Carbohydrate Polymers 96 (2013) 474–480

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Ultrasonic effect on the desizing efficiency of α-amylase on starch-sized cotton fabrics

Longyun Hao*, Rui Wang, Kuanjun Fang, Jingquan Liu

Chemistry and Environment College of Qingdao University, Qingdao 266071, China

ARTICLE INFO

Article history:
Received 14 January 2013
Received in revised form 4 April 2013
Accepted 5 April 2013
Available online 12 April 2013

Keywords:
α-Amylase
Enzymatic desizing
Ultrasound
Starch
Cotton fabrics

ABSTRACT

Enzymatic desizing by α-amylase and ultrasound irradiation are the two important clean technologies in the textile industry. In the present work, with the aim of giving a further insight to the influence of ultrasound on α-amylase activity and its desizing efficiency, the ultrasound-based experiments were afforded in two ways: (i) step-wise treatment of α-amylase by ultrasound and then enzymatic desizing, as well as; (ii) simultaneous utilization of ultrasound and α-amylase for the desizing. By the step-wise strategy, it is found that the ultrasound has negative impact on the α-amylase activity using soluble starch as substrate. However, the sonicated α-amylase possesses higher desizing efficiency because there are higher hydrophobic interactions between sonicated α-amylase protein and starch-sized cotton and thus intensifies its catalytic activity. By the simultaneous procedure, the enhancement to desizing efficiency is more pronounced than that by the step-wise procedure. This can be attributed to comprehensive actions of several reasons such as more effective stirring/mixing mechanism, damages or changes to substrate, more effective catalysis to hydrolytic reactions and faster removal of loosened products from the fabric bulk.

1. Introduction

Ultrasound (US) is a sound beyond human audible range of 18–20 kHz, which can be used for various purposes in diverse industrial fields(Kwiatkowska, Bennett, Akunna, Walker, & Bremner, 2011; Vankar, Shanker, & Verma, 2007). Low frequency (20–100 kHz) US is considered a green technology due to its economically viable performance, high efficiency and low instrumental requirements (Rokhina, Lens, & Virkutyte, 2009). It can be used for cleaning, welding and sonochemistry (Basto, Tzanov, & Cavaco-Paulo, 2007; Hao, Wang, Liu, & Liu, 2012b; Rokhina et al., 2009). US can promote a wide variety of chemical, physical and biological processes mainly through the cavitation phenomenon in the liquid medium. Acoustic cavitation is the formation, growth and explosive collapse of microscopic bubbles in liquid, which causes drastic increase in the local temperature and pressure, releases large amounts of highly localized energy and generates highly reactive hydroxyl radicals (Merdan, Akalin, Kocak, & Usta, 2004; Yachmenev, Blanchard, & Lambert, 2004). In heterogeneous systems, when microscopic cavitation bubbles collapse at the surface of the solid substrate, they generate powerful shock waves that cause effective stirring/mixing of the border layer of liquid at a solid/liquid interface (Yachmenev et al., 2004).

US has the potential to improve the performance of various enzyme in textile wet processing such as the hydrolysis of cellulose by cellulase, pectins by pectinase and/or starches by amylase (Hao, Wang, Liu, & Liu, 2012a; Imai, Ikari, & Suzuki, 2004; Wang, Yu, & Zhong, 2012; Yachmenev, Bertoniere, & Blanchard, 2001). α-Amylase, an important industrial enzyme for the removal of starch size from woven textiles, can hydrolyze starch, glucogen and related polysaccharides by randomly cleaving the internal α-1,4-glucosidic linkages (Dong & Lu, 2008; Muralikrishna & Nirnala, 2005). However, very little is known about the actual effect of US on α-amylase because contradictory results of inactivation and activation of enzymes upon US treatment have been reported. Apar, Turhan, and Ozbek investigated the effect of US (20 kHz) on the activity of alpha-amylase enzymes produced by *Bacillus subtilis* on corn, rice and wheat starch at 50 °C under pH 6.5. They found that the activity of alpha-amylase decreased from 89% to 75% by increasing the duty cycle from 10% to 80% at acoustic power 100 W (Apar, Turhan, & Ozbek, 2006). Kadhodaee and Povey confirmed that the sonication at a constant operating frequency of 30 kHz and maximum nominal power output of 50 W would result in the apparent inactivation of bacterial α-amylase. It was found that the inactivation rate varied depending on the temperature and the radiating face of the sonotrode used (Kadkhodaee & Povey, 2008). Yaldagard, Mortazavi, and Tabatabaie studied the effect of 20 kHz US on the germinated barley's alpha-amylase activity at different

* Corresponding author. Tel.: +86 532 85955529; fax: +86 532 85955529.
E-mail address: hlyqdu@hotmail.com (L. Hao).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.04.003

temperatures (30, 50 and 70 °C) and ultrasonic intensities (20, 60 and 100% setting from total power of 460 W) by using the Taguchi statistical method. They concluded that the US had a destructive effect on barley's alpha-amylase and caused apparent inactivation of this enzyme (Yaldagard, Mortazavi, & Tabatabaie, 2008). On the contrary, Souza et al. reported that the α-amylase activity determined in 40 kHz US bath was higher than that without sonication when temperature below 45 °C. In this work, the activation energies of amylase catalysis in the presence and absence of US irradiation were calculated to be 12.11 and 58.54 kJ/mol, respectively (Souza et al., 2013). Interestingly, positive and consistent results about sonication were obtained when α-amylase was used for the enzymatic desizing of woven textiles instead of the hydrolysis to soluble starch. Sahinbaskan and Kahraman treated the starch-sized 100% cotton plain woven fabric with α-amylase in conventional and ultrasonic bath procedures, respectively, and found that this US-based method brought about a significant increase in the starch-size removal. They attributed this effect to the acceleration of enzyme diffusion toward the fabric surface by the US (Sahinbaskan & Kahraman, 2011). Wang, Yu, and Zhong utilized 53 kHz US to improve the desizing efficiency of a commercial amylase. They suggested that the US assisted system could save half the processing time and improve about 5% point in desizing efficiency (Wang et al., 2012).

