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Chemical-physical characterization of complex systems: the case of pectins

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ABSTRACT

Checchetti Andrea, Chemical-physical characterization of complex systems: the case of pectins

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Key Words: Commerical pectins, centrifugation, extraction, intrinsic viscosity, hydrodynamic molecular weight, polymer compatibility, viscometric behaviors

In industrial process, separation of the hydrolyzed pectin solution is performed by centrifugal and filtration technologies. The effect of water addition to the hydrolyzed citrus peels slurry on the separation efficiency was investigated, with the aim to improve pectin recovery. Different dilution ratios were tested and it was found that the pectin recovery increases when increasing water addition passing through a maximum value. No relevant effects on pH and pectin yield were found when increasing the contact time between water and hydrolyzed slurry, confirming that no further progress of hydrolysis or extraction processes is caused by the dilution. Therefore, the increase in pectin recovery can be attributed only to the water addition.

Intrinsic viscosity properties of commercial pectins and their mixtures in dilute solutions were investigated by using capillary viscometer. Influence of degree of esterification on interaction polymer-solvent was studied in order to provide additional evidence that can be used to elucidate the mechanism of the intermolecular interaction between two pectins and to determine their compatibility in dilute solutions. The viscosity measurements of ternary system have been performed by two techniques:

1. The first was determined by putting the polymer A and B with certain weight ratio in a pure solvent

2. The second was determined by putting the polymer A (referred to as guest or probe polymer) in solution in which the polymer B (referred to as host or matrix polymer) is found at a constant concentration. This technique is called the method of polymer solvent.

By using the first method, two compatibility criteria, based on specific viscosity data and derived for synthetic polymers, have been applied to pectin mixtures at different degree of esterification (DE). Data revealed that positive molecular interaction take place when pectin in solution have a different DM (i.e. HM/LM mixtures), whilst an incompatible behavior was found for similar pectin (i.e. both HM and LM). In the case of incompatible pairs of pectin, also the apparent molecular weight of ternary solution showed an increase with respect to the additive mixing rule, due to repulsive forces. On the contrary, due to attractive forces in case of compatible pectin pairs, the molecular weight is lower than the calculated average.

By using the polymer-solvent method, the coefficient ($K_{AB)}$ of interaction between polymers has been calculated. The analyzing of this parameter K_{AB} allowed to justify the results obtained.

SOMMARIO

Nei processi industriali, la separazione di soluzioni di pectine idrolizzate è eseguita mediante la centrifugazione e la filtrazione. E' stato investigato l'effetto di aggiunta di acqua all'idrolizzato di bucce di agrumi sull'efficienza di separazione, con l'obiettivo di aumentare il recupero pectina. Diversi rapporti di diluizione sono stati testati e si è osservato che l'aumento nel recupero di pectina quando si aggiunge acqua passa per un valore massimo. Nessun effetto rilevante sul pH e e sulla resa di pectina è stato trovato quando si aumenta il tempo di contatto tra l'acqua e liquame idrolizzato, a conferma che nessun ulteriore processo di idrolisi o di estrazione è causato dalla diluizione. Pertanto, l'aumento nel recupero di pectina può essere attribuito solo all'aggiunta di acqua.
La viscosità intrinseca delle pectine commerciali e loro miscele in soluzioni diluite è stata studiata mediante viscosimetria capillare. L'influenza del grado di esterificazione sull'interazione polimero-solvente è stata studiata al fine di fornire ulteriori prove che possono essere utilizzate per chiarire il meccanismo di interazione intermolecolare tra due pectine e determinare la loro compatibilità nelle soluzioni diluite.

Le misure della viscosità dei sistemi ternario sono state eseguite tramite due tecniche.
La prima prende in considerazione la miscela tra il polimero A e B con preciso rapporto in peso in un solvente puro.

La seconda considera il polimero A in una soluzione di un polimero B che si trova ad una concentrazione costante. Questa tecnica è chiamata metodo del "polimero solvente".

Utilizzando il primo dei due metodi, due criteri di compatibilità, sulla base di dati specifici di viscosità e derivati per polimeri sintetici, sono stati applicati a miscele di due pectine a diverso grado di esterificazione (DM). I dati hanno mostrato che interazioni molecolari positive prendono posto quando

le pectine in soluzione hanno un diverso DM (HM/LM), mentre un comportamento incompatibile è stato trovato per pectine simili (ovvero entrambe HM o LM). Nel caso di coppie incompatibili di pectine, anche il peso molecolare apparente della soluzione ternaria ha mostrato un incremento rispetto alla regola additiva, a causa delle forze repulsive. Al contrario, a causa di forze attrattive, in caso di coppie di pectine compatibili, il peso molecolare è inferiore alla media calcolata. Utilizzando, invece, il metodo del polimero-solvente, il coefficiente (K_{AB}) di interazione per diversi valori di concentrazione della pectina B è stato calcolato. L'analisi di questo parametro K_{AB} ha permesso di stabilire la forza delle interazioni tra le pectine in soluzione e di giustificare i risultati ottenuti.

DEDICATION

This dissertation is dedicated to the memory of my mother.

A Nina

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These three years have been very rich scientifically and humanly.

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LIST OF PAPERS

This thesis is based on the following papers:

1. **EFFECT OF WATER ADDITION ON PECTIN RECOVERY FROM SOLUTION IN CENTRIFUGAL SEPARATION PROCESS**

   Massimo Migliori, Domenico Gabriele, Andrea Checchetti, Deborah Facciolo & Barbara Battipede

   Published on ***International Journal of Food Science and Technology*** **2010**

2. **COMPATIBILITY ANALYSIS OF PECTIN AT DIFFERENT ESTERIFICATION DEGREE FROM INTRINSIC VISCOSITY DATA OF DILUTED TERNARY SOLUTIONS**

   Massimo Migliori, Domenico Gabriele, Andrea Checchetti, Barbara Battipede

   Published on ***Reactive & Functional Polymers*** **70 (2010) 863–867**

3. **COMPATIBILITY CRITERIA COMPARISON FOR SOLUTION OF PECTIN AD DIFFERENT ESTERIFICATION DEGREE**

   Massimo Migliori, Domenico Gabriele, Andrea Checchetti, Barbara Battipede

   Written to be submitted to publication

4. **VISCOMETRIC STUDY ON THE INTERMOLECULAR INTERACTIONS BETWEEN PECTIN-PECTIN MIXTURES IN SOLUTION**

   Bruno de Cindio, Domenico Gabriele, Andrea Checchetti, Barbara Battipede

   Written to be submitted to publication

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8. Raudino A., Grasso D, La Rosa C., Dipasquale G., Chidichimo G., Checchetti A..

A THERMODYNAMIC AND NMR STUDY OF THE RIBBON LYOTROPIC MESOPHASES. AN INVESTIGATION OF THE POTASSIUM PALMITATE/POTASSIUM LAURATE/WATER SYSTEM.

Liquid Crystals**, 1989, 6(4):435-447**

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LIST OF ABBREVIATIONS

Abbreviation or Symbol Term

DA	Degree of Amidation
DE	Degree of Esterification
GalA	Galacturonic Acid
HG	Homogalacturonan
HM	High-Methoxy pectin
LM	Low-Methoxy pectin
MW	Molecular Weight
RGI	Rhamnogalacturonan I
RGII	Rhamnogalacturonan II

Quello di "*cercar* la regola" è il primo istinto di conoscere, mentre naturalmente per il fatto che sia trovata la regola, niente ancora è "conosciuto"! […] *Vogliono la regola* perché essa toglie al mondo il suo aspetto pauroso. *La paura dell'incalcolabile* come *istinto segreto* della scienza.

F. Nietzsche, *Frammenti postumi 1885-1887*

CHAPTER 1

GENERAL INTRODUCTION

HISTORY OF PECTIN

Pectin has a very long chemical history. Jams and jellies recipes were published in the “London Housewife’s Family Companion” of 1750 (International Pectin Producers Association, 2001). The discovery of the chemical compound was made by Vauquelin (1790). The term “pectin” originates from the Greek word “pektikos” meaning to gel or solidify, which describes the basic properties of these macromolecules to form gels with calcium ions (Pilnik, 1990). From a scientific point of view pectin was first isolated and described by Henry Braconnot in 1825. Braconnot reported about an acidic substance found in all plants and he predicted that it would have important functions, in particular as component in fruit responsible for gel formation (Braconnot, 1825). Commercially the first production of liquefied extract of pectin began in 1908 Germany and was quickly patented in the United States (Douglas, 1913).

SOURCES OF PECTIN

Pectin content in cell walls of most terrestrial plant can be up to 35% (Tombs, 1998). All green land plants contain pectin to a certain degree. Pectin content in dicotyledonous (flowering) plants is higher than that contained in monocotyledonous (seed-bearing) plants and grasses. See Table 1 for a comparison of the content of pectin in monocots versus dicots.

The plant cell wall (Figure 1.1) is composed of polysaccharides and proteins (Harholt, 2010). In all cases, the polysaccharides constitute the major part of the wall. The wall polysaccharides are often classified into cellulose, hemicelluloses, and pectin, and these three types are represented in almost all

cell walls in varying proportions. The structural elements of the cell wall polysaccharides and possible structural variations within these polysaccharides are listed in Table 2.

