Food Research International 42 (2009) 788–797

Contents lists available at ScienceDirect

Food Research International

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

Changes in texture, cellular structure and cell wall composition in apple tissue as a result of freezing

Sophie Chassagne-Berces [a], Cécile Poirier [b], Marie-Françoise Devaux [b], Fernanda Fonseca [a,*], Marc Lahaye [b], Giuseppe Pigorini [c], Christel Girault [c], Michèle Marin [a], Fabienne Guillon [b]

[a] UMR 782 Génie et Microbiologie des Procédés Alimentaires, INRA, AgroParisTech, F-78850 Thiverval-Grignon, France
[b] INRA-UR1268 Biopolymères Interactions Assemblages, F-44300 Nantes, Cedex 03, France
[c] AIR LIQUIDE, Centre de Recherche Claude Delorme, 78354 Les Loges-en-Josas, France

ARTICLE INFO

Article history:
Received 26 September 2008
Accepted 3 March 2009

Keywords:
Fruit
Freezing rates
Physical parameters
Macrovision
CLSM
Cryo-SEM
Structure
Cell wall
Image texture analysis
Granulometry

ABSTRACT

Apple texture is one of the critical quality features for the consumer. Texture depends on several factors that are difficult to control and which change with freezing. To better understand the mechanisms involved in the texture degradation of apple tissues following freezing/thawing, our approach was to combine mechanical properties, cellular structure and cell wall composition measurements on fresh and thawed apples (Granny Smith) after three different freezing protocols (at −20 °C, −80 °C and −196 °C). This work highlighted the interest of applying macrovision and image texture analysis to quantify the freezing effects on cellular structure and ice crystal size. Freezing at −20 °C and after immersion into liquid nitrogen were the protocols affecting the most fruit texture leading to cell membrane breakage resulting in cell wall collapse and tissue breakage, respectively, which accounted for the mechanical behaviour of the samples. All freezing protocols induced vacuole burst showing that the turgor pressure preservation remains critical during the freezing process.

1. Introduction

Freezing is used extensively to preserve food (fruit, vegetables, and meat). However, especially in the case of fruit, this preservation technique may result in textural changes leading to food softening (Brown, 1977). The quality of frozen/thawed fruit depends on a large number of factors including the type of fruit, the variety, raw quality and ripeness or time lapse between harvesting and processing (Marani, Agnelli, & Mascheroni, 2007; Phan & Mimault, 1980). Once the raw material and the harvesting conditions have been defined, the optimal freezing protocol for texture preservation has to be determined. In general, it is accepted that fast freezing better preserves local structure. It induces the production of a large number of small ice crystals that cause less migration of water and less breakage of cell walls, and consequently less texture deterioration (Brown, 1977; Delgado & Rubiolo, 2005; Marti & Aguilera, 1991). However, the kinetics of too fast a freezing can provoke breakage at the product level due to ice density differences with water which lead to texture modification. To improve stabilisation by freezing, a better understanding of the complex physical and chemical mechanisms taking place inside the fruit tissue during freezing/thawing is still needed.

Texture of fruit is determined by different physical characteristics that arise from the structural organization at different levels: from molecular to tissue level. The cell is the elementary unit within the tissue and its integrity strongly impacts textural quality. Among the many factors involved in fruit texture, the structural integrity of the cell components (cell wall and middle lamella) and cell turgor pressure determined by water content in the vacuoles are the most important (Waldron, Smith, Parr, Ng, & Parker, 1997). Several instrumental techniques are required to investigate the changes of fruit textural properties after freezing/thawing from different points of view: mechanical, microscopic and biochemical.

Mechanical measurements, such as the Kramer–Shear test (Mastrocola, Pittia, & Lerici, 1996; Phan & Mimault, 1980), compression tests (Chiralt et al., 2001; Kim & Hung, 1994), puncture tests (Marani et al., 2007; Phan & Mimault, 1980; Zhang, Duan, Zhang, & Peng, 2004) or back extrusion (Robbers, Singh, & Cunha, 1997) are widely used to evaluate the firmness at the organ scale. Such testing makes it possible to measure the mechanical properties of fruit tissue before and after freezing.

* Corresponding author. Tel.: +33 1 30 81 59 40; fax: +33 1 30 81 55 97.
E-mail address: fonseca@grignon.inra.fr (F. Fonseca).

0963-9969/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2009.03.001

Microscopy is a useful tool to visualize food structure at the tissular and cellular scales and to study the influence of freezing. Structural modifications associated with freezing/thawing have been studied in several plant tissues through light microscopy (Buggenhout et al., 2006a; Khan & Vincent, 1996; Otero, Martino, Zaritzky, Solas, & Sanz, 2000; Sterling, 1968) and scanning electron microscopy (SEM) without (Delgado & Rubiolo, 2005; Sousa, Canet, Alvarez, & Tortosa, 2006) or with cryo-system (Cryo-SEM) (Alonso, Tortosa, Canet, & Rodriguez, 2005; Bomben, King, & Hayes, 1983; Martinez-Monzo, Martinez-Navarrete, Chiralt, & Fito, 1998; Tregunno & Goff, 1996). The impact of freezing on the microstructure of apples (Bomben et al., 1983), carrots (Buggenhout et al., 2006a), peaches (Otero et al., 2000), raspberries and blackberries (Sousa et al., 2006), blueberries and wild blackberries (Marti & Aguilera, 1991) and strawberries (Delgado & Rubiolo, 2005) have already been studied. The main drawback of microscopic techniques is sample preparation that is time consuming and the reduced field of view observed in a single image. Confocal laser-scanning microscopy (CLSM) enables non destructive optical sectioning of samples and make sample preparation easier and more rapid (Gray, Kolesik, Hoj, & Coombe, 1999; Kalab, Allan-Wojtas, & Miller, 1995). The observation of a representative number of cells requires the acquisition of a large number of images or several adjacent images and the reconstruction of the whole region as a mosaic image (Guillemin, Devaux, & Guillon, 2004). An alternative technique to microscopy is to use stereomicroscope or macrovision systems making it possible to observe a field of view of about 1 cm^2. Plant tissue and cellular structures of tomato fruit can be characterised in this way (Devaux, Bouchet, Legland, Guillon, & Lahaye, 2008).

