



## Effect of barley antifreeze protein on thermal properties and water state of dough during freezing and freeze-thaw cycles



Xiangli Ding<sup>a</sup>, Hui Zhang<sup>a,\*</sup>, Li Wang<sup>a</sup>, Haifeng Qian<sup>a</sup>, Xiguang Qi<sup>a</sup>, Jianhui Xiao<sup>b</sup>

<sup>a</sup> State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi, 214122, PR China

<sup>b</sup> College of Food Science and Engineering, Jiangxi Agricultural University, 1011 Zhimin Road, Nanchang, 330045, PR China

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### ABSTRACT

This study examined the effects of barley antifreeze protein (BaAFP-1) on the thermal properties and water state of dough during freezing and freeze-thaw cycles. The thermal properties of treated and untreated fresh dough, including the apparent specific heat, freezing temperature, melting temperature, freezable water content and glass transition temperature were determined and compared. For frozen dough samples, the change in melting performance, freezable water content, pasting properties, moisture content, water mobility and water distribution during freezing and freeze-thaw cycles were analysed. The results demonstrated that the addition of BaAFP-1 increased the apparent specific heat of dough after freezing, increased the freezing temperature and the temperature range of the melting and glass transition temperatures, and decreased the melting enthalpy and freezable water content of fresh dough. The addition of BaAFP-1 also influenced the melting performance and gelation property of frozen dough after freeze-thaw cycles. It slowed the decrease in moisture content, weakened the influence of the freeze-thaw treatment on water mobility and influenced the water distribution in frozen dough.

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## 1. Introduction

Over the last two decades, there has been a growing demand for frozen dough, and it has been extensively studied in the last few years (Jia, Huang, Ji, et al. 2014; Jia, Huang, Rayas-Duarte, et al. 2014; Matuda, Chevallier, de Alcântara Pessoa Filho, LeBail, & Tadini, 2008). Both freeze processing and frozen storage has critical effects on the quality and stability of frozen dough. During the freezing process, the gluten network was deteriorated as a result of the initial exposure of the dough to extremely low freezing temperatures (Bhattacharya, Langstaff, & Berzonsky, 2003). An unfrozen phase existed in frozen dough, within which detrimental reactions that are diffusion-controlled can occur even during frozen storage

(Goff, 1995; Sim, Noor Aziah, Teng, & Cheng, 2012). During frozen storage, temperature fluctuations cause ice recrystallization, resulting in a decrease in gluten cross-linking. Dough strength is gradually lost and water redistribution is triggered by the modification of the water binding capacity of dough's constituents. These degradations of the dough molecules all lead to an accelerating reduction in quality and a shorter shelf life for frozen dough.

Antifreeze proteins (AFPs) are a family of proteins with thermal hysteresis activity that can lower the freezing point of solutions noncolligatively (Barrett, 2001). They can modify the growth of ice crystals, increase the stabilisation of ice crystals and inhibit the recrystallisation of ice during temperature fluctuations (Amornwittawat, Wang, Duman, & Wen, 2008). These distinct properties make AFPs promising additives for frozen food. AFPs have been isolated from polar fish, insects, fungi and plants. A few previous studies have evaluated their use in improving the quality and shelf life of frozen dough samples, ice creams, chilled and

\* Corresponding author. Tel.: +86 13921177990; fax: +86 510 853 29099.

E-mail address: [zhanghui@jiangnan.edu.cn](mailto:zhanghui@jiangnan.edu.cn) (H. Zhang).

frozen meat, gel and chilled actomyosin (Ding, Zhang, Chen, et al. 2015, Ding, Zhang, Liu, et al. 2014; Kontogiorgos, Goff, & Kasapis, 2008). Most of these studies focused on the effects of AFPs on the physicochemical, rheological, textural and baking characteristics of the products. And results showed that AFPs were highly effective in improving the rheofermentation capacity. With addition of AFPs, the residual gluten fibril was increased, the exposed starch granules was decreased, the gas production and retention of frozen doughs was enhanced (Jia, et al., 2012) and the decrease in elastic moduli and viscous moduli during frozen storage was slowed down (Jia, Huang, Ji, et al. 2014, Jia, Huang, Rayas-Duarte, et al. 2014). An increase in specific volume and a decrease in crumb hardness of baked frozen dough was resulted (Xu, Huang, Jia, Kim, & Liu, 2009). Little attention was paid to the influence of AFPs on the thermal properties and water state of the frozen food (Xu, Huang, Wang, & Rayas-Duarte, 2009).

Water is an integral part of food. The quality and shelf life of frozen foods are strongly dependent on their interaction with water, which can be described by three parameters: moisture content, water activity, and water dynamic mobility. The amount, physical state and location of water is crucial to frozen dough, because it is closely related to the formation of dough, specifically how it will hold gas and produce an open, aerated crumb structure in the final product (Loveday, Huang, Reid, & Winger, 2012). However, recent studies have indicated that it is not the water activity but the dynamic molecular mobility 'state' of the water in foods that directly affects the food stability (Li, Dickinson, & Chinachoti, 1998). During freezing and frozen storage, the water state plays an important role in the dough quality and stability. Despite recent progress in studies of water in food, the thermal properties of food are not yet well understood, although they have recently received considerable attention (Loveday et al., 2012). This type of information is crucial to our fundamental understanding of the role of water dynamics in frozen food quality and stability. However, a review of the recent literature shows that there are few quantitative studies of the physics governing ice crystal formation.

