Effect of electrolyzed water on enzyme activities of triticale malt during germination

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Abstract Triticale malt can be used as a source of enzymes or as a raw material for the production of functional foods. In this study, triticale malt was produced by soaking triticale seeds either in tap water (TW) or slightly acidic electrolyzed water (SAEW) and then rinsing with TW, SAEW, or alkaline electrolyzed water (AEW). We determined the length of the hypocotyl of triticale malt and the activities of α -amylase, phytase, proteases, and lipase during 4 days of germination. The electrolyzed water (EW) treatments promoted the growth of triticale malt. On the 4th day of germination, the hypocotyl length of triticale malt soaked in TW and watered with SAEW was 24.57% longer than that of triticale malt soaked and watered with TW. The α -amylase, phytase, acidic protease, and lipase activities of triticale malt soaked in SAEW and watered with AEW were high on the 4th germination day (0.11, 1.24×10^{-4} , 0.62, and 0.51 units/mg protein, respectively). The main finding of this study is that the use of EW, especially during the soaking procedure, may be a promising way to obtain triticale malt with high enzyme activity for use in the production of functional foods.

Keywords Electrolyzed water · Triticale · Soaking · Rinsing · Germination · Enzyme activity

Introduction

Triticale is a novel plant originating from a cross between wheat and rye. The global production of triticale has increased during the last two decades, from 6.4 million tons in 2006 to 13.7 million tons in 2009 and to approximately 17 million tons in 2014 (Zhu 2018). The chemical composition of triticale is more similar to wheat than to rye, and some triticale genotypes have a relatively high concentration of lysine, the limiting amino acid in cereals (McGoverin et al. 2011; Heger and Eggum 1991). Triticale is composed of carbohydrates, proteins, lipids, polyphenols, vitamins, minerals, phytic acid, and other micronutrients (Zhu 2018). Because of its composition and properties, triticale has high potential for food production. Various foods and beverages, including bread, cookies, pasta, malt, and spirits, have been developed from triticale (Zhu 2018). Therefore, triticale may have potential uses in the growing health food market and in the formulation of new cereal products.

Mature, sound cereal grains are characterized by very low levels of enzymatic activity. Under suitable conditions, cereal grains germinate and the activities of many enzymes increase to hydrolyze starch and other components. The malting process can increase the bioavailability of nutrients (Rimsten et al. 2002). The germination process increases enzyme activity in cereals, leading to decreased levels of antinutritional factors and better nutritional quality of grain (Centeno et al. 2001). Seed germination is accompanied by various metabolic reactions that alter the chemical composition compared with ungerminated seeds. Thus, the germination process has much value in practical applications. For example, increased protease activities during germination lead to the metabolism of proteins, which increases their nutrient bioavailability. The contents of

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essential amino acids (lysine, methionine, leucine, isoleucine, threonine, phenylalanine, and valine) also increase during germination, resulting in improved nutritional quality of proteins in triticale seeds (Sibian et al. 2017). In germinating rye and barley, higher phytase activity leads to reduced phytate content and increased free phosphorus content. Thus, the utilization of germinated rye and barley, which are rich in phytases, was found to increase the availability of phosphorus in animal feeds (Centeno et al. 2001). However, higher levels of enzymatic activity in sprouted grain may promote fungal growth during storage or negatively affect the food-processing characteristics of cereal (Mergoum et al. 2004). Contamination of seeds can occur pre- and post-harvest and during germination and sprouting when conditions are optimal for microorganism growth (Yang et al. 2013). Therefore, measures must be taken to eliminate pathogenic microorganisms from malted or sprouted seeds.

Electrolyzed water (EW) is usually generated by electrolysis of a dilute salt solution. This process yields acidic EW and alkaline EW simultaneously. Because many studies have shown that EW has strong disinfection properties and is safe for humans and the environment, it has been widely used in the food processing industry (Huang et al. 2008; Liu et al. 2011). Previous studies have shown that EW can reduce the microbial load in buckwheat sprouts, mung bean sprouts, and brown rice and also increase sprout lengths (Cao et al. 2012; Liu et al. 2011, 2013; Liu and Yu 2017). Therefore, the use of EW may be a promising method to produce malted or sprouted seeds.

