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Citrus Pectin: Structure and Application in Acid Dairy Drinks

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ABSTRACT

Pectin, a plant cell wall polysaccharide, is mainly used in food industries for its gelling and stabilizing properties. In industrial applications, pectin is usually widely extracted from citrus peels, and in some intances, apple pomace is also used. Lime and lemon are the preferred citrus species used in the extraction of pectin, while orange and grapefruit are used less often. In the food industry, pectin is widely employed in the production of jams and jellies, confectionary products and bakery fillings. The fine structure of pectin is affected by many parameters, such as the origin of raw material and extraction conditions. This structural variability impacts greatly on pectin functional properties. The other major use of pectin concerns the stabilization of acidified milk drinks and yogurts. With their refreshing natural taste and high nutritional value, acidified milk drinks enjoy great popularity. A large selection of different sour milk drinks, which vary according to the manufacturing process, ingredients and consistency, is available to meet the needs of every consumer. In all cases, protein flocculation and whey separation occur in the absence of stabilizers in acidified milk drinks. To prevent this behaviour and to stabilize milk drinks, citrus pectin can be added as a protecting colloid. This review presents the structure of citrus pectin and functionality, with a special emphasis on acid dairy drinks.

Keywords: gelation, polysaccharide stabilisation
Abbreviations: **ADD**, acid dairy drink; **ADI**, acceptable daily intake; **AFM**, atomic force microscopy; **Ara**, arabinose; **CCAFC**, codex committee on food additives and contaminants; **CSP**, Ca^{2+} sensitive pectin; **DA**, degree of acetylation; **DAm**, degree of amidation; **DB**, degree of blockiness; **DE**, degree of esterification; **DM**, degree of methoxylation; **EEA**, European economic area; ***endo*-PG**, *endo*-polygalacturonase; **EU**, European Union; **FAO**, food and agriculture organization of the United Nation; **Gal**, galactose; **GalA**, galacturonic acid; **GRAS**, generally recognized as safe; **HG**, homogalacturonan; **HM-pectin**, high methoxyl pectin; **INS**, international numbering system; **LMA**, low methyl-esterified amidated; **LM-pectin**, low methoxyl pectin; **MALDI-TOF MS**, matrix-assisted laser adsorption/ionization time of flight mass spectrometry; **MSNF**, milk solid non-fat; **MT**, metric ton; **NCSP**, non-Ca^{2+} sensitive pectin; **NMR**, nuclear magnetic resonance; **PME**, pectin methyl esterase; **RG-*I***, rhamnogalacturonan *I*; **RG-*II***, rhamnogalacturonan *II*; **Rha**, rhamnose; **USDA**, United States department of agriculture; **WHO**, world health organization; **XGA**, xylogalacturonan; **Xyl**, xylose

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Invited Review

INTRODUCTION

Pectin is a natural constituent of all terrestrial plants. It is one of the major plant cell wall components and is probably the most complex macromolecule in nature (Ridley *et al*. 2001; Coenen 2007). Pectin is a heterogeneous complex polysaccharide. Its composition and fine structure vary depending on the plant source and the extraction conditions applied. The fine structure of pectin deeply affects its functionality and applicability. Although most plant tissues contain pectin, only a few plant sources are currently used for the commercial pectin extraction. Commercial pectin is produced almost exclusively from citrus peel or apple pomace (Rolin 2002). The major constituent of pectin consists of linear sequences of (1→4)-linked α–D–galactopyranosyluronic acid (GalA) with some of the carboxyl groups esterified with methanol (Voragen *et al.* 1995). The proportion of GalA units that is methyl–esterified is one of the key parameters that determines the functional properties of pectin (Rolin *et al.* 1998; Ralet *et al*. 2001). Generally, commercial pectin preparations are divided into low-methoxyl (LM) (degree of methylation (DM) < 50%) and high-methoxyl (HM) (DM > 50%) pectin. The major outlet for pectin is through food applications, i.e., as a thickener and gelling agent in jams and jellies, bakery fillings, glazing, fruit and milk beverages (Voragen *et al*. 1995; May 2000; Rolin 2002). Recently, pectin utilization for healthcare applications – as drug delivery, gene repair and tissue repair – has offered food ingredient manufacturers as much as $ 890 million in sales to the healthcare industry (Kalomara information 2007). This review presents the structure of citrus pectin and functionality, with a special emphasis on acid dairy drinks.

CITRUS AND CITRUS PROCESSING

Citrus is the largest fruit crop worldwide. World citrus production in selected major producing countries in 2005/06 is estimated at 72.8 million MT (USDA Foreign Agriculture Service). Citrus is grown in two belts on both sides of the equator about 20 to 40 degrees of latitude. All citrus is thought to originate from the Himalayan region of south western China and northern India. Columbus brought citrus seeds to the western hemisphere in 1493 and planted them first on the island of Hispaniola, now called Haiti (FAO 2003). Citrus became commercialized in the Americas in the late 1800s. In the early to mid 1900s, the principal producing states were Florida, Texas and California, USA (Bates *et al.* 2001; FAO 2003). Following the devastating freeze in Florida in 1962; a group of Florida businessmen began to establish citrus groves, and later citrus industry, around São Paulo, Brazil (FAO 2003). In 2005/06, citrus production in Brazil exceeded 18 million tons followed by China and USA with around 15 million tons and 10 million tons, respectively (USDA Foreign Agriculture Service). In this regard around 14 million tons and 7 million tons of Brazil and USA production, respectively, went under processing last year. Presently, more than 55% of citrus production in developed countries and 22% of production in developing countries go under processing each year. About 75% of the processing belongs to citrus juice industry, 13% citrus canning industry and 7% as dried products (Bates *et al*. 2001; FAO 2003; Boriss 2006).

There is a great variety of citrus species and citrus fruits can be roughly classified as follows:

Orange-fruit types
- sweet orange
- bitter orange
- mandarin

Yellow-fruit types
- lemon
- lime, limetta
- grapefruit

Orange is the most important citrus product in the citrus industry, followed by grapefruits, lemons and mandarins (Schottler *et al*. 2002).

ECONOMICALLY FEASIBLE BY-PRODUCTS FROM CITRUS

Worldwide, industrial citrus waste is estimated at more than 15×10^6 tons per year, as the amount of residue obtained from the fruits accounts for 50% of the original whole fruit mass (Marin *et al.* 2007). By-products (sometimes called specialty products) are those saleable products made from citrus fruits besides juice. One of the opportunities of starting a new citrus processing operation is the unique opportunity to tailor the citrus processing plant's production of specialty products to the customer's needs. This needs to be done early in the design phase so that plants can be specially designed for the production of multiple products. Over 400 specialty-products can be made from citrus, in addition to juice. Many of these products are only research realities that lack either the backing or timing to be made profitable. It is vital to make plans on how to economically dispose of the peel and other solid wastes from operations before engaging in a fruit juice operation. There are presently 6 to 12 products that have established markets (Bates *et al.* 2001):

- Pectin
- Pectin pomace and dietary fibre
- Dried citrus peel
- Pulp wash
- Juice sacks and whole juice vesicles
- Beverage base and clouding agents
- Healthful, nutraceutical citrus beverages
- Fractionated citrus oils and D-limonene
- Citrus molasses and beverage alcohol base
- Flavonoids and limonin.