Clearly, the effect of US on enzyme performance is substrate dependent. What should be noted is that the starch size on the textile surfaces consists of non-soluble starch with higher molecular weight than the soluble starch, so the hydrolysis catalyzed by α-amylase for desizing is a heterogeneous procedure. As a heterogeneous system, much more factors are involved because it is a comprehensive impact of US on the amylase, fabric substrates and their mutual actions, and it is hard to tell which factor might be predominant and how to balance them. In this research, with the aim of giving a further insight to the influence of US on desizing efficiency of amylase, we performed the US-based experiments in two ways: (i) step-wise treatment of α-amylase by US and then enzymatic desizing, as well as; (ii) simultaneous utilization of US and α-amylase for the desizing. By this strategy, the effects of US on the α-amylase, fabric and their interactions will be thoroughly discussed. It will be clear as to how significant the sonication could be in reducing energy consumption and improving the starch size removal during the enzymatic desizing process by α-amylase.

2. Experimental

2.1. Materials

Untreated, starch-sized, 100% cotton plain woven fabrics used throughout this work were obtained from Binzhou Dyeing Factory, China. The fabric weight is 167 g/m^2 (47 ends/cm and 36 picks/cm) with 6.5% starch size add-on. A commercial α-amylase (HY-ktm, 40,000 U/mL) produced by selected strain of *B. subtilis* for desizing at medium and high temperature was supplied by Haiyi Chemical Company. The non-ionic wetting agent HWT (based on alkylaryl polyglycol ether) was also kindly provided by Haiyi Chemical Company The soluble starch (potato, AR) was bought from Tianjin Chemical Ltd. and used as substrate for estimating α-amylase activity.

3,5-Dinitrosalicylic acid solution was prepared for measuring the reducing sugar. 10 g of 3,5-dinitrosalicylic acid and 10 g of sodium hydroxide were dissolved in 500 mL distilled water. 200 g of sodium potassium tartrate was then added to the solution with constant mixing. Afterward by adding 2 g of phenol and 0.5 g of metabisulphite, the total volume of the solution was adjusted to 1 L with distilled water (Shukla & Jajpura, 2004).

2.2. Equipment

Experiments were performed in a Kudos (Japan) US cleaner with thermostatic water bath (temperature accuracy of ±1 °C) at a frequency of 49 kHz. The output power was set at 200 W and supplied by two transducers at the bottom horizontally along the length of the bath. The apparatuses have accurate digital time controller (0–90 min) and degas functions. All enzymatic reactions were carried out in a 250 mL cylindrical glass reactor (7 cm in diameter and 10 cm in height).

2.3. Methods

Desizing of woven fabrics was carried out in two ways:

(i) Two steps (step-wise): 50 mL of α-amylase (40 U/mL, pH 6.0) was filled in the glass tube and placed in the center of the 49 kHz ultrasonic field. Different temperatures (30 °C and 60 °C) were set during the sonication for various exposed time of 5, 10 and 15 min. After sonication, the residual activity of amylase toward soluble starch was immediately measured according to the method of reducing sugars. And then, the starch-sized cotton fabrics were desized according to following conditions: α-amylase dosage 2 mL/L, NaCl 5 g/L, nonionic wetting agent 2 g/L, pH 6.0, liquor ratio 30:1, temperature 50–90 °C and time 15 min. The binding ratio of amylase protein on fabrics was estimated by means of the loss of protein in solution during the process using the Bradford method (Bradford, 1976). Upon the completion of the reaction, the solution was drained, and then the treated samples were thoroughly washed with 95 °C water for 15 min. At last, the fabrics were rinsed using tap water and then dried at 105 °C to a constant weight.

(ii) One step (simultaneous): the cotton fabrics were desized in the presence of US by following conditions: α-amylase dosage 2 mL/L, NaCl 5 g/L, nonionic wetting agent 2 g/L, pH 6.0, liquor ratio 30:1, temperature 50–90 °C and time 15 min. Upon the completion of the reaction, the solution was drained, and then the treated samples were thoroughly washed with 95 °C water for 15 min. At last, the fabrics were rinsed using tap water and then dried at 105 °C to a constant weight.

2.4. Measurements

2.4.1. α-Amylase activity assay according to the method of reducing sugars

Amylase activity was determined by measuring the decrease/increase in absorbance at 540 nm at pH 6.0 and 25 °C. Two milliliters of starch solution (1%, w/v) was added to 2 mL of diluted α-amylase enzyme solution. The mixture was incubated at 50 °C for 10 min in a thermostated water bath and stirred during the incubation period. After the incubation, 2 mL of 3,5-dinitrosalicylic acid was added to the solution and then held in boiling water bath for 5 min. The intensity of the red-brown color developed during heating was measured spectrophotometrically against the blank solution at 540 nm after cooling to room temperature. The residual activity of α-amylase was calculated from the slope of the calibration curve prepared for standard solutions of glucose. All assays were performed in duplicate.

2.4.2. Determination of the desizing efficiency

For determining the desizing efficiency, samples were taken to test the residual starch content on them. The starch content on fabric was analyzed by referring to the perchloric acid method (Dong &

Lu, 2008; Wang et al., 2012). The desizing efficiency was calculated using Eq. (1):

$$\text{Desizing}\ \ (\%) = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

where W_0 and W_1 are the amount of starch for control and desized fabric, respectively.

2.4.3. The weight loss and retained strength of fabrics

Loss in weight (WL, %) by the enzymatic treatment was calculated as the percentage weight loss to the initial dry weight of fabric according to Eq. (2):

$$\text{WL}\ \ (\%) = \frac{W_{\text{before treatment}} - W_{\text{after treatment}}}{W_{\text{before treatment}}} \times 100 \quad (2)$$

The tensile strength of the cotton fabric was tested according to ISO 13934-1-1999 using YG-2 testing machine (Laizhou, China). Samples were tested five times and the average value was used. The retained strength (RS, %) was calculated according to Eq. (3):

$$\text{RS}\ \ (\%) = \frac{S_1}{S_0} \times 100 \quad (3)$$

where S_0 and S_1 are the tensile strength for control and desized fabric, respectively. All samples were conditioned at 20 °C in 65% RH for 24 h before testing weight and tensile strength.