The objective of this study was to determine the effect of EW on the growth of triticale malt and on the activities of various enzymes during triticale malt germination.

Materials and methods

Materials

The triticale ($\times$ *Triticosecale* Wittm.) cultivar used in this study was ‘Yun hei 14207’. Analytical grade chemicals and distilled water were used in all analyses.

Preparation of slightly acidic electrolyzed water (SAEW) and alkaline electrolyzed water (AEW)

SAEW and AEW were prepared using an EW generator (model XY-L-150; Xiniu Optics-Mechanics-Electricity Co. Ltd., Baoji, China). The SAEW and AEW were collected from the outlet in polypropylene containers and used immediately. SAEW with a certain available chlorine content (ACC) and pH was prepared by adding AEW and

distilled water to acidic electrolyzed water to obtain an ACC of about 30 mg/L. The pH of electrolyzed water was measured with a PHB-1 electrode (Ao Li Long Instrument Co. Ltd., Hangzhou, China). Oxidation–reduction potential (ORP) was measured with an ORP electrode (Lohand Biological Co. Ltd., Hangzhou, China). The ACC of EW was determined by iodometry (Block 1983). The physico-chemical parameters of all treatment solutions used in this study were as follows: for SAEW, pH = 5.32 ± 0.09 , ORP = 977 ± 6 mV and ACC = 30.14 ± 0.21 mg/L; for AEW, pH = 8.59 ± 0.11 and ORP = 182 ± 3 mV; tap water (used as the control treatment solution), pH = 7.80 ± 0.12 and ORP = 454 ± 4 mV.

Production of triticale malt

Triticale seeds (30 g) were washed five times with 300 mL treatment solution. After washing, the triticale seeds were soaked in the treatment solution (1:6, m/v) for 8 h, drained, and then spread on sterile cheesecloth in a plastic box with holes in the bottom. The box was kept in a constant temperature humidity chamber (model HWS; Ningbo Jiangnan Instrument Factory, Ningbo, China) at 20 °C and 85% relative humidity. The same treatment solutions (180 mL) were used to water the treated triticale seeds every day until the triticale malt was harvested.

Morphological measurements of triticale malt

The morphological measurements of triticale malt were taken at 24-h intervals after watering with EW or tap water (TW). The length of the hypocotyl of the malted triticale was measured with a Vernier caliper, and 30 malted triticale seeds from each treatment were measured.

Measurement of α -amylase activity

The 1st germination day was defined as 4–28 h after the soaking procedure. The α -amylase (EC 3.2.1.1) activity of triticale malt was investigated from the 1st to the 4th germination day using the method described by Wood et al. (2012) with modifications. Fresh samples were thoroughly ground with a mortar and pestle in distilled water, and the homogenate was centrifuged at 4000 r/min for 5 min. The supernatant served as the amylase extract and was heated at 70 °C for 15 min to inactivate β -amylase. The reaction solution contained 0.1 M citrate buffer, 1% (w/v) starch, and 1 mL enzyme extract. The reaction mixture was incubated at 40 °C for 5 min, and the reaction was then terminated by adding 4 mL of 0.4 M sodium hydroxide solution. The blank contained citrate buffer and starch but no enzyme extract. To the 2-mL reaction mixture, 2 mL 3,5-dinitrosalicylic acid (DNS) was added, and the mixture

was boiled at 100 °C for 5 min and cooled before measuring absorbance at 540 nm. A maltose solution was diluted appropriately and assayed as described above to construct a standard curve.

Measurement of phytase activity

The phytase (EC 3.1.3.8) activity of triticale malt was investigated from the 1st to the 4th germination day using the method described by Jongbloed and Kemme (1990) with modifications. Each sample (2.0 g) was finely ground and added to 100 mL sodium acetate buffer (0.25 M, pH 5.5). The mixture was stirred at high speed using a magnetic stirrer for 30 min to extract phytases. The reaction mixture (0.2 mL enzyme extract, 1.8 mL sodium acetate buffer, and 4 mL of 7.5 mM sodium phytate) was incubated at 37 °C in a water bath for 30 min. Then, 2 mL of 4.8 M nitric acid, 1 mL ammonium molybdate, and 1 mL ammonium metavanadate were added, and the mixture was incubated at room temperature for 10 min before reading absorbance at 415 nm. A potassium dihydrogen phosphate standard solution was diluted appropriately and assayed as described above to obtain a standard curve. Phytase activity was expressed as μmol free phosphate released $\text{min}^{-1} \text{g}^{-1}$ dry matter.