PECTIN STRUCTURE

Historical outline

The scientist Vauquelin discovered the existence of pectin in fruit juices some 200 years ago (Vauquelin 1790). At that time, however, it was not yet called by that name. The name pectin was first used in 1825, when Braconnot continued the research started by Vauquelin. He referred to the gel-forming substance as "pectic acid", from the Greek word *pektikos*, which means congealed, or solidified. Smolenski, in 1923, was the first to describe pectin as a polymer of GalA. In 1930, Meyer and Mark discovered the chain formation of the pectin molecule and Schneider and Bock, in 1937, established the first formula of the pectin molecule (as cited by Kertesz, 1951). The work of Kertesz (1951) defined pectin as a hetero-polysaccharide containing mainly GalA residues that are partly methyl-esterified. A certain amount of neutral sugars were detected, but the manner in which they were integrated in the pectin molecule was not determined. The work of De Vries and co-workers (De Vries *et al.* 1981, 1982) was instrumental in showing the existence of the two main pectic regions: "smooth" homogalacturonic regions and "hairy" rhamnogalacturonic regions encompassing neutral sugars side chains. From then, several other pectic domains have been described.

By 1965, pectin had been established in the industry essentially as a gelling agent in jams or jellies, though thereafter other application areas were identified. Presently, pectin is used in food systems as a fat substitute, dietary fibre and as stabilizer in acidified milk systems (Ralet *et al*. 2002).

Chemical structure of pectic domains

The term "pectin" is somewhat misleading since it implies the existence of a single well-defined macromolecule (Willats *et al*. 2006). In fact, pectin is currently strongly believed to contain different structural elements, the amount and fine structure of each varies widely with respect to plant origin (Schols and Voragen 1996), between different cell types, at different stages of cellular development, and even within the thickness of a given wall (Jauneau *et al*. 1998; Willats *et al*. 2001; Scheller *et al.* 2007).

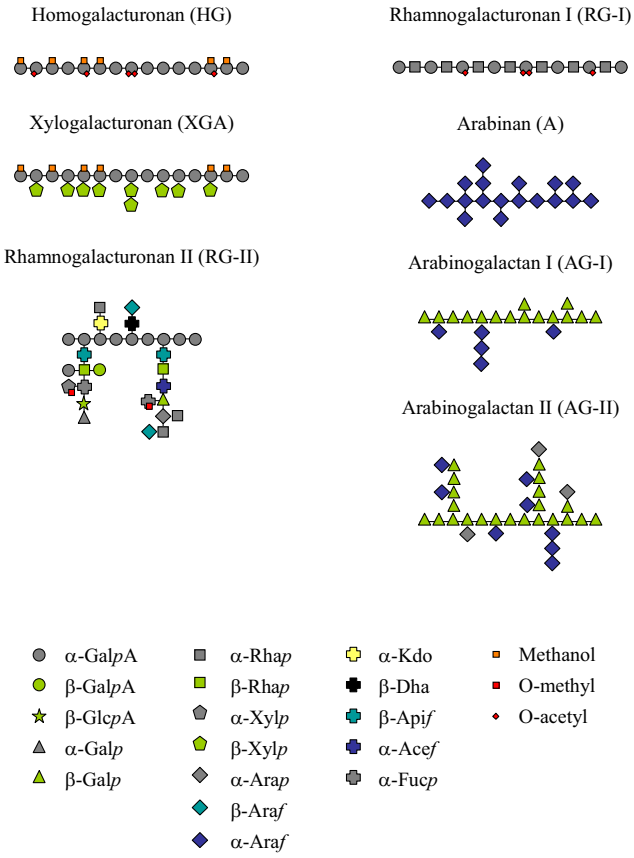

Fig. 1 Schematic representation of the structural domains of pectin. Structures are drawn with the reducing *terminus* to the right. Homogalacturonan and homogalacturonan-derived domains are shown in the first column; rhamnogalacturonan I and attached neutral sugars side-chains are shown in the second column. The symbols of the building units are shown in the accompanying legend and the predominant linkages are explained in the text. Adapted from Vincken *et al.* (2003).

The most commonly found structural elements are: (i) homogalacturonan, (ii) xylogalacturonan, (iii) rhamnogalacturonan I backbone, (iv) rhamnogalacturonan II, (v) arabinan, (vi) arabinogalactan I, and (vii) arabinogalactan II (Schols and Voragen 2002; Coenen 2007) (**Fig. 1**).

Homogalacturonan

Homogalacturonan (HG) is the simplest and most abundant pectic structural domain. It consists of a linear backbone of (1→4)-linked α-D-GalA residues (Ridley *et al*. 2001). The possible presence of single rhamnose (Rha) residues within HG regions was convincingly argued against by Zhan *et al*. (1998). The minimum estimated length of this domain is ~100 GalA residues (Thibault *et al*. 1993). For citrus, chemical as well as enzymatic approaches have led to the isolation of HG domains of narrow molar mass distribution with a degree of polymerization of 100-120 GalA residues (Thibault *et al.* 1993; Hellìn *et al*. 2005; Yapo *et al.* 2007). GalA residues are commonly partly methyl-esterified at C-6 (Voragen *et al.* 1995) and, in some plant species, partly acetyl-esterified at *O*-2 or *O*-3 (Ralet *et al.* 2005, 2008a). Both the degree of methyl-esterification (DM) (i.e. the number of methyl-esterified GalA residues for 100 total GalA residues) and degree of acetylation (DA) (i.e. the number of acetyl-esterified GalA residues for 100 total GalA residues) have a profound impact on functional properties. Lime pectin is particularly rich in HG, which accounts for ~80-90% of raw pectin mass (Ralet and Thibault 1994; Hellín *et al*. 2005; Yapo *et al*. 2007). Native citrus pectin, and its constitutive HG domains, is highly methyl-esterified and lowly acetyl-esterified (Ralet and Thibault 1994; Ros *et al.* 1996, 1998).

Xylogalacturonan

Xylogalacturonan (XGA) consists of a HG backbone with mostly single-unit substituents of β-D-xylopyranose (Xyl) linked at *O*-3 of GalA residues. In lemon albedo pectin, the presence of very limited amounts of XGA has been reported (Ros *et al.* 1998).

Rhamnogalacturonan I backbone and neutral sugars side-chains

The rhamnogalacturonan I (RG-I) backbone consists of [→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→] repeats (McNeill *et al*. 1980, 1984; Albersheim *et al*. 1996). RG-I domains isolated from several plant species, including citrus, were shown to be highly acetylated at *O*-2 and/or *O*-3 of GalA units (Komavilas and Mort 1989; Ralet and Thibault 1994; Schols and Voragen 1994; Ros *et al.* 1996; Ralet *et al.* 2005). So far, no evidence has been published that GalA units in RG-I domains are methyl-esterified. The Rha residues of RG-I backbone are substituted, mainly at *O*-4, with α-L-arabinofuranose (Ara)- and β-D-galactopyranose (Gal)-containing side-chains (Voragen *et al.* 1995). Lemon pectins were shown to contain arabinan structures with a central core of (1→5)-linked Ara*f* residues carrying essentially single Ara*f* substituents at C-3 (Ralet and Thibault 1994; Ros *et al.* 1996). Both types of galactans: (1→4)-linked type I (arabino)-galactans and (1→3),(1→6)-linked type II arabinogalactans were also detected (Ralet and Thibault 1994; Ros *et al.* 1996) as well as single D-Gal*p*-(1→4) substitutions (Ros *et al.* 1996, 1998). Rhamnogalacturonans I was recently recovered from citrus peels pectins after extensive degradation by homogalacturonan-degrading enzymes (Yapo *et al.* 2007). Further use of side-chains-degrading enzymes allowed the recovery of a high molar mass RG-I backbone (Yapo *et al.* 2007).