2.4.4. Drop absorbency test

Drop absorbency of the samples was calculated as the AATCC Test Method 79-2000 (Saravanan, Prakash, & Jagadeeshwaran, 2011).

2.4.5. Staining test

The fabric samples were submerged in a 0.005 M iodine/iodide potassium solution for 3 min to stain the residual starch on them. Subsequently, the samples were taken out and sucked using filter paper, and then dried at room temperature. The photos of stained samples were taken using a light microscopy (Nikon E600) fitted with a digital camera.

2.4.6. Fluorescence measurement

Intrinsic fluorescence measurement of amylase was performed with a Cary Eclipse spectrophotometer using quartz cuvette thermostated at an excitation wavelength of 280 nm. Emission spectra were corrected using buffer as a blank and recorded at the range of 300–450 nm. Excitation and emission slit widths are 5 and 10 nm, respectively. The scan rate was maintained constant at 600 nm/min for all experiments.

2.4.7. Scanning electron microscope

A scanning electron microscope (SEM) examination was carried out by mounting the fabric samples on sub with double stick adhesive tape and coated with gold in Sputter Coater Unit. The samples were then viewed using a JSM-6390LV Scanning Electron Microscope (JEOL, Japan) with accelerating voltage of 20 kV and magnification 600×.

3. Results and discussion

3.1. Step-wise strategy

3.1.1. Effect of US on α-amylase activity

In order to investigate the effect of US on α-amylase performance, the enzyme solutions were sonicated for different time at a constant frequency of 49 kHz under 30 and 60 °C, respectively, and the data related to the residual activity shown in Fig. 1. To examine the effect of thermo-sonication on amylase, the inactivation due to heat treatment only was also plotted at the same time. The result indicates the thermal inactivation of the enzyme is negligible at 30 °C, but it is revealed that the sonication lead to obvious decrease in amylase activity at this temperature. Fig. 1 also shows that at higher temperature, greater inactivity can be observed due to the heat-induced denaturation of amylase. However, deactivating efficiency of US becomes lower at elevated temperatures probably because of the cushioning effect by the higher vapor pressure of water to weaken the pressure inside the cavitation bubbles at the moment of adiabatic collapse (Kadkhodaee & Povey, 2008).

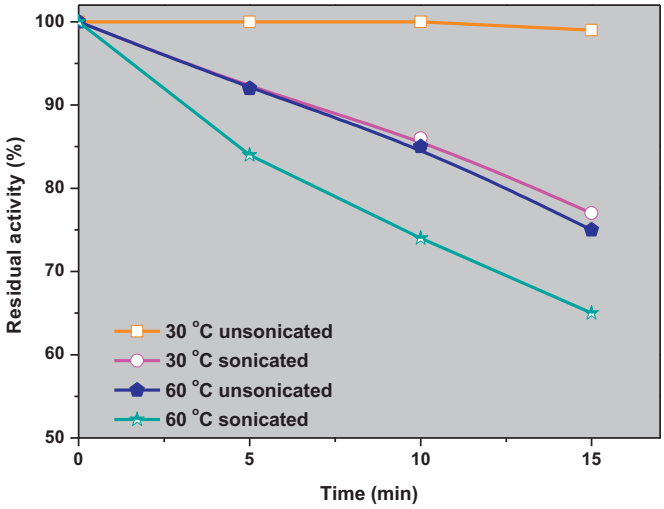

Fig. 1. Effect of sonication time on α-amylase activity.

As known, the physical, chemical and biological functions of amylase are ultimately determined by its native structure as well as the changes in its molecular associations caused by variations of the temperature, pH value, ionic strength, mechanical and chemical forces in the environment (Liu, Chen, & Chou, 2003). Inactivation of amylase by sonication can be mechanically attributed to mechanical stresses or shear forces created by micro-streaming and shock waves, which can change the secondary and tertiary structure of the enzyme protein by breaking various bonds and increase its hydrophobicity by unfolding and revealing of hydrophobic amino acids (Ozbek & Ulgen, 2000). Fluorescence emission spectra of amylase sonicated for different time were recorded at excitation wavelength of 280 nm and shown in Fig. 2, where it is observed that the fluoresce intensity is increased with the increasing sonication duration. The ultrasonic cavitation in the liquid medium can disperse the amylase molecules and thus reduce the physical-linked aggregations. However, the maximum fluorescence emission wavelength of the sonicated amylase is found to red-shift slightly about 2–3 nm. With an excitation of 280 nm, most of a protein's intrinsic fluorescence can be attributed to tryptophan residues (Bakkialakshmi, Shanthi, & Bhuvanapriya, 2012; Hodgson & Plaxton, 1995). The red-shift of emission wavelengths means that the tryptophan side chains are transferred to the outer part of a protein structure and exposed in a more hydrophilic environment, revealing some unfolding of the amylase protein (Andreaus, Azevedo, & Cavaco-Paulo, 1999). Apart from the mechanical forces, the generation of free hydroxyl radicals through sonolysis of water is also an important factor for the activity loss of amylase. The hydroxyl radicals, with unpaired electron and highly reactive activity, possess a very high redox potential and strong oxidation characteristic to make them react very rapidly with many amino acids, especially the tyrosine, by a combination of addition, hydrogen abstraction or electron transfer reactions (Kadkhodaee & Povey, 2008). Moreover, the crosslink between some amino acids,

