasound and antioxidant treatments are listed in Table 1. In each batch, suspensions of WWF were prepared by stirring 25 g of WWF and 250 mL of distilled water or antioxidant solution (1 g/100 g ascorbic acid or 0.5 g/100 g glutathione of WWF, w/w) in a 500 mL beaker. The ultrasonication experiments were carried out on a 750W/20KHz ultrasonic processor (vcc750, Sonics and Materials Inc., Newtown, USA) equipped with a probe of 13 mm diameter. The probe was submerged to a depth of 25 mm in the suspensions, and the sonication amplitude was set at 80 g/100 g energy input with 25 s on and 5 s off in each 30 s when the samples were treated. This 30 s cycle was repeated until the desired treatment time was reached. The temperature of the suspensions was maintained below 50 °C with a circulating water bath and was monitored by a glass-stem thermometer submerged at the same depth as the ultrasonic processor probe. During the treatment, the suspension of WWF was constantly stirred with a magnetic stirrer. After the treatment, the suspension was filtered through two layers of quantitative filter paper by using an aspiration pump, and the obtained filtrate was collected and recycled for the next measurement. The concentrations of ascorbic acid and GSH in the filtrates were measured according to the AOAC method 987.21 (AOAC, 1999) and the method of Moro et al. (2012), respectively. The residue over the filter paper was dried in a vacuum drying oven (ZK-82, Shanghai Laboratory Instrument Works Co., Ltd., Shanghai, China) until the moisture content was reduced to 14 g/100 g and then ground in a laboratory mill (Q-200A2, Shanghai Bingdu Electrical Co., Ltd., Shanghai, China), and passed through a 150 μm sieve. Multiple batches of ultrasound treatment of WWF suspensions were conducted to obtain sufficient amount of flour for quality analysis and noodle preparation.

2.3. Assay of PPO activity

PPO activity was determined following the method of Anderson and Morris (2001) with some modifications. Trials were conducted to prepare proper flour/substrate ratios to ensure the absorbance readings from the spectrophotometer were in the confidence range (0.2–0.8). Untreated or treated WWF (0.5 g) was placed in a 50 mL microcentrifuge tube with 7.5 mL of 10 mM L-DOPA in 50 mM MOPS buffer (pH = 6.5), which was incubated at room temperature (20 °C) for 1 h with a rotating mixer (10 rpm) and then centrifuged at 8000 g for 3 min. The absorbance of the supernatant was measured using a spectrophotometer (UV-1800, Shanghai MAPADA Instrument Co., Ltd., Shanghai, China) at 475 nm. The control flour was assayed without L-DOPA substrate. The PPO activity was calculated as the difference in the absorbance of test sample and control and expressed as Δ475/min g flour. The PPO activity in the obtained filtrates after the treatments was measured following the same procedure with the reactant/substrate ratio (v/v) of 0.2.

2.4. Determination of free phenolics content

The free phenolics content in the treated and untreated WWF was determined using the method described by Singleton, Orthofer, and Lamuela-Raventos (1999), with some modifications. Briefly, 5 g of WWF was blended with 20 mL of 80 g/100 g chilled ethanol for 10 min. After centrifugation at 3000 g for 10 min, the supernatant was collected and extraction was repeated once. Supernatants were pooled, evaporated at 45 °C to 10 mL, and reconstituted with distilled water to a final volume of 25 mL. Then 125 μL of properly diluted extract was mixed with 0.5 mL of distilled water in a test tube, followed by the addition of 125 μL of Folin-Ciocalteu reagent (FCR). The sample was well mixed and allowed to stand for 6 min before 1.25 mL of a 7 g/100 g sodium carbonate aqueous solution was added. Distilled water was added to adjust the final volume to 3 mL. After incubation for 90 min at room temperature, the absorbance of the reaction solution was measured at 760 nm against the blank solution using a spectrophotometer (UV-1800, Shanghai MAPADA Instrument Co., Ltd., Shanghai, China). Using gallic acid as standard, the free phenolics content was expressed as micromoles of gallic acid equivalent per gram of flour (dry basis). Three replicates were performed for each test.