Rhamnogalacturonan II

Rhamnogalacturonan II (RG-II) is a highly conserved structure in the plant kingdom. It is a low molar mass (5-10 kDa) highly complex macromolecule with a short HG-like backbone substituted by four different side-chains encompassing several unusual sugar residues (Schols and Voragen 2002). This structural element, although present in very limited amounts, plays a key role as it is strongly believed to be involved in the cross-linking of two pectin molecules within the cell wall through a borate di-ester (Ishii *et al.* 1999; Ishii and Matsunaga 2001). RG-II was recently isolated from citrus peels pectin after extensive degradation by homogalacturonan-degrading enzymes (Yapo *et al.* 2007).

Pectin models

Although the structure of the different pectic domains is now quite well known, the way they are connected one with another to form a macromolecular structure is still a matter of debate. Two pectic models are considered nowadays: the "smooth and hairy regions" model (Schols and Voragen 1996) (**Fig. 2**) and the "RG-I backbone" model (Vincken *et al*. 2003) (**Fig. 3**). In the first one, "smooth regions" (HG) alternate with "hairy regions" (XGA and RG-I encompassing neutral sugars side chains) to form the pectin molecule backbone, RG-II being an integral part of some HG domains. In the second one, HG, XGA, arabinan and galactan would occur as side-chains of RG-I backbone, forming a "*molecular brush*" (Vincken *et al*. 2003), RG-II being again an integral part of some HG domains.

Macromolecular features

The macromolecular characteristics of pectin mainly include its molar mass and conformation. These are determinants of pectin industrial applications as the strength of pectin gels is positively correlated with apparent molar mass

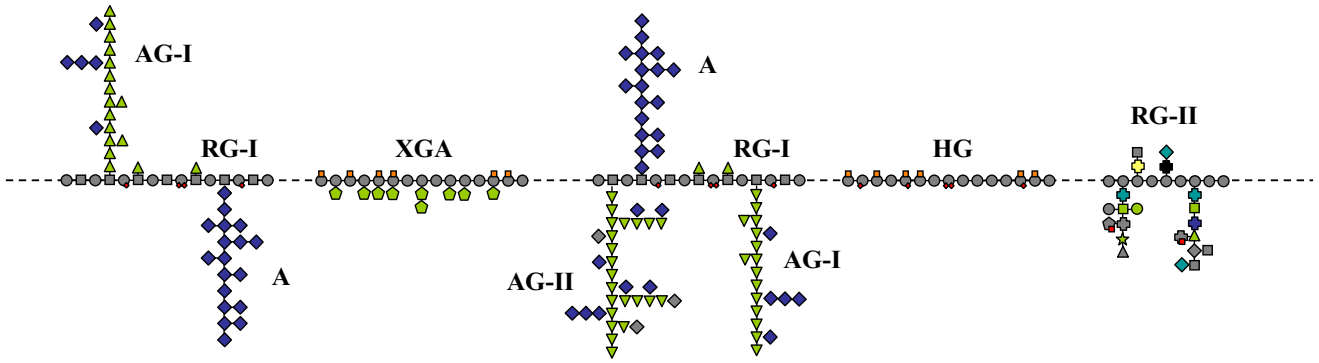

Fig. 2 Schematic representation of the smooth and hairy model of pectin. In this "smooth and hairy regions" model (Schols and Voragen 1996) the pectic backbone consists of alternating homogalacturonan (HG) (smooth regions), xylogalacturonan and rhamnogalacturonan-I (RG-I). Rhamnogalacturonan-II (RG-II) is considered an integral part of HG domains. Arabinan (A) and arabinogalactan I and II (AG-I, AG-II) make up the "hairy" part of the macromolecule.

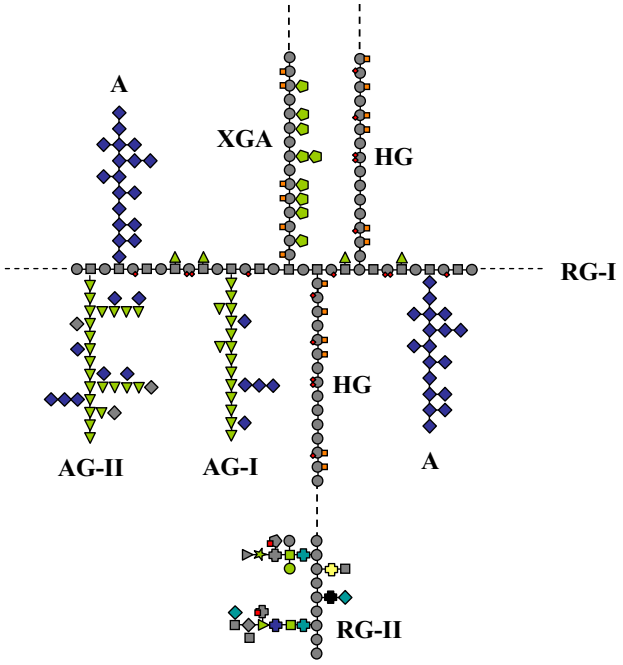

Fig. 3 Schematic representation of the RG-backbone model. In this "RG-I backbone" model (Vincken *et al.* 2003), the RG-I backbone is decorated, not only with arabinan and arabinogalactan I and II side chains, but also with homogalacturonan (HG) and xylogalacturonan (XGA) domains. Rhamnogalacturonan-II (RG-II) is considered an integral part of HG domains.

(Rolin 2002). Pectin molar mass vary with plant source, raw material stage of ripening and extraction conditions. It should be emphasized that a large number of factors, such as charge density, neutral sugars content and solvent quality can affect pectin aggregation and molecular state in solution. It was also shown that pectin molecules of similar molar masses may exhibit different hydrodynamic properties due to differences in DM, branching and neutral sugars content which make their solution behaviour even more complex (Rolin *et al.* 1998). Molar mass determination is still a challenge due to problems of heterogeneity and aggregation in addition to the usual broad molar mass distribution. Developments in high-performance size-exclusion chromatography coupled with laser light scattering and/or viscometric detection, led to an improvement in pectin characterization, although aggregation and poly-dispersity can disturb light-scattering data (Ralet *et al.* 2002).

Pectin conformation is a matter of complexity. As the individual sugar rings are essentially rigid, the overall conformation of the chain is primarily determined by the bridge angle and the relative orientations of the component sugars as defined by the rotational angles. The linkages between monomers in HG are axial-axial, giving the polymer an intrinsic stiffness due to the severe conformational constraints imposed by this type of linkage (Burton and Brant 1983). A rather extended conformation with a persistence length of 4.5 to 13 nm was determined on isolated HG domains or HG-rich citrus pectin (Cros *et al.* 1996; Morris *et al.* 2008; Ralet *et al.* 2008b). Some contradictory conclusions have been reported concerning the influence of DM on the conformation of the pectin macromolecules. Molecular modelling, nuclear magnetic resonance and small angle neutron scattering have shown that methoxyl groups have no significant influence on the flexibility of the linkages between GalA residues (Cros *et al.* 1992, 1996). However, other studies have shown a general decrease in the hydrodynamic volume of the pectin molecules and an increase in chain stiffness with decreasing DM, with both steric and electrostatic interactions playing an important role in conformational changes (Morris *et al.* 2000). Using another experimental approach combining intrinsic viscosity, sedimentation coefficient and weight-average molar mass determinations, the same authors recently concluded that all citrus pectin molecules tested were of similar conformation, whatever their DM (Morris *et al.* 2008). Isolated RG-I domains or RG-I-rich pectin appear very flexible (Axelos and Thibault 1991; Hourdet and Muller 1991; Ralet *et al.* 2008b). A complex set of different factors may influence the conformation of the pectin molecules and may partly explain some of the discrepancies found in literature about this subject.