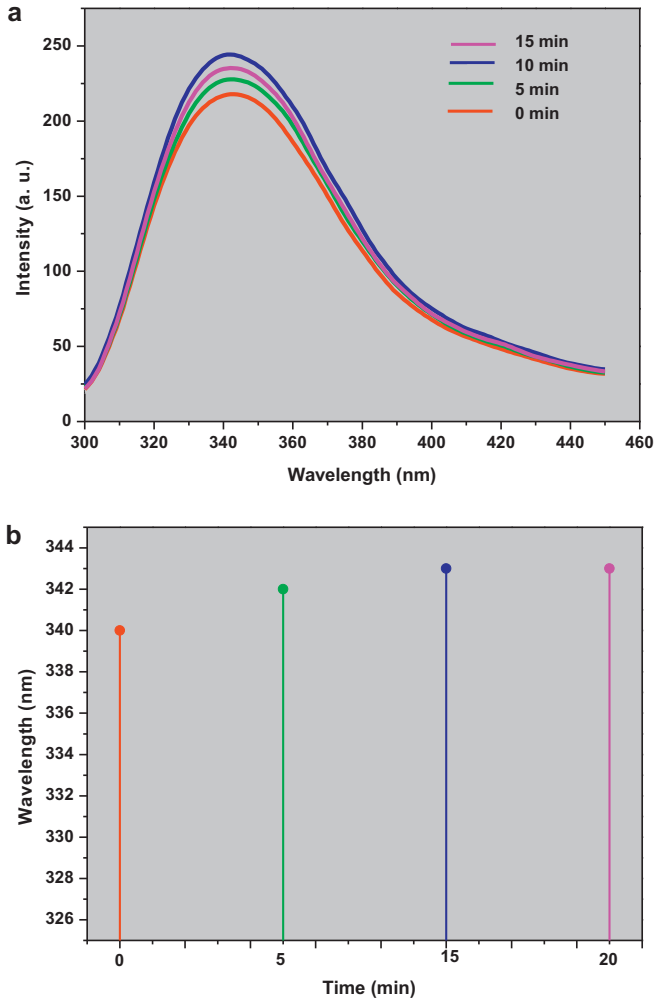

Fig. 2. Effect of sonication on fluorescence intensity (a) and emission wavelength (b) of α-amylase (sonication temperature: 30 °C).

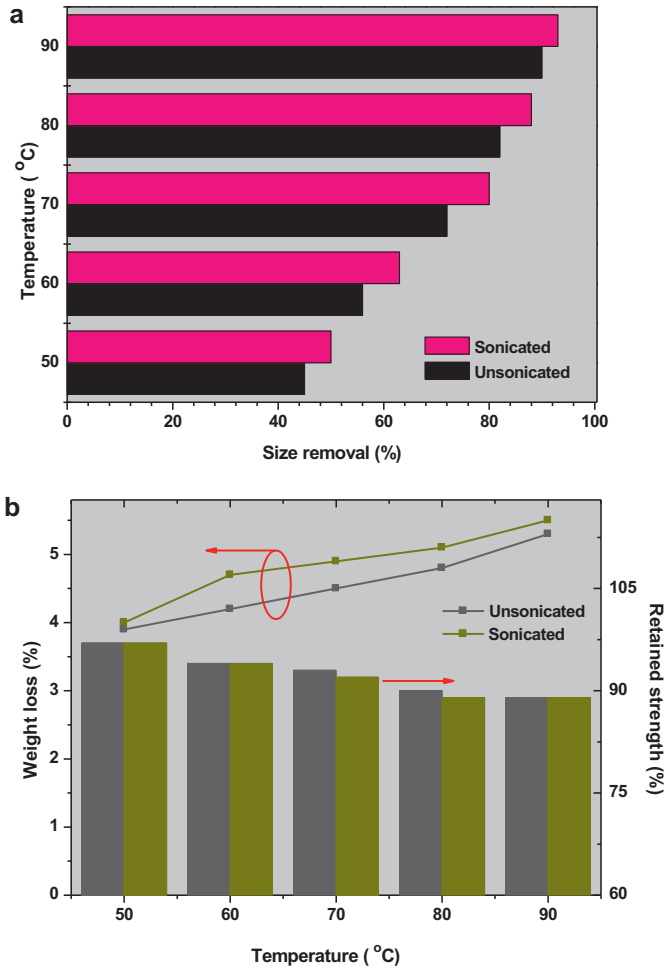

Fig. 3. Effect of sonication on size removal (a), weight loss and retained strength (b) of fabrics (sonication time: 10 min, sonication temperature: 30 °C).

especially the cysteine residue, is also responsible for the activity loss of amylase by severe altering the enzyme active site geometries (Avivi & Gedanken, 2007). Generally, the contribution of physical and chemical mechanisms toward the activity loss of amylase is not equal and strongly dependent on the experimental conditions, the amino acid compositions and the conformational structure of the amylase in application.

3.1.2. *The desizing performance of sonicated α-amylase*

Modern production processes for woven fabrics introduce some sizes, usually the starch, on the warp yarns for preventing them from breaking. After weaving, the woven fabrics are usually treated for efficient removal of starch sizes using α-amylase by its ability to cleave α-1,4 glycosidic bonds present in the endo part of amylose or amylopectin molecules. The desizing efficiencies of original and sonicated α-amylase within the temperature range from 50 to 90 °C were investigated, respectively, and the results about size removal, weight loss as well as retained strength shown in Fig. 3. It is observed that raising the temperature up to 90 °C brings a remarkable improvement to the desizing efficiency in terms of the higher size removal, higher weight loss and lower retained strength. High temperature can intensify the desizing efficiency of α-amylase by accelerating the catalytic rate, enhancing the enzyme molecular mobility on the substrate surface and improving the swellability of the size film (Ibrahim, El-Hossay, Morsy, & Eid, 2004). These favorable factors, at this experimental condition, can counteract the activity loss induced by heat. Interestingly, the pre-sonicated amylase has higher desizing efficiency with respect to the original one at the same temperature although it possesses some activity loss tested by soluble starch as substrate. As a homogenous system, the enzymatic hydrolysis of soluble starch molecules absolutely depends on the function of amylase, so the dysfunction of amylase by sonication will result in obvious activity loss in the solution. On the occasion of desizing, the system is heterogeneous with respect to the insoluble starch size film on fabric, so the hydrolysis of starch molecules is also regulated by the adsorption properties of enzyme protein on the substrate besides the function of amylase. The adsorption of amylase protein on starch-sized cotton is a solid/liquid process advancing by the movement of the protein from aqueous phase to the solid surface of the fabric and determined by the structure of the enzyme, the surface area and the number of sub-units held by the substrate (Nithiyanantham & Palaniappan, 2010). First, amylase protein is bound by virtue of affinity after effective contact with the cotton surface resulting in forming an enzyme–substrate (E–S) complex. Then, the water molecules are transferred to the active sites of the E–S complex and surface reaction between water and starch size is catalyzed by the E–S complex (Sarkar & Etters, 2001). Time-amylase uptake isotherms of original and sonicated amylase are plotted in Fig. 4. It can be noted the residual amylase decreases rapidly after short-time contact with both the substrates, and then the slope of the adsorption isotherms decreases. In general, the adsorption of amylase protein on substrate is a quick process. By contrast, the

