International Food Research Journal 23(5): 1960-1964 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Influences of sulfhydryl oxidase isolated from *Aspergillus niger* on physicochemical properties of starch and rheological properties of wheat dough

[1*]Siswoyo, T.A, and [2]Morita, N.

[1]*Department of Agronomy, Faculty of Agriculture, and Center for Development of Advanced Sciences and Technology (CDAST), University of Jember, Kalimantan 37, Jember 65121, Indonesia*
[2]*Department of Food Packing Technology, Toyo College of Food Technology , 4-3-2, Minami Hanayashiki Kawanishi, Hyogo 666-0026, Japan*

Article history

Received: 1 October 2015
Received in revised form: 13 January 2016
Accepted: 28 January 2016

Keywords

Sulfhydryl oxidase
Physicochemical
Starch
Rheological

Abstract

The sulfhydryl oxidase (SHOxase; EC. 1.8.3.2) which catalyzes the oxidation of sulfhydryl groups to disulfides was purified from Aspergillus niger using a combination of chromatographies. The influence of SHOxase on starch properties was tested by rheometer, farinographand scanning electron microscopy (SEM). Moreover, the gelatinization and retrogradation were studied by differential scanning calorimetry (DSC). The data showed that the SHOxase blended on dough formula increased the stability time of dough in a farinograph and all parameters of viscoelastic properties compared to the control. Furthermore, SEM image of SHOxase on dough showed that the gluten matrix was distinctly developed. The peak temperature (T_p) of the starch gelatinization and enthalpy value (ΔH_g) of control during mixing were compared with the SHOxase blended wheat flour. Mixing increased the T_p of the starch gelatinization and the ΔH_g. The addition of SHOxase in the blend increased the T_p and decreased the ΔH_g of the starch gelatinization. According to the Avrami equation, the retrogradation rate of starch during storage was slower than that of the control. The abilities of SHOxase to strengthen, decrease of ΔH_g and retard of retrogradation of wheat dough are likely attributable to its ability to catalyze formation of disulfide bonds in the systems.

Introduction

Wheat protein (gluten) is quite unique among other cereal and plant proteins in its ability to form dough with viscoelastic properties for making bread, biscuit, pasta, and other cereal products (Bushuk, 1998; Edwards *et al*., 2003; Anjum *et al*., 2007). Though gluten comprises around 10–14% of wheat flour, and consists of two major groups: the gliadins and glutenins and also contains about 2–3% of cysteine and cystine (Lasztity, 1986), they are responsible for the viscosity and elasticity of dough to a great extent (Sliwinski *et al*., 2004; Joye *et al.*, 2009). The gliadins consist of a mixture of many protein of low and medium molecular weight with little contains of disulfide bonds or inter-chain disulfide bonds. Many researchers have investigated the physicochemical properties of gliadins and glutenins, also their relationships to the bread-making potential of flour. Studies on the effect of heat on wheat protein interactions have shown that the sulfhydryl-disulfide interchange reaction was responsible for the protein network formation upon thermosetting (Weegels *et al.*, 1994; Lagrains *et al.*, 2010; Pepels *et al*., 2013). Furthermore, it has been shown that disulfide cross-links played a significant role in the protein network formation in dough. Disulfide reducing agents (cysteine, glutathione, dithiothreitol, and sulfite), sulfhydryl oxidizing agents (bromate and iodate), and sulfhydryl blocking agents (N-ethylmaleimide) had been considered the changes of physical properties of dough (Sarwin *et al.*, 1993; Goesaert *et al.*, 2005; Pecivova *et al*., 2010).

Sulfhydryl oxidase (SHOxase) was catalyzed formation of disulfide bond on protein network. This enzyme could impart selected on functional properties and the physicochemical aspect into food properties. In wheat dough, sulfhydryl oxidases can act on the cysteine residues of gluten proteins and form additional disulfide bonds in the protein network, thus strengthening the matrix and secondly producing hydrogen peroxide, which can non-enzymatically crosslink wheat biopolymers (Buchert *et al*., 2010). However, few researches have been carried out on SHOxase as a natural additive which considered to improve dough properties. The information about the influence of SHOxase on thermal and rheological properties of dough was poor. It is important to predictive of dough and bread properties during storage. The present study examined the influences

*Corresponding author.
Email: *triagus.faperta@.unej.ac.id, siswoyo.triagus@gmail.com*

of SHOxase isolated from *Aspergillus niger* on rheological and thermal properties of starch in dough and related with the retrogradation of starch during storage.