Tab.1 content of pectin in monocots versus dicots

Components	Monocots (%)	Dicots (%)
Cellulose	30	30
Pectin	5	35
Arabinoxylan	30	5
Xyloglucan	4	25
β-(1,3),(1,4)-Glucans	30	0
Glycoproteins	1	5

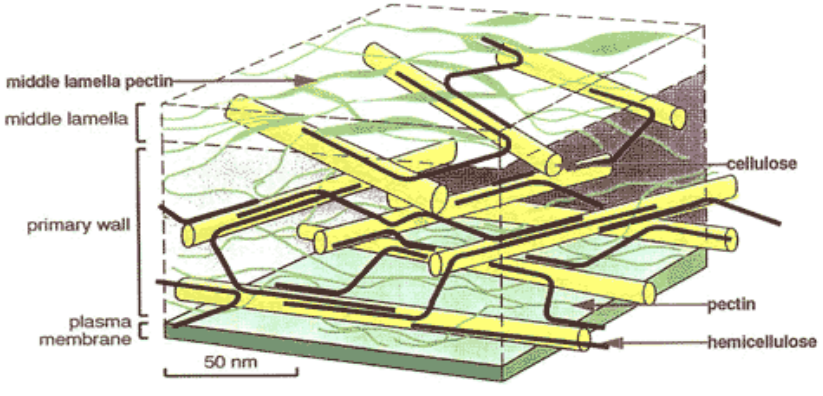

Figure 1.1 Plant Wall Cell Structure

	Structural element	Diversity within the structural elements
(Hemi)celluloses	Xyloglucan	Distribution of side chains over the β-(1,4)-glucose backbone Sugars in the side chains Degree of acetylation Hydrogen bonds to cellulose Present as free loops, trains or in crosslinks? Entrapped in amorphous cellulose?
	(Glucurono)-arabinoxylan	Degree of arabinose and (OMe)-glucuronic acid substitution Distribution of substituents Degree of methyl esterification and acetylation Degree and distribution of ferulic acid Presence of xylose, galactose, rhamnose and galA at the reducing end Entrapped in amorphous cellulose?
	Cellulose	Amorphous or crystalline structure (Non)-covalent linkages towards pectic elements?
Pectic structural elements	Homogalacturonan	Lentgh of homogalacturonan segments in between two RG-I units Degree of methyl esterification Degree of acetylation Degree of blockiness Connection to other structural elements
	Xylogalacturonan	Degree and substitution of xylose Length of xylose side chains Degree of methyl esterification Other sugars than xylose present in side chains (e.g. fucose) Presence and level of acetylation Location of xylogalacturonan within pectic macromolecule
	Rhamnogalacturonan I	Length of RG-I backbone Length of arabinan and arabinogalactan side chains Distribution of the side chains over the RG-I backbone Degree and localization of acetylation Presence of methyl esters Connection to other structural elements
	Arabinan	Branching of the side chains Level and localization of ferulic acid Presence of terminal arabinose-pyranose
	Arabinogalactan I	Size, linkages present, degree and type of branching Distribution of branching of the side chains over the galactan backbone Ratio of arabinose to galactactose Level and localization of ferulic acid Distribution of the side chains over the galactan backbone Presence of arabino-furanosyl residues in galactan backbone Ratio of the β-(1,3)-linked galactose in the β-(1,4)-galactan backbone Presence of terminal arabinopyranosyl residues
	Arabinogalactan II	Size, linkages present, degree and type of branching Ratio of arabinose to galactactose Degree and localization of ferulic acid Terminal arabinopyranosyl-, glucuronopyranosyl and/or rhamnosyl residues present? Distribution of the side chains over the galactan backbone Attachment to other pectin structural elements
	Rhamnogalacturonan II	Small differences in structure Level of borate crosslinking Distribution of RG-II chains over the pectic molecule

α-L-Ace*f* A, β-D-Api*f*, α-L-Ara*f*, β-L-Ara*f*, α-L-Arap, β-D-Dha*p*, α-L-Fuc*p*, α-D-Gal*p*, β-D-Gal*p*, α-D-Gal*p* A, β-D-Gal*p* A, β-D-Glc*p*, α-D-Glc*p* A, β-D-Glc*p* A, α-D-Kdo*p*, α-L-Rha*p*, β-L-Rha*p*, α-D-Xyl*p*, β-D-Xyl*p*, Ferulic acid, O-acetyl, O-methyl

Table 2 Schematic structures of plant cell wall polysaccharides and possible variation of each structural element (adapted from Schols, H.A.; Voragen, A.G.J., 2002).

Cellulose is the main supporting wall structure and is composed of β-1,4-linked glucan chains organized in more or less crystalline microfibrils. Hemicelluloses include several different polymers, chiefly xylans, xyloglucans, and (gluco)mannans, which are characterized by having a backbone of β-1,4-linked sugars with an equatorial linkage configuration (Scheller, 2010). Pectin is the third group of polysaccharides, characterized by relatively high extractability using acid or chelators and a high content of galacturonic acid (GalA). Together, the hemicelluloses and pectin constitute the matrix in which cellulose microfibrils are embedded.

Pectin is probably the most complex polysaccharide in nature as it can be composed of 17 different monosaccharides, some of them being esterified by methyl, acetyl or feruloyl groups (Ridley, 2001; Vincken, 2003). By degrading cell wall materials or extracted pectin by purified pectolytic enzymes, it clearly appeared that the different monosaccharides were not randomly distributed along the pectin macromolecule but were concentrated within different pectic structural domains.

CHEMISTRY OF PECTIN

Pectin structure

The term ‘pectin’ is a somewhat misleading since it rather implies one molecule. In fact pectin describes a family of oligosaccharides and polysaccharides that have common features, but are extremely diverse in their fine structures. (Ridley, 2001). However, all pectins are rich in galacturonic acid, and the FAO and EU stipulate that ‘pectin’ must consist of at least 65% GalA. Three major pectic polysaccharides are recognized, all containing GalA to a greater or lesser extent.

Pectins, as a compound, are linear polysaccharides composed primarily of D-galactopyranosyluronic acids joined via α(1→4) glycosidic linkages. The regular structure is intradispersed with L-rhamnopyranosyl units, or “hairy regions”, methyl ester groups and rarely, neutral-sugar side-chains.

The galacturonic acid units contained within pectin can be either partially methyl-esterified, acetylated or both. Figures 1.2-1.4 are typical representations. Pectin, as naturally found, generally have an average molecular weight (MW) of approximately 200 kDa (with 300 kDa being a normal occurrence) and form strong gels in the presence of cations (divalent or monovalent).

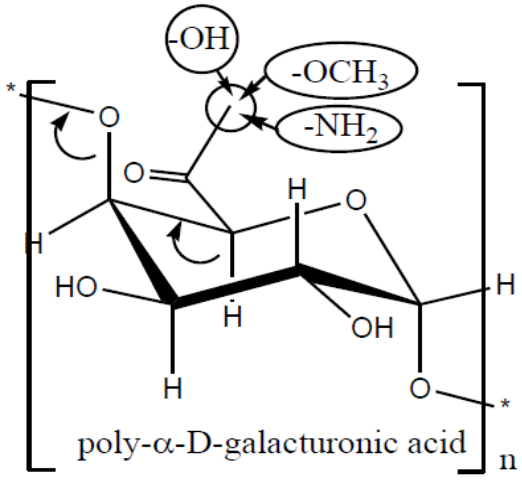

Figure 1.2 Naturally occurring forms of D-galacturonic acid residues where arrows indicate possible β-elimination in the ester form.

