Innovative Food Science and Emerging Technologies 38 (2016) 349–355

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Comparison of the application of high pressure and pulsed electric fields technologies on the selective inactivation of endogenous enzymes in tomato products

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ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form 24 June 2016
Accepted 27 July 2016
Available online 29 July 2016

Keywords:
Tomato juice
Pectin methylesterase
Polygalacturonase
High pressure processing
Pulsed electric fields

ABSTRACT

Application of novel technologies such as high pressure (HP) or pulsed electric fields (PEF) on the remaining activity of endogenous tomato pectinolytic enzymes such as Pectinmethylesterase (PME) and Polygalacturonase (PG), responsible for tomato products texture was studied. HP combined with temperature (200–800 MPa @ 55–75 °C), PEF (5.5–12.5 kV/cm, 0–12 ms treatment time) and thermally treated (55–75 °C) samples were studied. After thermal treatment, PG appeared to be more resistant than PME. Opposite behavior was observed for HP treated samples. For PME inactivation more intense P-T process conditions were necessary compared to PG. For PEF treatment, 98% inactivation was observed at 12.5 kV/cm and 6 ms for PME, and at 5.5 kV/cm and 11 ms for PG. PME appeared to be more HP and PEF resistant compared to PG. The results support the potential application of HP and PEF to selectively inactivate PG while partially retaining PME in tomato juices, aiming in improved tomato products' textural characteristics.

Industrial relevance: The aim of the tomato industry is to produce tomato products of desired textural and sensorial characteristics while increasing the yield by decreasing the evaporated water. This can be achieved by applying novel technologies such as high pressure (HP) processing or pulsed electric fields (PEF) that affect the remaining activity of the endogenous pectinolytic enzymes such as Pectinmethylesterase (PME) and Polygalacturonase (PG), responsible for the final texture leading to products with improved quality characteristics such as viscosity, color and consistency. However, HP treatment is a batch process and makes it difficult for the treatment of large quantities (production of small quantities of superior products could be the target of the application of HP technology), while PEF technology could be applied in line with the typical production flow of that kind of products before the cold break step.

1. Introduction

Condensed tomato products are widely appreciated for their key quality characteristics such as flavor, color and texture. Viscosity plays a very important role in such products, as it determines texture and consistency (Alviar & Reid, 1990). One of the major factors that affects consistency is the presence of tomato cell walls and cell wall polysaccharide modifying enzymes (Espachs-Barroso, Van Loey, Hendrickx, & Martín-Belloso, 2006; Kalamaki, Stoforos, & Taoukis, 2012, chap. 12). Concentrated tomato product viscosity has been found to be correlated with the activity of Pectin Methylesterase (PME, EC 3.1.1.11) and Polygalacturonase (PG, EC 3.2.1.15) (Barrett, Garcia, & Wayne, 1998). PME de-esterifies pectin yielding methanol and free carboxyl moieties whereas PG depolymerizes the de-methoxylated pectin, hydrolyzing α-1,4-glycosidic bonds of the polygalacturonic acid chain, resulting in polygalacturonic acids of lower molecular weight and free galacturonic acid monomers. These two enzymes, when acting synergistically, lead to the depolymerization of cell wall pectin chains and a drastic drop in viscosity (Fachin et al., 2003). However, when PME acts alone, the resulting free carboxyl group moieties on the pectin side chains can interact with bivalent cations such as Ca^{+} and form a cross-linked network increasing viscosity (Crelier, Robert, Claude, & Juillerat, 2001; Errington, Tucker, & Mitchell, 1998). It is therefore of high relevance to the tomato industry to control the activity of the two pectinolytic enzymes. Thermal treatment has been used for decades in the food industry and aims at inactivating microorganisms and enzymes that are detrimental to food quality. The tomato industry also commonly employs thermal treatment to inactivate PME and PG in tomato (Gould, 2013). More specifically, PG has been found to be more heat resistant than PME (Crelier et al., 2001; Stoforos, Crelier, Robert, & Taoukis, 2002) making their selective thermal inactivation impossible.

High hydrostatic pressure (HP) processing of foods is a non-thermal technology aiming at inactivation of microorganisms and enzymes in

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http://dx.doi.org/10.1016/j.ifset.2016.07.026
1466-8564/

Table 1
Pulsed electric field (PEF) treatment conditions for the study of PME and PG inactivation by PEF.

Treatment parameter	Conditions
Electric field strength (kV/cm)	5.5–12.5
PEF treatment time (ms)	0–12.0
Input temperature (°C)	15
Output temperature after PEF treatment (°C)	25–60
Pulse width (μs)	15
Frequency (Hz)	300
Electrode distance (mm)	4.0

foods while minimally affecting sensory and nutritional attributes (Farr, 1990; Knorr, 1993). The pressure inactivation of tomato pectinolytic enzymes has been studied (Crelier et al., 2001; Kalamaki et al., 2012, chap. 12). The inactivation of both enzymes was expressed by first order kinetics. The effect of pressure on the inactivation rate constants is very different for the two enzymes. The inactivation of PG happens at moderate pressures and temperatures, while inactivation of PME exhibits an antagonistic effect between temperature and pressure (Fachin, Loey, Oey, Ludikhuyze, & Hendrickx, 2002; Kalamaki et al., 2012, chap. 12; Shook, Shellhammer, & Schwartz, 2001). PME inactivation rates decrease as pressure rises from 0.1 to 100 MPa, then remain constant at pressures between 100 and 500 MPa and finally increases again for pressures exceeding 500 MPa (Kalamaki et al., 2012, chap. 12). This effect enables the use of high pressure processing to completely inactivate PG while retaining PME activity almost unaffected (Boulekou, Mallidis, Taoukis, & Stoforos, 2011), making this technology relevant to the improvement of the texture of tomato products.