Materials and Methods

Materials

The crude enzymes from *Aspergillus niger* were provided by Amano Pharmaceutical Co., Ltd. (Nagoya). The wheat flour used was a hard type "Hermes", provided by Okumoto Flour Milling Co., Ltd. (Osaka). The protein and ash contents of wheat flour were 11.8 and 0.38%, respectively; both were expressed on a 13.5% moisture basis.

Purification of SHOxase

Thirty grams of the crude enzyme was applied to the column chomatography of DEAE-Sepharose CL-4B (Pharmacia), Phenyl Toyopearl650C (Tosoh), and Hydroxyapatite (Nihon Chemical) in this order. The activity of SHOxase was based on the total RS- group using 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) (Chan and Wasserman, 1993).One unit of enzyme activity was defined as the enzyme quantity which oxidizer one µmol of cysteine per min. Measurement of protein was carried out according to Lowry *et al.* (1951). Protein concentrations were also estimated from the absorbance at 280 nm. The molecular weight of the purified SHOxase is estimated to be 55 kD a by SDS polyacrylamide gel electrophoresis in comparison with standard proteins of appropriate molecular weight, such as myosin, *β*-galactosidase, albumin, aldolase, carbonic anhydrase and myoglobin (Daiichi Chemical).

Preparation of sample

For the physicochemical measurement of dough, wheat flour (280 g) and water (210 mL) containing enzyme was used after mixing for 30 min in a home-baker (SD-BT-3, Matsushita Electric Co., Ltd, Osaka) to clearly determine the effect of enzyme (Morita *et al*., 1994) and this sample was used for differential scanning calorimetry (DSC) and scanning electron microscopy (SEM).

Rheological test

The amounts of SHOxase used for determination of physical properties of dough were 8 units/kg of flour, unless otherwise stated. Farinograph data were obtained in a Brabender farinograph equipped with a 50 g stainless steel mixer; the standard speed of mixing was 63 rpm at 30°C.The farinograph data including arrival time, development time, stability time, the percentage water absorption and valorimeter value were obtained on maximum resistance, centered on 500 B.U.

Viscoelastic properties of dough were measured using a Fudoh rheometer (Rheotech Co., Ltd., Tokyo). The dough sample obtained by mixing for 30 min in the bread baker, a 2-cm diameter plunger for viscoelastic measurement and a 5-cm i.d. x 5 cm sample were used, and the penetration depth was controlled at 2 cm and the data were processed using the computer program Rheosoft TR-06 (Rheotech Co., Ltd.).

Scanning electron microscopy (SEM)

SEM was performed using a small portion of dough which had been mixed for 30 min, as describe by Morita *et al.* (1996). The dough sample was frozen with liquid nitrogen, and then fractured into a piece of about 1x1x0.5 cm^3. After the sample was lyophilized, the sample was fixed with solution of 2% OSO_4. Then, the sample was washed thoroughly with deinoized water, freezed and lyophilized again. Next, the sample was put on the surface of silver paste on SEM metal stubs, and coated with a thin layer (about 150 µm) of palladium/platinum, after that viewed at 10 kV and photographed at a speed of 100 s/picture at 17 mm working distance in a Hitachi scanning electron microscopic apparatus model S-800.

Differential scanning calorimetry (DSC)

The DSC measurements were done with a Shimadzu DSC apparatus (Model DSC-60, Kyoto, Japan), controlled by TA-60 WS software and connected to a thermal analysis. The calorimeter was calibrated with indium (melting point, 156.7°C, ΔH = 27.6 J/g) and reference used was liquid paraffin as reported by Siswoyo and Morita (2001). Gelatinization temperatures of onset (T_o), peak (T_p), finally (T_f) and the gelatinization enthalpies (ΔH_g) of starch were measured to characterize the thermal properties of starch in dough. The starch in dough samples was analysed for starch retrogradation after storage for 0-12 days in aluminum capsules at 22°C under a hermetically sealed condition. The temperature was raised from room temperature to 120°C at the rate of 5°C per min.

