



## Effect of high-pressure/high-temperature processing on chemical pectin conversions in relation to fruit and vegetable texture

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### ABSTRACT

Heat sterilization of plant derived food products entails considerable organoleptic and nutritional quality losses. For instance, texture loss of fruits and vegetables occurs, next to turgor pressure losses, mainly due to chemical changes in the cell-wall pectic polysaccharides. High-pressure sterilization, i.e. the combination of high temperature ( $\geq 90$  °C) with high pressure ( $\geq 500$  MPa), could present a positive alternative assuring safety while minimizing quality losses. In this study, the potential of high-pressure sterilization in preserving fruit and vegetable texture was evaluated by investigating the effect of combined high-pressure/high-temperature (HP/HT) treatments on two texture related chemical pectin conversions in model systems. First, a protocol was developed to perform reproducible kinetic studies at HP/HT under constant processing conditions. Subsequently, apple pectin solutions at pH 6.5 were subjected to different HP/HT combinations (500, 600 and 700 MPa/90, 110 and 115 °C) and the extent of chemical demethoxylation and  $\beta$ -eliminative depolymerization was determined. At atmospheric pressure, both zero-order reaction rate constants increased with increasing temperature. At all temperatures, demethoxylation showed a higher rate constant than  $\beta$ -elimination. However, a temperature rise resulted in a stronger acceleration of  $\beta$ -elimination than of demethoxylation. When combining high temperature with high pressure,  $\beta$ -elimination was retarded or even stopped, whereas demethoxylation was stimulated. These results are very promising in the context of the texture preservation of high-pressure sterilized fruits and vegetables, as  $\beta$ -elimination is accepted to be one of the main causes of thermal softening and low methoxylated pectin can enhance tissue strength by forming cross-links with calcium ions present.

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### 1. Introduction

Consumer demand is increasing for high quality, fresh tasting foods free from additives, microbiologically safe and with an extended shelf life. The most commonly used food preservation method has been thermal processing, including pasteurization and sterilization. However, these processes adversely affect organoleptic, textural and nutritional qualities. Food scientists and the food industry are therefore continuously searching for novel, less degradative processing technologies (Mertens & Knorr, 1992). A technology that has shown such potential is high-pressure processing (Ludikhuyze, Van Loey, Indrawati, & Hendrickx, 2001). High-pressure processing applies pressures of 400–600 MPa at ambient temperature to inactivate enzymes and vegetative micro-organisms. At the same time, it offers an advantage in minimal

deleterious effects on food quality attributes (e.g. colour, flavour, and nutritional value). Pressure is transmitted uniformly and instantaneously throughout the food, which results in a very homogeneous processing impact. Currently, high-pressure processing is successfully applied on a commercial scale for pasteurization of a whole range of food products, e.g. fruit juices, guacamole, oysters and ham. To achieve a complete inactivation of enzymes, vegetative micro-organisms, as well as spores, high pressure must be combined with a second inactivating factor. In high-pressure sterilization, this second factor is elevated temperature. In general, sterilization with high pressure is possible by starting high-pressure treatments at elevated temperatures, e.g. 60–90 °C, and using the compression heat for rapid and uniform heating to higher temperatures (de Heij et al., 2003). Depending on the nature of the product, the initial product temperature, and the applied pressure, the adiabatic temperature increase may vary from 3 to 9 °C/100 MPa. Various quality aspects of high-pressure sterilized food products are superior to conventionally heat sterilized products (Matser, Krebbers, van den Berg, & Bartels, 2004). However, data on the influence of combined high-pressure/high-temperature

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processing on the texture of fruits and vegetables are rather sparse.

Texture is probably one of the most important quality characteristics of edible fruits and vegetables. Texture is a characteristic at the plant organ level, depending on a structural hierarchy (Waldron, Parker, & Smith, 2003). The polysaccharides that make up the plant cell wall (cellulose, hemicellulose and pectin) form the basis of this hierarchy. Changes in texture during ripening, processing and storage are mainly related to (bio-)chemical conversions in pectin as pectin is principally abundant in the plant middle lamella, plays a crucial role there in cell–cell adhesion, and, moreover, is brought into solution more easily and is more chemically reactive than the other cell-wall polymers (Van Buren, 1979). One of the main structural components of pectin is homogalacturonan, a linear chain of  $\alpha$ -(1,4)-linked galacturonic acid residues which can be methoxylated (Ridley, O'Neill, & Mohnen, 2001). Enzymes responsible for degradation of this pectin component include pectin methylsterases and polygalacturonases (Rexova-Benkova & Markovic, 1976). At elevated temperatures, high methoxylated pectin is prone to non-enzymatic conversions: depolymerization and demethoxylation. The  $\beta$ -eliminative depolymerization is mainly responsible for the extensive softening of low-acid fruits and vegetables during heat treatments (Sila, Smout, Elliot, Van Loey, & Hendrickx, 2006). This reaction proceeds on uronic acids, which possess a glycosidic linkage on C-4 in the  $\beta$ -position of the carboxyl group at C-5 (Kiss, 1974). A prerequisite is the presence of a methyl-ester group at C-6, rendering H-5 sufficiently acidic to be removed by an alkali. This results in the formation of unstable, intermediary anions that are stabilized by losing the C–O linkage in the  $\beta$ -position. Consequently, a double bond appears between C-4 and C-5 at the non-reducing end. This depolymerization leads to pectin solubilization and, consequently, to decreased cell–cell adhesion, resulting in tissue softening. The reaction rate is strongly dependent on the degree of methoxylation of pectin, pH and the presence of ions (Keijbets & Pilnik, 1974; Sajjaanantakul, Van Buren, & Downing, 1989; Sajjaanantakul, Van Buren, & Downing, 1993). Chemical demethoxylation takes place at the same time as it proceeds under the same temperature and pH conditions and influences the  $\beta$ -elimination (Kravtchenko, Arnould, Voragen, & Pilnik, 1992). In order to improve or preserve the texture of fruits and vegetables limited demethoxylation is desirable because demethoxylated pectin is less susceptible to  $\beta$ -elimination and can be ionically cross-linked by divalent cations such as calcium forming fortifying networks. The aim of this study was to investigate the effect of combined high-pressure/high-temperature treatments on the chemical demethoxylation and  $\beta$ -eliminative depolymerization of pectin to gain insight in the possible impact of HP/HT treatments on the texture of fruits and vegetables.

