Journal of Food Engineering 122 (2014) 1–7

Contents lists available at ScienceDirect

Journal of Food Engineering

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

Two micro-mechanical techniques for studying the enzymatic maceration kinetics of apple parenchyma

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ARTICLE INFO

Article history:
Received 13 June 2013
Received in revised form 2 August 2013
Accepted 9 August 2013
Available online 21 August 2013

Keywords:
Micro-mechanics
Apple
Enzyme degradation
Kinetics
Diffusion

ABSTRACT

The enzymatic texture loss during apple maceration was studied by two micro-mechanical techniques. The first technique consisted of a 5% strain compression cycles at a strain rate of $4.5 \times 10^{-4}\ s^{-1}$. The second technique consisted on micro-puncture of the apple parenchyma with a small needle. The first technique led to the peripheral tissues degradation modelling with a first order kinetic reaction or a more pertinent Weibull function. The second technique evidenced that the jagged part of the load *vs* penetration curve corresponded to an interaction between the needle tip and the turgescent apple texture and the fractal dimension of this jagged part was chosen as the texture parameter. Modelling the enzyme diffusion phenomenon with the second Fick's law and taking into account the model previously established on peripheral tissues allowed the estimation of an equivalent enzyme diffusivity through the apple parenchyma varying between 3.5×10^{-11} and $5.5 \times 10^{-11}\ m^2\ s^{-1}$.

1. Introduction

Apple juice is the second more consumed fruit beverage in Europe (www.aijn.org 2012 Liquid Fruit Market Report) and contains bioactive secondary plant substances such as polyphenols which are responsible of many health benefits (Kujawska et al., 2011). During apple juice processing enzymes are frequently used for enhancing yield and for juice clarification (Ceci and Lozano, 2010). The effect of enzyme types, concentration and operating conditions on the final quality of apple juice has been the subject of much studies, for example Poll (1988), Will et al. (2000, 2002), Mihalev et al. (2004), Sorrivas et al. (2006), Markowski et al. (2009), Jinghua et al. (2011), Oszmianski and Wojdylo (2006), Oszmianski et al. (2009, 2011) and Sandri et al. (2011).

The reaction kinetics was also studied and the main contributions are presented in Table 1. This table presents the studies performed on apples, pumpkin, potato and carrot which can be considered as fleshy solids. The reaction kinetics was followed by different methods: weighing the liquefied part of the fruit adsorbed on a filter paper (Tanchev et al., 1989, 1990a,b, Tantchev et al., 1993), weighing the non-macerated part of the vegetable (Biekman, 1992; Biekman et al., 1993), final product viscosity loss (Struebi et al., 1978; Mutlu et al., 1999; Sarıoğlu et al., 2001), alcohol-insoluble solids in three fractions: residual tissue, single cells (pulp) and juice (Missang et al., 2001a,b), yield and juice viscosity (Sharma et al., 2005); Sun et al., 2006). When a first order kinetic is applied to these results, the reaction constants varied between 0.18 and 86.4 h^{-1} and the activation energies varied between 30 and 62 kJ mol^{-1}.

Although the texture, and particularly the microstructure, of foods plays an important role in the fruit quality (Mebatsion et al., 2008), the nutrients bioavailability (Parada and Aguilera, 2007) or plant-based foods promoting nutritional quality (Van Buggenhout et al., 2012) the microstructure was poorly studied in relation with enzyme maceration. Grazyna et al. (1999) observed by Scanning Electronic Microscopy (SEM) the changes in microstructures of apple tissue treated by four different enzymes and they observed qualitative differences between the enzyme actions on the apple cells. Sorrivas et al. (2006) studied the mechanisms of enzymatic clarification of apple juice by SEM and Transmission Electronic Microscopy (TEM). The role of amylase and pectinase enzymes on the cloudy juice stability was partly explained by these techniques.

The apple tissues were disintegrated by the different enzyme actions leading to a more softened texture. Among the different studies on the fleshy fruits micromechanics, the tensile/compression test and the micro-puncture were pertinent to determine the softening effect of the enzymes. Recent works involving a

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0260-8774/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jfoodeng.2013.08.017

Table 1
Kinetics of fruits and vegetables enzymatic maceration as reported in the literature.