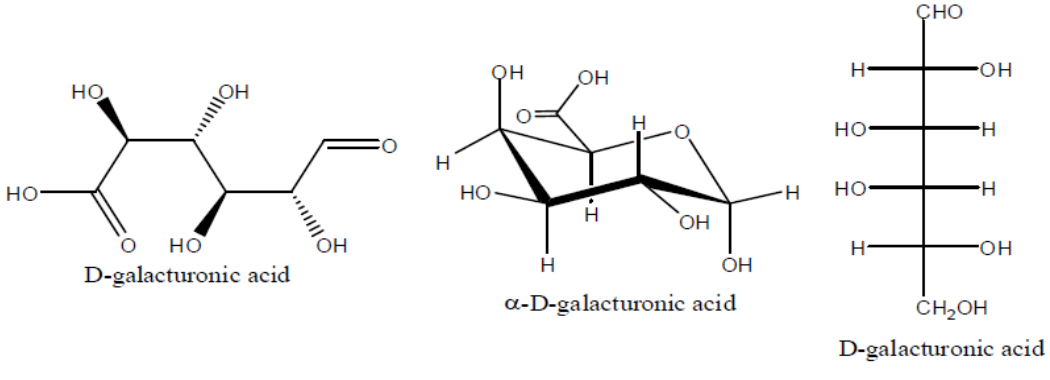

Figure 1.3 Alternative representations of D-Galacturonic Acid

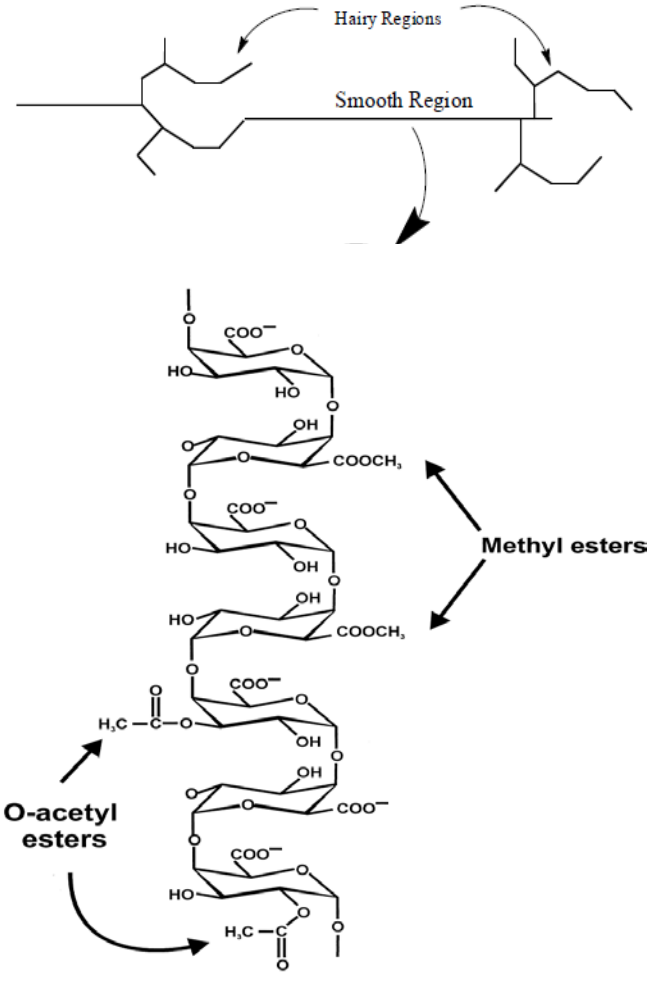

Figure 1.4 Overview of the pectin structure. Homogalaturonan with partially methyl-esterified α-(1→4)-linked D-Galacturonic acids comprises the backbone in the smooth region

A general scheme of pectin structure

A general structure of pectin polysaccharides is given in many papers, e.g., in (Willats, 2001; Neil, 2003). GalA occurs in two major structural features that form the backbone of three polysaccharide domains found in all pectin species: homogalacturonan (HGA), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) (Willats, 2006). It is thought that these three polysaccharide domains

can be covalently linked to form a pectic network throughout the primary cell wall matrix and middle lamellae (Ovodov, 2009).

The linear region of homogalacturonan consists of 1,4-linked α-*D*-galactopyranosyluronic acid residues. These regions are joined by one or two α-*L*–rhamnopyranose residues which are involved in the linear chain by a 1,2-linkage. The backbone of many pectins has this structure: they differ only by the length of the chain (Willats, 2001; Neill, 2003; Oosterveld, 1996).

The ramified region consists of three subunits:

- RG-I
- Arabinogalactan
- Xylogalacturonan

They can be present in different ratios, as it is shown for apple pectin (Schols, 1995). The data obtained for pectins from a peach, carrot, onion, leek, and potato are fully consistent with this structure . In some cases the polymer contains an apiogalacturonan fragment (Ovodov, 1998).

The RG-I fragment considerably varies in different pectins (See figure 1.5).

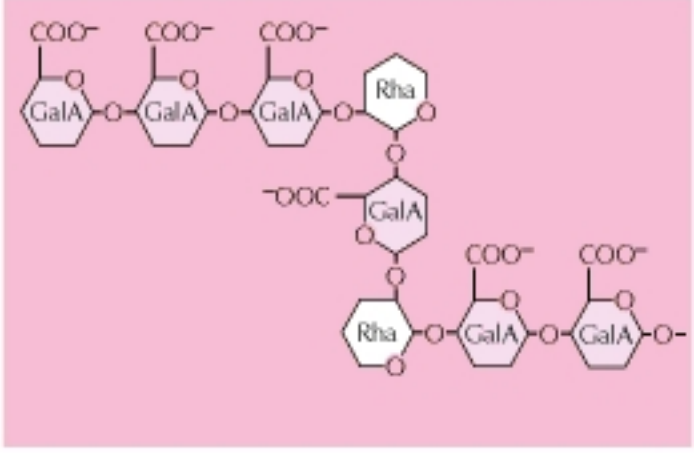

Figure 1.5 The backbone of rhamnogalacturonan contains galacturonic acid (GalA) and rhamnose (Rha) residues, to which numerous side chains are also attached.

Its backbone consists of alternating residues of 1,4-linked galacturonic acid residues and 1,2-linked rhamnose residues partially substituted for by single galactose residues linked to rhamnose residues by 1,4-linkages.

Also, the RG-I subunit can have long arabinan and galactan side chains (See figure 1.6). Arabinose residues can be terminal 1,3- and 1,3,5-linked residues. RG-I can also contain either a xylogalacturonan unit in which single xylopyranose residues are 1,3-linked to the backbone, as it is in apple pectin, or an apiogalacturonan fragment in which single or 1,3-linked *D*-apiose residues are linked to the *D*-galacturonic acid of the core by 1,2- and/or 1,3-linkages. A general model for apple, citrus, and beet pectins has been suggested which has an alternating linear 1,4-linked α–*D*-galacturonan chain and a branched region containing most of the neutral monosaccharides (Novosel'skaya, 2000) (See figure 1.7).

Original pectins from these sources differ by molecular mass (citrus > apple > beet) and content of rhamnose (beet > apple > citrus). From apple, beet, and citrus pectins, homogalacturonan, with a polymerization degree, of 72–120, 91–108, and 114–138 kDa, respectively, were isolated. Sugar beet pectin was found to contain ferulic acid (Fer) residues which are linked to the neutral monosaccharides of side chains (mainly to *L*-arabinofuranose residues) by an ester bond (Guillion, 1989). Figure 1.8 presents a schematic diagram of the structure of HGA, RG-I and RG-II and lists the major potential variations within their fine structure.

An unknown aspect of pectic network structure is the distribution of HGA, RG-I and RG-II within pectin chains (Willats, 2001). The presence of covalent linkages between the pectic structural elements has been hypothesized already for a long time based on co-extraction and co-elution. Mainly two models dealing with the pectic network have been established (See Figure 1.9). The so-called

hairy regions consisting of RG-I (with neutral side chains attached) and XGA are interspersed with so-called smooth (HG) regions.