2.5. Thermal properties

The thermal properties of flour samples were measured using a DSC-Pyris Diamond (Perkin-Elmer Corp, Norwalk, CT, USA). The calorimeter was calibrated with an indium standard. About 3 mg of ultrasound-treated or untreated WWF samples were accurately placed into aluminium DSC pans (the accuracy was 0.1 mg), and 6 μL of distilled water was added by micropipette in order to achieve a flour-water weight ratio of 2:1. The sample pans were sealed and
formed at 30°C, Milford, MA). The mobile phase consisted of 0.05 M sodium acetate and the supernatant was filtrated through a 0.45 μm microfiltration membrane. The resultant filtrate was used for the HPLC determination of thiamine and riboflavin. The separation was carried out by using a μBondapak C18 column (150 mm × 4.6 mm, Waters Corporation, Milford, MA) by a HPLC system with a 1525 Alliance fluorescence detector (Waters Corporation, Milford, MA). The mobile phase consisted of 0.05 M sodium acetate-methanol (30:70, v:v; pH 6.0). The separation was performed at 30°C at a flow rate of 1 mL/min and the injection volume was 20 μL. The fluorescence detector was operated at 365 nm and 435 nm as excitation and emission wavelengths, respectively, for thiamine and riboflavin. The thiamine and riboflavin contents were expressed in μg/g DM.

2.8. Noodle preparation

The preparation of noodle was conducted according to the method of Niu, Li, Wang, Chen, and Hou (2014). Flour (300 g), water (105 g) and salt (6 g) were mixed into dough by using a KitchenAid mixer (KitchenAid, St. Joseph, MI). After resting for 20 min, the dough was passed through a Laboratory noodle machine (Mode JMTD-168/140, Beijing, China) seven times with the roller gap reduced gradually to obtain the desired dough sheet thickness. The width and thickness of the resultant noodle strands were 2 ± 0.08 mm and 1 ± 0.03 mm, respectively. The noodle strands were placed in an airtight plastic bag to prevent drying and to make noodle handling easier.

2.9. Color measurement of noodle sheets

A Chroma meter (Konica Minolta CR-400, Japan) equipped with D65 illuminant was used to measure the noodle sheet L* values at 0, 2, 4, 8, 12 and 24 h after preparation, according to the method described by Hou (2010b). Two noodle sheets were stacked together and placed on a black background. Three readings were taken on each side of the noodle sheet, and an average of 12 readings was calculated. The preparation of noodle sheets was done on two different days.

2.10. Texture profile analysis

Textural profile analysis (TPA) of cooked noodles was measured using a TA-XT2i Texture Analyzer (Stable Micro Systems, England). The noodle sheets were cut into 2.5 cm long pieces, and the measurements were carried out exactly 5 min after cooking. Each noodle strand was cut to a length of 2.5 cm, three short noodle strands were selected and placed on the base plate, then compressed with a cylindrical probe (3.5 cm dia.) by using a 10 kg load cell. The compression strain was 85% of the total noodle strand thickness. Means were based on at least eight measurements. Four textural parameters (Hardness, Springiness, Cohesiveness, and Resilience) were reported from the TPA.

2.11. Statistical analysis

All measurements were performed at least in triplicate. All statistical analyses were carried out by using SPSS 16.0 software for windows. p < 0.05 was considered to be significant.

