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In situ pectin engineering as a tool to tailor the consistency and syneresis of carrot purée

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ABSTRACT

To investigate whether *in situ* pectin engineering would be a helpful tool in tailoring the consistency and syneresis of vegetable purées, carrot was selected as a plant tissue in which the textural properties are largely influenced by pectin methylesterase-induced pectin changes. The effect of low-temperature and high-temperature blanching, as well as the effect of two types of mechanical disruption, blending and high-pressure homogenisation, on the flow properties of carrot purée was explored. The influence of these different purée preparation steps on pectin was examined via physicochemical analysis of fractionated walls and isolated polymers, and via anti-pectin antibodies entailing *in situ* and *ex situ* analyses. Purée prepared by blending non-pretreated carrots showed a rather high consistency and pronounced syneresis. Treatments that solubilise pectin, such as high-pressure homogenisation and, in particular, high-temperature blanching, limited syneresis phenomena. In contrast, when the intercellular adhesion in carrot tissue was strengthened via low-temperature blanching, the degree of syneresis increased. High-pressure homogenisation was useful to reduce the carrot tissue particle size and, consequently, resulted in a lower consistency carrot purée. Low-temperature blanching on the other hand increased the consistency of carrot purée as the higher level of intercellular adhesion presumably led to an increased resistance to particle disintegration upon blending or high-pressure homogenisation.

1. Introduction

In today's food industry, a global trend towards the manufacture of healthier and more natural fruit and vegetable food products, such as soups, smoothies and sauces, is ongoing, as well as the incorporation of puréed vegetables in other food products (Blatt, Roe, & Rolls, 2011). The creation of these products involves the mechanical disruption of parenchyma-rich plant tissues. The resulting plant-food dispersions are a combination of a liquid phase (serum), containing pectic polysaccharides and other soluble substances, and a dispersed phase (pulp), containing the plant insoluble solids such as cell walls (Lopez-Sanchez et al., 2011). High-pressure homogenisation, a more intense shear treatment to further mechanically disrupt the plant material compared with conventional blending, has recently been introduced in the context of vegetable processing as a tool to further exploit the natural structuring potential of different plant sources. The deliberate application of particular thermal and mechanical processes on raw plant material makes it possible to design naturally structured/textured food products without the addition of texture-controlling agents such as starches, gums and stabilizers.

The rheological properties of plant-food dispersions are often related to parameters such as particle size, morphology and volume (Day, Xu, Oiseth, Hemar, & Lundin, 2010; Lopez-Sanchez et al., 2011). Detailed research towards the role of pectin on the flow properties of purées on the other hand is lacking. However, it is known that *in situ* pectin structural modifications during food processing remarkably alter the textural/rheological properties of plant-based foods (Sila et al., 2009) and, in addition, this polysaccharide occurs in both the liquid and dispersed phase of plant-food dispersions due to its solubility characteristics (Van Buren, 1979). Hence, the role of pectin on purée consistency and syneresis (i.e. the spontaneous separation of serum and pulp) should not be neglected.

One of the most abundant building blocks of pectin is homogalacturonan (HG), a linear chain of galacturonic acid (GalA) residues in which some of the C-6 carboxyl groups are methyl-esterified. HG

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in general and its methyl-esterification (degree and pattern) in particular strongly determine the functionality of pectin in plant-based food products (Willats, Knox, & Mikkelsen, 2006). During processing, HG is prone to chemical and/or enzymatic conversion reactions leading to pectin depolymerisation and/or demethoxylation. In low-acid plant tissues, pectin depolymerisation occurs at high temperatures (>80 °C) through a β-elimination reaction which is favored by hydroxyl ions and methyl-esterified GalA residues (Van Buren, 1979). The depolymerisation and solubilisation of pectic polymers involved in cell–cell adhesion has been demonstrated to cause serious texture deterioration in processed plant tissues (De Roeck, Sila, Duvetter, Van Loey, & Hendrickx, 2008). Pectin demethoxylation, by the action of cell-wall-bound pectin methylesterase (PME), can improve the intercellular adhesion since the increase in free pectic carboxyl groups provides a greater opportunity for pectic polymers to be cross-linked with divalent ions such as Ca^{2+}. Endogenous PME activity is enhanced during conventional low-temperature blanching, typically 15–45 min at 50–60 °C (Ni, Lin, & Barrett, 2005; Sila, Smout, Vu, Van Loey, & Hendrickx, 2005), whereas high-temperature blanching inactivates PME. In some fruits and vegetables however, demethoxylated pectin can act as a substrate for the action of the depolymerising enzyme polygalacturonase, which results in texture/viscosity loss (Sila et al., 2009).

