



# The effect of high pressure homogenization and endogenous pectin-related enzymes on tomato purée consistency and serum pectin structure



Jihan Santanina J. Santiago, Zahra Jamsazzadeh Kermani, Fei Xu, Ann M. Van Loey, Marc E. Hendrickx\*

Laboratory of Food Technology, Leuven Food Science and Nutrition Research Centre (LForCe), Department of Microbial and Molecular Systems (M<sup>2</sup>S), KU Leuven, Kasteelpark Arenberg 22, PO Box 2457, 3001 Leuven, Belgium

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## ABSTRACT

The influence of mechanical tissue disintegration techniques (i.e. blending and high pressure homogenization) and the stimulation of endogenous pectin-related enzymes (i.e. pectin methyl-esterase and polygalacturonase) on tomato purée consistency, serum composition and serum pectin structure were investigated. Serum pectin structure was characterized in terms of degree of methyl-esterification, acetylation, neutral sugar composition and molecular weight ( $M_w$ ) distribution.

Endogenous pectin methyl-esterase and polygalacturonase stimulation resulted in the lowest purée consistency and highest serum yield. However, when such purée was homogenized, a higher purée consistency and a low serum yield were observed. Moreover, the  $M_w$  of serum pectin was exceptionally high for the homogenized purées. The low methyl-esterified, linear and remarkably high  $M_w$  tomato serum pectin of the homogenized purées partly explains their increased consistency. This work demonstrated that high pressure homogenization can at least partially restore the consistency of tomato purée despite an initial consistency loss ascribed to enzymatic pectin degradation.

**Industrial relevance:** The synergistic action of endogenous pectin-related enzymes causes serum pectin de-polymerization that consequently results in consistency loss of tomato purée. Nevertheless, intense high pressure homogenization showed to influence serum pectin molecular weight and at least restore the consistency loss. This means that a high tomato purée consistency can still be achieved regardless of the initial action of endogenous enzymes in the tomato pieces or puréed tomato food system using high pressure homogenization. This offers an additional processing alternative in the production of tomato-based dispersions with a targeted functionality.

## 1. Introduction

Tomatoes, amongst fruits and vegetables, are commonly processed into dispersed food systems such as soups, sauces, juices and purées. The consumption of these tomato-based dispersions has been suggested due to the increased bioavailability of bioactive health promoting compounds (e.g. carotenoids) compared to the intake of raw tomatoes (Gartner, Stahl, & Sies, 1997; Porrini, Riso, & Testolin, 1998). These dispersions are produced involving a combination of processing unit operations such as the indispensable mechanical tissue disintegration by blending and high pressure homogenization that alters the microstructure of the tomato matrix and the widely used thermal treatments

for preservation purposes (Gould, 1992; Moelants, Cardinaels, Jolie, et al., 2014; Moelants, Cardinaels, Van Buggenhout, et al., 2014). These unit operations result in a complex food system composed of insoluble pulp/particles dispersed in a continuous liquid/serum phase (Barrett, Garcia, & Wayne, 1998). Both serum and particle phases influence the sensory, nutritional and/or rheological properties of these dispersions (Barrett, Garcia, & Wayne, 1998; Hayes, Smith, & Morris, 1998; Diaz, Anthon, & Barrett, 2009; Lopez-Sanchez et al., 2011; Moelants, Cardinaels, Jolie, et al., 2014; Moelants, Cardinaels, Van Buggenhout, et al., 2014). Specifically, the consistency of tomato dispersions can be influenced by the presence of pectin in both serum and particle phases (Sánchez et al., 2003; Anthon, Diaz, & Barrett, 2008; Tibäck et al., 2009;

**Abbreviations:** DAC, degree of acetylation; DM, degree of methyl-esterification; GalA, galacturonic acid; HG, homogalacturonan; HPH, high pressure homogenization; HP, high pressure treatment; HT, high temperature treatment; LT, low temperature treatment;  $M_w$ , molecular weight; MWCO, molecular weight cut off; PG, polygalacturonase; PLNS, pectin-linked neutral sugars; PME, pectin methyl-esterase; RG, rhamnogalacturonan; UA, uronic acid

\* Corresponding author.