3. Results and discussion

3.1. Effects of ultrasound and antioxidants on PPO activity and free phenolics content of WWF

The PPO activity of WWF is shown in Fig. 1. The results indicated that the PPO activity in all ultrasound-treated groups significantly decreased as compared to the untreated group, which was similar to the effect of ultrasound on other foods such as fruits and dairy products (Ercan & Soysal, 2011; Villamiel & De Jong, 2000). The results also showed the residual activity of PPO decreased significantly with the increase of ultrasonic time. When ultrasound was applied for 50, 100, and 150 s without antioxidants, the residual activity of PPO was 94.53%, 75.74%, and 43.06%, respectively. Denaturation of protein is mainly responsible for the ultrasonic inactivation mechanism of enzymes, either by free radicals in sonolysis of water molecules or from the shear force resulting from the formation or collapse of cavitating bubbles (Mason, Lorimer, Baters, & Zhao, 1994). Longer treatment time can increase ultrasonic inactivation efficacy and promote more protein denaturation. Lower levels of PPO activity were observed in the samples treated with combined ultrasound and antioxidants (ascorbic acid or GSH) compared to the samples treated only with ultrasound. The results were in agreement with Jang and Moon (2011), who observed that simultaneous treatment with ultrasound and ascorbic acid had a synergistic inhibitory effect on PPO in fresh-cut apple. Fig. 1 also shows that the synergistic effect of ascorbic acid with ultrasound was more pronounced than that of GSH with ultrasound. When PPO substrates (both O₃ and phenolics) are absent, ascorbic acid irreversibly inactivates PPO, probably by binding to its...
active site. A direct relationship was observed between enzyme inactivation by ascorbic acid and the disappearance of histidine residues to which Cu²⁺ are bound in the active site of PPO (Arias et al., 2007). It is probable that cavitation generated by ultrasound created a suitable microenvironment for ascorbic acid to bind directly to the active site in which no PPO substrate was present. As a result, the interaction of PPO substrates with the active site of PPO was blocked. The inhibition mechanism of GSH on PPO probably occurred because the redox active thiol group in the GSH trapped the o-quinone as additional compounds to form the cysteine-quinone group and prevented the browning reaction. This process was enhanced when ultrasound was combined with GSH. The better synergistic effect of ascorbic acid with ultrasound than with GSH might be explained by the more affinity of ascorbic acid with the active site of PPO.

In addition, the results showed that PPO activity in the filtrates obtained after treatments of WWF was close to zero, which indicated that the decrease of PPO activity in WWF was due to the effect of ultrasound and antioxidants on enzyme deactivation of PPO rather than a washing out of PPO into the water. The results also indicated that the concentration of ascorbic acid in the filtrates after treatments was 0.99 g/100 g (1 g/100 g in the original solution), and 0.49 g/100 g for GSH (0.5 g/100 g in the original solution), which demonstrated that little residual of ascorbic acid or GSH remained in the treated flour.

Phenolics, such as caffeic, chlorogenic, gallic, protocatechuic, p-coumaric acids, and catechin, have been found in wheat bran. Those phenolic compounds are easily oxidized in the presence of PPO and other enzymes (Fuerst et al., 2006). Most phenolic acids in wheat grain are insoluble and bound by ester and ether linkages with polysaccharides in the cell wall. These phenolic compounds are called bound phenolic acids; a smaller portion of these compounds is soluble and called free phenolic acids (Verma, Huc, & Chibbar, 2009). Jiang et al. (2011) reported that free phenolic acids could contribute more to the darkening, and bound phenolic acids generated less color by being less accessible to the PPO. The effect of ultrasound and antioxidants treatments on free phenolics content in WWF is shown in Fig. 1. The results showed that free phenolics content decreased as ultrasound time increased, which was probably due to the fact that ultrasound promoted the extraction of free phenolic acids into the WWF suspension. The decline of free phenolic acids may retard the process of darkening to some extent. The results also indicated that antioxidants exhibited little effect on the extraction of free phenolic acids, and there was no synergistic effect between ultrasound and antioxidants on the washing out of free phenolics into the water.

Furthermore, considering that the free phenolics fraction is only a very small portion of the total phenolic acids, as well as the close cross-linking of bound phenolic acids with other components in the cell wall, ultrasound treatment exhibited a slightly adverse effect on the antioxidant capacity of the phenolic acids in WWF.

### 3.2. Effect of ultrasound and antioxidants on darkening of WWN

The ΔL* value of noodle dough sheets made from WWF are shown in Fig. 2. The results showed that the rate of darkening decreased over time in all groups, and the darkening was most rapid during the first 0–2 h period. The differences in the value of ΔL* among the groups were minimum at 2 h after preparation, which was in agreement with Aasenstorfer, Appelbee, and Mares (2010), who reported that the amount of darkening during the early stage was highly dependent on protein concentration and independent of PPO activity. The amount and rate of darkening over a 24 h period in groups B and C, which were treated with ascorbic acid or GSH alone, showed no significant change compared to the untreated sample; however, other groups treated with both ultrasound and antioxidants indicated significantly different trends compared to the untreated sample and showed a close correlation between the rate of darkening during the 2–24 h period and PPO activity (r > 0.90, p < 0.05).