Measurement of protease activity

The protease (EC 3.4.2.21) activity of triticale malt was investigated from the 1st to the 4th day of germination using the method described by Drapeau (1976) with modifications. Each fresh sample was thoroughly ground with a mortar and pestle in buffer (pH 3.0 lactate buffer, pH 7.5 phosphate buffer, and pH 10.5 boric acid buffer for determination of acidic, neutral, and alkaline protease activities, respectively). The homogenate was centrifuged at 4000 r/min for 5 min, and the supernatant served as the protease extract. A 1-mL aliquot of the protease extract was mixed with the corresponding buffer; substrate (1 mL casein solution) was then added, and the reaction mixture was incubated at 40 °C for 10 min. The reaction was stopped by adding 5 mL of 0.4 M trichloroacetic acid (TCA). After standing for 10 min, the mixture was filtered through a Whatman No. 42 filter paper, and then absorbance at 680 nm was read against a blank. The blank was prepared by mixing the casein solution with TCA and then adding the enzyme solution to the casein-TCA mixture. A tyrosine standard solution was diluted appropriately and analyzed using the method described above to obtain a standard curve.

Measurement of lipase activity

The lipase (EC 3.1.1.3) activity of triticale malt was investigated from the 1st to the 4th day using the method described by Humbert et al. (1997) with modifications. The fresh samples were thoroughly ground with a mortar and pestle in 50 mM Tris-HCl (pH 7.0), and the homogenate was then centrifuged at 10,000 r/min for 10 min. The supernatant served as the lipase extract. The lipase extract (0.2 mL) was mixed with 4.6 mL Tris-HCl buffer, pH 8.0, and incubated at 37 °C for 5 min. Substrate (0.2 mL of 50 mM *p*-nitrophenyl butyrate in acetonitrile) was then added, and the reaction mixture was shaken and incubated at 37 °C for a further 30 min. The reaction was terminated by boiling the mixture. The mixture was centrifuged at 8000 r/min for 5 min, and the absorbance of the supernatant was read at 410 nm. The blank was prepared by boiling the mixture before adding the substrate. Lipase activity was calculated from the change in absorbance (0.1 units/min).

Protein content determination

The protein content was measured using the Kjeldahl procedure (Jung et al. 2003), and the amylase, phytase, protease and lipase activities were calculated based on the protein content.

Statistical analysis

For each treatment, data from three independent replicate trials were pooled and the mean values were calculated. One-way analysis of variance (ANOVA) was used to determine the significance of differences among treatments. Statistical analyses were conducted using SPSS 16.0 software (SPSS Inc. Chicago, IL, USA). Statistical significance was set at a *P* value of < 0.05.

Results and discussion

Effect of electrolyzed water on the hypocotyl length of triticale malt

The hypocotyl length of triticale malt was measured beginning 24 h after soaking until the triticale malt was harvested (Fig. 1). During the growth of triticale malt, the hypocotyl grew faster from seeds soaked in SAEW than from seeds soaked in TW from the 1st to the 3rd germination day. However, the hypocotyl of triticale malt soaked in TW and watered with SAEW and AEW was longer than those in other treatments on the 4th germination day. The hypocotyl of triticale malt soaked in TW and watered with

Fig. 1 Effect of electrolyzed water on length of hypocotyl of triticale malt. Key: STWT-triticale seeds were soaked and watered during germination with tap water; STWS-triticale seeds were soaked in tap water for 8 h and watered with slightly acidic electrolyzed water during germination; STWA-triticale seeds were soaked in tap water for 8 h and watered with alkaline electrolyzed water during germination; SSWT-triticale seeds were soaked in slightly acidic electrolyzed water for 8 h and watered with tap water during germination; SSWS-triticale were soaked and watered during germination with slightly acidic electrolyzed water; SSWA-triticale were soaked in slightly acidic electrolyzed water for 8 h and watered with alkaline electrolyzed water during germination

SAEW and AEW was 24.57% and 16.0% longer, respectively, than that of triticale malt soaked and watered with TW on the 4th germination day ($P < 0.05$).