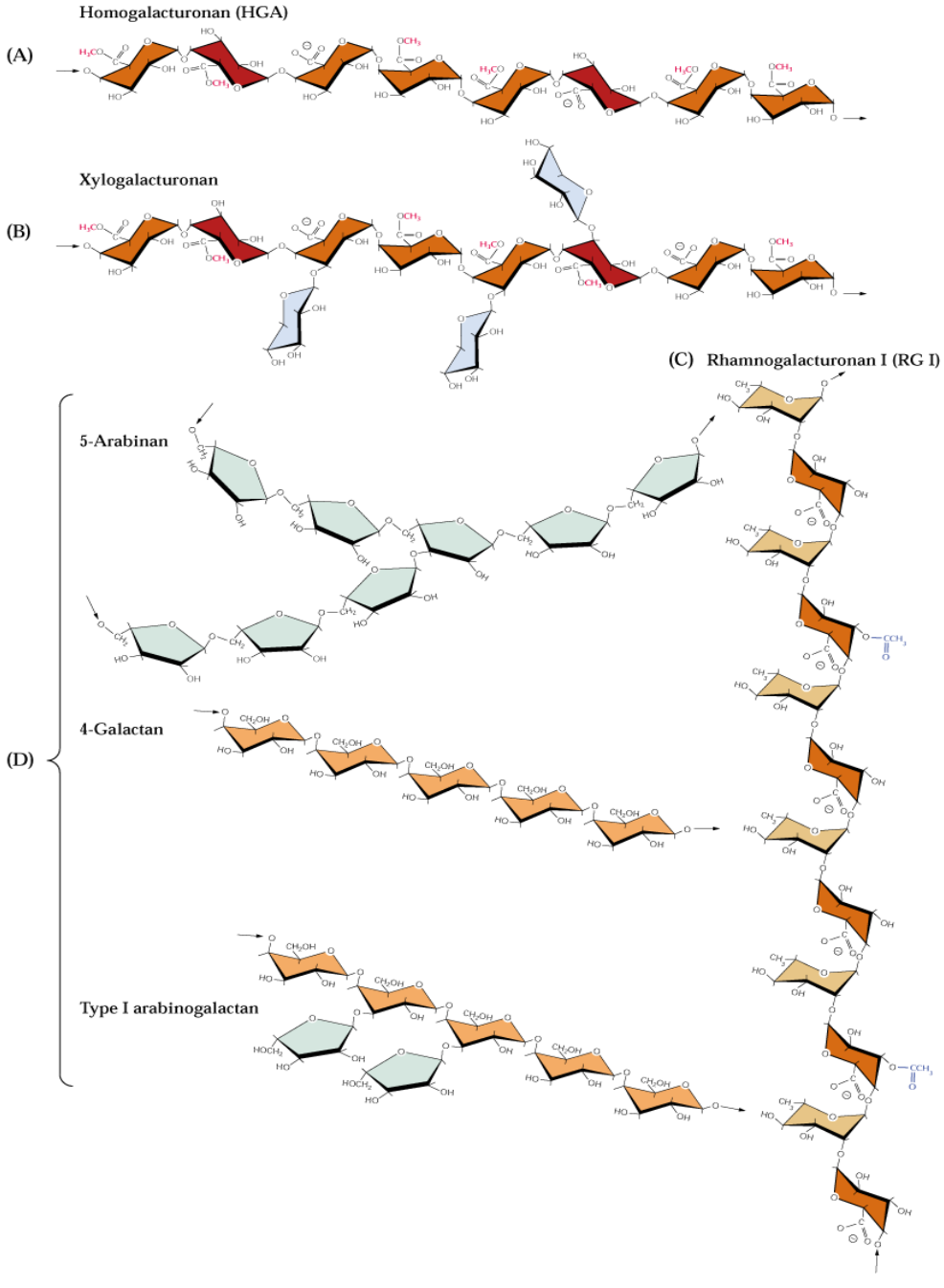

Figure 1.6 Different components of network of pectin structure

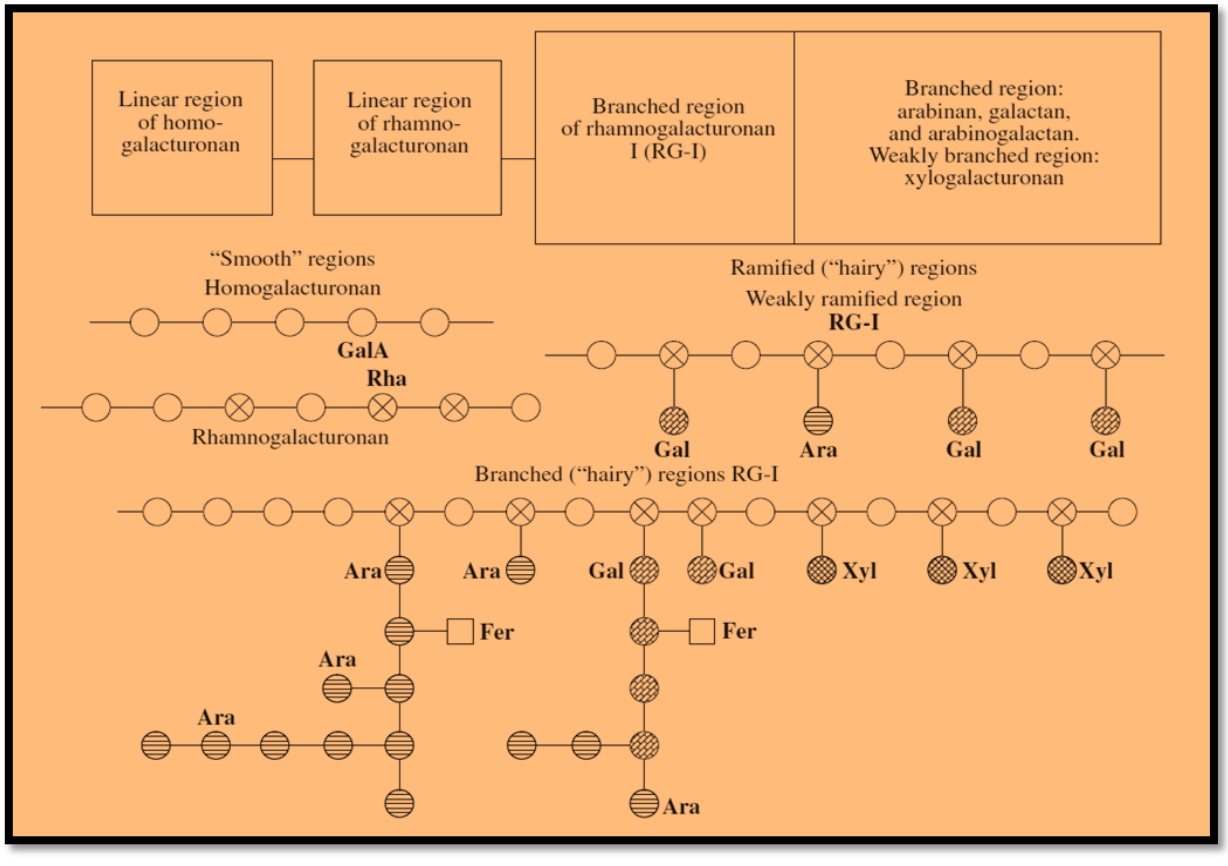

Fig. 1.7 Schematic structure of pectin polysaccharides. Fer, ferulie acid residue

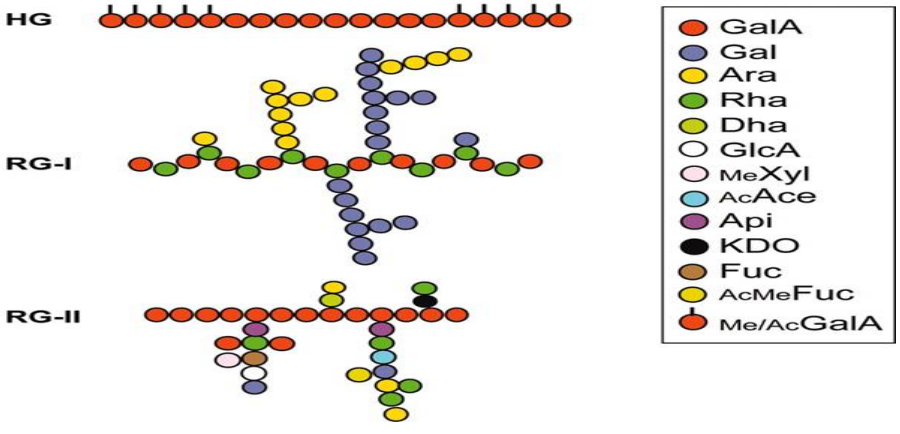

Figure 1.8 Simplified schematic diagram to indicate some of the features of the three major polysaccharide domains of pectin: homogalacturonan (HGA), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II).

The second model, the so-called rhamnogalacturonan backbone model, was proposed by Vincken et al., (2003), which locates HG as a side chain of RG-I. RG-II is proposed to be attached to the homogalacturonan segments as RG-II is liberated from the pectic macromolecule by endo-polygalacturonase treatment.

Pectin macromolecule is one of the most complex polysaccharide, if not the “most complex polymer in nature” (Vincken, 2003). Its functionalities are strongly related to this complex structure, which can vary largely inside a plant species as well as between species.

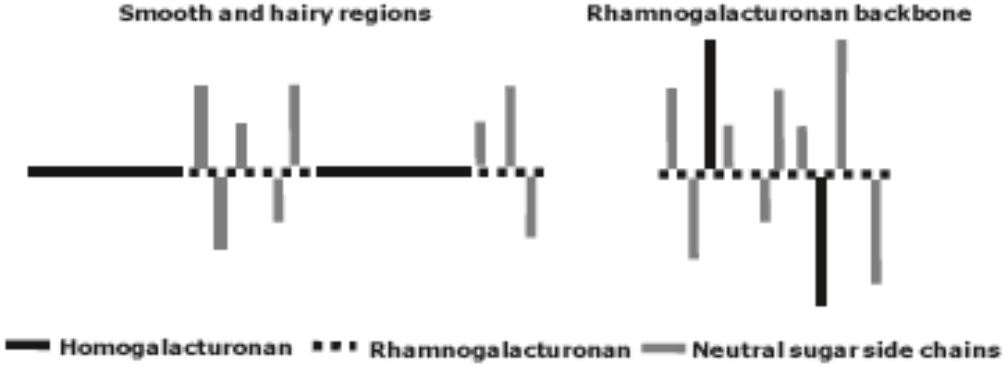

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Figure 1.9 Schematic representation of two different models describing the hypothetical pectin structure, adapted from Vincken et al. (Vincken et al., 2003).

FINE STRUCTURE MODIFICATION

Extraction from the plant

Three steps are required to extract pectin from the plants:

1. Aqueous extraction from the plants starting material
2. Purification of the liquid extract
3. Isolation of the extracted pectin from the liquid

Extraction of pectin may be made by aqueous acid or base, (Joyea, 2000). The classical way for the food industry is to perform the operation in hot mineral acid (70-80°C, pH = 2) which separates pectin from the cellulose plant materials. The acid extraction process generally yields a pectin of high degree of esterification (high DE pectin), approximately equal to the naturally occurring DE.

The basic extraction process yields a pectin of low degree of esterification (low DE pectin) as a result of saponification of the ester groups. The extraction conditions are generally chosen to preservate the molecular weights of pectins.

Modification of the fine structure

Pectin extracted from apple and citrus tipically have a DE value of 75-80 %. As all functionalities are linked to its fine structure, pectin is often modified and tuned regarding both the DE and the pattern of the acidic residues. In that aim, pectin can be de-esterified by different ways leading to different patterns. Chemical methods include mostly working under alkali conditions where the methyl groups can be liberated, while biochemical tools implies the use of enzymes extracted from the plant cell itself. By playing with these different techniques it is possible to image the built of a huge variety of pectin patterns.