In samples treated with ultrasound and GSH, lower values in ΔL* with a higher rate of darkening between 12 and 24 h were observed than those treated with ultrasound only. The results suggested that GSH acted as a reducing agent for oxidized PPO reaction products; however, its reducing ability gradually decreased towards the end of the 24 h period. On the contrary, the combined treatment of ultrasound and ascorbic acid showed the lowest values in ΔL* and effectively inhibited the darkening of fresh noodles during the 24 h period. In general, the results suggested that combined treatment of ultrasound with ascorbic acid or GSH had the inhibitory effect on the darkening of WWN by the inactivation of PPO, and ascorbic acid was more effective than GSH when combined with ultrasound in deactivating of PPO and retarding fresh raw noodle darkening.

### 3.3. Effect of ultrasound and antioxidants on the thermal properties of WWF

The thermal properties of treated WWF are present in Table 2. The onset temperature (T₀), peak temperature (Tₚ) and end
temperature ($T_D$) of WWF were not significantly affected by the treatments; the enthalpy of melting of the treated groups showed little decrease compared to the untreated sample. The thermal properties of wheat starch have been reported to be considerably influenced by the stability of the granular structure in starch (Baek, Yoo, & Lim, 2004). Heat-gelatinization is a phase transition of granules from an ordered state to a disordered state during heating in excess water, which involves melting of ordered regions, both on the crystallite and on the level of double-helical order. Ultrasonic treatment distorts the crystalline region in starch granules prior to the crystallite and on the level of double-helical order. Ultrasonic treatment used in the study was a processing aid. The results showed that the contents of thiamine and riboflavin decreased as the ultrasonic time increased, but only the riboflavin content was slightly affected. This can probably be attributed to the fact that after grinding wheat kernels into WWF, the vast majority of B vitamins were still trapped in the cells of the scutellum and aleurone layer, both of which were difficult to reduce into fine particles, and the ultrasound treatment used in the study was a short time and insufficient to disrupt the cells and release the B vitamins into the solution.

3.4. Effects of ultrasound and antioxidants on the mineral contents and B vitamins of WWF

In order to evaluate the influence of treatments on the nutritive substances in WWF, the contents of Fe, Zn, and Ca in the control and treated samples were measured. Fig. 3 shows that ascorbic acid or GSH (group B or group C) exhibited little effect on the mineral contents, and no synergistic effect of antioxidants with ultrasound was found. A general decrease was observed for each of the three minerals contents with the increase of ultrasonic time, but the decrease was insignificant. According to a previous report, minerals may be encapsulated within cell walls that must be broken down by the combined effects of dilute acid attack and ultrasonication in order to bring minerals into the liquid extractant (Filgueiras, Capelo, Lavilla, & Bendicho, 2000). Prat, Lopez-Gonzalez, Ruiz, and Barbas (2009) also reported that the best conditions for the extraction of Zn, Cu, Fe, Mg, and Mn from rat liver were 10 g/100 g HNO$_3$ and 8 min of sonication with 19 kHz frequency, and the ultrasound served as a processing aid. The results of these studies were in agreement with Nascentes, Korn, and Arruda (2001), who also found that the recovery of Ca and Zn from vegetables with ultrasound only were much lower than those treated with combined acid and ultrasound. These results probably suggest that the efficiency of ultrasound extraction of minerals from organisms is limited, which also explains the little loss of minerals in WWF after ultrasonic treatments observed in our study.