INDUSTRIAL PECTIN PRODUCTION

The citrus industry produces three intermediate products (percentage values in relation to the raw product mass):

Juice/pulp	45-55%
Peel	45-55%
Essential oil	0.2-0.5% (Schottler *et al.* 2002)

Apple pomace, that contains 15-20% pectin, and citrus peel, which contain 30-35% pectin, are currently the only raw materials of importance to the manufacture of pectin. Historically, the major part of the pectin industry developed from utilization of apples (Rolin 2002; Mesbahi *et al.* 2005), but today, dried citrus peel is the largest source. Among the citrus, lime and lemon are preferred and orange and grapefruit are less often used. Indeed, lime and lemon yield pectin of higher specific viscosity and lower Ca^{2+} sensitivity than orange and grapefruit (Rolin 2002).

Pectin has been manufactured from citrus peel for more than 50 years. Pectin manufacture involves leaching, acid extraction, precipitation, purification and standardization (**Fig. 4**).

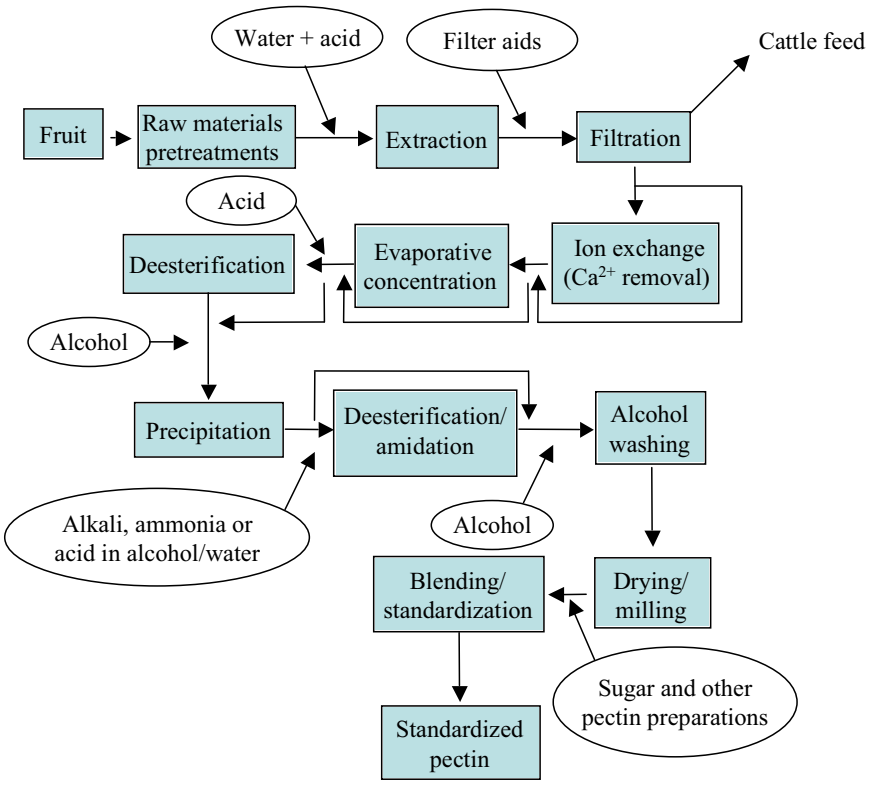

Fig. 4 Industrial pectin production.

Process

Pre-treatment and extraction

Most pectin extraction in industry is done in Europe using lime peels imported from Mexico and South America. Before industrial extraction, the raw materials are generally subjected to a pre-treatment (blanching, washing, drying) to inactivate enzymes that otherwise would rapidly degrade the pectin molecules and to increase the product stability during transportation. Washing in water, in order to leach out sugars, is necessary prior to drying so as to minimize caramelisation. Washing may even be preferred when the raw material can be used without drying, because the leachable material has to be separated from the pectin at a later stage to reach a pure product. Citrus peel is extensively leached with water and this leach water has the potential for large pollution problems.

Extraction of pectins from raw materials is usually performed by acid treatment (pH 1 to 3) at high temperature (50-90°C), with nitric acid for 3-12 hours (Rolin 2002). The extraction conditions (pH, temperature, time) must be optimized to provide good yields of pectin that also has the desired DM (typically 55-80). Acid extraction has at least two pitfalls: (i) it may degrade pectin structure, and (ii) it does not meet environmental safety standards. Acid-extracted pectins often contain a mixture of both Ca^{2+}-sensitive (CSP) and non-Ca^{2+}-sensitive (NCSP) molecules. Separation, from extracted juice, of these two types of pectin by selective precipitation has been described by Glahn (1995a, 1995b). Selective precipitation is achieved by a solution of water and alcohol that contains a dissolved polyvalent cation such as Ca^{2+} (usually nitrate salt), which causes the CSP to gel. It is also possible to separate CSP and NCSP directly *in-situ* by fractional extraction at differential pH. At pH around 3, NCSP is separated and at pH 2 (typical of normal pH for pectin extraction) extracted pectin turns out to be CSP.

Extraction technology is being continually studied and extraction processes using steam injection under pressure, microwave heating, enzymatic and microbial tools have been proposed (Fishman *et al.* 2000, 2003, 2006; Panouillé *et al.* 2006). It has been shown that flash extracted pectin from orange albedo by microwave heating under pressure exhibited increased molar mass, size and intrinsic viscosity compared to pectin extracted by conventional heating techniques (Fishman *et al.* 2000). Moreover, gel forming properties of orange and lime pectin prepared by rapid microwave heating were better than those of commercial citrus pectin (Fishman *et al*. 2003). The USA is one of the largest producers of oranges in the world and 90% of this huge production is used to manufacture juice. Adapted extraction processes could result in the growth of pectin extraction industry in the USA using source-orange-peels that now go to waste (Walker 2003).

An enzyme-hydrolytic technology would be environmentally safer and potentially more effective in terms of pectin yields (Panouillé *et al.* 2006). Analysis of the scientific and patent literature shows that a number of research centers have been conducting studies to develop a biotechnological method for pectin extraction, but these studies are of exploratory nature only and their results are still far from industrial application (Ptichkina *et al*. 2008).

