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The response of watercress (*Nasturtium officinale*) to vacuum impregnation: Effect of an antifreeze protein type I

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ABSTRACT

The setting up of methodologies that reduce the size of ice crystals and reduce or inhibit the recrystallisation phenomena could have an extraordinary significance in the final quality of frozen products and consequently bring out new market opportunities. In this work, the effect of an antifreeze protein type I (AFP-I), by vacuum impregnation (VI), on frozen watercress was studied. The VI pressure, samples' weight, *Hunter Lab* colour, scanning electron microscopy (SEM), and a wilting test were analysed in this work.

The water intake of watercress samples augmented with vacuum pressure increase. The results also showed that, independently from the vacuum pressure used, the *Lab* colour parameters between raw and impregnated samples were maintained, showing no significant differences ($P > 0.05$).

A VI of 58 kPa, during 5 min, allowed impregnating the AFP-I solution (0.01 mg ml^{-1}) into the watercress samples. The scanning electron microscopy (SEM) analysis showed the AFP-I impregnated frozen samples with better cell wall definition and rounded cell shape with smaller ice crystals compared with the control samples.

The wilting test results corroborated that AFP-I is a valuable additive, since the leaves impregnated with AFP-I showed higher turgidity compared to the control samples.

The present findings will help to better understand the effect of AFP-I, particularly, on frozen watercress microstructure and its importance as valuable food additive in frozen foods and mainly in leafy vegetables.

1. Introduction

The major objective of the freezing process is to extend the shelf-life of foodstuffs. Moreover, frozen products are able to reach the consumer in different parts of the World with a good quality, being this feature one of the most valuable for producers, food retailers and consumers. Nevertheless, if the temperature of the freezing process and the storage conditions for product preservation are not appropriate, problems can arise. In frozen food production, the ice formation should be as fast as possible to minimise structural damages. Also, the temperature fluctuations that may occur during distribution and storage should be minimal, since they can lead to product deterioration and reduce drastically its quality and consequently its commercial value.

The ice formation and recrystallisation phenomena, due to temperatures fluctuations along the cold chain, induce changes, not only in the size and number of ice crystals, but also in their shape and orientation. Recrystallisation corresponds to water migration as a result of local water motions allowing molecular diffusion from one ice crystal to another, more often without change in ice content (Blond and Le Meste, 2004).

The development of innovative and more efficient pretreatments in the freezing process are always in research in order to achieve better food materials, and consequently, higher quality frozen products, satisfying the consumer demands and market requirements.

Setting up methodologies that could reduce the size of ice crystals and reduce or inhibit the recrystallisation phenomenon could have an extraordinary significance in the product final quality and consequently bring out new market opportunities.

Antifreeze proteins (AFPs) or thermal hysteresis proteins (THPs) are able to depress the freezing point of aqueous solutions below the melting point, inhibit ice recrystallisation, and suppress or modify ice crystal growth. The difference between the freezing and the melting point is termed thermal hysteresis. AFPs are found in a large number of organisms, such as fish, bacteria, insects and plants (Yang and Sharp, 2004). These proteins help to protect these organisms in very cold environments by lowering the temperature

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at which ice crystals grow and changing the size and shape of the ice crystals (Atıcı and Nalbantoğlu, 2003; Baardsnes et al., 1999; Banasiak, 2006; Breton et al., 2000; Carpenter and Hansen, 1992; Chapsky and Rubinsky, 1997; Deng and Laursen, 1998; Evans and Fletcher 2001, 2004; Feeney and Yeh, 1998; Fletcher et al., 2001; Gómez and Sjöholm, 2004; Graether et al., 2001; Grandum et al., 1999; Griffith and Ewart, 1995; Griffith et al., 2005; Jarząbek et al., 2009; Kristiansen and Zachariassen, 2005; Kuiper et al., 2003; Li and Sun, 2002; Lu et al., 2002; Robles et al., 2007; Scotter et al., 2006; Smallwood et al., 1999; Strom et al., 2005; Tomczak et al., 2003; Wathen and Jia, 2005; Wu et al., 2001; Yang and Sharp, 2004; Yu and Griffith, 1999; Zhang et al., 2008).

It is thought that AFPs inhibit the development and recrystallisation of intercellular ice by adsorbing onto the surface of ice crystals via van der Waals interactions and/or hydrogen bonds (Yeh et al., 2000). The basis for adsorption specificity lies in a hydrogen-bonding match between groups on the ice-binding site of the AFP and oxygen atoms on the ice lattice. In the winter flounder α-helical AFP type I, the regularly spaced hydrophilic threonines (Thr) have been suggested to be the principal ice binding residues (Deluca et al., 1998; DeVries and Lin, 1977; Sicheri and Yang, 1995; Wen and Laursen, 1992). On the other hand, other studies (Chao et al., 1997; Haymet et al., 1998, 1999; Zhang and Laursen, 1998) in which the AFP-I hydrophilic amino acids were replaced with hydrophobic ones, showed that hydrogen bonding is not necessary for the antifreeze effect. Others authors (Davies et al., 2002; Sonnichsen et al., 1996; Yang et al., 1998) reported that the binding mechanism is principally due to the entropic effects of docking a relatively hydrophobic, flat protein surface to ice, as well as van der Waals contacts and the formation of some hydrogen bonds. Wierzbicki et al. (2007) proposed that the AFPs interactions with the ice–water interfacial region "poisons" it and thus stops the ice from growth, and do not bind to ice, but accumulate at the ice–water interface. Therefore, the AFPs ice-binding mechanism details are not yet well established and more studies are still required (Ewart et al., 1999; Wathen and Jia, 2005).