Vitamins are biologically active compounds that reduce damage by free radicals and retard degenerative diseases for people's normal health and growth (Jacab & Sotoudeh, 2002). Wheat grains are a good source of water-soluble vitamins (B-group), such as thiamine, riboflavin, and pyridoxine. These B vitamins are primarily located in the scutellum and aleurone layer of wheat grain (Piironen, Lampi, Salmenkoilo-Marttila, & Liukkonen, 2009). The effects of ultrasound and antioxidant treatments on the thiamine and riboflavin contents in WWF are presented in Fig. 4. The results showed that the contents of thiamine and riboflavin decreased as the ultrasonic time increased, but only the riboflavin content was slightly affected. This can probably be attributed to the fact that after grinding wheat kernels into WWF, the vast majority of B vitamins were still trapped in the cells of the scutellum and aleurone layer, both of which were difficult to reduce into fine particles, and the ultrasound treatment used in the study was a short time and insufficient to disrupt the cells and release the B vitamins into the solution.

![Fig. 3. Changes of Fe, Zn, and Ca contents in whole-wheat noodle (WWN) after ultrasound and antioxidant treatments. Data shown are means ± standard deviation of 3 measurements.](image)

![Fig. 4. Changes of thiamine and riboflavin contents in whole-wheat noodle (WWN) after ultrasound and antioxidant treatments. Data shown are means ± standard deviation of 3 measurements.](image)
Effects of ultrasound and antioxidants on the textural properties of raw whole wheat noodles.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Quality parameters</th>
<th>Optimal cooking time</th>
<th>Cooking yield (g/100 g noodle)</th>
<th>Cooking loss (g/100 g noodle)</th>
<th>Hardness (g)</th>
<th>Springiness</th>
<th>Cohesiveness</th>
<th>Resilience</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>3'40 ±10NS</td>
<td>174.0 ± 1.9</td>
<td>11.35 ± 0.37NS</td>
<td>304.5 ± 30.5</td>
<td>0.859 ± 0.012NS</td>
<td>0.379 ± 0.015NS</td>
<td>0.116 ± 0.006NS</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>3'40 ±10</td>
<td>174 ± 1.9</td>
<td>11.25 ± 0.40</td>
<td>301.2 ± 25.9</td>
<td>0.851 ± 0.010</td>
<td>0.377 ± 0.018</td>
<td>0.118 ± 0.007</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>3'40 ±10</td>
<td>174 ± 1.9</td>
<td>11.19 ± 0.45</td>
<td>299.7 ± 35.4</td>
<td>0.857 ± 0.013</td>
<td>0.382 ± 0.013</td>
<td>0.119 ± 0.007</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>3'40 ±10</td>
<td>174 ± 1.9</td>
<td>11.47 ± 0.32</td>
<td>304.0 ± 27.8</td>
<td>0.851 ± 0.009</td>
<td>0.375 ± 0.012</td>
<td>0.113 ± 0.005</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>3'40 ±10</td>
<td>174 ± 1.8</td>
<td>11.58 ± 0.28</td>
<td>302.4 ± 24.6</td>
<td>0.847 ± 0.014</td>
<td>0.378 ± 0.015</td>
<td>0.110 ± 0.005</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>3'40 ±10</td>
<td>174 ± 1.8</td>
<td>11.42 ± 0.25</td>
<td>304.7 ± 32.2</td>
<td>0.850 ± 0.013</td>
<td>0.380 ± 0.017</td>
<td>0.112 ± 0.006</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>3'40 ±10</td>
<td>175 ± 1.6</td>
<td>11.59 ± 0.30</td>
<td>307.3 ± 34.3</td>
<td>0.850 ± 0.010</td>
<td>0.362 ± 0.010</td>
<td>0.112 ± 0.005</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>3'40 ±10</td>
<td>175 ± 1.7</td>
<td>11.65 ± 0.28</td>
<td>306.5 ± 28.4</td>
<td>0.846 ± 0.011</td>
<td>0.357 ± 0.013</td>
<td>0.114 ± 0.006</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>3'30 ±10</td>
<td>175 ± 2.0</td>
<td>11.68 ± 0.25</td>
<td>306.2 ± 30.9</td>
<td>0.852 ± 0.008</td>
<td>0.349 ± 0.016</td>
<td>0.114 ± 0.003</td>
</tr>
<tr>
<td>J</td>
<td></td>
<td>3'40 ±10</td>
<td>176 ± 2.0</td>
<td>11.85 ± 0.30</td>
<td>310.5 ± 34.5</td>
<td>0.845 ± 0.010</td>
<td>0.352 ± 0.014</td>
<td>0.109 ± 0.004</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td>3'40 ±10</td>
<td>175 ± 2.0</td>
<td>11.78 ± 0.35</td>
<td>309.5 ± 27.7</td>
<td>0.847 ± 0.013</td>
<td>0.355 ± 0.015</td>
<td>0.110 ± 0.005</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>3'30 ±10</td>
<td>175 ± 1.9</td>
<td>11.74 ± 0.28</td>
<td>311.3 ± 28.5</td>
<td>0.842 ± 0.016</td>
<td>0.358 ± 0.014</td>
<td>0.110 ± 0.006</td>
</tr>
</tbody>
</table>