Filtration and purification

The pectin raw extract is separated from the plant residue by filtration and/or centrifugation processes. The solid: liquid ratio has to be well defined for an efficient liquid/solid separation. Ratios of 1:17 for apple and 1:35 for citrus are often used. Efficient filtration requires reasonably low viscosity, but the more water added to the process, the more energy is needed to remove it. Currently, rotary drum vacuum filtration is commonly used in the industry. Insoluble filter aids, such as wood cellulose and perlite, may be used to facilitate this process. Extracts are rapidly brought to pH 3-4, whereupon the temperature is lowered to avoid pectin demethylation and depolymerisation. Weak bases, like sodium carbonate or ammonia are used to minimize β-elimination reactions. The clarified extract may be passed through a column with cation-exchange resin to remove Ca^{2+} ions and generate the sodium form of pectin, which is better adapted for food application. Next, the solution is concentrated to 3-4% dry material by evaporation or membrane filtration. Pectin is then precipitated by pouring the extract into an appropriate alcohol (usually iso-propanol). The precipitate obtained is pressed, washed in a fresh bath of alcohol, pressed and finally dried and milled to a desired particle size. To recover HM-pectin, an alternative to alco-

hol precipitation is precipitation by adding appropriate metal salts to the extract. Pectin forms insoluble salts with, for example, Cu^{2+} and Al^{3+}. Removal of metal ions from the precipitated pectin is done by washing in acidified aqueous alcohol. Pectin suspended in alcohol is in a very suitable form for further modification. The LM-pectins are generally obtained by controlled acid de-esterification or by alkali de-esterification. The temperature should not exceed 50°C to avoid depolymerisation reactions. This treatment can yield HM-pectin with DM values in the range of 55-75% or LM-pectin in the DM range of 20-45%. Ammonia may convert methyl-esterified carboxylate groups of pectin to primary amides. This is done industrially by suspending precipitated pectin in a mixture of alcohol and water with dissolved ammonia. By choosing proper conditions with respect to ammonia concentration, pectin with various proportions of amidated, methyl-esterified and free carboxylate groups can be produced. Regulation requires that the degree of amidation (DAm) does not exceed 25% (Voragen *et al*. 1995; Rolin *et al*. 1998; May 2000).

Standardization

Standardization is a current industrial practice to produce pectin samples with consistent properties. Diversity of natural raw material, climatic conditions to which the plants have been exposed, ripeness and peel pre-treatment processes may lead to large differences in pectin properties and functionalities. HM-pectin is therefore diluted and standardized with sucrose, dextrose, glucose or lactose to a given gelling power defined as degree sag. The standard of 150° sag means that 1 g of pectin is able to gel 150 g of sucrose under defined conditions of pH and temperature. Due to their large application range, LM-pectins are not necessarily standardized. For pharmaceutical purposes, pectin without admixed sugar is also available from the major manufacturers (Voragen *et al*. 1995; Rolin *et al*. 1998; May 2000). So, when using pectin, it is obviously important to choose a type, which has been standardized in a way that reasonably corresponds to the intended use.

World market and regulations

Pectin extraction is an intensive energy utilizing process that requires sophisticated operations and control. Tropical developing countries may have a locally owned pectin manufacturing operation, but it is typically hard pressed to compete with imported pectin unless the native operation is given governmental protection. Typically, pectin operations are co-located with large-scale juice operations that run at least 300,000 MT of fruit per year (Bates *et al.* 2001).

The pectin industry is dominated by large multinational firms, which have undergone major changes in the past five years. The world leaders include CP Kelco (owned by I.M. Huber Corporation since 2005), Danisco sugar (Denmark), Cargill (acquired Citrico and Degussa Food Ingredients) and Herbstreith and Fox (Germany) (USDA Foreign Agricultural Service 2007). Approximately 35000 tons of pectin are produced and used each year in the world (Daniells 2007; USDA Foreign Agricultural Service 2007;). CP Kelco has around 40% of the market (35-40% of the world market and 35-45% of European economic area (EEA) market) followed by Danisco (20-30% of the world market and 15-25% of EEA market), Degussa Food Ingredients (10-20% of the world market and 15-25% of the EEA market), Herbsteith and Fox (5-15% of the world market and 10-20 of EEA market), Cargill and Obipektin (10% of the world and EEA market, separately). The remaining 5% is held by small processors (USDA Foreign Agricultural Service 2007).

Pectin is an essential additive in many food applications, such as beverages, protein drinks, yogurts, jams, jellies and desserts. In addition to these general applications, pectin may also hold applications as a prebiotic, potential source of soluble fiber and fat replacer in functional food and nutraceutical applications. Recent *in vitro* tests have shown that pectin acts as a prebiotic, by preventing pathogens from binding to the intestine and increasing the growth of probiotic bacteria in the large intestine (Iisakka 2003). In regard to fiber additives, Functional Food and Nutraceuticals estimates that besides oat bran, psyllium and soya fiber, pectin will fare the best in nutraceutical application based on a combination of cost, quality, performance and versatility advantages. In addition, a physically modified version of pectin is in the market as a fat replacer. Pectin also holds medical applications for colon specific drug delivery, which may face a potential $1 billion market in the medical field (Kalorama Information 2007). The market of pectin has been estimated to be growing at a rate of 3-5% annually since 2001. The average price of HM and LM-pectins are approximately $16000 per ton since 2006 (USDA Foreign Agricultural Service 2007).

Although the FAO/WHO joint Expert Committee on Food Additives and the EU, have not established a numerical acceptable daily intake (ADI) and consider pectin as safe, some national regulations may limit the amount of pectin added in some applications. In the US, pectin is GRAS-Generally Recognized as Safe. In most food it can be used according to good manufacturing practice in the levels needed for its application *quantum satis*. In the International Numbering System (INS), that provides an agreed International numerical system for identifying food additives, created by the Codex Committee on Food Additives and Contaminants (CCFAC), pectin has the number 440. In Europe, it is differentiated into E440(i) for non-amidated pectin and E440(ii) for amidated one (USDA Foreign Agricultural Service 2007).

PECTIN GELLING PROPERTIES AND APPLICATIONS

In many food products, gelation of polysaccharides is critical to the formation of structures with the desired texture. On a molecular level, an aqueous gel consists of three elements:

- Junction zones where polymer molecules are joined together;
- Inter-junction segments of polymers that are relatively mobile;
- Water entrapped in the polymer network.

The properties of pectin gels are strongly influenced by several factors. Extrinsic factors, such as pH, ionic strength, co-solute concentrations and temperature, influence the strength, texture and general viscoelastic properties of pectin gels (Rao and Lopes da Silva 2006). Intrinsic variables of the pectin macromolecules, including the molar mass, sequence of sugars along the chain, DM and distribution of the methyl groups along the chain, greatly impact on gelling properties of this polymer. Among them, DM plays the most critical role (Rolin *et al.* 1998; Cardoso *et al.* 2003).

Pectin with a high degree of polymerization is more viscous in solution than otherwise comparable pectin of lower degree of polymerization. The dependence of gel strength on the molar mass is more pronounced with breaking strength method than with non-destructive test methods. It seems highly plausible that non-homogalacturonan portion kinks the molecules and prevents aligning as well as the formation of a precipitate (Rolin *et al*. 1998).

