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Pectin-interactions and *in vitro* bioaccessibility of calcium and iron in particulated tomato-based suspensions

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ABSTRACT

In this study, the pectin structure–function relationship in tomato-based suspensions was explored. Particularly, electrostatic pectin-interactions were investigated and these interactions were subsequently linked to the *in vitro* bioaccessibility of Ca and Fe-ions in tomato-based suspensions. Process tomatoes were either treated at high-pressure (HP) or blanched at high temperature (HB). Ca^{2+} mediated pectin–pectin interactions in reconstituted HP tomato-based systems were stronger than in reconstituted HB tomato-based systems. These interactions were influenced by the molecular structure of pectin present in the serum fraction of the tomato suspensions, and improved with increasing Ca^{2+} concentration and pH. Investigation of the essential mineral content showed that HP and HB tomato-based suspensions contained similar amounts of Ca and Fe-ions (9.8 ± 0.8 and 0.39 ± 0.1 mg/100 g of purée, respectively). The Ca and Fe-ions in HB tomato-based suspensions were two times more bio-accessible than the Ca and Fe-ions in HP tomato-based suspensions. Finally, it could be concluded that the bioaccessibility of Ca and Fe-ions decreases with increasing electrostatic pectin-interactions.

1. Introduction

Owing to their nutritional and health benefits, fruits and vegetables should constitute an important part of everyday diet. In particular, tomato, which is one of the world's most consumed fruits, contains complex carbohydrates, dietary fibre, vitamins such as A (β-carotene) and C, and minerals including manganese, phosphorous, iron and calcium. With the increasing demand for fruits and vegetables and because they are perishable, edible portions are frequently processed into a variety of more convenient, easy to transport quality products. Specifically, tomato is processed into widely consumed tomato-derived particulated products such as juices, soups, ketchup and tomato sauce (Thakur, Singh, & Handa, 1996). During processing, these particulated products are susceptible to chemical and/or enzymatic reactions leading to changes in quality (e.g. color, texture) as well as changes in nutritional value in terms of both nutrient concentration (amount of nutrients present) and nutrient bioaccessibility (amount of nutrients available for absorption) (Parada & Aguilera, 2007). In fruits and vegetables, important changes that occur during storage and processing are related to changes in cell wall polysaccharides, in particular pectin (Voragen, Coenen, Verhoef, & Schols, 2009).

Pectin is a heteropolysaccharide predominately containing galacturonic acid (GalA) residues, in which varying proportions of acid groups are present as methyl esters, while a specific amount of neutral sugars might be present as side chains (Voragen et al., 2009). Three major pectic polysaccharide building blocks (homogalacturonan (HG), rhamnogalacturan-I (RG-I) and rhamnogalacturan-II (RG-II)) have been isolated from plant cell walls and structurally characterized (Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011; Ridley, O'Neill, & Mohnen, 2001). HG is the predominant pectin building block in the primary cell wall and middle lamella. This polysaccharide is a homopolymer of α-1,4-linked GalA residues within which moieties of GalA may be partially methylesterified at C-6 and/or O-acetylated at O-2 and/or O-3 (Mohnen, 2008; Sila et al., 2009; Voragen et al., 2009). RG-I on the other hand is a branched and compositionally heterogeneous polysaccharide generally comprising a backbone of the repeating

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disaccharide [→4)-α-D-GalA-(1 → 2)-α-L-rhamnose-(1→]. This polysaccharide is partially substituted at the C-4 position of rhamnose with single neutral glycosyl residues or polymeric side chains which are principally composed of L-arabinose and/or D-galactose. Finally, RG-II is composed of at least eight α-(1,4)-linked D-GalA residues to which four structurally conserved chains are attached (O'Neill & York, 2003; Yapo, Lerouge, Thibault, & Ralet, 2007). It is widely accepted that these polysaccharide building blocks (HG, RG-I and RG-II) are covalently interconnected to each other, thereby forming complex pectic composites (Yapo, 2011). Furthermore, pectin chains interact with each other by non-covalent cross-links such as Ca^{2+}-bridges, hydrogen bonds and hydrophobic interactions (Caffall & Mohnen, 2009; Sanchez, 2011). In the food industry, ionic cross-linking of pectin has been exploited in intact plant tissues to preserve texture upon processing (Fraeye et al., 2009) as well as in *ex situ* gelling applications, whereby blocks of non-methylesterified HG polymers are electrostatically linked together by calcium-ions allowing gel formation (Thakur, Singh, & Handa, 1997). Changes in the pectin structure, especially alterations related to the HG polymer, are believed to affect the polyvalent ion binding capacity of pectin, which may in turn alter pectin functionality. For instance, non-digestible carbohydrates such as pectin have been shown to impair the absorption of minerals and trace elements in the small intestine because of their binding and/or chelating effect (Bosscher, Van Caillie-Bertrand, Van Cauwenbergh, & Deelstra, 2003). The major determining factors for this phenomenon are the number of ionizable functional groups such as free hydroxyl groups and carboxyl groups and their physical structures during small intestinal digestion (Bosscher et al., 2003). For pectin, intrinsic factors such as pectin degree of methylesterification (DM), distribution of non-methylesterified GalA residues, molar mass and linearity, as well as extrinsic factors such as pH greatly influence its ion binding capacity and hence the absorption of minerals and trace elements. For example, by decreasing the DM of pectin, more groups of non-methylesterified GalA residues are present and can form complexes with polyvalent metal ions (Bosscher et al., 2003) such as Fe and Ca-ions. Of course, a pH above the pKa of pectin (3.38–4.10) is required to ensure ionization of the non-methylesterified groups of pectin (Sriamornsak, 2003). Polyvalent ions that are electrostatically bound, are consequently inaccessible during human absorption. As a result, Ca deficiency leads to poor bone formation and limited bone mass accumulation in infants and adolescents, as well as bone loss and osteoporosis in the elderly (Peacock, 2010) while Fe-ion deficiency leads to iron deficiency-induced anemia (Lieu, Heiskala, Peterson, & Yang, 2001).