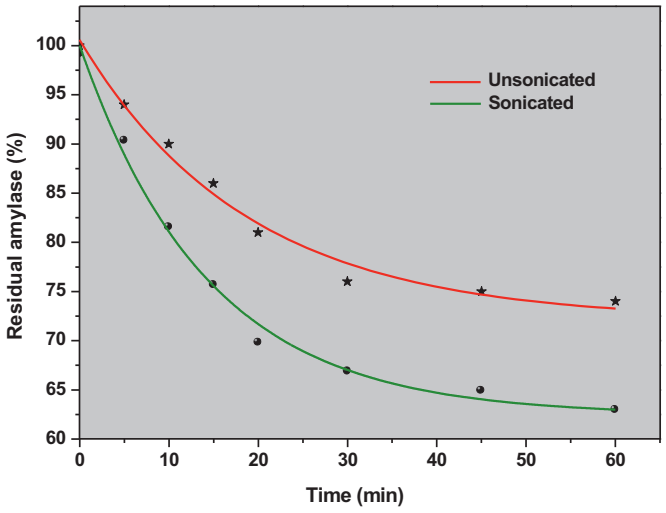

Fig. 4. Effect of sonication on the adsorption of amylase on the fabrics (sonication time: 10 min, sonication temperature: 30 °C).

equilibrium adsorption of sonicated amylase is higher than that of original one resulting from a higher substantivity (Warren, Royall, Gaisford, Butterworth, & Ellis, 2011). As demonstrated above, the molecular chains of amylase can be partly unfolded and more hydrophobic tryptophan residues formerly hidden inside the 3D structure are relocated to the outer side after sonication, which will lead to the increase in hydrophobic interactions between sonicated amylase protein and starch-sized cotton. Clearly, the higher adsorption ability of sonicated amylase protein would improve its accessibility to starch-sized cotton substrate and intensify its catalytic activity toward insoluble starch-size by increasing the effective enzyme concentration on the cotton substrate. In contrast, the activity loss by the US is relatively weak and can be counteracted in this condition so the overall desizing efficiency is improved for the sonicated one.

3.2. Simultaneous procedure

From the above discussion, it is known that the sonication is a beneficial means for the enzymatic desizing when applied in a pretreatment way. In the real industrial production, it is a more economic and convenient method to apply US accompanying the enzymatic desizing process. Moreover, using this simultaneous procedure, the US energy can modify the structural properties of starch size at the same time, which will probably further improve the desizing efficiency. To evaluate this effect, the enzymatic desizing was carried out in the presence of US and the results about desizing efficiency at different temperatures exhibited in Fig. 5. It is suggested that the operating temperature can be lowered by 10 °C with the assistance of US to acquire the same desizing quality with the conventional condition. By comparison, the enhancement to desizing efficiency using the simultaneous method is more pronounced than that with the stepwise procedure.

The enzymatic desizing involves the following stages: (i) transferring the amylase molecules from aqueous phase to fabric surface through border layer; (ii) binding of amylase molecules by fabric bulk; (iii) catalyzing the hydrolytic reaction of starch size; and (iv) transporting the products of hydrolytic reaction to the aqueous phase (Yachmenev et al., 2004). Theoretically, the favorable effect of US on desizing efficiency can be attributed to comprehensive actions of several reasons. First of all, providing a far more effective stirring/mixing mechanism for the immediate, border layer of liquid at the solid–liquid interface, the US can propel

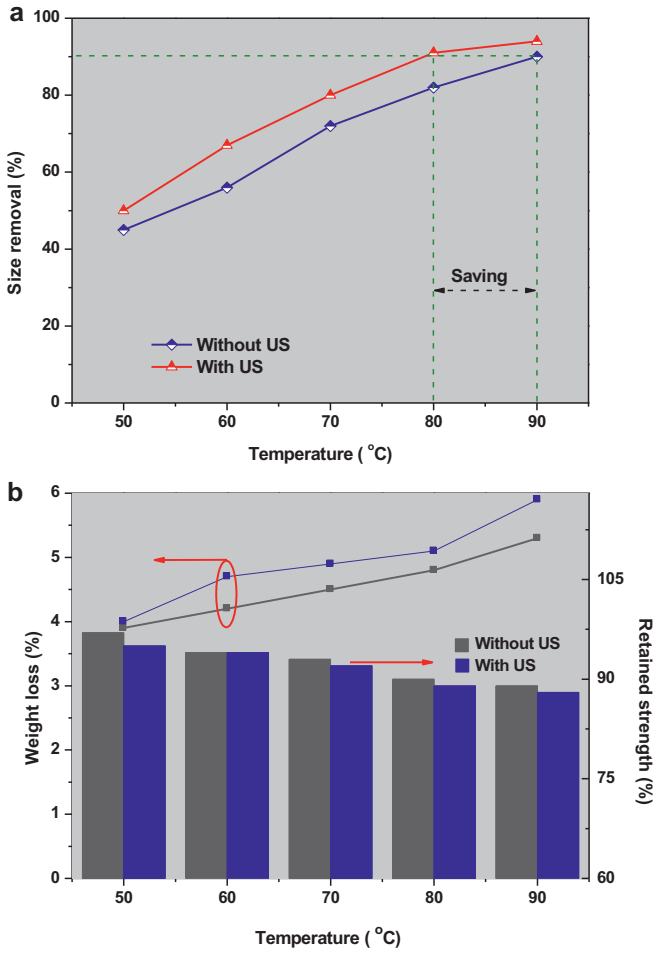

Fig. 5. Effect of US on size removal (a), weight loss and retained strength (b) of fabrics.