Native citrus pectin is highly methylated (HM-pectin). LM pectin is generally obtained by controlled acid or alkali de-esterification but other means, namely enzymes and ammonia, can be used. Treatment of pectin with acid, alkali or microbial (*Aspergillus niger*, *Aspergillus japonicus*, *Aspergillus foetidus*) pectin methyl esterase (PME) leads to pectin with a random distribution of free carboxyl group, whereas the action of alkaline PMEs from higher plants (tomato, orange, alfalfa, apple) and from fungi (*Trichoderma reesei*) results in a blockwise arrangement of carboxyl groups on the pectin molecule (Kohn *et al.* 1983; Thibault and Rinaudo 1985; Denes *et al.* 2000). The distribution pattern of free and esterified carboxyl groups has a profound

effect on gelling properties (Ralet *et al.* 2001). A random distribution of methyl esters gives a low Ca^{2+} sensivity, while a blockwise distribution generates a local charge concentration, which may hold Ca^{2+} ions in place in gel structure. CSP can gel in the presence of Ca^{2+} ions without sugars and are therefore useful for low-fat or sugarless, acidic food stuff formulations (Joye and Luzio 2000). Several methods, such as conductimetry and NMR, allow the determination, to a certain extent, of the distribution of methyl-esters along HG domains (Kohn *et al.* 1983; Thibault and Rinaudo 1985; Grasladen *et al.* 1988). More recently, an enzymatic method was developed (Daas *et al*. 1998, 1999, 2000; Limberg *et al.* 2000a, 2000b; Daas *et al*. 2001a, 2001b). Pure pectolytic enzymes were used and the generated oligosaccharides were identified and quantified by a combination of mass spectrometry and high-performance anion-exchange chromatography at pH 5. From their work, Daas and co-workers introduced the concept of "degree of blockiness" (DB) in pectin. The DB increases when the GalA residues are distributed in a more blockwise way over the pectin molecule.

The deesterification method using ammonia produces a different type of LM pectin in which some carboxylic group have been amidated. Amidated pectin has been claimed to have a blockwise distribution pattern of the amide groups and a random distribution of the free carboxyl groups (Racapé *et al*. 1989). For amidated pectin, it is possible to identify the distribution of methyl esters and amide groups by using off-line coupled high-performance anion-exchange chromatography at pH 5 and mass spectrometry (Guillotin *et al*. 2006).

HM-pectin

Jam manufacture is the main user of industrially extracted pectin, utilizing the ability of HM pectin to form a gel with sugar and acid – the so-called low water-activity gels or sugar-acid-pectin gels. Such a gel is considered a three-dimensional network of pectin molecules in which the solvent (water) encompassing the co-solutes (sugar 55-75% w/w, and acid pH 2.5-3.5) is immobilized, resulting in a system resisting deformation and showing a stress/strain relationship for small deformations. The high sugar concentration creates conditions of low water activity, which in turn promote chain-chain rather than chain-solvent interactions; whereas the acid lessens the negative charges on the carboxyl groups, thus diminishing electrostatic chain repulsion. The fact that sucrose can be replaced by other polyols, that less acid is necessary for pectin with higher DM and that a completely methoxylated pectin will gel without any acid, are considered as proof of these functions (Voragen *et al.* 1995). Junction zones formation is made possible through the smooth regions of pectin. To avoid turbidity, syneresis and precipitation, there must be junction-zone – terminating structural elements present in the chain, and "hairy regions" are thought to play such a role (Ralet *et al.* 2002). Gels formed under these conditions are stabilized by aggregated helices supported by hydrogen bonds and grouping of methyl-ester groups through hydrophobic interactions within a cage of water molecules. Recently, global structures of HM pectin in gels were visualized by atomic force microscopy (AFM) (Fishman *et al*. 2004, 2007). Rods, segmented rods, rings, branched molecules, and dense circular areas of pectin were visible.

Many factors influence the conditions of gel formation and strength, among them DM is a key factor. DM controls the rate of gelation and the gelling temperature. In general, increasing the DM leads to a faster gelation and higher gelling temperature. Moreover, at constant pH and co-solute concentrations, the final gel strength increases with increasing DM. As the DM of HM-pectin decreases, a lower pH is required for gelation, although the apparent pk_a increases due the decrease of DM (Rao and Lopes da Silva 2006). DM correlates with the gel setting rate and gel texture under otherwise similar conditions, which means that very highly esterified pectin gels quicker at higher temperature than HM pectin of lower DM. Very highly methylated pectin will also form a more elastic and brittle gel texture compared to pectin of lower DM (Herbstreith and Fox KG Corporate Group 2003).

HM-pectin is also used in the confectionary industry for making fruit jellies and jelly centers, in fruit juices and fruit drink concentrates as a stabilizer and/or to provide mouth-feel. It is also used in fermented and directly acidified dairy drinks (Ralet *et al.* 2002).

LM-pectin

Ionic-mediated gelation through divalent cations, of which the most relevant is Ca^{2+}, is the classical mechanism of LM-pectin gelation. The mechanism of Ca^{2+} binding to the ionized carboxyl groups on the pectin chains is similar to the egg-box proposed for alginate. The Ca^{2+} ions occupy the electronegative cavities in a two-fold buckled ribbon structure of the GalA residues (Rao and Lopes da Silva 2006).

Because of the electrostatic nature of the bonds, LM-pectin is very sensitive to intrinsic parameters that can modify the environment of the carboxyl groups, such as the nature, distribution and amounts of substituents along the galacturonic backbone. Thus, the gel forming ability increases with decreasing DM. Furthermore, LM-pectin with a blockwise distribution of free carboxyl groups are very sensitive to low Ca^{2+} levels (Kohn *et al.* 1983; Thibault and Rinaudo 1985; Ralet *et al.* 2001). For LM blockwise pectin at low level of Ca^{2+}, the gel structure will be less elastic, rather pasty with lower breaking strength compared to a gel prepared with an optimum level of Ca^{2+} (Herbstreith and Fox KG Corporate Group 2003; Dixon 2008). An overdose of Ca^{2+} will lead to pectin precipitation, also called pre-gelling. This is reversible only to a limited extent, even when the gel is once more heated above its setting temperature and cooled down without destruction (Herbstreith and Fox KG Corporate Group 2003). The traditional application of LM-pectin is in jams with soluble solids below 55% (low-calorie jams, jelly preserves and conserves). This is the limit for HM-pectin. The heat reversibility of LM-pectin gels may be utilized in bakery jams and jellies for glazing purpose. LM-pectin also finds application in the production of fruit preparations for yogurt and fruit/milk desserts (Ralet *et al*. 2002).

Amidated LM-pectin can gel under the same conditions as LM-pectin. It has a lower gelling temperature than non-amidated pectin of the same DE. Amidated LM-pectin accounts for the major part of today's use of LM-pectin. Compared to non-amidated LM-pectin, the gels are less prone to syneresis and their texture is easier to control due to functional saturation with Ca^{2+} ions in most applications (Rolin 2002). Amidated pectin needs less Ca^{2+} to gel and is less prone to precipitation at high Ca^{2+} levels, suggesting that its gelation cannot be fully explained by the "egg-box model". Indeed, blocks of amide groups along the chain promote association through hydrogen bonding (Racapé *et al*. 1989; Alonso-Mougan *et al.* 2002; Rolin 2002).

System parameters

At a pH of roughly 3.6 (depending on pectin type), around half of the carboxyl groups of pectin are dissociated. Repulsion between the charged groups contributes to solubility and decreases the tendency for gelation. At low pH, fewer charges are present and solubility declines, whereas at a high pH, more charges are present and solubility increases. If Ca^{2+} salt is added, Ca^{2+} ions may form bridges between molecules (LM-pectin mechanism) and this increases the viscosity or causes gelation. Protons compete with Ca^{2+} especially at low pH. In the absence of Ca^{2+}, the viscosity is inversely related to pH. That is why pectin of very low DM can gel at pH values below 2.5 (Löfgren *et al.* 2005).

In the presence of monovalent salts, such as NaCl, less Ca^{2+} is needed for pectin to pass sol-gel transition points.

More Ca^{2+} ions are indeed able to establish junction zones, while the charge of the polymer is increasingly screened by monovalent ions. The combined effect of pH and sugars promotes gelation at lower Ca^{2+} levels. pH and co-solute effects on chain-solvent interactions counterbalance the decrease in the number of carboxyl groups available for Ca^{2+} binding and chain-chain interactions are promoted (Voragen *et al.* 1995).