Pectin chain characteristics

Pectin is a weak polyelectrolyte with pK_a of about 3.5 (depending on the DE and degree of dissociation), and is not fully charged in solution. Its fractional charge can be modified by changing the solution pH, counter ion concentration, or the ionic strength, and this charge influences the conformation of the polymer in aqueous solution: the pectin being more extended when charged, i.e. at pH above its pK_a. Subsequently, the relative charge quantity (DE) and the distribution will affect the persistence length. In literature pectin has reported to behave from a rod-like polymer to a semi-flexible coil of different stiffness (Anger, 1985; Axelos, 1989) with the most recent studies giving a

persistence length LP ranging from 6.7 to 9.0 nm (Axelos, 1991; Malovikova, 1993) obtained by experimental viscometry.

Some authors have reported that the persistence length of the pectin chains could depend on the DE (Deckers, 1986). Persistence length has been determined for pectin with DE going from 28 to 73% (Perez, 2000). Viscosity measurements gave values ranging from 5.9 to 12.6 nm. As general conclusion we can consider pectin as semiflexible coil like polymer with a persistence length of the order of 10 residues.

INDUSTRIAL PRODUCTION OF PECTINS

As an abundant raw material, apple pomace and citrus peels are the sources from which most industrial pectin are derived. However, raw materials are highly dependent upon local crop sources. In some parts of the world, sugar beet pulp, sunflower heads, or potato pulp are used. May (May, 1990) and Voragen (Voragen, 1995) previously described, as summarized in Figure 1.10, the industrial process for pectin extraction in detail.

The source materials are refluxed with dilute mineral acid (~ pH 2) at 60-100°C for 1-10 hours. The hot pectin extract is separated from the solid residue and pectinase-free α-amylase is added to hydrolyze starch if the source is apple or peach pomace. The clarified extract is concentrated under vacuum to ~ 4% pectin content and precipitated with 2-propanol. The precipitate is washed, dried, and ground to a powder form. Desired yield and DE determine extraction temperature and time (de-esterification proceeds faster than de-polymerization at lower temperatures). Low DE types of pectin are produced via acidic treatments at various stages during the extraction process. When ammonia is used for this purpose, amidated pectin are obtained as a final product. To get a product that is consistent over a range of properties, blending batches and dilution with sugars is customary (May, 1990).

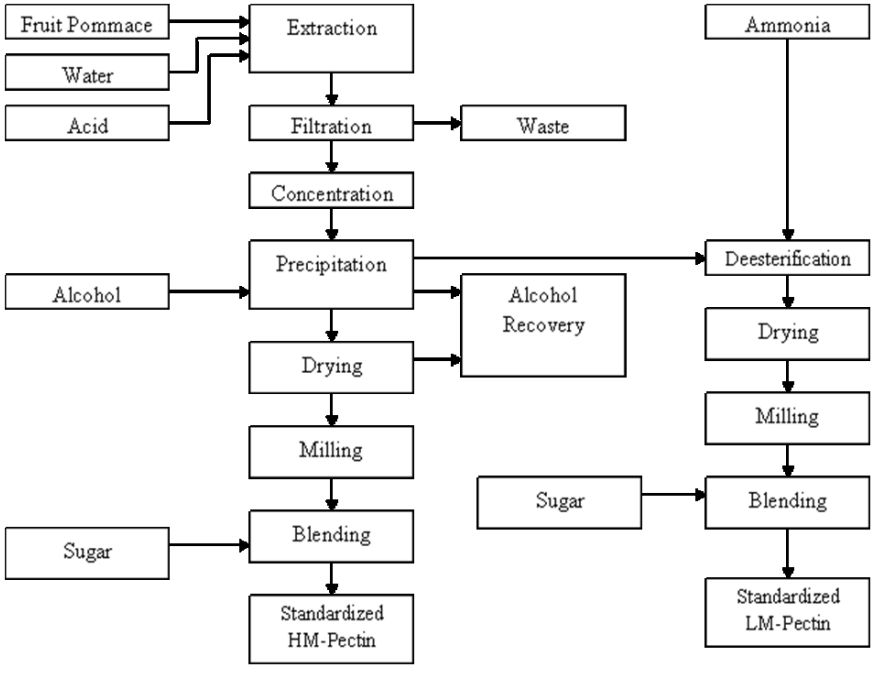

Figure 1.10 Industrial production of pectins

IMPORTANT USES

Historically, pectins have found use as general texture modifiers and gelling agents. Among the more common applications, pectin is used extensively in jams, jellies, confectionaries, deserts, yogurts, and anti-diarrheal agents. Some of the more important modern uses include:

1. Ca^{2+} sequestering agent in detergents
2. fillers in low calorie food products
3. edible acidifying agents
4. rheology modification
5. biodegradable surfactants and emulsifiers
6. edible packaging
7. dairy stabilizers

8. dietary fat replacements

Recently, new applications of pectin have become very important. Most prominent is the treatment of wastewater effluents where pectin has found extensive use in treatment regimens involving contamination with heavy metals. Pectins of most configurations show affinity for complexation with metal ions in aqueous solutions. Also, as an excipient, pectin efficiently encapsulates many pharmaceutical actives that are expatriated in the human large intestine and colon thereby greatly increasing drug efficacy.

Commercially, pectin has found widespread use in both the food processing and pharmaceutical industries owing to its gelling capacity.

GELLING PROPERTIES OF PECTINS

Pectins are divided into three groups on the basis of their different gelling properties.

High Ester Pectins (HM Pectins)

These pectins have usually a more than 50% s of esterified polygalacturonic acid units (DE) (figure 1.11), thus practically no reaction with calcium ions occurs. The gel strength depends, among others, on acid content, type of pectin, concentration and soluble solids content, which generally has to be more than 55%.

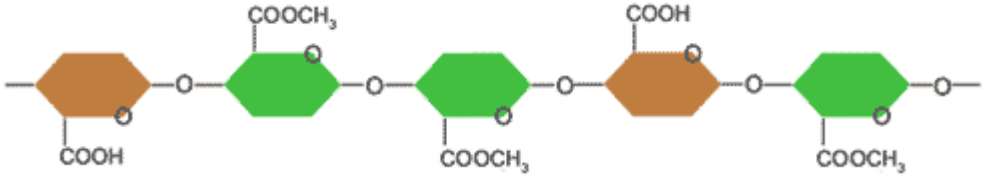

Figure 1.11 HM pectin formula

The degree of esterification correlates with the gel setting rate and gel texture under otherwise similar conditions. Very high esterified pectins jellify quicker at higher temperatures and form more elastic and brittle gel textures than less esterified pectins.

This accurate correlation requires a very homogeneous inter- and intramolecular carboxyl group distribution. Due to a blockwise distribution of carboxyl groups classic citrus pectin with the same degree of esterification will form gels with a slightly higher setting temperature and a more elastic-brittle texture than Classic Apple Pectin.

Low Ester Pectins (LM Pectins)

Pectin with less than 50% of esterified polygalacturonic acid units (DE) can jellify with calcium ions (figure 1.12). LM pectin thus do not only form gels with sugar and acids, but at less soluble solids mainly with calcium ions. The resulting gel strength is determined by pectin concentration, type of pectin, soluble solids content, pH range and the concentration of buffer salts and calcium ions.

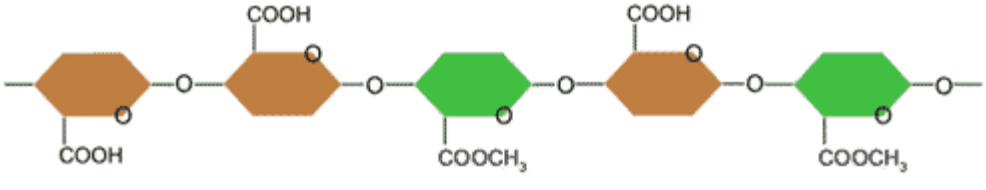

Figure 1.12 LM pectin formula

A well matched balance between pectin and calcium concentration will lead to an optimal texture. Exceeding the calcium optimum will produce a brittle gel with tendency towards syneresis (loss of water from the gel) or, in the end, to the formation of calcium pectinate, the insoluble calcium salt of pectin. Since gel setting with LM pectins is also possible with a low soluble solids content and at a high pH-value, this opens up numerous application possibilities in dietetic and dairy products.

Amidated Pectins

In case of amidated pectin ammonia instead of acids is used for de-esterification and with that part of the ester groups is replaced by amide groups (figure 1.13). This process modifies the gelling properties in comparison to acid de-esterified pectins. LM amidated pectin, just like non-amidated pectin, require calcium ions for gelling. They will already set sufficiently with only minor calcium amounts present. Furthermore, the gel setting temperature of amidated pectins is less influenced by the calcium dosage.

