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Enhanced electrostatic interactions in tomato cell suspensions

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ABSTRACT

The natural consistency of processed tomato products arises from cell wall particles and the interactions between them. In this study, ion exchange resins were used to investigate these interactions. Two types of resins were used, a hydrogen form cation exchange resin and an anion resin in the hydroxide form. Serum phase of tomato suspensions were treated with either a cationic or an anionic resin to exchange various ionic compounds with hydrogen and hydroxide ion respectively. The treated serum was then reconstituted to the tomato pulp and the suspension was re-suspended with shear. The linear storage modulus varied with the different types of resin treatment. Samples treated with the anion exchange resin resulted in a higher modulus than the untreated tomato puree and the puree treated with the cation resin. Effect of the resins was dependent on the concentration of resin used. The anion treated sample resulted in a network formation which was quite sensitive to pH and was attributed to long range electrostatic interactions caused by protein–pectin interactions. Using Infrared spectroscopy the conformational changes in the protein structure as a result of resin treatment was detected by analyzing the amide-I and amide-II regions.

1. Introduction

Processed tomato products in the form of purees and sauces are a primary source of tomatoes in the modern diet. Considerable research has been conducted in the past to elucidate the reason for natural consistency and structure of tomato products. Tomato as a fruit primarily consists of cellulose, pectins, hemicelluloses, proteins and sugars. Functional role of these polysaccharides in cell wall suspensions arise inherently from their roles in the plant itself. Complex interactions among these building blocks are responsible for the structuring of tomato products (Barrett, Garcia, & Wayne, 1998; Tanglertpaibul & Rao, 1987a, 1987b; Beresovsky, Kopelman, & Mizrahi, 1995). During the various stages of processing many changes occur in these building blocks which in turn affect the characteristics of the end product (Augusto, Ibarz, & Cristianini, 2012; Lopez-Sanchez et al., 2011; Lopez-Sanchez, Svelander, Bialek, Schumm, & Langton, 2011; Sanchez, Valencia, Gallegos, Ciruelos, & Latorre, 2002). A better understanding of these natural interactions enables the food industry to create better products with desired rheological properties.

Cellulose is the primary component in vegetable cell wall suspensions and also the primary contributor to the rheology by forming the backbone of the particles. Pectins are grafted naturally onto the cellulose backbone and are also found in the serum phase. They are known to contribute significantly to the structure depending on the processing conditions and extrinsic factors such as pH, ionic conditions etc. (Beresovsky et al., 1995; Takada & Nelson, 1983). Pectins are a diverse class of polysaccharides that are chemically heterogeneous. This heterogeneous nature of pectins arises from the various possible branchings and the chemical diversity of the backbone. Pectins play a vital role in the quality of processed foods. Many processes may alter the structure of pectins which in turn change the tangible properties of foods. The unique characteristics of pectins allow for an interesting interplay with other components through hydrogen bonding, hydrophobic interactions, ionic and maybe even covalent coupling (Sila et al., 2009). Pectin–pectin interactions have been studied quite well, the most exploited use of these interactions are in making gels. Two main mechanisms of gelling have been identified depending on the

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degree of methoxylation (DM) of pectins. Pectins with a high degree of methoxylation (DM > 50%) gel only at a pH lower than 3.5 and in the presence of high sugar content (higher than 55%) (Thakur, Singh, Handa, & Rao, 1997; Willats, Knox, & Mikkelsen, 2006). The high sugar content promotes hydrophobic interactions between the methoxyl groups and the low pH keeps the carboxyl groups from disassociating, hence reducing electrostatic repulsion. This effect, combined with hydrogen bonding between the carboxyl groups and secondary alcohol groups promotes gelation (Thakur et al., 1997). On the other hand, low methoxylated pectins (DM < 50%) may interact with other pectin chains to form a gel, in the presence of Ca^{2+}ions. In such conditions homogalacturonan (HG) chains present in pectins interact with each other according to the "egg box model" (Grant, Morris, Rees, Smith, & Thom, 1973). The carboxyl groups present on the demethoxylated galacturonic acid molecules creates a negative charge that is capable of holding in place a calcium ion (Braccini, Grasso, & Perez, 1999). Extrinsic factors such as pH, sugar content, presence of ions and temperature play an unequivocal role in pectin–pectin interactions (Fraeye, Duvetter, Doungla, Van Loey, & Hendrickx, 2010).