In the pH range where gelation of HM-pectin usually occurs (~2.0-3.7), gel strength, setting temperature and setting rate increase with the reduction in pH, when other conditions, such as total solids content and ionic strength, remain unaltered. This is due to the enhancement of macromolecular interaction resulting from the reduction of the pectin charges. Generally, the upper limit of pH for HM-pectin gelation is raised if, either the DM, total solid concentration or pectin concentration, increases. pH is a factor that is not so critical in the development of LM-pectin gels (2.0-6.0). However, it has a significant role in the final properties of the gels. A decrease in pH leads to different kinetics of gel formation and to a decrease in shear modulus, due to the decrease in the number of ionized carboxyl groups necessary for ionic complexation and gel formation. At a low pH, more Ca^{2+} is needed to induce gelation than at a neutral pH. Compared to a salt-free solution, less Ca^{2+} is necessary to form gels when the ionic strength increases. However, gels in salt-free solution are formed more rapidly, but the final modulus is lower (Rao and Lopes da Silva 2006). When pH is below 3.5, there is the predominance of non-dissociated acid groups, which leads to more hydrogen binding in the gel network. This gives rise to a more rigid, non-shear-reversible gel network. When pH is above 3.5, there is a predominance of ionized acid groups, which favours Ca^{2+} cross-linking, leading to the formation of a more spreadable, shear reversible gel network. With LM-pectin, as the soluble solids increase, the requirement for Ca^{2+} decreases and the Ca^{2+} bandwidth decreases (Ralet *et al.* 2002).

The affinity of pectin for Ca^{2+} increases with decreasing average DM and increasing length of unsubstituted galacturonan stretches. The affinity of HM-pectin for Ca^{2+} is generally not high enough to lead to sufficient chain association for gelation to occur. Generally, increasing the concentration of sugar increases the gel strength, gelling temperature and gelling rate. The minimum concentration of sugar or polyol required for gelation depends on its ability to sufficiently stabilize hydrophobic interaction. For LM-pectin, the presence of sugar or other co-solutes is not necessary, but there is an increase in gelling temperature and gel strength and a decrease in syneresis as the total soluble solids increase (Rao and Lopes da Silva 2006).

ACID-DAIRY-DRINKS – A GROWTH SECTOR WITH A SIGNIFICANT POTENTIAL

Acid dairy drinks (ADD) are worldwide products existing in many variations, e.g. fruit milk drinks, yoghurt drinks, soy milk, butter-milk, whey drinks, kefir among others. These beverages can be described as an acidified protein liquid system with stability and viscosity similar to natural milk. Such drinks are usually composed of an acid dairy phase (fermented base) or a neutral base (milk, soy milk) with an acidic medium (fruit phase) (Laurent and Boulenguer 2003).

According to DMI Dairy Management Inc™ (2007), drinkable yoghurt stands out as the fastest growing seller, not just in dairy, but in the food and beverage industry as a whole. China topped the list of producers with a whopping 49% year-over-year growth rate. Annual sales of drinkable yoghurt grew by 18.4% to $7.76 billion from mid-2005 to mid-2006 (DMI Dairy Management Inc™ 2007). Numerous opportunities for functional ingredients, such as Omega-3, phytosterols and probiotics in drinkable yoghurt, have made the products an attractive area for innovation.

Stabilizing properties (pectin-protein interactions at interfaces)

A substantial portion of pectin today is used for the stabilization of low-pH dairy drinks, including fermented drinks and mixture of fruit juice and milk. The drinks may be heat-treated in order to increase their shelf life. Low viscosity and homogenous appearance are preferred characteristics. Casein, however, is prone to aggregation at low pH, particularly when subjected to heat treatment. Thus, in the absence of a stabilizer, high viscosity, whey exudation and sandy mouth feel are likely quality defects in these types of drinks (Rolin *et al.* 1998). In the late 1950s, it was proven that addition of HM-pectin to acidified milk drinks prevented the formation of sediment. Casein in milk, at its natural pH of approximately 6.6, is in the form of stable sub-micron particles, often called micelles (Ye 2007). At this pH, caseins are negatively charged. They are mutually repellent, which prevents precipitation. During acidification, electrostatic repulsion decreases and casein particles tend to aggregate. At the isoelectric point (pH 4.6), casein particles are uncharged and exhibit the weakest hydration. Below the isoelectric point, casein particles exhibit a net positive charge.

The detailed changes of the micelles above the isoelectric point can be summarized as followed:

- Between pH 5.8 and 5.5, the zeta potential decreases, leading the micelles to form clusters (particle size change from 180 to 1300 nm at pH 5.5).

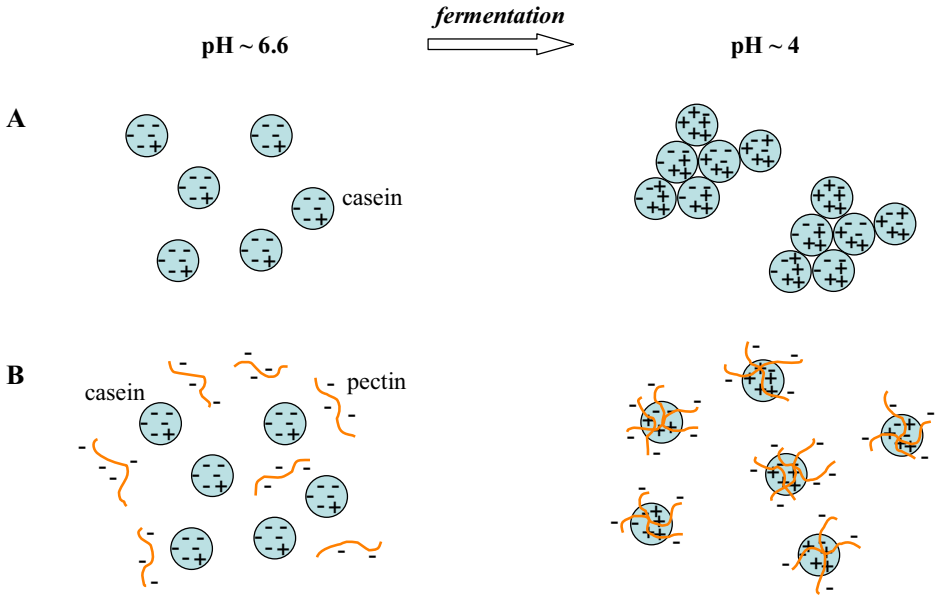

Fig. 5 Schematic representation of pectin stabilizing properties in acidified dairy drinks. (**A**) Casein in milk at its natural pH (~ 6.6) is in the form of stable sub-micron particles that are globally negatively charged. During fermentation, acidification occurs and casein particles become globally positively charged. Below pH 5, aggregation of the casein is irreversible. (**B**) At pH > 3.5, pectin is negatively charged due to the presence of carboxylic functions. At pH 6.6, both polymers are negatively charged and repel each other. At pH ~ 4, pectin electrostatically stick to the positively charged areas of casein particles producing a highly hydrated coating, which prevents casein aggregation. Such pectin-stabilized particles are often depicted as “fuzzy golf balls”.