Calculation of retrogradation kinetics

Retrogradation of starch during storage was estimated from a DSC thermogram. The Avrami model was employed to describe the kinetics ofstarch retrogradationin the starch. The model (McIver *et al*., 1968) can be expressed as:

$$\theta = \frac{\Delta H_\infty - \Delta H_t}{\Delta H_\infty - \Delta H_0} = \exp(-kt^n) \quad (1)$$

Where θ is the fraction of uncrystallized starch at time t, ΔH_0 and ΔH_t are the enthalpy changes at time 0 and time t, respectively, ΔH_∞ is the limiting enthalpy change, k is the rate constant, and n is the Avrami exponent. ΔH_∞ was taken to be the limiting enthalpy change at infinite time ($t \rightarrow \infty$) obtained from the plot of $1/\Delta H_t$ against 1/t (Mita, 1992).The rate constants *(k)* and exponents *(n)* for the starch (where ΔH_0 was zero in this study) were obtained from linear regression of the retrogradation enthalpy data as:

$$\text{Log}\{-\ln(\frac{\Delta H_\infty - \Delta H_t}{\Delta H_\infty})\} = \log k + n \log t \quad (2)$$

Results and Discussion

Rheological properties of dough

The influences of SHOxase on rheological properties of dough were determined using a rheometer and farinograph. As shown in table 1, additions of SHOxase increased of the viscoelastic properties of dough such as stress, modulus of elasticity, and viscosity coefficient compared with the control, whereas the relaxation time was similar than that of the control. The farinograph data: arrival time of the dough containing 8 units SHOxase was strongly delayed compared with that of the control. But, the stability times of the dough prepared with SHOxase was longer than those of the control. Additions of SHOxase caused the flour to absorb a little more water than the control and did not change in the valorimeter value. Likewise, figure1 shows the effect of purified SHOxase on the dough properties, as observed by SEM. In the control, starch granules in the dough are uniformly covered with gluten, however, addition of purified SHOxase to dough seems to fibrous gluten and more fibrous than control. From these data, it seems that the hardness of dough containing SHOxase was related to the oxidation of SH group, which possibly affected the inter- or intramolecular SH-SS interchange reaction that would contribute to the structure of protein. In the oxidation of SH groups of protein and the SH-SS interchange reaction might have taken place, and the formation of intermolecular protein SS bonds might have occurred consequently. According to the continuous network model of protein structure (Pepels *et al*., 2013), the elasticity of dough is at least partly due to disulfide cross links in the network of protein molecules.

Table 1. Rheological properties of dough*

	Control	SHOxase
Rheometer[a]		
Stress (10^3 Nm^{-2})	7.6 ± 0.8	10.3 ± 0.6
Modulus of elasticity (10^4 Nm^2)	3.0 ± 0.3	3.9 ± 1.6
Relaxation time (sec)	0.5 ± 0.1	0.5 ± 0.2
Viscosity coefficient (10^4 Nsm^{-2})	1.5 ± 0.2	2.2 ± 1.0
Farinograph[b]		
Arrival time (min)	1.5	1.3
Development time (min)	2.5	2.5
Stability time (min)	3.0	4.6
Water absorption (%)	52.76	52.30
Valorimeter value (unit)	87.0	87.0
Weakness (B.U)	88.0	85.0

*Amount of enzyme added: SHOxase (8 units).
[a]Data are average of 3 replications.
[b]Data are average of 2 replications.

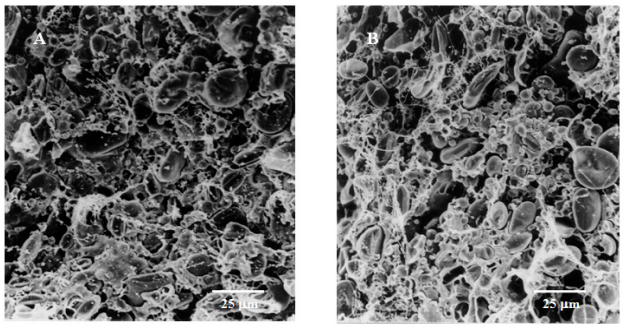

Figure 1. Scanning electron microscopy images of starch control (A); and SHOxase (B). Images were taken at 10 kV and photographed at a speed of 100 s/picture at 17 mm working distance

Thermal properties of starch in dough

The effect of SHOxase during mixing on starch gelatinization parameter peak *(T_p)* and the gelatinization enthalpies *(ΔHg)* are shown in Figure 2A and B. As shown in Figure 2A that the T_p was tended to increase but the addition of SHOxase into the dough formulation caused the ΔHg to decrease than the control (Figure 2B). It seems that the addition of SHOxase could increase the formation of disulfide bonds in protein during dough mixing, apparently limiting the water absorption on starch granular. Samples with less water available for starch gelatinization due to protein water absorption showed higher T_p and lower ΔH of starch gelatinization (Mohamed and Duarte, 2003).