E-mail address: [marceg.hendrickx@kuleuven.be](mailto:marceg.hendrickx@kuleuven.be) (M.E. Hendrickx).

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Houben et al., 2014).

Pectin is one of the most interesting cell wall polysaccharides due to its poly-anionic nature and solubility characteristics (Thakur et al., 1997). It largely comprises a linear chain of 1,4 linked  $\alpha$ -D-galacturonic acid (GalA) residues and a branched chain with a repeating disaccharide [ $-\alpha$ -D-GalA-1,2- $\alpha$ -L-Rha-1-4-] $_n$  backbone containing individual, linear, or branched oligosaccharide side chains attached to the rhamnose residues (Voragen et al., 2009). The most abundant building blocks of pectin is the linear homogalacturonan chain which can be methyl-esterified on the C-6 carboxyl groups up to 70% to 80% and O-acetylated at O-3 or O-2 depending on the plant source (Voragen et al., 2009). Changes on the molecular structure of pectin due to biochemical reactions can influence the consistency of tomato dispersions (Sánchez et al., 2003; Anthon, Diaz, & Barrett, 2008; Tibäck et al., 2009; Houben et al., 2014). The consistency loss of tomato dispersions has been related to enzymatic pectin degradation caused by the synergistic action of pectin methyl-esterase (PME) and polygalacturonase (PG) (Verlent et al., 2006; Tibäck et al., 2009; Houben et al., 2014). PME catalyzes the removal of the methyl groups in the linear galacturonic acid-rich domain and PG subsequently de-polymerizes the de-esterified pectic molecule (Duvetter et al., 2009). To circumvent the consistency loss due to pectin enzymatic conversion, hot break processing of tomatoes that typically refers to a chopping temperature of 85 to 90 °C is being commercially used (Goodman, Fawcett, & Barringer, 2002). However, the detrimental effect of high temperature on some sensory properties of tomatoes (e.g. color and flavor) is inevitable. Moreover, Sánchez et al. (2003) and Tibäck et al. (2009) inferred that prolonged heating also entails pectin breakdown resulting in consistency loss. The emergence of non-thermal technologies such as high pressure processing may provide additional alternatives to the food industry for the manufacture of tomato dispersions. High pressure treatment can selectively inactivate PG thereby preventing pectin de-polymerization (Duvetter et al., 2009; Houben et al., 2013). Therefore, the consistency and the overall quality (e.g. color) of tomato dispersions can be improved by high pressure pre-treatment of tomato pieces (Duvetter et al., 2009).

Recently, high pressure homogenization (HPH) of tomato dispersions presented higher consistency than non-homogenized dispersions (Thakur, Singh, & Handa, 1995; Bayod et al., 2007; Colle et al., 2010; Panozzo et al., 2013; Palmero et al., 2016). Different mechanisms, including turbulence, shear and cavitation, were proposed to cause cell wall disruption during HPH (Stang, Schuchmann, & Schubert, 2001; Floury et al., 2004). The influence of HPH on the particles and its consequent effect on the rheological properties of the dispersion were extensively investigated. The concentration of the particles, their size, size distribution, morphology, deformability and surface properties largely influence the flow behavior of the high pressure homogenized dispersions, depending on the plant matrix (Bayod et al., 2007; Bayod & Tornberg, 2011; Lopez-Sanchez et al., 2011; Moelants, Cardinaels, Jolie, et al., 2014; Moelants, Cardinaels, Van Buggenhout, et al., 2014). However, specific properties of the serum phase and the molecular characteristics of the solubilized biopolymers are scarcely known. Although it has been reported that the serum had less influence on the rheology of dispersions, the serum was suggested to be essential for the overall structure organization of the dispersion and its interaction with the particles (Moelants et al., 2012; Moelants, Cardinaels, Jolie, et al., 2014; Moelants, Cardinaels, Van Buggenhout, et al., 2014). Therefore, profound investigation of the serum phase composition may provide a better insight on the solubilization of polymers (e.g. pectin) during processing. These solubilized pectic polymers may play a role in the interactions of (in)soluble constituents influencing the structural and physical stability of the dispersions (Christiaens, Van Buggenhout, Chaula, et al., 2012; Christiaens, Van Buggenhout, Houben, et al., 2012; Moelants, Cardinaels, Jolie, et al., 2014; Moelants, Cardinaels, Van Buggenhout, et al., 2014; Kyomugasho, Willemsen, et al., 2015). Moreover, pectin has been recognized as a ubiquitous component in the serum phase of plant-based dispersions due to its solubility. When