The characterisation of samples by imaging techniques is completed by applying image analysis to quantify the structure observed. Cell size and shape can be measured from microscopic images after a segmentation of each individual cell. For macroscopic images, techniques based on image texture analysis can be envisioned to quantify information on object size. Image texture refers to local variation of grey levels and several methods have been proposed to quantify these variations. Grey level granulometry from mathematical morphology (Serra, 1982) has been successfully applied to extract quantitative information related to cell size in tomato tissues (Devaux et al., 2008).

Biochemical changes of the cell wall (Waldron et al., 1997) are also related to texture changes. Fruit cell walls are composed of cellulose and hemicellulose embedded in a matrix of pectins (Kunzek, Kabbert, & Gloyna, 1999; Muhlethaler, 1967). During fruit ripening or storage, softening occurs as a result of enzymatic degradation of cell walls (Johnston, Hewett, & Hertog, 2002). Few authors (Alonso et al., 2005; Buggenhout et al., 2006b) have studied biochemical changes following fruit freezing.

In this work, two hypotheses of fruit tissue degradation during freezing were kept. Freezing process influences ice crystal formation resulting in (1) vacuole rupture causing loss of turgor pressure and (2) structural damage of cells and cell walls, and, hence, the modification of tissue architecture. The aim of this work is to investigate the changes of textural properties during freezing/thawing at different levels of observation to better understand the mechanisms involved in the softening of apple tissue following freezing and thawing and to quantify the changes. Texture was measured by mechanical techniques at the organ scale. The cellular structure was investigated at the tissue scale using macrovision. The vacuolar integrity was observed at the cell scale using confocal microscopy. Ice crystals were analysed by cryo-scanning electron microscopy. The global composition of the cell wall was studied using biochemical techniques. Three freezing protocols were applied to study the different hypotheses of degradation. The apple was taken as a model of fruit due to its macroscopic flesh homogeneity.

2. Materials and methods

2.1. Samples

The apple variety Granny Smith was selected due to its availability and its good stability during storage conditions. The variety exhibits a lower texture degradation than other apple varieties (personal communication). Fruits were purchased from an agricultural cooperative (Dorléane, Saint Hilaire Saint Mesmin – Loiret, France), where they were stored at 1 °C under a modified atmosphere. Apples were studied at commercial maturity. In the lab, they were stored in a cold chamber at 4 °C, for a maximum duration of one month, until the moment of the experiment.

2.1.1. Sample preparation

The apples were placed overnight at ambient temperature (21 ± 1 °C) before sampling. A 2 cm thick transverse cross section was cut at the equatorial level of each apple. Cylinders (1.2 cm in diameter and 2 cm in height) were taken equidistant from the surface and the seed sacs in the parenchyma region using a circular punch.

2.1.2. Freezing and thawing

Three freezing protocols were applied: at −20 °C in a cold chamber, at −80 °C in gas nitrogen convection (Silversas, Air Liquide, Paris, France) and by immersion in liquid nitrogen (LN2, boiling point = −196 °C) until the core temperature reached the equilibrium value with the freezing temperature. The three protocols correspond to slow (0.9 °C/min), intermediate (8.1 °C/min) and very fast (310 °C/min) freezing rate, respectively. Once frozen, the samples were packed in polyethylene bags and thawed in a cold chamber at 4 °C overnight. They were finally placed at room temperature (21 ± 1 °C) until the sample cores reached room temperature.

Fresh samples were used as reference samples for each experimental technique. The three freezing protocols and fresh samples are referred to as protocols in the following.

2.2. Mechanical properties

The texture of apple cylinders was measured with a TA.XT2i texture analyser (Stable Micro Systems Ltd, Godalming, UK). All experiments were conducted at 21 °C. The fruit cylinders were kept at the same orientation because of fibrous non-isotropic properties of apple flesh (Khan & Vincent, 1993). For each protocol, two cylinders per fruit and ten fruit were analysed resulting in 20 measurements. Penetrometry tests were carried out as in Mehinagic et al., 2003 but with a 2 mm diameter cylindrical probe, penetrat-

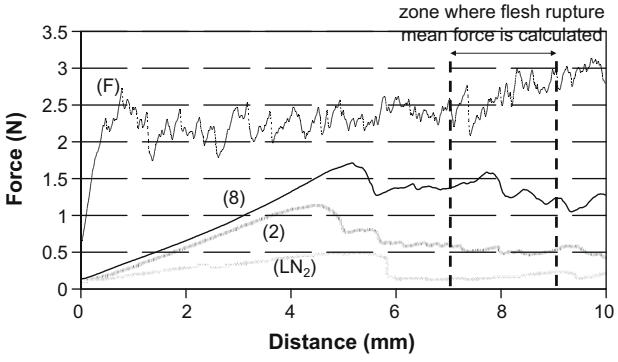

Fig. 1. Force–distance curves obtained during puncture test for fresh apples (*F*) and thawed apples after freezing: at −20 °C (2), at −80 °C (8) and immersion in liquid nitrogen (LN_2).