To study pectin structure–function relationships, a selective extraction of pectic fractions from cell wall material is commonly performed followed by a physicochemical analysis of the fractionated cell walls and isolated polymers (Christiaens et al., 2012a). Recently, analytical techniques that are less time consuming and less invasive to the sample have been implemented for exploring this structure-function relation. In this research, the relationship between pectin-interactions and pectin functionality is investigated without prior isolation of pectin from the plant tissues. By exploring *in situ* the interactions between fluorescently labeled exogenous pectins of different DMs and pectin with different structural features created at the surface of tomato tissue particles or in the liquid phase through selective processing, insight into pectin-interactions in tomato-based suspensions was obtained. Thereafter, the bioaccessibility of Ca and Fe in tomato purées was investigated in an *in vitro*-simulated digestion of the tomato purées. By examining Ca and Fe bioaccessibility in relation to pectin-interactions, the pectin structure–function/bioaccessibility relationship was explored.

2. Materials and methods

Tomatoes were processed and the resulting suspensions were investigated for pectin-interactions and *in vitro* bioaccessibility of essential minerals.

2.1. Processing of tomato tissues

Process tomatoes (*Lycopersicum esculentum* cv. CLX 38 197) at the red-ripe stage were purchased from Italy and processed to create different enzyme populations. On the one hand, whole tomatoes were vacuum sealed and high-pressure pretreated (10 min, 7 °C and 550 MPa) to selectively maintain endogenous PME activity while inactivating polygalacturonase (PG) activity. According to Fachin et al. (2003), under conditions of combined high-pressure/thermal processing, 200–550 MPa/5-50 °C, PG inactivation can be achieved. On the other hand, tomatoes were cut into one cm slices, vacuum sealed and blanched at 95 °C for 8 min, to inactivate the pectin degrading enzymes (PG and PME) (Christiaens et al., 2012b; Houben et al., 2014). Purée was subsequently prepared using a mixer (Büchi mixer B-400, Flawil, Switzerland). The purées obtained were then sieved to remove the seeds and peels, and incubated at 25 °C for 1 h to allow action of PME (if present). Thereafter, the purées were blanched at 95 °C for 30 min. The blanched purées were then cooled and used in further sample preparation steps. High-pressure pretreated tomato samples were denoted as HP while high temperature blanched tomato samples were denoted as HB. The purées were divided into three parts:(i) one part for separation of the purée into tissue particles with different sizes, (ii) one part for extraction of the liquid phase (serum), and (iii) one part for determination of *in vitro* bioaccessibility of essential minerals (see Sections 2.2.1, 2.2.2 and 2.3, respectively).

2.2. Determination of pectin-interactions in reconstituted tomato-based suspensions

2.2.1. Separation of tomato purée particles based on size

Using a sieve shaker (Retsch, Aartselaar, Belgium) equipped with sieves of different pores sizes (40, 80, 125, 250, 500 and 1000 μm), purée was separated into fractions with different sizes by the wet sieving technique. The pulp collected on each sieve was then assembled and drained over a filter to remove excess water as described by Moelants et al. (2014). Thereafter, the particles obtained were either used in pectin characterisation (see Section 2.2.3) or stored over 70% ethanol for at least two weeks before further use in microscopy examinations (see Sections 2.2.4 and 2.2.6).

2.2.2. Extraction of the liquid phase (serum)

Tomato purée was centrifuged (30 min, 20 °C and 12400 g) and the supernatant obtained was filtered under vacuum to completely separate particles from the liquid phase (serum). One part of this serum was lyophilised and used for pectin characterisation while the other part was used for reconstitution experiments with tissue particles for microscopy studies.

2.2.3. Pectin characterisation

Cell wall material of tomato tissue particles was isolated as alcohol insoluble residue (AIR) and the AIR was evaluated for the DM of pectin. In the case of tomato serum, the pectin present was