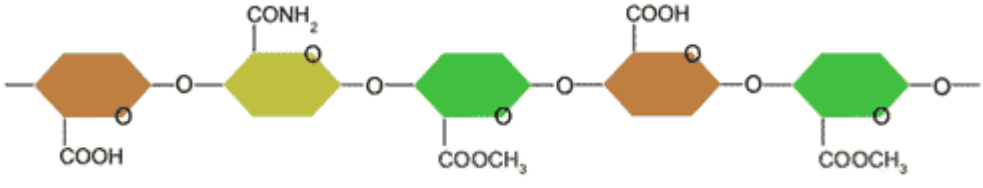

Figure 1.4 Amidated pectin formula

The different properties of pectins

The most important function of pectin commercially is that it acts as a gelling or thickening agent in processed foods (Schols, 2002). Thus, there has been great interest in the structure and function of gels of pectin. Current thoughts on the structure of pectin gels can be found in work reported elsewhere. (Tsoga, 2004; Löfgren, 2002; Löfgren, 2005). It is believed that pectin gel networks form due to polymer-polymer interactions stabilized by a combination of hydrophobic interactions and hydrogen bonds and enabled by the formation of junction zones. Low-acid pH promotes hydrogen bonding between non esterified carboxyl groups on the polysaccharide, high degrees of methyl-esterification of the polysaccharide promote intermolecular hydrophobic interactions, and high sugar content desiccates the polysaccharide of water enabling closer inter-polymeric contacts. In general, the DE determines the gelling mechanism of pectin. HM pectin form gels mainly by hydrophobic interactions

and hydrogen bonds at acidic pH values because of the reduction in electrostatic repulsion and in the presence of more than 55% sugar or a similar cosolute, which decreases polymer–water interactions leading to junction zone stabilization by promoting hydrophobic interactions between ester methyl groups (Oakenfull, 1984) On the other hand, LM pectins have the ability to form gels in the presence of Ca^{2+} ions over a wider range of pH values, with or without sugar, by the "egg-box" mechanism (Axelos, 1991) (figure 1.14). In this case, sections of two pectic chains, which must be free of ester groups, are held together by calcium ions. For LM pectins, the combined effect of pH and sugar promotes gelation at a lower calcium level despite the decrease in the number of sequences of carboxyl groups for calcium binding because of the specific effect of sugar on the water activity, promoting hydrophobic interactions (Thakur, 1997). Although not completely understood, it is believed that the gelation of LMA pectins also occurs by the "egg-box" mechanism, in addition to the formation of hydrogen bonds by amide groups, which are responsible for stabilizing the junction zones between different pectin molecules (Alonso-Mougan, 2002).

The gelling properties of pure LM or HM pectins in systems that favor gelation of these polysaccharides (with Ca^{2+} or different cosolutes and low pH, respectively) have been widely explored in the literature (Löfgren, 2005, Tsoga, 2004; Evageliou, 2000, Grosso, 1998) More recently, mixed HM/LM pectin-gelled systems were studied in the presence of more than 30% sucrose and Ca^{2+}, which favored HM and LM pectin gelation, respectively (Löfgren, C., 2002, Löfgren, 2007), but the strongest synergistic effect in the rheological properties was obtained for a mixed HM/LM pectin gel in the presence of Ca^{2+} and 60% sucrose at pH 3. In this way, the study of mixtures of different pectin may be of great interest in food applications, to obtain products with determined functionalities, without the addition of excess co-solute, just exploiting a possible synergy between both polysaccharides.

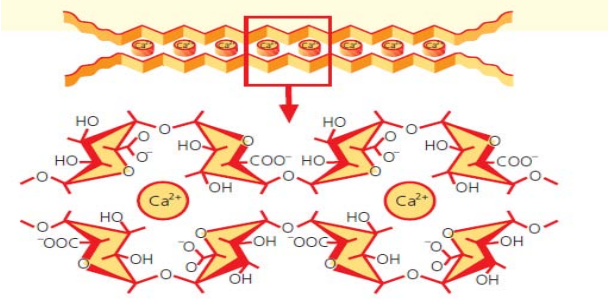

Figure 1.14: “egg box” gelling model

RESEARCH IUSSUES

Pectin used in the food industry to obtain products with desired texture and gelling characteristics, are frequently pectin mixtures. This wide use of pectin mixtures need further studies to better understand the ability to form gels. Actually their characterization is made only with DE. But it is known that different gels are obtained with pectins having the same DE and similar gels with pectins having different DE. For these reasons we considered necessary to characterize the pectins used to form gels by measuring also the hydrodynamic molecular weight and by studying the compatibility of pectin mixtures.

The compatibility of polymers can be measured experimentally by Fourier transform infrared spectroscopy (FTIR), polarized optical micrograph (POM, scanning electron microscopy (SEM), viscometry and thermogravimetry analysis (TGA), light scattering, size esclusion chromatography (SEC) and others.

Although the viscometry requires no expansive equipment, it is sensitive; viz. the viscosity method can accurately reflect the change of compatibility of two polymers with different molecular weight of corresponding constituent polymers. In recent years, considerable attention has been devoted to using the dilute-solution viscosity (DSV) measurement as a technique to predict polymer-polymer

compatibility. The effectiveness of the viscometric method rely on the assumption that mutual interactions of macromolecules (polymer-polymer interactions) in solution have a great influence on the viscosity in ternary system (polymer A – polymer B – solvent). Since the two polymers are dissolved in the common solvent and their hydrodynamic volume and configuration are greatly affected by the solvent selected, polymer – solvent interactions may also play a key role in characterizing the viscosity behavior in ternary systems to which little attention has been paid. In general, the viscosity measurement of ternary system can be performed by two techniques:

- the viscosity measurement of ternary system can be determined either by putting the polymers A and B with certain weight ratio in a pure solvent
- the viscosity measurement of ternary system can be determined either by putting the polymer A (referred to as guest or probe polymer) in solution in which the polymer B (referred to as host or matrix polymer) is found at a constant concentration. This later technique, referred to as the method of polymer solvent (Tewari, 1992)

Solution viscosity properties

Solution viscosity allows to obtain a measure of the size of polymer molecules and hereupon can be used as an empirical measure of molecular weight. A change in viscosity of a solution is due to the introduction of relatively large solute molecules into an environment of smaller solvent molecules. To test the effect that causes a biopolymer solute when it is introduced into a solvent it is first necessary to measure the viscosity of the solvent alone by determining the force required to produce a given rate of shear. The measurement of the viscosity after the addition of solute particles indicates that a greater force is required to maintain the same shear rate, i.e., there is an increase in viscosity.

The ratio of the two viscosities yields:

$$\eta / \eta_s = 1 + \nu\phi \qquad (1)$$

Where η is solution viscosity, η_s is solvent viscosity, ϕ is the volume fraction of the solute molecules and ν is a numerical constant whose value has been previously calculated for various shapes of macromolecules.

The greater force required for deformation is due to the smaller volume of fluid in which the overall deformation must take place as the solute particles have limited deformation yet they occupy a fraction of the total volume of the fluid (hence the relationship between viscosity and volume fraction of solute). This applies only to dilute solutions where the solute particles are considered as individual moieties and does not take into account concentration effects and solute-solute interactions.

The ratio of solution viscosity to solvent viscosity is called the relative viscosity, η_{rel}

$$\eta_{rel} = \eta / \eta_s \qquad (2)$$

It is more convenient to express data in terms of the specific viscosity where unity is subtracted from relative viscosity:

$$\eta_{sp} = \eta_{rel} - 1 = (\eta - \eta_s) / \eta_s \qquad (3)$$

This is a measure of the fractional change in viscosity produced by adding the solute. Considering a macromolecular solution to be equivalent to a suspension of particles within the solvent, it is obviously proportional to the concentration of the solute *C*. The ratio gives the reduced viscosity, η_{red};

$$\eta_{red} = \eta_{sp} / C \qquad (4)$$

In order to eliminate the interaction effects and consider each polymer coil being sufficiently distanced from its behavior so as not to interfere with the flow of surrounding polymers, the reduced viscosity is extrapolated to zero concentration. The limit of η_{sp}/C as $c \rightarrow 0$ is called the intrinsic viscosity and depends only upon the properties of isolated molecules.

The unit of the reduced viscosity is defined via the used concentration unit, which is usually [g ml^{-1}] for viscometric measurements. With this, the unit for the reduced viscosity is [ml g^{-1}].

The intrinsic viscosity [η] has the same unit, [ml g^{-1}], as the reduced viscosity η_{red}. For a better understanding, the intrinsic viscosity can be considered as a measure for the volume demand of the single polymer coil in ideally diluted solution. The intrinsic viscosity is proportional to the reciprocal density of the polymer coil in solution and therefore directly related to the polymer dimensions. Determination of the intrinsic viscosity is expressed by the Huggins equation, which yields the intrinsic viscosity via extrapolation of the reduced viscosity to zero:

$$\eta_{sp}/C = [\eta] + K_H[\eta]^2 C \qquad (5)$$

A plot of η_{sp}/C versus polymer concentration yields a straight line, described by the Huggins equation. The slope $K_H[\eta]^2$ describe the intermolecular interactions between polymer and solvent (Figure 1.15). Alternatively, intrinsic viscosity may be obtained by plotting $(\ln \eta_{rel})/C$ versus polymer concentration, yielding a straight line described by the Kraemer equation.

$$(\ln \eta_{rel})/c = [\eta] + k_K [\eta]^2 C \qquad (6)$$

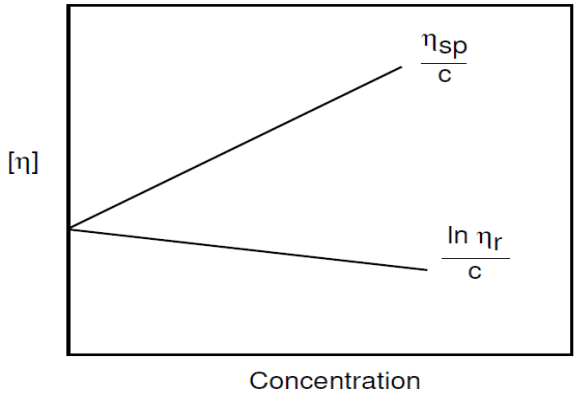

Figure 1.15 Typical plots of η_{sp}/C and $\ln \eta_r/C$ as a function of concentration. The curves extrapolate to the same [η] at zero concentration, but approach zero concentration with different slopes.