Retrogradation of starch

The enthalpy value of re-gelatinization of starch stored at 22°C during 0-12 days was observed in the range from 50 to 75°C. The peak is identified as the melting peak of amylopectin crystallites (Zhang and Jackson, 1992; Lai *et al*, 2000). The ΔH_t at 0 day of

Table 2. Effect of SHOxase on thermal properties of retrogradation and avrami parameter of dough

	Retrogradation (22°C, 12 days)			Avrami parameter[a]			
	T_o	T_p	ΔH_t	ΔH_∞	n	k (day^{-1})	r^2
Control	47.5	60.1	6.46	10.96	0.8	0.141	0.98
SHOXase	53.0	61.2	5.33	10.85	0.7	0.131	0.96

[a]Avrami exponent *(n)*, rate constant *(k)*, and regression coefficient (r^2) were obtained from linear regression of experimental data, as described by Siswoyo and Morita (2001).

all the samples tested was undetectable after heating, because starch was completely gelatinized during heating. The enthalpy for melting of starch increased logarithmically with storage time (Figure 3A). Rapid retrogradation behavior as exemplified by the control, showed a rapid increase in ΔH commonly at the 8th day of storage, resulting in maximal ΔH by the 12th day. Slow retrogradation rates as exemplified by addition of SHOxase, showed an increase in ΔH that was detectable on the 2nd to 6th day and ΔH gradually increased during storage. The addition of SHOxase showed less ΔH than that of the control after 12 days of storage. In the course of storage, SHOxase reduced greatly the value in the ΔH than that of the control. This result suggests that the retrogradation of starch was retarded by the coexistence of SHOxase.

The Avrami model was found to give a reasonable description of starch retrogradation during the starch gelatinization, with a regression coefficient of 0.96–0.98 (Figure 3B), and ΔH_∞ values were in the ranges of 10.96 (control) and 10.85 (SHOxase) J/g (Table 2). The Avrami exponent (n) for retrogradation kinetics had a range of 0.8 (control), which was higher than that of SHOxase (0.7). The difference in values between the control and SHOxase in starch suggests that the mechanism for recrystallization of starch might be different as effect of SHOxase on intermolecular formation of protein, but the n value of control coincided perfectly with the n value of wheat starch, which is in the range of 0.78-1.26 (Zhang and Jackson, 1992). For the rate constant (k), the addition of SHOxase slowed the retrogradation rate by factors of 0.93 (kc/kSHOxase) with respect to the control (kc).

This result suggests that the addition of SHOxase is better at retarding the retrogradation of starch during storage than the control. The disulfide bond increase on intermolecular formation of the protein might prevent the reorganization of amylopectin molecules during storage.

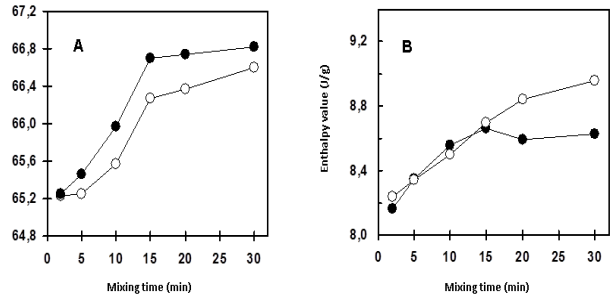

Figure 2. Effect of SHOxase during mixing on thermal properties of dough.Peak temperature (A); Enthalpy value of gelatinization (B). ○, Control; •, SHOxase

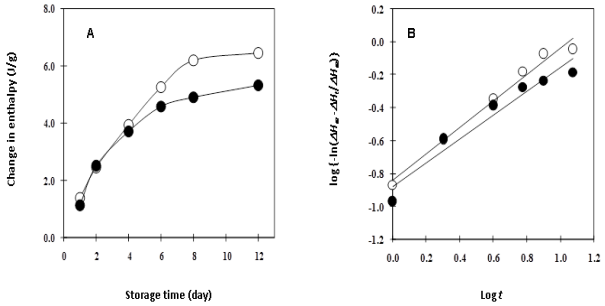

Figure 3. Gelatinization enthalpy of retrogradated starch as function of storage at 22°C (A); Plots of logarithmic fraction of crystallization (B), ○, Control; •, SHOxase

Acknowledgement

This research was supported by the Ministry of Research, Technology and Higher Education, Indonesia.