Author	Year	Product	Operating conditions	Measurements	k (h^{-1}); E_a (kJ mol^{-1})
Struebi et al.	1978	Apple nectars	1 Commercial enzyme; 63–1000 mg/kg; 0–4 h, 38–40 °C	Viscosity loss	0.24–1.81 h^{-1}
Tantchev et al.	1989	Grated apples and pumpkins	1 Commercial enzyme; 200 mg/kg; 0–4 h 20–40 °C	Weighing the liquefied part of the fruit adsorbed on a filter paper	0.21–1.12 h^{-1} ≈ 62 kJ mol^{-1}
Tantchev et al.	1990	Grated carrot	4 Commercial enzymes; 100 or 500 mg/kg; 0–6 h 20–50 °C	Weighing the liquefied part of the fruit adsorbed on a filter paper	0.21–0.61 h^{-1} ≈ 30 kJ mol^{-1}
Biekman	1992	Potato cubes	1 Commercial enzyme, 18.5 and 15.4 mg/g; 0–25 h; 40 °C	Weighing the non macerated potato cubes	0.18 h^{-1}
Biekman et al.	1993	Potato cubes	1 Commercial enzyme (cellulase and hemicellulase activity); 20–160 mg/g; 0–2 h; 38 °C; rotating drum	Weighing the non macerated potato cubes	0.54–0.75 h^{-1}[c]
Tantchev et al.	1993	Apple cubes	12 Commercial enzymes; 200–2000 mg kg^{-1}; 0–3.5 h; 20–40 °C	Weighing the liquefied part of the fruit adsorbed on a filter paper	0.21–1.81 h^{-1} 37.6–83 kJ mol^{-1}
Metlu et al.	1999	Pectin solutions	1 Commercial enzyme (pectinase); 0.05–2.00% v/v; 0–0.17 h, 15–45 °C	Viscosity loss	3.6–86.4 h^{-1} 38.94 kJ mol^{-1}
Missang et al.	2001a	Apple cubes	1 Commercial enzyme (PG[a], PME[b] and cellulase activities); 0–26 h 25 °C	Alcohol-insoluble solids in three fractions: residual tissue, single cells (pulp) and juice	1.55 & 0.27 h^{-1}[d]
Missang et al.	2001b	Apple cubes	1 Commercial enzyme (PG, PME[b] and cellulase activities); 0–26 h 25 °C, different apple ripeness	Alcohol-insoluble solids in three fractions: residual tissue, single cells (pulp) and juice	2.79–3.94 h^{-1} & 0.39–0.28 h^{-1}[d]
Sartoglu et al.	2001	Pectin solutions 0.5–3.5% w/v	1 Commercial immobilized enzyme (pectinase); 0.1 g/ml particle; 0–0.33 h, 20–90 °C	Viscosity loss	15.08 h^{-1} 50 kJ mol^{-1}
Sharma et al.	2005	Grated carrot	Pectolytic and cellulolytic enzymes; 50–650 mg/kg, pect:cellulo ratio 3:7–7:3, 30–150 min, 25–65 °C	Yield, juice viscosity	2.1 h^{-1}[c]
Sun et al.	2006	Carrot pulp	4 Commercial enzymes; 0.025–0.1 mg/g, 32–88.3 min 45 °C	Yield, juice viscosity, β-carotene content	2.5 h^{-1}[c]
Diano et al.	2008	Pectin solution Apple juice	1 Commercial pectolytic enzyme immobilized on different supports; 0.3–0.58 mg/g; 0–1.5 h, 10–70 °C	Viscosity loss	4.8–47.4 h^{-1}

[a] Polygalacturonase.
[b] Pectinmethylesterase.
[c] Estimation from reported data.
[d] Two primary mechanisms.

miniature tensile device were performed by Oey et al. (2007) and Alamar et al. (2008) to determine respectively the influence of the turgor and the storage conditions on the mechanical behaviour of apple tissues. These works gave very pertinent information at a meso-scale, typically $3 \times 11 \times 5$ mm^3, on the mechanical resistances of the apple tissues and their evolutions with turgor, storage conditions and cultivars. The tensile stage was placed under a stereomicroscope and the cells deformations were analysed and quantified by image analysis.

The examination of mechanical noise produced during cutting of potato tuber parenchyma tissue and a micro-penetration (probe diameter 20 μm) was applied by Hiller et al. (1996). These micro-mechanical tests allowed the authors to propose pertinent values of the cell sizes and cell wall stiffness in place without disassembly the parenchyma tissue. A combined acoustic–mechanical profiling was also performed on 86 different apple cultivars by Costa et al. (2011) demonstrating a good performance of their approach in measuring apple crispiness and sensory evaluation.

The aim of the present work was to study the feasibility of two micromechanical tests, a compression and a micro-puncture, to follow the softening of apple tissues when soaking in enzyme solutions.

2. Materials and methods

Granny Smith apples were purchased in a local supermarket and stored at 4 °C.

The enzyme used in this work, Endozym® Pectofruit XL (Spindal AEB Group, Gretz-Armanville, France) was extracted from *Aspergillus niger* and was a mix of Polygalacturonases, Pectinesterases and Pectinlysases. The enzyme solutions were prepared with deionized water at 0.5, 1.0 and 2.0 ml/100 ml concentrations for the compression tests and the enzyme concentrations were 1.0 and 2.0 ml/100 ml for the puncture tests.

The micro-mechanical tests were carried out on a miniature tensile stage DB-T200Petri (Deben Microtest, Suffolk, UK). This equipment is similar to the tensile stage used by Oey et al. (2007) and Alamar et al. (2008) with an additional Petri dish allowing mechanical tests with immersed pieces.

All the experiments are carried on at room temperature about 24 °C.

2.1. Compression tests

The apples were longitudinally cut in frites (cross section 7×7 mm^2) and immersed overnight in a 0.6 M mannitol solution buffered with K_2HPO_4 (0.02 M) and KH_2PO_4 (0.02 M) to minimise the osmotic pressure effect (Oey et al., 2007). Before performing the compression test, one frite is removed from the beaker and a cubic specimen ($7 \times 7 \times 7$ mm^3) is prepared with parallel blade razor and immersed in a 0.6 M mannitol solution with or without enzyme in the Petri dish of the tensile device (Fig. 1A). The cubic samples were cut in the parenchyma avoiding the skin and the core, the compression is carried on in the radial direction. A preload of 3 N was applied before the compression phase to ensure a good contact between the apple cube and the grips. The compression test consisted on cycles of compressions; the load was measured with a 100 N full scale cell. The strain and the speed were respectively 5% (0.35 mm) and 0.2 mm min^{-1} corresponding to a