the transferring of amylase macromolecules (usually 50–60 kDa) from aqueous phase to fabric surface and thus promote the adsorption of enzyme macromolecules onto the substrate (Gupta, Gigras, Mohapta, Goswami, & Chauhan, 2003). Second, sonication can definitely affect the physicochemical properties of starch size in different ways. The intense mechanical forces and hydroxyl radicals attacks coming from the cavitation of US could generate rich pores or crevices in the starch size, make the exposed surface rougher, promote the disintegration of starch molecule aggregates, swell the starch size, increase the solubility of the starch molecules and even lead to the depolymerization of starch by the scission of the macromolecules (Czechowska-Biskup, Rokita, Lotfy, Ulanski, & Rosiak, 2005; Imai et al., 2004; Lima & Andrade, 2010; Zuo, Hebraud, Hemar, & Ashokkumar, 2012; Zuo, Knoerzer, Mawson, Kentish, & Ashokkumar, 2009). Directly, the SEM photos of fabrics were taken and revealed in Fig. 6. The original starch-sized cotton fabric presented in Fig. 6(a) possesses a lot of starch sizes on its surface, which should be reduced after enzymatic desizing. From Fig. 6(b), it can be seen that most starch sizes are removed by the enzymatic attack of amylase. Apparently in Fig. 6(c), with the assistance of US, the starch sizes are totally eliminated during the enzymatic desizing process. The improvement in desizing efficacy by sonication can also be directly demonstrated by staining fabric samples with a solution of iodine/potassium iodine and shown in Fig. 7. Dramatically, the sample treated by amylase together with sonication is least stained to form purple complex by the iodine reagent indicating a more sufficient removal of starch from textiles. Moreover, impurities or any non-starch components in

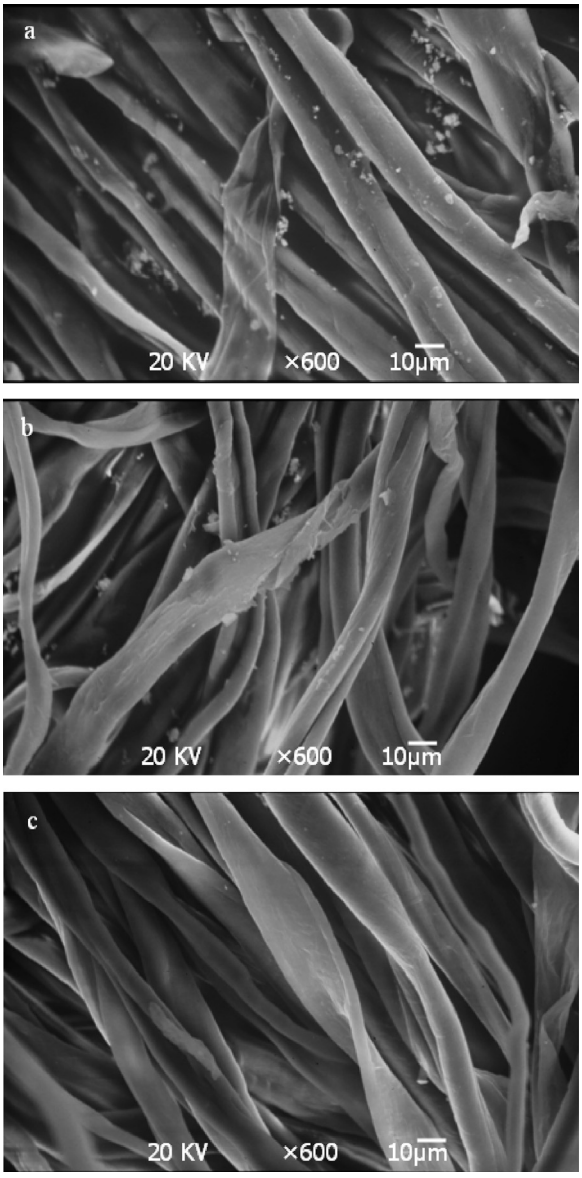

Fig. 6. SEM photos of fabrics: untreated (a), enzymatically desized (b), and sonicated and enzymatically desized (c).

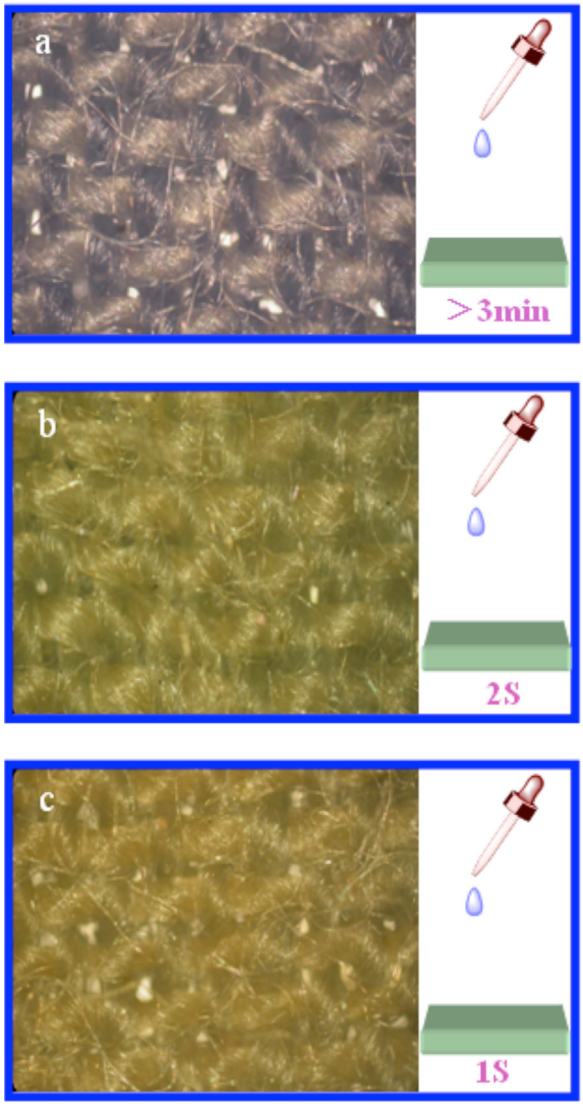

Fig. 7. Staining photos of the fabrics with a solution of iodine/potassium iodine: untreated (a), enzymatically desized (b), and sonicated and enzymatically desized (c).