BaAFP-1 is a new member of the plant AFPs family. It can be extracted from cold acclimated malting barley (*Hordeum vulgare* L.) and purified to electrophoretic purity. Mass fingerprinting and sequencing studies have indicated that it has a high homology with the alpha-amylase inhibitor BDAI-1 (*Hordeum vulgare*). Relatively high thermal hysteresis activity (THA = 1.04 °C, 18.0 mg mL<sup>-1</sup>) and hydrophilicity was observed with it. In this study, the effect of BaAFP-1 on the thermal properties and water state of dough during freezing and freeze-thaw cycles was evaluated systematically and comprehensively. The aim was to clarifying the relationship between the thermal properties and water state of dough and the cryopreservation mechanism of BaAFP-1.

## 2. Materials and methods

### 2.1. Materials

Flour, sugar, salt and shortening were purchased from the local market. BaAFP-1 was obtained from cold acclimated malting barley (*Hordeum vulgare* L.) use the method described before (Ding, Zhang, Chen, et al. 2015, Ding, Zhang, Liu, et al. 2014).

### 2.2. Dough preparation and storage

Samples of dough, with and without addition of BaAFP-1, were prepared using the slightly modified methods of Xu (Xu, Huang, Wang, & Rayas-Duarte, 2009). Hereafter, they were called control doughs and BaAFP-1 doughs, respectively. The BaAFP-1 dough formulation contained 0.5% AFPs (flour basis). No yeast was added

to eliminate the effect of fermentation. All of the ingredients were mixed at the same time. After mixing, the dough was rested, divided, molded and wrapped in a polyethylene sheet. Part of these fresh dough samples were studied immediately after they were made and the others were made into frozen dough samples and pan-sealed dough samples. The frozen dough samples were made by immediately freezing fresh dough samples with a refrigerator (Sanyo Electric Co., Ltd, Osaka, Japan) at -30 °C for 2 h, and then stored at -18 °C. Pan-sealed dough samples were made by sealing fresh dough samples with differential scanning calorimeter (DSC) aluminium pans and then frozen them with a refrigerator (Sanyo Electric Co., Ltd, Osaka, Japan) at -30 °C for 2hr, and then stored at -18 °C (Bot, 2003). Freeze-thaw treatments were applied to frozen dough samples and pan-sealed dough samples to mimic the temperature fluctuations that occur during frozen storage. A freeze-thaw cycle consisted of partially thawing the frozen doughs at room temperature until the centre temperature was 15 °C, then subjected to frozen storage again at -18 °C for 24 h. Frozen dough samples and pan-sealed dough samples after freeze-thaw cycles were named C0, C1, C2, C3 and C4, respectively, in which the number represents freeze-thaw times that dough samples endured.

### 2.3. Apparent specific heat (*C<sub>app</sub>*) determination

The apparent specific heat (*C<sub>app</sub>*) refers to the specific heat of food during phase change (Sweat, 1986). It is closely related to the structure and energy of food, and provide basic parameter for the frozen storage device design and the analysis of thermal stresses during freezing (Rubinsky, 1982). *C<sub>app</sub>* of dough samples was determined on fresh dough samples using the methods given in a previous study (Xu, Huang, Wang, & Rayas-Duarte, 2009). A Q200 DSC (TA Instruments, New Castle, Delaware, USA) was used and the collected data were analysed using the TA instrument universal analysis software (TA Instruments, New Castle, Delaware, USA). The procedure was as follows: samples (~7 mg) were cooled to -60 °C at the rate of 5 °C/min and then subjected to heating at the rate of 1 °C/min up to 10 °C. The freeze-thaw procedure was conducted on blank pan, standard material (KCl) and doughs, respectively. The *C<sub>app</sub>* of fresh dough was calculated using Formulas (1):

$$C_{app} = \frac{m_{std}}{m_s} \cdot \frac{DSC_s - DSC_b}{DSC_{std} - DSC_b} \cdot C_{p, std}$$

Where *C<sub>app</sub>* is the apparent specific heat of dough, J/g·°C; *m<sub>std</sub>* is the weight of standard material, mg; *m<sub>s</sub>* is the weight of sample, mg; *DSC<sub>s</sub>* is the heat flow of sample, Mw; *DSC<sub>b</sub>* is the heat flow of blank, Mw; *DSC<sub>std</sub>* is the heat flow of standard material, Mw; *C<sub>p, std</sub>* is the apparent specific heat of standard material, J/g·°C (Liu, 2000).

### 2.4. Determination of the freezing and melting properties

The freezing and melting properties, including the freezing temperature (*T<sub>f</sub>*), melting temperature (*T<sub>m</sub>'*), freezable water content (*F<sub>w</sub>*) and glass transition temperature (*T<sub>g</sub>'*), were determined on fresh dough samples using the methods given in a previous study (Ding, Zhang, Chen, et al. 2015, Ding, Zhang, Liu, et al. 2014). The procedure was as follows: use an empty pan as reference, dough sample (~10 mg) was cooled to -90 °C at 10 °C min<sup>-1</sup>, and equilibrated for 5 min. It was then scanned initially from -90 °C to 20 °C. Samples were cooled to -90 °C at 10 °C min<sup>-1</sup> again, heated at 10 °C min<sup>-1</sup> to -20 °C and annealed for 30 min, cooled at 5 °C min<sup>-1</sup> to -90 °C, and scanned from -90 °C at 2 °C min<sup>-1</sup> to 20 °C. *T<sub>g</sub>'* was calculated from the last melting curve. *T<sub>f</sub>* was taken as the peak temperature at exothermic curve, and *T<sub>m</sub>'* was taken as the onset

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