NS: values in the same column are not significantly different.

a: Results are presented as means ± S.D. (n = 3 for cooking quality analysis; n = 8 for textural property analysis). Means with different small letter superscripts within the same rows are significantly different at p < 0.05.
b: As described in Table 1.
c: Cooking loss was calculated on dry basis.

3.5. Effect of ultrasound and antioxidants on the textural properties of WWN

Texture of cooked noodles is one of the major quality attributes that determine noodle eating quality. Many efforts have been made to determine reproducible and reliable instrumental methods to determine noodle texture (Baik, 2010; Hatcher, 2010; Ross, 2006; Ross & Crosbie, 2010). However, each method has its limitation that it does not truly measure sensory attributes (Ross, 2006; Ross & Crosbie, 2010). Textural profile analysis (TPA) has been proposed by many researchers and is one of the most widely accepted instrumental methods to estimate the sensory texture attributes of cooked noodles (Baik, 2010; Hatcher, 2010).

High-power ultrasound caused damage to the rheological and physical properties of starch (Zuo, Hebraud, Hemar, & Ashokkumar, 2012) and negatively affected the appearance and quality properties of noodles. In another study, Jambrak, Mason, Lesla, Herceg, and Herceg (2008) reported that ultrasound altered the functional properties of whey proteins (solubility and foaming ability) as samples were exposed to high temperatures caused by sonication. However, little is known about the effect of short-time and low-frequency ultrasound on wheat flour characteristics and products. The textural properties of the cooked noodles are summarized in Table 3. No significant differences were found in springiness, cohesiveness, and resilience among the samples with different treatments. Additionally, there was little negative influence from the two antioxidants and synergistic effect of the ultrasound and antioxidants on the three textural parameters of cooked WWN. However, the hardness of cooked noodles treated with ultrasound for 3 min was significantly greater than that of the control, which had the lowest hardness value. The samples treated for 1 min or 2 min were slightly harder than the control but not significantly. This can probably be attributed to the fact that the ultrasound decreased the pasting peak viscosity of the wheat starch, which resulted in harder noodle texture (Li et al., 2012). Antioxidants exhibited little effect on the hardness of noodle texture when applied alone or with ultrasound. It can be concluded that combined treatment of short-time and low-frequency ultrasound with antioxidants used in the study had little effect on the textural properties of WWN with the exception of the hardness value when treated for 3 min with ultrasound.

4. Conclusion

The effects of combined treatment of ultrasound with ascorbic acid or GSH on the inactivation of PPO in WWF and inhibiting enzymatic darkening of WWN were investigated. The results showed that the simultaneous treatments of ultrasound and antioxidant were effective in reducing the PPO activity and preventing the discoloration of WWN during a 24 h period, and the combined use of ultrasound and ascorbic acid appeared to be a more effective approach. In addition, the thermal properties, representative mineral elements, and B vitamins in WWF, as well as textural attributes of cooked WWN, were little affected by the treatments. These results suggest that the combined treatment with ultrasound and antioxidants is a promising technique in the inactivation of PPO and preserving the freshness of WWN. In view of this, further application of this new technique can be extended to other wheat-based products, such as dumpling skins and refrigerated dough, and some fresh vegetables such as potatoes.

Acknowledgment

The authors would like to thank the seed station in the city of Shandong Province, China for providing wheat seeds. We are grateful for the state key laboratory of food science and technology in Jiangnan University for performing atomic absorption spectrometer analyses.

References