Previous studies have reported the use of EW for producing sprouts. The length of buckwheat sprouts was promoted by SAEW with an ACC of 50 mg/L (Cao et al. 2012). Previously, we reported that EW could promote the growth of mung bean sprouts (Liu et al. 2011). In previous studies, SAEW enhanced the growth of germinated brown rice (Liu et al. 2013), and soybean sprouts soaked and watered with SAEW were longer than those soaked and watered with TW (Liu et al. 2014a). In the present study, EW used either in soaking or rinsing procedures promoted the growth of triticale malt, consistent with previous research (Liu et al. 2014a). This may be due to the sanitizing properties of EW (Huang et al. 2008), which were beneficial for the growth of triticale malt and for seed vigor.

Effect of electrolyzed water on α -amylase activity during growth of triticale malt

The α -amylase activity of triticale malt soaked in TW changed little during germination (Fig. 2a), while that of triticale malt soaked in SAEW was significantly increased on the 4th germination day. The α -amylase activity of triticale malt soaked in SAEW and watered with TW,

SAEW, or AEW was 0.066, 0.11, and 0.11 units/mg protein, respectively, while that of triticale malt soaked in TW and watered with TW, SAEW, and AEW was lower (0.050, 0.028, and 0.016 units/mg protein, respectively).

Amylases are a class of hydrolases, and α -amylase plays an important role in starch metabolism and germination. α -Amylase releases a large amount of oligosaccharides along with α -D-maltose, which is used in the brewing industry. Wheat malt is one of the main sources of amylases. Liu et al. (2014b) found that the abscisic acid content of mung bean sprouts was lower in those treated with EW than in those treated with TW. Abscisic acid has been shown to inhibit α -amylase synthesis at the transcriptional and translational stages (Muralikrishna and Nirmala 2005). Our results showed that on the 4th germination day, the α -amylase activity of triticale malt was higher in those treated with SAEW than in those treated with TW, which could be due to a lower abscisic acid content in triticale malt in the SAEW treatments.

Effect of electrolyzed water on phytase activity during triticale malt growth

The phytase activity of triticale malt in the different treatments first decreased and then increased during the monitoring period (Fig. 2b). On the 1st germination day, phytase activity was relatively high in triticale malt soaked in TW and watered with TW or SAEW (1.07×10^{-4} and 0.97×10^{-4} units/mg protein, respectively). On the 2nd and 3rd germination days, phytase activity was relatively low in all treatments. On the 4th germination day, the phytase activity of triticale malt soaked in SAEW and watered with AEW or SAEW was 1.24×10^{-4} and 1.13×10^{-4} units/mg protein, respectively, significantly higher than in other treatments ($P < 0.05$).

Phytate is hydrolyzed by phytase, releasing free inorganic phosphate and myo-inositol. Extensive phytate removal has been shown to improve the absorption of iron and zinc in humans. Thus, malting might be a feasible way to obtain cereal products with high phytase activity and low phytate content (Rimsten et al. 2002). In our study, the phytase activity of triticale malt was higher on the 4th germination day than on the 1st germination day in all treatments except for STWT (triticale soaked and watered with TW). Fluctuations in phytase activity have also been reported for germinating rye and barley, i.e. phytase activity in germinated rye was higher on day 3 than on day 5, and that in germinated barley was higher on day 5 than on day 3 (Centeno et al. 2001). Therefore, changes in phytase activity may depend on the seed species, germination conditions, and the germination time.

Fig. 2 Effect of electrolyzed water on activities of enzymes during growth of triticale malt. Key: STWT-triticale seeds were soaked and watered during germination with tap water; STWS-triticale seeds were soaked in tap water for 8 h and watered with slightly acidic electrolyzed water during germination; STWA-triticale seeds were soaked in tap water for 8 h and watered with alkaline electrolyzed

water during germination; SSWT-triticale seeds were soaked in slightly acidic electrolyzed water for 8 h and watered with tap water during germination; SSWS-triticale were soaked and watered during germination with slightly acidic electrolyzed water; SSWA-triticale were soaked in slightly acidic electrolyzed water for 8 h and watered with alkaline electrolyzed water during germination

Effect of electrolyzed water on protease activity during growth of triticale malt

The acidic protease activity of triticale malt increased to higher levels after soaking in SAEW than after soaking in TW (Fig. 2c). On the 4th germination day, the acidic protease activity of triticale malt soaked in SAEW and watered with TW, AEW, or SAEW was 0.67, 0.62, and 0.53 units/mg protein, respectively, significantly higher than in other treatments ($P < 0.05$).