- Between pH 5.5 and 5, a re-organization of the different kinds of caseins occurs in the micelle by solubilization of α and β casein. Micelles are then associated in multi-strands and are no longer spherical.
- Below pH 5, Ca^{2+} is completely soluble, the aggregation of casein is irreversible and a three-dimensional network is formed by the cluster of aggregated strands (Laurent and Boulenguer 2003).

A mechanism of stabilization by pectin, under acidic conditions, was proposed in which the adsorption of the pectin on the casein micelle surface throughout carboxylic-rich zones of HG domains is involved. The highly methyllated (uncharged) pectin segments would form entropy-rich loops that extend into the solution. These loops result in the repulsive interaction between the micelles at low pH in the same way as κ-casein chains do at milk pH (Rao and Lopes da Silva 2006) (**Fig. 5**). The stabilization of casein with pectin is only effective in the pH interval 3.2-4.5. By extension, pectin is not sufficiently dissociated at a pH below 3.5. It then either does not efficiently anchor or there is weak repulsion between "hairs" in an interpenetration zone. At a pH above 4.5, casein does not possess enough positively charged areas and there is no longer an attraction between pectin and casein (Rolin *et al*. 1998). In more concentrated acidified milk systems, it was suggested that stability is associated with the existence of a network of pectin-coated casein micelles, but a large fraction of pectin does not interact directly with acidified milk gel (large casein micelles). It was shown that less than 20% of the pectin added directly interacts with casein particles (Syrbe *et al.* 1998). The remaining 80% is involved in a network with casein-pectin complexes, but plays no role in stabilizing the final product. This excess fraction seems however necessary to produce a stable system (Janhøj *et al.* 2008).

Recently, it was shown that (i) stabilization in ADD might be caused by a combination of depletion interaction between pectin coated casein micelles and a pectin network, (ii) that 50-90% of all pectin is bound to casein and (iii) that stability is not affected by the remaining non-bound fraction (Boulenguer and Laurent 2003; Tromp *et al.* 2004).

Pectin dosage is a determinant factor in acidified milk drink stabilization. Too little pectin destabilizes the product compared to no pectin addition. If a series of milk drinks is prepared with different pectin additions, the viscosity first increases until a certain concentration is reached. A further increase in pectin amount causes a sharp decline in viscosity. A point of minimum viscosity is then reached, beyond which further addition of pectin causes a new increase in viscosity. The tendency for sediment formation grossly follows viscosity until the point of minimum viscosity. It is believed that the de-stabilization at low pectin dosages is because the adsorbing pectin molecules tend to wrap around casein particles when the surface is not crowded with pectin. Electrostatic repulsion is at a minimum because pectin has balanced the initial casein charge rather than increased numeric net charge. At full stabilization, only those pectin molecular areas, which interact strongly with casein, are anchored at the casein particles surface, whereas those areas, which are less strongly attached, are forced away from the surface (Rolin *et al.* 1998). Viscosity overshoot and final viscosity reduction become particularly pronounced when the casein particle size is low. It is striking that, independent of casein particle size, the maximum of low shear viscosity occurs at about half the pectin concentration at which the viscosity minimum (and product stability) is observed. This could point to bridging by weakly adsorbed polymers, expected to be the most pronounced at about 50% surface coverage. In contrast to the results at low shear rates, the limiting high-shear viscosity increased steadily with pectin concentration. This was interpreted as an increase in particle volume by the absorbed pectin layer, matching the results on the increased sediment volume after centrifugation. Pectin addition also changes the flow behaviour of ADD from shear-thinning and thixotropic to quasi-newtonian. This means that the system undergoes a transition from a flocculated state with partial surface coverage into a free-flowing state, where surface coverage is completed and particle attraction minimized. This conclusion is corroborated by microscopy and particle size analysis (Rolin *et al.* 1998).

In industry, the pectin dosage required for optimum acidified milk stabilization depends on the formulation and production technology of the product. The stability of acidified milk drinks is evaluated on the basis of the viscosity, the amount of sediment after defined centrifugation and by microscopic examination of the protein particles (Herbstreith and Fox KG Corporate Group 2003).

Important parameters are:

- pH value of the drink (optimum range 3.9-4.1)
- protein content and particle size (1-2 μm~ smooth, 10-20 μm~ chalky, 40-60 μm~ grainy)
- fermentation conditions during yoghurt preparation (temperature, time, bacteria culture used to produce uniform-sized protein particles)
- conditions during direct acidification using juice or acid (direct acidification makes large protein particles)
- heat treatment during the production process (the more intense the heat treatment, the greater the risk of thermal agglomeration of protein particles).
- homogenization (10-20 MPa, to destroy caseinate gel)
- heat treatment of the finished products (it has adverse effect because of the weakest bound molecules dislodge from the complex)
- Addition of Ca^{2+} (more Ca^{2+} reacts with more pectin) (Syrbe *et al.* 1998; Boulenguer and Laurent 2003; Herbstreith and Fox KG Corporate Group 2003; Lucey 2004; Tromp *et al.* 2004; Sedlmeyer *et al.* 2004; Mosteller 2006; Sejersen *et al.* 2007; Ye 2007; Janhøj *et al.* 2008).

Dilution drastically hampers the stabilization of ADD. In fact, except for Ca^{2+} enriched drinks, water-dilution modifies the ionic strength, which induces a decrease in stability (Ye 2007). A high Ca^{2+} concentration inhibits stabilization since it strongly enhances caseinate self-association (Syrbe *et al.* 1998). ADD milk solid non-fat (MSNF) can vary from 1 to 8.5% in commercial application (Laurent and Boulenguer 2003).

Types of pectin used for acid dairy drink (ADD) stabilization

ADDs are commonly stabilized by HM-Pectin (Syrbe *et al.* 1998; Boulenguer and Laurent 2003; Herbstreith and Fox KG Corporate Group 2003; Lucey 2004; Tromp *et al.* 2004; Sejersen *et al.* 2007; Ye 2007; Janhøj *et al.* 2008). LM-pectin with more numerous carboxyl groups should associate more strongly with the positively charged casein particles than HM-pectin. However, LM-pectin exhibits lower stabilization properties than HM-pectin. Tighter binding of pectin molecules could be the reason, leading to a flatter configuration and less polymeric stabilization, but without detailed information on the molar mass and the distribution of carboxyl residues, all this remains speculation (Syrbe *et al.* 1998). HM-apple pectin is especially suited for this stabilization mechanism when a high viscosity ADD is assumed. If a low-viscosity end product is required, HM-citrus pectin is mainly used. Only HM-pectin within a very specific range of esterification (approx. 68-72%) is suited for the stabilization of ADDs. For optimum stabilization, pectin requires a high molar mass and defined Ca^{2+} reactivity (Herbstreith and Fox KG Corporate Group 2003).

CONCLUSION

Pectin is a high value functional food ingredient widely used as a gelling agent and stabilizer. Food scientists and plant scientists therefore share a common goal to better understand the structure and functionalities of pectic polymers at the molecular level. The basic properties of pectin have been known for nearly 200 years, but recently there has been tremendous progress in our understanding of the

very complex fine structure of pectic polymers and pectinolytic enzymes. This has been made possible by synergies between plant and food research and by the application of a range of state-of-the-art techniques including enzymatic fingerprinting, mass spectrometry, NMR, molecular modelling, and monoclonal antibodies. With this increased knowledge, there are increased opportunities for novel applications. Producers are beginning to develop a new generation of sophisticated designed pectins with specific functionalities. However, it will be important that these advances are carefully managed, so that pectin maintains its deserved reputation as a natural product.

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