Assessing the influence of processing on pectin in food matrices has predominantly been performed using *ex situ* analysis techniques and more specifically, via physicochemical analysis of fractionated walls and isolated polymers (Sila et al., 2009). Anti-pectin antibodies now provide a new range of opportunities as they allow the precise localisation of defined structural pectic domains in intact plant cell walls (Christiaens, Van Buggenhout, Ngouémazong, et al., 2011; Willats et al., 2006). Of particular interest for food technologists are a set of antibodies that bind to HG domains of pectin, including JIM5, JIM7, LM18, LM19, LM20, PAM1 and 2F4 (Knox, Linstead, King, Cooper, & Roberts, 1990; Liners, Letesson, Didembourg, & Van Cutsem, 1989; Verhertbruggen, Marcus, Haeger, Ordaz-Ortiz, & Knox, 2009; Willats, Gilmartin, Mikkelsen, & Knox, 1999). Knowledge on the binding specificities of these antibodies is at hand: antibodies LM18 and LM19 need a stretch of unesterified GalA residues for recognition, while methyl-esterified residues are required for the binding of LM20 (Christiaens, Van Buggenhout, Ngouemazong, et al., 2011; Verhertbruggen et al., 2009). In contrast, the epitope of JIM5 contains both methyl-esterified and non-methyl-esterified GalA residues. JIM7 can be used as a general anti-pectin probe as it recognises HG with very diverse degrees and patterns of methyl-esterification (Christiaens, Van Buggenhout, Ngouemazong, et al., 2011). PAM1, on the other hand, is a much more 'specific' antibody since it only binds to long blocks of approximately 30 non-esterified GalA residues (Willats et al., 1999). Finally, localisation of Ca^{2+}-cross-linked pectin, important in cell–cell adhesion, is possible with monoclonal antibody 2F4 (Liners et al., 1989).

The objective of the current study was to explore the opportunities of *in situ* pectin engineering in tailoring the consistency and syneresis of vegetable purées. Carrot (*Daucus carota*), in which PME-induced pectin changes play an important role in determining the structural/textural properties of the tissue, was selected as a commercially important plant material. The effect of low-temperature and high-temperature blanching, as well as the effect of two types of mechanical disruption, blending and high-pressure homogenisation, on the flow properties and pectin structure of carrot purée was investigated. Pectin was examined, not only via the traditional physicochemical analysis of fractionated walls and isolated polymers, but also via anti-HG antibodies entailing *in situ* (microscopy) and *ex situ* (immuno-dot assays) analyses.

2. Materials and methods

A schematic overview of the experimental set-up is presented in Fig. 1.

2.1. Preparation of carrot purées

2.1.1. Plant material

Fresh carrots (*D. carota* cultivar Nerac) were bought in a local shop and stored at 4 °C for a maximum period of 48 h before use. The carrots were peeled and cut into non-calibrated pieces (around 1 cm^3) before further purée preparation. For each sample, pieces of at least four different carrots were used to reduce variability from individual specimens.

2.1.2. Pretreatments

The non-calibrated carrot pieces were either not pretreated (raw sample) or subjected to conventional low-temperature blanching (LTB = 40 min, 60 °C) or high-temperature blanching (HTB = 5 min, 95 °C) (Sila et al., 2005). The thermal pretreatments were performed in a temperature-controlled water bath in which the carrot samples, vacuum-packed in a polyethylene bag, were immersed completely. After the pretreatment, samples were immediately cooled to ambient temperature in an ice-water bath.

2.1.3. Blending and high-pressure homogenisation (HPH)

Deionised water was added in a 1:1 (w/w) ratio to the (non-) pretreated carrot pieces. Subsequently, the pieces were mechanically disrupted using a laboratory blender (Waring Commercial, Torrington, Connecticut, USA), operating the first 20 s at low speed and the next 40 s at high speed. Selected samples were further disrupted using a Panda 2k high-pressure homogeniser (Niro Soavi, Parma, Italy), of which the in- and outlet were thermostated at 4 °C using a cryostat (Haake, Karlsruhe, Germany), via a single pass at 100 bar. Purée samples intended for chemical pectin characterisation was frozen with liquid N_2 and stored at −40 °C.

2.2. Bostwick consistency index

The consistency of the fresh carrot purées was measured using a Bostwick consistometer. In this empirical test, a sample is allowed to flow under its own weight along a level surface for 30 s at room temperature. The distance travelled by the purée was recorded as the Bostwick consistency index (in centimetres) and a distinction was made between the pulp and the serum fraction. A high Bostwick consistency index for the pulp fraction corresponds with a low consistency purée which has a low resistance to flow, while a low Bostwick consistency index for the pulp fraction characterises high consistency purées which have a high resistance to flow. For each sample, the Bostwick consistency index was measured in duplicate.

2.3. Generation of carrot tissue particle fractions of different sizes

A sieve shaker (Retsch, Aartselaar, Belgium), equipped with six sieves (40, 80, 125, 250, 500 and 1000 μm), was used to fractionate the carrot purées into carrot tissue particle fractions of different sizes. After loading the sieve shaker with 200 g of the fresh sample, wet sieving was performed in 2 min (shaking amplitude 0.5 mm). Afterwards, each sieve was manually dried with paper and the fractions retained on the sieves were weighed. The relative amounts of the different particle fractions allowed for an estimation of the particle size distribution. Carrot tissue particle fractions intended for microscopic analysis were stored in 70% (v/v) ethanol.