The intrinsic viscosity [η] represents the most relevant variable to describe the viscous behavior of a polymer solution and most viscometric measurements have the aim of its determination. The knowledge of the dimensions of a single polymer coil allows for the calculation of the solution volume filled with polymer. A matter of particular interest is the polymer concentration where the solution is completely filled with polymer coils and the coils start to interpenetrate as shown in Fig. 1.16.

This concentration is denoted as the critical concentration c*. The critical concentration marks the transition from a dilute to a semi-concentrated solution. This transition is accompanied by great changes in the flow properties of a polymer solution. At concentrations above c* the flow behavior is dominated by the intermolecular interactions of the polymer coils whereas below c* mainly the polymer-solvent interactions determine the flow properties.

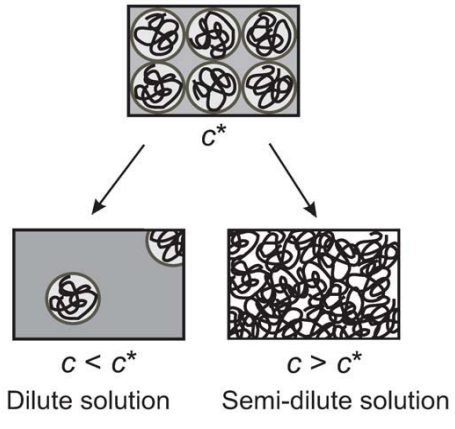

Figure 1.16 Definition of the critical concentration c*.

[η]-Molecolar weight relationship

The intrinsic viscosity [η] of a polymer in a certain solvent can also be empirically correlated with the molar mass M:

$$[\eta] = K\,M^{a} \qquad (7)$$

In the literature, this dependence is referred to as the [η]-M-relationship or the Mark-Houwink Sakurada-relationship (KMHS-relationship). *K* and *a* are constant for a given solvent and temperature. The exponent *a* is a measure for the solvent quality and therefore for the solution structure of the dissolved polymer. Values of *a* are typically between 0.5 and 0.8. Close to 0.5 represents near theta solvent conditions. Farther away from 0.5 represents large deviations from a theta solvent. The knowledge of K and *a* allows for an easy determination of the molar mass of a polymer by measuring the intrinsic viscosity. The determination of the molar mass from Eq. 9 yields the viscosity average molar mass M_η.

Criteria of polymer-polymer compatibility

Methods for the experimental study of polymer compatibility are numerous and very diverse, and may be divided into several main groups:

- methods based on the determination of optical homogeneity of the mixture
- methods for the determination of glass transition temperatures
- methods for the direct determination of interactions on molecular level
- indirect methods for the miscibility estimation

The viscometric method, based on the study of the interactions in diluted solutions of two polymers in a common solvent is one of the indirect methods. Although the method is conceptually and experimentally very simple it is relatively seldom applied and provides information about polymer-polymer interactions and polymer-solvent interactions in solution. A lot of work has been reported on systems involving neutral or uncharged polymers in organic solvents, complex mixtures of polymers, neutral and water-soluble polymers in water and mixtures of polyanions in aqueous solvents (Kavlak, 2004). In this study, different commercial pectin (HM and LM) were selected to examine their compatibility.

Based on the fact that within liquid medium the attractive interactions causes expansion of polymer chains while the repulsive interactions lead to the shrinkage of polymer coils, several models were proposed to investigate the polymer-polymer compatibility (Mbareck, 2008). Krigbaum and Wall model permits the estimation of polymer compatibility by the comparison of the experimental and theoretical values of polymer-polymer interaction parameter. According to this model many criteria come Δb, $\Delta[\eta]$, thermodynamic parameters α and β ,were proposed to determined polymer-polymer compatibility by viscometry, (Wanchoo, 2003; Lewandowska, K., 2005; Aroguz, 2006).

The aim of this thesis was to utilize these criteria and study the compatibility behavior of pectins in different compositions and with different DE using a dilute solution viscometric method. This study permitted to find some thermodynamic and hydrodynamic parameters of pectins mixtures in aqueous solutions and to explain the compatibility in terms of these parameters.

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CHAPTER 2

EFFECT OF WATER ADDITION ON PECTIN RECOVERY FROM SOLUTION IN CENTRIFUGAL SEPARATION PROCESS

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Abstract

Pectin is extracted from plant residues frequently available as by-product of economically more valuable food processing and is widely used for different applications. In industrial process, separation of the hydrolyzed pectin solution is performed by centrifugal and filtration technologies. In this paper, the effect of water addition to the hydrolyzed citrus peels slurry on the separation efficiency was investigated, with the aim to improve pectin recovery. Different dilution ratios were tested and it was found that the pectin recovery increases when increasing water addition passing through a maximum value. No relevant effects on pH and pectin yield were found when increasing the contact time between water and hydrolyzed slurry, confirming that no further progress of hydrolysis or extraction processes is caused by the dilution. Therefore, the increase in pectin recovery can be attributed only to the water addition.

Keywords Centrifugal separation, dilution, efficiency maximization, pectin recovery.

INTRODUCTION

Pectin is a group of polysaccharides extracted from the cell wall of many fruits and plants. Production and characterization processes are well reported (Voragen et al., 1995; Fishman, 2000) because of the wide range of applications ranging from food to pharmaceuticals. Common steps of the production process are (May, 1990) extraction of pectin by hydrolyzing the organic material (usually using mineral acid), separation of pectin solution from the resulting slurry and pectin precipitation and purification. Different extraction procedures have been proposed on laboratory scale, depending upon the type of pectin source and expected characteristics of the final product (Sun & Hughes, 1998; Yeoh et al., 2008; Seggiani et al., 2009).

Despite its impact on efficiency of pectin recovery, solid–liquid separation of the hydrolyzed slurry after the extraction step has not been carefully investigated. Separation of the solution from the exhaust solid on laboratory-scale test has been reported in the literature. It usually involves, in variable order, a filtration (either with cloth or under vacuum) and a centrifugation step. Shi et al. (1996) used a double layer of cheesecloth for the filtration of a prewashed slurry. Cho et al. (2003) filtered through cheesecloth before centrifuging and then filtering under vacuum the clarified suspension. Sing- thong et al. (2005) used a vacuum filter in series with a centrifuge step. Seggiani et al. (2009) used a Buchner filter thermo stated at 70 °C with a diatomaceous earth precoat, followed by a press. Joye & Luzio (2000) used a large vacuum crock, equipped with nylon filter cloth and a rubber diaphragm to press the cake under vacuum. This set-up allowed simulation of the action of a rotary drum vacuum filter, typically used in industrial pectin extraction. On the other hand, many authors have focused on pectin extraction from different substrates using different processes by centrifugation (with wide range of rotational speed and time) or a single step of filtration of the hydrolyzed slurry (Kar & Arslan,

1999; Kalapathy & Proctor, 2001; Thomas et al., 2003; Iglesias & Lozano, 2004; Fishman et al., 2006; Kurita et al., 2008; Sirisakulwat et al., 2008; Yapo, 2009).

In industrial applications, before the separation of pectin solution, the pH is increased above three to promote pectin extraction (Seggiani et al., 2009) and to stabilize the slurry against further hydrolysis (Yapo, 2009). The suspension containing solubilised pectin and colloidal particles attached to the solid residual material is then fed through a separation scheme that may differs from process to process. A typical sequence of industrial separations is described in the following. The slurry is feed through a series of a decanter and a centrifuge separating the liquid fraction from the exhausted solid (with an average moisture higher than 80%). After this step, the solution is treated through a filter press, generally using diatomaceous earth as filter aid, and separating the clarified solution from a waste colloidal solid suspension. This sequence of separation operation is very important for efficient production process and, because of the colloidal nature of the solubilised pectin; the separation of the polymeric solution cannot be treated as a classical solid–liquid extraction. In fact, during separation, pectin has to move out from the porous cellulosic matrix by a complex transport mechanism also affected by the centrifugal force. The mass transfer is promoted by concentration difference and it may be enhanced by decreasing the pectin bulk concentration and positively affecting the process yield. In addition, it is well known that pectin in aqueous solution forms aggregates that can affect solution viscosity and consequently the mobility of the molecules (Fishman et al., 2001). Fishman et al. (2007) demonstrated that the transition concentration from diluted to aggregate macromolecules holds above 10 $\mu g\ mL^{-1}$, much lower than that of an industrial process (order of 10 $mg\ mL^{-1}$). Nevertheless, it is known that pectin behaves as flexible chains (Morris et al., 2008) and hydrodynamic properties can be significantly affected by the degree of methyl-esterification more than molecular weight (Morris et al., 2000). Therefore, with the aim of increasing the mass transfer from the solid core to the solution bulk

during the separation step, the addition of water to the slurry before centrifugation could dilute pectin aggregates and improve hydrodynamic properties of pectin molecular chains. In this paper, this effect was studied for improved recovery of pectin in a laboratory- scale process. Hydrolyzed suspension of citrus peel was separated in a laboratory-scale centrifuge at different levels of water dilution. Pectin was precipitated by using ethanol, and process efficiency was calculated as recovery of pectin per unit of treated hydrolyzed suspension.