the starch size, such as surface proteins, lipids, waxes and additives, should be considered for explaining the intensified desizing effect of US. Presence of hydrophobic impurities in the dried film of starch increases the hydrophobicity of the substrate and thus undermines the wetability of substrate and the accessibility of amylase molecules. With the ability to generate powerful shock waves by acoustic cavitation the US could directly remove some impurities from the substrate or break the distribution continuity of impurities on the substrate surface, obviously improving the wetability and susceptibility of the starch size. Drop absorbency time of fabric after enzymatic desizing can be largely reduced from over 3 min to 2 s (Fig. 7(a) and (b)). By the sonication plus amylase, the instantaneous drop absorbency time is further reduced to 1 s (Fig. 7(c)), also revealing a more effective desizing efficiency. As we know, amylase is highly substrate specific in its action, so the damages or changes in the substrate would definitely make the enzymatic attack larger and deeper into the starch size (Chen, Huang, Tang, Chen, & Zhang, 2011; Dhital, Shrestha, & Gidley, 2010), resulting in far more extensive hydrolysis and higher desizing efficiency. Third, a more effective catalysis to hydrolytic reactions of amylase can be produced by the US due to more rapid diffusion ability of amylase macromolecules on the substrate to find a new productive position and establish a more productive contact. Last but not least, the loosened products such as oligosaccharides with a DP of 2–12 derived from the multiple attacks of amylase could be easily removed from the substrate to the aqueous phase under the ultrasonic condition to reduce the competitive/non-competitive inhibition by the accumulated hydrolytic products (Muralikrishna & Nirnala, 2005; Sarkar & Etters, 2001). Concluded from above discussions, we can propose that although the US would inactivate some α-amylase due to its damage to the intricate structure of enzyme protein, the enzymatic desizing efficiency could still be effectively build up by the beneficial impacts of ultrasonic energy on the substrate and interactions between amylase–substrate complexes.

4. Conclusion

The ultrasonic energy was introduced in the enzymatic desizing processes of woven cotton fabrics for higher desizing efficiency. By the step-wise strategy, it is found that the US has negative impact on the α-amylase activity when using soluble starch as substrate.

However, the sonicated α-amylase possesses higher desizing efficiency because US leads to the increase in hydrophobic interactions between sonicated amylase and substrate by unfolding and revealing of hydrophobic amino acids and thus intensify its catalytic activity toward insoluble starch size. About 2–3 nm red-shift of the maximum fluorescence emission wavelength can be recorded for the sonicated amylase. By the simultaneous procedure, the enhancement to desizing efficiency is more pronounced than that with the step-wise procedure. It is suggested that the operating temperature can be lowered by 10 °C with the assistance of US to acquire the same desizing quality with the conventional condition. This can be attributed to comprehensive actions of several reasons such as more effective stirring/mixing mechanism, damages or changes to substrate, more effective catalysis to hydrolytic reactions and faster removal of loosened products from the fabric bulk. The US improvement for desizing can be directly demonstrated by the SEM photos and staining tests using iodine/iodine potassium solutions.

Acknowledgements

We acknowledge Shandong Provincial Natural Science Foundation (ZR2010EQ034), National Natural Science Foundation of China (51173086) and Taishan Scholars Program of Shandong Province, China for financial support of this project.

References

Andreaus, J., Azevedo, H., & Cavaco-Paulo, A. (1999). Effects of temperature on the cellulsoe binding ability of cellulase enzymes. *Journal of Molecular Catalysis B: Enzymatic*, *7*, 233–239.

Apar, D. K., Turhan, M., & Ozbek, B. (2006). Enzymatic hydrolysis of starch by using a sonifier. *Chemical Engineering Communications*, *193*, 1117–1126.

Avivi, S., & Gedanken, A. (2007). Are sonochemically prepared aylase protein microspheres biologically active? *Ultrasonics Sonochemistry*, *14*, 1–5.

Bakkialakshmi, S., Shanthi, B., & Bhuvanapriya, T. (2012). Study of fluorecence quenching of barley α-amylase. *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy*, *90*, 12–17.

Basto, C., Tzanov, T., & Cavaco-Paulo, A. (2007). Combined ultrasound-laccase assisted bleaching of cotton. *Ultrasonics Sonochemistry*, *14*, 350–354.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, *72*, 248–254.

Chen, Y., Huang, S., Tang, Z., Chen, X., & Zhang, Z. (2011). Structureal changes of cassava starch granules hydrolyzed by a mixture of α-amylase and glucoamylase. *Carbohydrate Polymers*, *85*, 272–275.

Czechowska-Biskup, R., Rokita, B., Lotfy, S., Ulanski, P., & Rosiak, J. M. (2005). Degradation of chitosan and starch by 360-kHz ultrasound. *Carbohydrate Polymers*, *60*, 175–184.

Dhital, S., Shrestha, A. K., & Gidley, M. J. (2010). Relationship between granule size and in vitro digestibility of maize and potato starches. *Carbohydrate Polymers*, *82*, 480–488.

Dong, S., & Lu, D. (2008). Kinetics of the thermal inactivation of *Bacillus subtilis* amylase and its application on the desizing of cotton fabrics. *Journal of Applied Polymer Science*, *109*, 3733–3738.