pectin is extracted from commercial sources, it is an important ingredient owing to its functional properties (e.g. thickening, gelling, stabilizing, emulsifying) ascribed to its chemical structure. Therefore, it is interesting to investigate the structure of serum pectin that may offer the use of a naturally existing constituent in tomato dispersions as a functional component. In this view, changes on the chemical structure of serum pectin and its possible relation to consistency can be investigated. Furthermore, the likelihood of restoring the consistency loss of tomato dispersions, which is due to the uncontrolled enzymatic activities and/or prolonged heating, using HPH can be explored. Understanding the influence of a combination of unit operations on the chemical structure of serum pectin and its potential functional properties allows a targeted processing of tomato dispersions. In addition, a holistic approach in processing tomato-based dispersed products considering the nature of the soluble serum components (e.g. pectin) could probably be considered.

Therefore, the present work was aimed to characterize the structure of serum pectin as influenced by mechanical tissue disintegration techniques (i.e. blending and high pressure homogenization) and the stimulation of endogenous pectin-related enzymes (i.e. PME and PG) during purée preparation. The serum phase composition, the chemical structure of serum pectin and the physico-chemical properties of the differently prepared tomato purées were examined.

## 2. Materials and methods

### 2.1. Raw material

A batch of red-ripe tomatoes (*Solanum lycopersicum* cv. Prince) was purchased from a local shop in Belgium, stored at 4 °C for maximum 3 days and utilized in preparing six different purées. These tomatoes were washed, dried, and then cut into slices or quarters. Except for the tomato quarters which were subjected to high pressure pre-treatment, the tomato slices ( $\pm 1$  cm) were immediately vacuum-packed in a single layer using polyethylene bags (DaklaPack® Lamigrip Stand-up Pouch Transparent; 220 mm  $\times$  300 mm + 65 mm bottom fold), frozen in liquid nitrogen and then stored at  $-40$  °C. Upon use and to facilitate the blending, the frozen tomatoes were thawed in a temperature-controlled water bath at 25 °C for 5 min.

### 2.2. Preparation of tomato purées

A schematic overview of the purée preparation is presented in Fig. 1. The various purée processing conditions were generally selected based on the aim of each specific treatment. High pressure pre-treatment was performed to selectively inactivate polygalacturonase (PG), while maintain the pectin methyl-esterase (PME) activity (Christiaens, Van Buggenhout, Chaula, et al., 2012; Christiaens, Van Buggenhout, Houben, et al., 2012). For the heat treatment at 95 °C for 30 min, enzyme inactivation is reportedly achieved at this temperature at a shorter time (Moelants et al., 2012; Houben et al., 2013). Since we aimed to obtain high amounts of pectin in the serum, 30 min heat treatment was chosen that could result in higher pectin solubilization (Moelants et al., 2012). For the same reason, we chose 100 MPa for high pressure homogenization to enhance the amount of pectin in the serum (Moelants et al., 2012). In the case of low temperature treatment at 40 °C for 30 min, this was previously reported as an optimum condition for the activities of tomato PME and PG enzymes (Houben et al., 2014).

#### 2.2.1. High pressure pre-treatment

To selectively inactivate the endogenous PG enzyme, tomato quarters were pre-treated at 550 MPa for 10 min in a single-vessel, laboratory-scale high pressure equipment with the cryostat pre-set at 4 °C (Engineered Pressure Systems International, EPSI, Temse, Belgium). Tomato quarters were vacuum-packed in a double-film polyethylene bag and then placed into the vessel. After the treatment and

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