Structure formation in sugar containing pectin gels - Influence of gel composition and cooling rate on the gelation of non-amidated and amidated low-methoxylated pectin

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ABSTRACT

Gel structure formation and gel properties of low-methoxyl pectin (LMP) and low-methoxyl amidated pectin (LMAP) with similar degree of methoxylation have been investigated by oscillatory rheological measurements. The gelling process was examined in a sugar-acid environment matching the conditions in jams and jellies. Factors studied included cooling rate, calcium content and pH. Parameters derived from the rheological measurements comprised the gel point, structuring velocity, initial and critical structuring temperature, average structuring developing rate and loss factor ($\tan\delta_{end}$).

The influence of the cooling rate on the gelling process of LMP was moderate and the influence on the final gel properties was significant, $\tan\delta_{end}$ decreased with increasing cooling rate. The calcium content significantly affected the structuring process of LMAP and the final gel properties. At high calcium content, the gelling process started at a higher temperature but the resulting gels were less strong. The pH had a significant but partly opposite effect on the gelation of LMP as well as LMAP. The differences in gelation behavior between LMP and LMAP can be explained by the lower number of available blocks of free carboxyl groups in LMAP as well as by the formation of additional hydrogen bonds through the amide groups.

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1. Introduction

Pectin is a polysaccharide and extracted from plant cell walls. It is used in the food industry for its gelling, thickening, stabilizing, and emulsifying properties. Variations in pectin structure enable this broad range of commercial applications. Depending on the degree of methoxylated carboxyl groups (degree of methoxylation, DM) pectins are traditionally classified as high-methoxylated pectins (HMP) with DM > 50% and low-methoxylated pectins (LMP) with DM < 50%. Chemical de-esterification of HMP in presence of ammonium ions results in low-methoxylated amidated pectin (LMAP).

For understanding of the gelling process of pectin it is helpful to review the types of junction zones in pectin gels. A limited number of hydrophobic interactions may be formed between the methyl ester groups immediately at the beginning of the cooling process and induce the gelation (Oakenfull & Scott, 1984). These

interactions have a rather low energy, a limited working range of about 2 nm (Walstra, 2002, Chapter 3) and become weaker with decreasing temperature. Upon further cooling, at lower temperatures hydrophilic interactions between undissociated carboxyl groups of the galacturonic acid and/or hydroxyl groups of carboxyl, hydroxyl or amide groups can develop via hydrogen bonds (Oakenfull & Fenwick, 1977; Oakenfull & Scott, 1984). These bonds are also of low energy and with 0.2 nm their working range is even smaller than that of the hydrophobic interactions. That means that the pectin molecules have to come in close contact in order to form a gel network. This can be achieved by a high soluble solid concentration (>50%) since the resulting reduced water activity allows the approach of pectin chains (Evageliou, Richardson, & Morris, 2000; Kastner et al., 2014; Thakur, Singh, Handa, & Rao, 1997). The influence of the hydrogen bonds gains more importance upon temperature reduction and supports inter-chain association during network formation. These two types of junction zone formation occur during gelation in all types of pectin and are typical for a cold-set gelation (Burey, Bhandari, Howes, & Gidley, 2008). However, gelation of LMP is governed by ionic interactions between dissociated carboxyl groups, typically via calcium ion bridges (Thibault &

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Ralet, 2003) in an ionotropic gelation (Burey et al., 2008). Calcium bridges represent a third type of junction zone formation and start to form immediately after gel preparation. They are much stronger than hydrophobic and hydrogen bonds and with about 20 nm their working range is rather long (Walstra, 2002, Chapter 3). Pectin gels with combined or dominating ionic junction zones require less soluble solids than HMP gels based on hydrophobic or hydrogen bonds, and can be formed also in sugar-free systems. The ionotropic gelation can take place in pectin solutions without heating (Ström & Williams, 2003; Vincent & Williams, 2009) and can be performed also as isothermal titration at room temperature (Fang et al., 2008). It seems to be possible, however, that ionic interactions formed at higher temperature tend to be initially not stable but their stability will increase during cooling (Cárdenas, Goycoolea, & Rinaudo, 2008; Garnier, Axelos, & Thibault, 1993). In LMAP, junction zones are additionally stabilized by hydrogen bonds involving the amide group (Alonso-Mougán, Meijide, Jover, Rodrı;guez-Núñez, & Vázquez-Tato, 2002; Black & Smit, 1972; Löfgren, Guillotin, & Hermansson, 2006).

Structure formation and properties of pectin gels strongly depend on intrinsic and extrinsic factors (Endress & Christensen, 2009; Rolin, Chrestensen, Hansen, Staunstrup, & Sørensen, 2009; Yapo & Gnakri, 2015). Intrinsic factors affecting a system are those related to the composition, e.g. type of pectin and concentration, agent used for pH adjustment, presence of divalent cations or co-solutes like sugar. Important extrinsic (technological) factors are e.g. heating and cooling conditions.