Pulsed electric fields is another non-thermal technology that aims in processing liquid foods and maintaining sensory and nutritional characteristics. Several studies have been performed on the use of PEF for microbial inactivation (Min, Jin, & Zhang, 2003; Nguyen & Mittal, 2007) as well as enzyme inactivation from tomato (Aguiló-Aguayo, Odriozola-Serrano, Quintão-Teixeira, & Martín-Belloso, 2008; Espachs-Barroso et al., 2006; Min et al., 2003). The inactivation of PME and PG has been described at various processing conditions. Aguiló-Aguayo, Soliva-Fortuny, and Martín-Belloso (2007) observed an 82% inactivation of PME and a 12% inactivation of PG after a treatment of 35 kV/cm, treatment time of 1500 μs and a frequency of 100 Hz. Nguyen and Mittal (2007) observed an inactivation of PME up to 55% at 87 kV/cm, 40 pulses while preheating tomato juice to 50 °C. Giner et al. (2000) observed a 93.8% PME inactivation after treatment at 24 kV/cm, 400 pulses and a pulse width of 20 μs. The different sensitivity of the two enzymes at different processing conditions may allow for their selective inactivation using pulsed electric fields.

The objective of this work was to comparatively assess and model the different inactivation behaviors of PME and PG in tomato juice caused by high pressure, pulsed electric fields and thermal treatment. This selective inactivation results in yield increase of concentrated tomato products in terms of producing products of higher viscosity with less water evaporation, mainly attributed to the action of remaining PME.

2. Materials and methods

2.1. Tomato juice preparation

Fresh, ripe tomatoes (*Alamanda* cv.) were collected, washed, peeled and cut. Tomatoes were blended using a household food processor and filtered through a 0.5 mm mesh cloth to remove peels and seeds.

The resulting homogenous juice (soluble solids = 7.0 ± 0.1 °Brix) had a pH value equal to 4.32 ± 0.33, while the initial color value was a/b = 1.26 (L^* = 37.7 ± 1.8, a^* = 19.8 ± 2.5 and b^* = 15.9 ± 1.9). The juice was stored at −40 °C, until further use.

2.2. Thermal processing

Tomato juice was thermally treated at temperatures in the range of 55–75 °C. Preliminary experiments were conducted to determine which range of temperatures had a measurable inactivation effect on the enzymes. The experiments were conducted in triplicate. Ten (10) ml of juice were placed in a test tube (10 mm diameter) and immersed in a water bath set at the desired temperature. The time required for the temperature to reach the target value (<30 s in all experiments) was considered as time zero of the process and two samples were removed from the water bath and immediately dipped into iced water until PME and PG activity was measured. The rest of the samples were removed at predetermined time intervals according to the experimental design, following the same procedure.

2.3. High pressure processing

High pressure treatments were achieved using a laboratory scale HP equipment with a maximum operating pressure and temperature of 1000 MPa and 90 °C, respectively (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Holland) consisting of a high pressure unit with a pressure intensifier a vessel of 1.5 l and a multivessel system consisting of six vessels of 45 ml capacity each. The pressure transmitting fluid used was polyglycol ISO viscosity class VG 15 (Resato International BV, Roden, Holland). Process temperature in the vessels was achieved by liquid circulation in the outer jacket controlled by a heating-cooling system. The desired value of pressure was set and after pressure build up (20 MPa/s), the pressure vessels were isolated. This point defined the time zero of this process. Pressure of the vessel

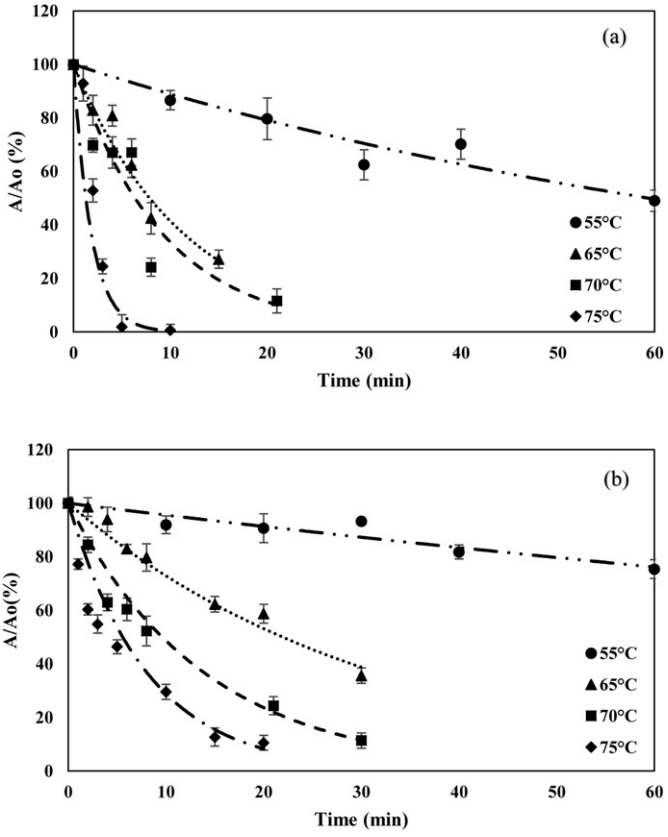

Fig. 1. Thermal inactivation of PME (a) and PG (b) at 55 °C (●), 65 °C (▲), 70 °C (■) and 75 °C (♦) (ambient pressure). Lines represent the fit of Eq. (3) to experimental data. Error bars represent standard deviation from multiple treatments and measurements.