The effect of EW on neutral protease activity during the growth of triticale malt is shown in Fig. 2d. During the monitoring period, neutral protease activity remained at a higher level in triticale malt soaked in TW than in triticale malt soaked in SAEW. On the 4th day of germination, the neutral protease activity of triticale malt soaked in TW and watered with AEW, SAEW, or TW was 0.33, 0.24, and 0.22 units/mg protein, respectively, significantly higher than in other treatments ($P < 0.05$).

The effect of EW on alkaline protease activity during the growth of triticale malt is shown in Fig. 2e. The alkaline protease activity of triticale malt soaked in SAEW and watered with AEW (SSWA treatment) remained at a relatively high level during the monitoring period. On the 3rd germination day, the alkaline protease activity of triticale malt in the SSWA treatment was 0.12 units/mg protein.

Plants accumulate protein reserves in developing seeds, and storage proteins are mobilized during seed germination and subsequent seedling growth (Müntz et al. 2001). Protein breakdown and recycling, which depend on the activity of proteolytic enzymes, are essential parts of the plant response to environmental stress (Hameed et al. 2008). We observed increased acidic protease activity during germination, and this may account for the breakdown of large, insoluble storage proteins into soluble proteins, peptides, and amino acids. Gibberellins produced in the embryos of germinating seeds may enhance protease activity (Li et al. 2011). Generally, acidic protease activity was higher than neutral and alkaline protease activity in triticale malt soaked in SAEW, consistent with the results of a previous study (Ramakrishna and Rao 2005). On the 4th germination day, triticale malt soaked in SAEW had higher acidic protease activity, while triticale malt soaked in TW had higher neutral protease activity. This result indicates that a soaking solution close to the optimum pH of protease affected protease activity more strongly than did the rinsing solution.

Effect of electrolyzed water on lipase activity during growth of triticale malt

The effect of EW on lipase activity in triticale malt is shown in Fig. 2f. On the 4th germination day, the lipase

activity of triticale malt soaked in TW and watered with TW, SAEW, or AEW was 0.27, 0.30, and 0.38 units/mg protein, respectively. However, lipase activity was higher in triticale malt soaked in SAEW and watered with TW, SAEW, or AEW (0.57, 0.49, and 0.51 units/mg protein, respectively).

Lipase catalyzes the hydrolysis of glycerol esters to yield free fatty acids (Opute 1975). Autoxidation of fatty acids produced by lipase can cause rancidity in grain (Kermasha et al. 1993). Rancidity is not usually a problem in intact grains stored at normal temperature and moisture levels (Peterson 1999). However, the malting process involves germination, during which lipase activity increases. As a result, triticale malt powder has the potential to spoil during storage. Previous studies have shown that the higher the water content of seeds, the higher the lipase activity, as the increased water content may enhance the solubilization process necessary for enzyme action (Opute 1975). In our previous study, we found that the water absorption capacity of triticale seeds was higher in those treated with SAEW than in those treated with TW (Liu et al. 2016). In this study, on the 4th germination day, lipase activity was significantly higher in triticale malt soaked in SAEW than in triticale malt soaked in TW, possibly because the seeds treated with SAEW absorbed more water during soaking.

Conclusion

Our results indicate that EW can promote the growth of triticale malt. On the 4th germination day, the hypocotyl of triticale malt soaked in TW and watered with SAEW was 24.57% longer than that of triticale soaked and watered with TW. Compared with triticale malt soaked in TW, triticale malt soaked in SAEW had higher α -amylase, phytase, acidic protease, and lipase activities on the 4th germination day. Enzyme activities were particularly high in triticale malt soaked in SAEW and watered with AEW (activities of α -amylase, phytase, acidic protease, and lipase were 0.11, 1.24×10^{-4} , 0.62, and 0.51 units/mg protein, respectively). Our results provide an overview of enzyme activity characteristics during triticale malt germination and lay a strong theoretical foundation for further research on the application of triticale malt in functional food.

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