Gupta, R., Gigras, P., Mohapta, H., Goswami, V. K., & Chauhan, B. (2003). Microbial α-amylases: A biotechnological perspective. *Process Biochemistry*, *38*, 1599–1616.

Hao, L., Wang, R., Liu, J., & Liu, R. (2012a). The adsorptive and hydrolytic performance of cellulase on cationised cotton. *Carbohydrate Polymers*, *89*, 171–176.

Hao, L., Wang, R., Liu, J., & Liu, R. (2012b). Ultrasound-assited adsorption of anionic nanoscale pigment on cationised cotton fabrics. *Carbohydrate Polymers*, *90*, 1420–1427.

Hodgson, R. J., & Plaxton, W. C. (1995). Effect of polyethylene glycol on the activity, intrinsic fluorescence, and oligomeric structure of castor seed cytosolic fructose-1,6-bisphosphatase. *FEBS Letters*, *368*, 559–562.

Ibrahim, N. A., El-Hossay, M., Morsy, M. S., & Eid, B. M. (2004). Optimization and modification of enzymatic desizing of starch-size. *Polymer-Plastics Technology and Engineering*, *43*(2), 519–538.

Imai, M., Ikari, K., & Suzuki, I. (2004). High-performance hydrolysis of cellulose using mixed cellulase species and ultrasonication pretreatment. *Biochemical Engineering Journal*, *17*, 79–83.

Kadkhodaee, R., & Povey, M. J. W. (2008). Ultrasonic inactivation of *Bacillus* α-amylase. I. Effect of gas content and emitting face of probe. *Ultrasonics Sonochemistry*, *15*, 133–142.

Kwiatkowska, B., Bennett, J., Akunna, J., Walker, G. M., & Bremner, D. H. (2011). Stimulation of bioprocesses by ultrasound. *Biotechnology Advances*, *29*, 768–780.

Lima, F. F., & Andrade, C. T. (2010). Effect of melt-processing and ultrasonic treatment on physical properties of high-amylose maize starch. *Ultrasonics Sonochemistry*, *17*, 637–641.

Liu, H.-L., Chen, W.-J., & Chou, S.-N. (2003). Mechanisms of aggregation of alpha- and beta-amylases in aqueous dispersions. *Colloids and Surfaces B: Biointerfaces*, *28*, 215–225.

Merdan, N., Akalin, M., Kocak, D., & Usta, I. (2004). Effects of ultrasonic energy on dyeing of polyamide/Lycra blends. *Ultrasonics*, *42*, 165–168.

Muralikrishna, G., & Nirnala, M. (2005). Cereal α-amylases – An overview. *Carbohydrate Polymers*, *60*, 163–173.

Nithiyanantham, S., & Palaniappan, L. (2010). Ultrasonic study of adsorption in disaccharide metabolism. *Applied Acoustics*, *71*, 754–758.

Ozbek, B., & Ulgen, K. O. (2000). The stablility of enzymes after sonication. *Process Biochemistry*, *35*, 1037–1043.

Rokhina, E. V., Lens, P., & Virkutyte, J. (2009). Low-frequency ultrasound in biotechnology: State of the art. *Trends in Biotechnology*, *27*(5), 298–306.

Sahinbaskan, B. Y., & Kahraman, M. V. (2011). Desizing of untreated cotton fabric with the conventional and ultrasonic bath procedures by imobilized and native α-aylase. *Starch*, *63*, 154–159.

Saravanan, D., Prakash, A. A., & Jagadeeshwaran, D. (2011). Optiization of thermophile *Bacillus licheniformis* α-amylase desizing of cotton fabrics. *Indian Journal of Fiber & Textile Research*, *36*, 253–258.

Sarkar, A. K., & Etters, J. N. (2001). Kinetics of the enzymatic hydrolysis of cellulose. *AATCC Review*, (3), 48–52.

Shukla, S. R., & Jajpura, L. (2004). Estimating amylase activity for desizing by DNSA. *Textile Asia*, *11*, 15–20.

Souza, M., Mezadri, E. T., Zimmerman, E., Leaes, E. X., Bassaco, M. M., Prá, V. D., et al. (2013). Evaluation of activity of a commercial amylase under ultraound-assisted irradiation. *Ultrasonics Sonochemistry*, *20*, 89–94.

Vankar, P. S., Shanker, R., & Verma, A. (2007). Enzymatic natural dyeing of cotton and silk fabrics without medal mordants. *Journal of Cleaner Production*, *15*, 1441–1450.

Wang, W., Yu, B., & Zhong, C. (2012). Use of ultrasonic energy in the enzymatic desizing of cotton fabric. *Journal of Cleaner Production*, *33*, 179–182.

Warren, F. J., Royall, P. G., Gaisford, S., Butterworth, P. J., & Ellis, P. R. (2011). Binding interactions of α-amylase with starch granules: The influence of supramolecular structure and surface area. *Carbohydrate Polymers*, *86*, 1038–1104.

Yachmenev, V. G., Bertoniere, N. R., & Blanchard, E. J. (2001). Effect of sonication on cotton preparation with alkaline pectinase. *Textile Research Journal*, *71*(6), 527–533.

Yachmenev, V. G., Blanchard, E. J., & Lambert, A. H. (2004). Use of ultrasonic energy for intensification of the bio-preparation of greige cotton. *Ultrasonics*, *42*, 87–91.

Yaldagard, M., Mortazavi, S. A., & Tabatabaie, F. (2008). The effect of ultrasound in combination with thermal treatment on the germinated barley's alpha-amylase activity. *Korean Journal of Chemical Engineering*, *25*(3), 517–523.

Zuo, Y. Y. J., Hebraud, P., Hemar, Y., & Ashokkumar, M. (2012). Quantification of high-power ultrasound induced damage on potato starch granules using light microscopy. *Ultrasonics Sonochemistry*, *19*, 421–426.

Zuo, J. Y., Knoerzer, K., Mawson, R., Kentish, S., & Ashokkumar, M. (2009). The pasting properties of sonicated waxy rice starch suspensions. *Ultrasonics Sonochemistry*, *16*, 462–468.