The influence of calcium ions depends on the stoichiometric ratio between calcium ions and the dissociated free carboxyl groups. This ratio is calculated as $R = 2[Ca^{2+}]/[COO^-]$ (Axelos & Kolb, 1990; Capel, Nicolai, Durand, Boulenguer, & Langendorff, 2006; Cárdenas et al., 2008; Garnier et al., 1993; Ström et al., 2007). A theoretical saturation threshold of the R exists at which every calcium ion in the gel is bound to two dissociated carboxyl group. This threshold is affected on one hand by the degree of dissociation of the carboxyl groups and, thus, by the pH in the gel system. The pK_a of pectin is about 3.5 (Ralet, Dronnet, Buchholt, & Thibault, 2001), at this pH 50% of the carboxyl groups are dissociated. On the other hand the binding of calcium to pectin chains also depends on the distribution of these groups (block-wise or random). Ionic interactions require a certain number (blocks) of about 6—14 subsequent dissociated carboxyl groups (Liners, Thibault, & Cutsem, 1992; Luzio & Cameron, 2008; Powell, Morris, Gidley, & Rees, 1982) in order to form junction zones named as "egg-boxes". Vincent and Williams (2009) therefore suggested a modified R_{eff}, in which only dissociated carboxyl groups in blocks are considered. The calculation of their exact number requires, however, detailed knowledge of the pectin molecular structure. Single randomly distributed dissociated carboxyl groups may also interact with calcium ions. In case they are oriented to the outside of the egg-boxes, they could form larger dimer aggregates and even an extended network (Braccini & Pérez, 2001; Fraeye et al., 2009, 2010). Moreover, excess calcium ions may be located in the gap between galacturonic acid molecules and interact with other C-atoms than C6 (Siew, Williams, & Young, 2005), they might course a certain electrostatic repulsion. The number of rather unspecific or random calcium crosslinks will increase with increasing calcium ion content.

The degree of methoxylation and the distribution of the carboxyl groups in a more random or more block-wise way can influence the gelling process especially with respect to the ionotropic gelation (Fraeye et al., 2009; Ngouémazong et al., 2012). According to Fraye et al. (2009, 2010) pectin with a dominating block-wise distribution of the dissociated carboxyl groups is able to gel at a lower calcium concentration (R) than pectin with a more

random distribution of free carboxyl groups. At higher calcium content (R > 1) pectin gels were found to become more cross-linked and elastic and a plateau of the storage modulus (G′) was reached only at very high calcium contents with R up to 5.0. Additional crosslinks might result from random interactions of calcium ions with single carboxyl groups as described above. When the calcium content becomes too high, precipitation and/or syneresis can occur and the gel strength may be reduced (Fraeye et al., 2010; Grosso & Rao, 1998). Gels prepared from amidated pectins were found to be less sensitive to syneresis (Thakur et al., 1997; Thibault & Ralet, 2003) than those of non-amidated pectins.

The complex gelling process and gel properties of pectin systems as well as the influence of different intrinsic and extrinsic factors on the gelation have been successfully examined by a variety of methods including a wide range of rheology-based methods. In the majority of studies, however, only one or two intrinsic factors have been varied, like e.g. pectin concentration, co-solutes, ion concentration, type of ions or acid and alkaline media to adjust the pH (Evageliou et al., 2000; Fraeye et al., 2010; Gigli, Garnier, & Piazza, 2009; Guillotin, Van Kampen, Boulenguer, Schols, & Voragen, 2006; Lopes da Silva & Gonçalves, 1994; Löfgren & Hermansson, 2007; Löfgren et al., 2006; Rao & Cooley, 1993; Sousa, Nielsen, Armagan, Larsen, & Sørensen, 2015; Ström et al., 2007; Tsoga, Richardson, & Morris, 2004). As a consequence the results of some of these studies are hardly comparable because of differences in the intrinsic factors of the model system as well as extrinsic factors in the experimental setup, methodology or measuring equipment. In addition, often only one parameter, the classical gel point (GP) defined as crossover of storage modulus G′ and loss modulus G″ at a certain frequency, was reported (Gigli et al., 2009; Holst, Kjøniksen, Bu, Sande, & Nyström, 2006; Iglesias & Lozano, 2004). In recent years, the rheological characterization of a gelling process was significantly improved by using new parameters like the initial structuring temperature (IST), defined as the temperature at which the first derivation of G′ as a function of time (dG′/dt) differed from zero for the first time, and the critical structuring temperature (CST) as the extrapolated temperature of the first strong increase of dG′/dt (Kastner, Einhorn-Stoll, & Senge, 2012a, 2012b; Einhorn-Stoll, Kastner, & Senge, 2012; Einhorn-Stoll, Kastner, Hecht, Zimathies, & Drusch, 2015; Kastner et al., 2014). This method allows the evaluation of the gelling kinetics and the final gel properties and, thus, gives information about systems without clear gel point. These new parameters are now generally accepted and have been used by several other groups (Garrido, Lozano, & Genovese, 2015; Sousa et al., 2015; Wang, Hua, Yang, Kang, & Zhang, 2014).

For these reasons, in the last years the overall aim of our group was the investigation of the pectin gelling process and the final gel properties in a broad study, covering all major factors such as pectin type, content of calcium ions, pH and cooling rate. Several results have already been achieved and published and complementary examinations are the subject of the present study. The kinetics of structure formation of HMP gels, a typical cold-set gelation, as well as the properties of the final gels were investigated before at varying cooling rates and pH (Kastner et al., 2014). A high cooling rate promoted early structure formation and resulted in a less elastic gel compared to a low cooling rate. Varying the pH by differences in the acid concentration showed that an optimum range for the structure formation as well as for the final gel properties at intermediate acid concentration exists. In the present study the influence of the cooling rate on the gelation of LMP will be investigated because it is assumed that here the temperature-independent ionic interactions (ionotropic gelation) will dominate the gelation process and reduce the influence of the cooling rate (cold-set gelation). Moreover, the influence of pH on the