Polysaccharides are also known to interact with proteins to form complexes. These interactions play a role in the texture, rheology and micro-structure of many food systems (Doublier, Garnier, Renard, & Sanchez, 2000; de Kruif & Tuinier, 2001; Tolstoguzov, 1991). Depending on various factors, there could be three possible scenarios for protein-polysaccharide interactions in mixtures. The interactions could result in the formation of two distinct phases, behaving like two immiscible liquids due to the incompatibility of the polymers. Alternatively the interaction may results in n homogeneous stable solution of macromolecules, because of co-solubility (no interaction). In this case, they are not stabilized by electrostatic interactions nor form complexes. Co-soluble complexes are the third type of interaction that could occur, when two types of macromolecules exist in the same phase and result in the formation of protein-polysaccharides complexes. Bernal, Smajda, Smith, and Stanley (1987) used a model system to investigate the gelling properties of pectin–protein gels. It was found that the addition of proteins to calcium-pectin gels improved the gel strength due to protein-pectin interactions. Protein-pectin interactions have also been observed in tomato suspensions by Takada and Nelson (1983). The highest viscosity of a tomato puree and the serum phase was at a pH of 4.40. The pH is an important factor as it determines the nature of interactions depending on the pKa of pectins and the isoelectric point of the proteins. In another work, an addition of 1% denatured soy protein to tomato juice increased the consistency and changed the rheological properties considerably (Thakur, Singh, & Handa, 1996; Tiziani & Vodovotz, 2005). This system exhibited a weak physical gel behavior, stabilized by non-covalent interactions with a complex rheological response, indicating a network formation or aggregation among the proteins and pectins (Tiziani & Vodovotz, 2005).

In this work, the electrostatic interactions present in tomato cell wall suspensions are investigated. A novel method for processing tomato suspensions was explored with the use of ion exchange resins. A cation and an anion resin were employed to substitute ionic compounds with H^+ and OH^- respectively. It will be shown that processing with different resins can change the physical functionality of tomato suspensions by altering protein-pectin interactions.

2. Materials and experimental methods

2.1. Resin treatment

Ion exchanger I (Catalogue number 104765), a strong cation exchange resin in the H^+ form and Ion exchanger III (Catalogue number 104767), a strong anion exchange resin in the OH^- form were obtained from Merck Millipore International. The cation and anion resins were loaded by incubating 100 g of resin material in 100 ml of 4 M HCl or NaOH respectively for 3 h. Excess acid/base was washed away with de-ionized water until the filtrate was at neutral pH. A tomato suspension was prepared from an industrial supply of 28° brix tomato paste obtained from Unilever. The paste was hot breaked by the suppliers and contained no enzyme activity. This paste was diluted to 8.5% total solid content as measured by dry weight basis which is referred henceforth as the control or reference sample. This tomato suspension was centrifuged at 12,000 *g* for 20 min at room temperature of 20–25 °C. Serum phase was separated from pulp by decanting. Different concentrations (% w/v) of each resin was added to this serum phase and incubated for a period of 1 h at room temperature. After filtering off the resin material, the treated serum phase was reconstituted back into the tomato pulp. Using a high speed mixer Ultra-turrax T25 (IKA laboratory equipment), the mixture was re-suspended by shearing at a speed of 13,000 rpm for 1 min at room temperature (20–25 °C). All samples were left in a refrigerator at 5 °C overnight before performing any measurements. Experiments were repeated in duplo.

2.2. Enzyme treatment

Two classes of enzymes were used to treat tomato suspensions, a protease (Promod 144GL, Biocatalysts Ltd UK) and a pectinase. The pectinase being a mixture consisting of a pure polygalacturonase (Endo-polygalacturonase M2, EC 3.2.1.15, Megazyme International Ireland) and pectin methylesterase (pectin methylesterase, EC 3.1.1.11, Novozyme International Denmark). The mix was made up of ten parts Endo-polygalacturonase to one part of pectin methylesterase by volume. The tomato suspensions were incubated with the enzymes either before or after treating with the anion resin treatment, to qualitatively identify the nature of the interactions. Both enzymes were used at a concentration of 0.1% w/w in tomato suspensions. All enzyme treatment was performed at pH 4.5 and a temperature of 45 °C over a period of three hours. After enzyme treatment the samples were heated to 90 °C for 5 min to irreversibly inactivate the enzymes.

2.3. Rheology

A stress controlled rheometer (AR-2000ex sold by TA-instruments) with a parallel plate geometry of 40 mm diameter was used for all measurements. Wall slip occurs commonly in two-phase systems due to steric, hydrodynamic, viscoelastic and chemical constraints acting near the boundaries (Barnes, 1995). This wall slip is apparent for concentrated suspensions with a smooth measuring surface. To avoid this, the plates were stuck with a 3 M 800 grit sand paper. A preliminary check was done by flow sweeps at gaps of 2000 μm, 1800 μm and 1500 μm. There were no differences in the steady state flow curves at different gaps, thus wall slip did not occur with the sand paper stuck on the plates (Yoshimura & Prud'homme, 1988). All measurements were done with the temperature of the peltier plate set to 25 °C. A gap of 1500 μm was chosen as it was an order of magnitude larger than the mean particle size and was small enough to avoid any flow effects arising from large gaps.

The sample was subjected to a pre-shearing step at a shear rate of 5 1/s for 30 s and subsequently was allowed to equilibrate for 10 min prior to measuring. Rheology of various samples was thixotropic, hence very sensitive to the pre-conditioning of the material. To ensure confidence in the rheological measurements, all samples were strictly subjected to the same pre-conditioning procedure, including the loading procedure in the rheometer. All