References

Anjum, F. M., Khan, M.R., Din, A., Saeed, M., Pasha, I. and Arshad, M. U. 2007. Wheat gluten: high molecular weight glutenin subunits-structure, genetics, and relation to dough elasticity. Journal of Food Science 72(3): R56-R63.

Bushuk, W. 1998. Wheat breeding for end-product use. Euphytica 100: 137-145.

Buchert, J., Cura, D. E., Ma, H., Gasparetti, C., Monogioudi, E., Faccio, G., Mattinen, M., Boer, H., Partanen, R., Selinheimo, E., Lantto, R. and Kruus, K. 2010. Crosslinking food proteins for improved functionality. Annual Review of Food Science and Technology 1: 113–138.

Chan, K.Y. and Wasserman, B. P. 1993. Direct colorimetric assay of free thiol groups and disulfide bonds in suspensions of solubilized and particulate cereal proteins. Cereal Chemistry 70: 22-26.

Edwards, N. M., Mulvaney, S. J., Scanlon, M. G. and Dexter, J. E. 2003. Role of gluten and its components in determining durum semolina dough viscoelastic properties. Cereal Chemistry 80: 755–763.

Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Gebruers, K. and Delcour, J.A. 2005. Wheat flour

constituents: how they impact bread quality, and how to impact their functionality. Trends in Food Science and Technology 16: 12–30.

Joye, I.J., Lagrain, B. and Delcour, J.A. 2009. Endogenous redox agents and enzymes that affect protein network formation during breadmaking – A review. Journal of Cereal Science 50: 1–10.

Lagrain, B., Goderis, B., Brijs, K. and Delcour, J.A. 2010. Molecular basis of processing wheat gluten toward biobased materials. Biomacromolecules 11(3): 533-541.

Lai, V.M.F., Lu, S. and Lii, C. 2000. Molecular characteristic influencing retrogradation kinetics of rice amylopectins. Cereal Chemistry 77: 272-278.

Lasztity, R. 1986. Recent results in the investigation of the structure of the gluten complex. Food/Nahrung 30(3-4): 235-244.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. Journal of Biology Chemistry 193: 265-275.

McIver, R. G., Axford, D. W. E., Colwell, K. H. and Elton, G. A. H. 1968. Kinetic study of the retrogradation of gelatinized starch. Journal of Science and Food Agriculture 19: 560-563.

Mita, T. 1992. Structure of potato starch paste in the aging process by the measurement of their dynamic moduli. Carbohydrate Polymers 17: 269-276.

Mohamed, A. A. and Duarte, P.R. 2003. The effect of mixing and wheat protein/gluten on the gelatinization of wheat starch. Food Chemistry 81: 533-545.

Morita, N., Nakamura, M., Hamauzu, Z. and Toyosawa, I. 1994. Effect of calcium gluconate on physical properties of wheat flour dough and breadmaking. Oyo Toshitsu Kagaku 41: 407-412.

Pecivova, P., Pavlinek, V. and Hrabe, J. 2010. The effect of the combination of reducing and oxidising agents on the viscoelastic properties of dough and sensory characteristics of buns. Journal of Science and Food Agriculture 90: 1681-1687.

Pepels, M., Filot, I., Klumperman, B. and Goossens, H. 2013. Self-healing systems based on disulfide–thiol exchange reactions. Polymer Chemistry 4: 4955-4965.

Sarwin, R., Laskawy, G. and Grosch, W. 1993. Changes in the levels of glutathione and cysteine during the mixing of doughs with L-threo- and D-erythro-ascorbic acid. Cereal Chemistry 70: 553-557.

Sliwinski, E.L. Kolster, P. Prins, A. and van Vliet, T. 2004. On the relationship between gluten protein composition of wheat flours and large-deformation properties of their doughs. Journal of Cereal Science 39: 247–264.

Siswoyo, T.A. and Morita, N. 2001. Influence of acyl chain lengths in mono and diacyl-sn-glycerophosphatidylcholine on gelatinization and retrogradation of starch. Journal of Agricultural and Food Chemistry 49: 4688-4693.

Weegels, P. L., Groot, A. M. G., Verhoek, J. A. and Hamer, R. J. 1994. Effects on gluten of heating at different moisture contents. II. Changes in physico-chemical properties and secondary structure. Journal of Cereal Science 19: 39-47.

Zhang, W. and Jackson, D.S. 1992. Retrogradation behavior of wheat starch gels with differing molecular profiles. Journal of Food Science 57: 1428-1432.