## 2. Materials and methods

### 2.1. Pectin

Apple pectin (degree of methoxylation 70–75%, Fluka, Switzerland) was used for all experiments.

### 2.2. Thermal treatments

A 0.3% (w/v) solution of apple pectin in 0.1 M Na-phosphate buffer pH 6.5, divided over screw-capped test tubes (2 ml per tube), was heated at 70, 80, 90, 100, 110 and 120 °C in a thermostated oil bath. After an equilibration period of 5 min, allowing the solution to reach the desired temperature, a first sample (treatment time 0 min) was withdrawn. The rest of the samples were removed after preset time intervals. The samples were immediately

cooled in an ice water bath, and subsequently analyzed for unsaturated uronides and methanol content. Thermal treatments were carried out once.

### 2.3. High-pressure/high-temperature treatments

A 0.3% (w/v) pectin solution in 0.1 M MES–NaOH pH 6.5 (MES = 2-(N-morpholino)ethanesulfonic acid) was divided over flexible microtubes (500  $\mu$ l), and subsequently treated at 90, 110 and 115 °C in combination with 500, 600 and 700 MPa for different time intervals. The HP/HT treatments were carried out in a custom-made laboratory scale high-pressure unit (Resato, The Netherlands), consisting of six individual vessels (6  $\times$  43 mL, internal diameter = 20 mm), each surrounded by a heating coil connected to a thermostat. The pressure medium consisted of propylene glycol. This equipment allows computer-controlled pressure build-up, data logging of both pressure and temperature, and processing conditions up to 1000 MPa and 120 °C.

First, a protocol had to be developed to treat samples in a reproducible way at semi-constant HP/HT. As an example, in Fig. 1 the temperature and pressure history of a pectin sample during treatment at 110 °C and 600 MPa is shown (starting from pressure build-up). The samples were inserted in cylindrical, polyoxymethylene acetate (POM) sample holders (85 mm long, 12 mm internal diameter, and 3 mm thickness), filled up with water (excluding air), and closed with a movable stopper sealed with a ring. These sample holders were tailor-made to fill the vessels optimally, so the ratio of sample volume to pressure medium volume is as large as possible and constant. The sample holders (containing the samples) were cooled to 10 °C in a cryostat. Subsequently, the sample holders were transferred to the pressure vessels already equilibrated at the desired process temperature. The vessels were closed and the temperature in the sample holder was allowed to rise to an initial temperature (e.g. up to  $\sim$ 66 °C) which was dependent on the desired process temperature after pressure build-up. This preheating was the result of heat transfer from the pressure medium to the samples. This initial temperature was beforehand experimentally determined for each pressure/temperature combination under study. Sample holders with a 1.6 mm hole were used to allow temperature measurement inside the sample holder using a 36.8 mm type J thermocouple attached to the pressure vessel stopper. Subsequently, pressure was built up very fast; increasing in 5 s from 0.1 to 150 MPa and then from 150 MPa to the set pressure (e.g. 600 MPa) at a rate of 10 MPa/s. This was accompanied by a temperature rise (e.g. up to  $\sim$ 107 °C) due to compression heating. After attaining the desired pressure, the individual vessels were isolated.

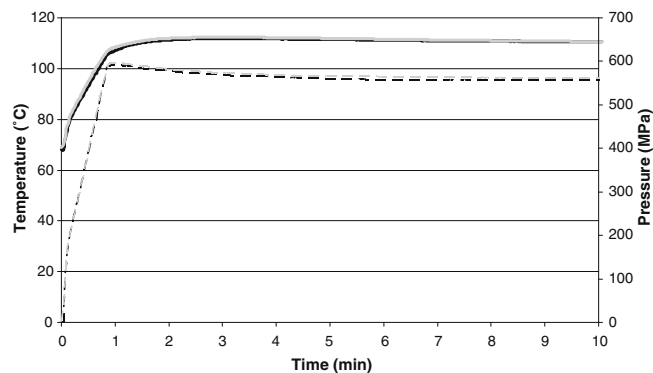


Fig. 1. Temperature and pressure history of a pectin sample during treatment at 110 °C and 600 MPa, starting from pressure build-up (—: temperature profile first run, - - -: pressure profile first run, ···: temperature profile second run, - · - ·: pressure profile second run).

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