AFPs application in frozen foods may inhibit recrystallisation during freezing, storage, transport and thawing, thus preserving food texture by reducing cellular damage and, by reducing drip also minimise the loss of nutrients (Griffith and Ewart, 1995).

The application of antifreeze proteins in food are reported in the literature. Boonsupthip and Lee (2003) showed the ability of antifreeze proteins to preserve gel-forming functionality of food muscle proteins in frozen conditions. They also conclude that AFP still provides better protection than a conventional cryoprotectant sucrose–sorbitol mixture. Other studies concerning antifreeze proteins have been reported, in frozen meat (Payne et al., 1994; Payne and Young, 1995) and ice cream (Regand and Goff, 2006). Moreover, Khanna and Daggard (2006) showed that the antifreeze proteins can be effective even at low concentrations such as 0.6 μg ml^{-1}. Holmberg et al. (2001) also reported antifreeze activity in applications with very low concentrations of AFPs.

Wang et al. (2008) reported that in order to transfer antifreeze protection to a plant, it is crucial to introduce AFPs into the apoplast space to confer an optimal antifreeze effect. This phenomenon occurs since ice forms preferentially in the apoplast where the solute concentration is the lowest. Furthermore, the cellular dehydration and disruption of cell integrity occurs since intracellular water is lost and extracellular ice grows.

Nevertheless, very few studies were found reporting the effect of antifreeze proteins in vegetables (Cutler et al., 1989), and none at all in watercress.

Vacuum impregnation (VI) is a useful process that allows the introduction of valuable additives directly into foodstuffs throughout its pores, protecting natural tissue composition, thus improving texture quality and lowering drip loss, and, in some cases, reduces the need for heat treatment, preserving the product characteristics and heat labile elements. VI, as a pre-treatment step, has been widely used in processes such as freezing, drying and canning, due to its ability in quality improvement (Bolin and Huxsoll, 1993).

In this process (VI), the penetration of external liquid is caused by the combined effect of capillary action and a pressure gradient (i.e., the hydrodynamic mechanism, HDM) (Fito, 1994; Fito and Pastor, 1994). After product immersion in a closed tank containing the liquid phase, VI is carried out in a two steps procedure: first, the vacuum pressure (p_1) is imposed on the system for a short time (t_1), promoting the expansion and outflow of internal gas in the product (the product pore native liquid is released due to the internal gas outflow); second, the atmospheric pressure (p_2) is restored for a certain time (usually $t_2 = t_1$) with compression leading to a great reduction in the pores' remaining gas, and subsequent influx of the external liquid into the porous structure (Fito et al., 2001; Gras et al., 2002, 2003).

Fruits and vegetables are suitable for developing high quality vacuum impregnated products, since their porous structure, containing a gas or liquid phase, is susceptible for impregnation with an external solution (Zhao and Xie, 2004).

In view of the fact that there are only a few studies on the AFPs applications in foodstuffs, the objectives of this work were to optimise the VI process in order to mechanically introduce an AFP-I on watercress and test its benefits on the quality of this frozen leafy vegetable microstructure and texture.

2. Materials and methods

2.1. Raw material

Raw watercress (*Nasturtium officinale*) was kindly supplied from a local producer. The leaves were selected (dia = 1.4 cm), washed thoroughly and analyzed within 24 h.

2.2. Vacuum impregnation experiments

The vacuum impregnation experiments were run in order to select the vacuum pressure value that could guarantee AFP-I solution influx with uniform distribution, and on the other hand preserve watercress initial characteristics.

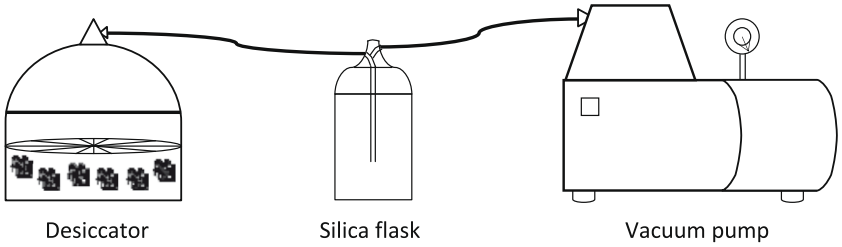

Fig. 1. Vacuum impregnation apparatus.