MATERIALS AND METHODS

Extraction from citrus peels

Citrus peels (essential-oils free) from dried lemon peels, obtained from Tucuman (North Argentina), were used as raw material; 130 g of dried peels (moisture 12% in weight) was mixed at 70 _C with 1950 mL of distilled water, and 33 mL of hydrochloric acid (33%⁄w) was added. The pH of the suspension was 1.5 ± 0.2 and the hydrolysis was carried out for 4 h. The hydrolyzed slurry was then treated with 160 mL of sodium carbonate (Na_2CO_3 9.0%w⁄v) and 1950 mL of distilled water (Seggiani et al., 2009). The resulting slurry was left at 65 °C under continuous stirring at 375 rpm (Heidolph mod RGL 100; Germany) for 4 h, and the final pH was in the range 2.5–3.0.

Pectin recovery and determination

After the extraction, the suspension was kept in a temperature controlled bath (Julabo) at 65 °C, continuously stirred to avoid slurry sedimentation. Pectin suspension was separated from the exhausted solid using a laboratory-scale centrifuge (Eppendorf mod.5810, Germany) equipped with a rotor A-4-62. Different process parameters were prescreened:

• Centrifugation time: 5, 10 and 15 min.

• Centrifugation rotational speed: 1500, 2500, 3500 rpm, equivalent to 453, 1258 and 2465 g, respectively.

• Samples mass: 40, 50 and 60 g.

The effect of dilution was investigated by adding water at 10%, 20% and 30% (w⁄w) of the native suspension. After centrifugation, the weight of both the resulting phases, the supernatant pectin solution and the exhausted solid, was measured using a laboratory balance (Mettler Toledo, Germany, mod. PG403-s). Pectin was precipitated from the supernatant solution by adding double volume of ethyl alcohol under vigorous stirring. The insoluble pectin fraction was separated by using a vacuum water pump and then dried in a Binder (USA) oven at 55 °C for 18 h to obtain dry pectin. Galacturonic acid content was determined according to the procedure recommended by the Joint FAO⁄WHO Expert Committee on Food Additives (JECFA, 2009): 5 g of sample was stirred for 10 min with a mixture of hydrochloric acid and ethanol (60% v⁄v), then filtered and washed with ethanol until the filtrate is free of chlorides. The obtained material is dried in oven at 105 °C for 2.5 h and one-tenth of it (m_0 weight) is mixed with 100 mL of distilled water, then 5 drops of phenolphthalein are added and the obtained solution is titrated with 0.1 m sodium hydroxide and the result is recorded as initial titre (V1). Afterwards, 20 mol of 0.5 M sodium hydroxide and (after 15 min rest) 20 mL of 0.5 M hydrochloric acid were added, and the mixture is shaken until the pink colour disappears; finally, the system is titrated with 0.1 M sodium hydroxide to a pink colour persisting after vigorous shaking, and the obtained value is recorded as saponification titre (V_2). For non amidated citrus pectin, the galacturonic acid content is determined as weight per cent on a moisture-free basis:

$$GC = 19.41 \cdot 10^3 \cdot (V_1 + V_2)/m_0 \quad (1)$$

where m_0 is the weight of the washed and dried sample used for the titration procedure. The degree of esterification (as % of total carboxyl groups) can be determined as

$$DE = 100\ V_2/(V_1+V) \quad (2)$$

Any preparation was independently repeated three times, and data showed throughout the paper are the arithmetic mean ± standard deviation.

Centrifugation: nomenclature

Figure 1 presents a scheme of the laboratory-scale centrifugation process.

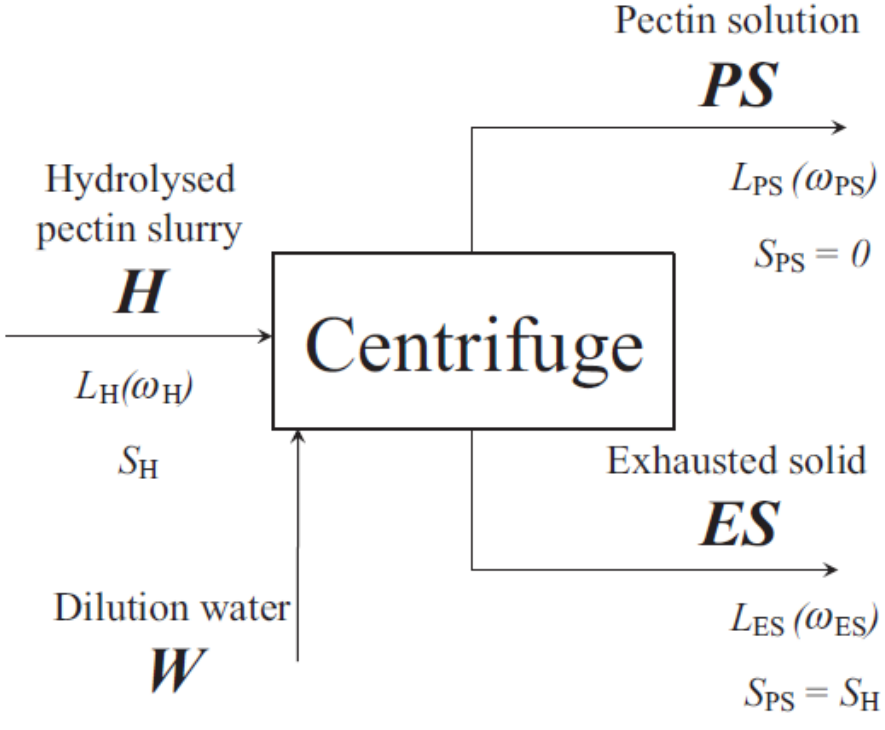

Figure 1 Laboratory-Scale centrifugation scheme

The main process feed is the hydrolyzed suspension H, containing the exhausted solid SH and the pectin solution LH (solute fraction ω_H including all soluble compounds). When added, the dilution water is W and two streams came out from the process: the supernatant pectin solution PS (solid fraction ω_{PS}) and the exhausted solid ES. It is assumed that ES contains all the solid of the inlet suspension (SES = SH) and a part of pectin solution LES is entrapped with the same solute content of the liquid outlet ($\omega_{ES} = \omega_{PS}$).

Centrifugation process relevant parameters

When dealing with solid–liquid separation, the attention is usually focused on the solid recovery (Rousseau, 1987; Svarovsky, 1990) and the definition of recovery and separation efficiency are referred to the solid fraction. In our case, the main process stream is the liquid part of the slurry; therefore, the classic efficiency definition can be modified with reference to the recovery of the liquid fraction. The separation yield ε is, then, defined as the ratio between the outcoming liquid stream LPS and the overall process inlet:

$$\varepsilon = LPS/ (H + W) \quad (3)$$

To analyze the effect of dilution on the overall amount of supernatant, the ratio ω can be defined as:

$$\omega(W) = L_{PS}(W\neq0)/ L_{PS}(W=0) \quad (4)$$

This parameter quantifies the increase of the liquid stream LPS as a function of dilution, in comparison with the process without water addition. In addition, the separation efficiency gE can be calculated as the ratio between the liquid stream coming out and the liquid feed of the process that for diluted solutions leads to the following:

$$\eta_E = L_{PS}/ (L_H + W) \quad (5)$$

This definition clearly establishes the fraction of liquid recovered into the supernatant, with respect to the total amount of liquid feed to the centrifuge, including the dilution water W. In principle, it would be more interesting to verify the dilution effect in terms of recovery of liquid solution coming from the hydrolyzed feed H (where pectin are solubilized). Therefore, depending on the water stream W and for a constant hydrolyzed amount H, a liquid recovery efficiency gR can be defined as:

$$\eta_R (W) = L_{PS} (W\neq0) / (L_{PS} (W=0)+W) \quad (6)$$

Where L_{PS} (W≠0) is the liquid stream recovered when dilution water W is added and L_{PS} (W =0) the

same stream recovered without dilution. In case of $\eta_R < 1$, the amount of recovered liquid, in presence of dilution, is lower than the sum of the non diluted liquid stream and dilution water W. This implies a negative effect on the recovery of LH on adding dilution water. On the contrary, when $\eta_R > 1$ the recovered liquid L_{PS} is increased with respect to the sum of the same stream without dilution and the dilution water. This means that water dilution increases the recovery of liquid from hydrolyzed H into the liquid stream LPS.

RESULTS AND DISCUSSION

Effect of sample mass

Before proceeding with the investigation of the dilution effect, some preliminary evaluations were performed on the centrifugation process. To investigate if there was an effect of the centrifuged mass on the separation yield ε, tests with 40–60 g of the sample were conducted. ε did not depend on the treated mass and therefore, it was decided to keep 60 g of hydrolyzed slurry H throughout the study (data not given).

Effect of centrifugation time and rotational speed

With a constant H value, the effect of centrifugation time and speed was preliminarily investigated. From now on, lines shown into the charts are a guide for the reader and not result of modeling or interpolation. In Fig. 2, values of centrifugation yield are reported at different time and speed. Centrifugation time did not affect the process performance in the investigated time range and hence a centered value of 10 min was set in the rest of the experimental plan. On the contrary, the rotational speed of the centrifuge was positively correlated to the separation efficiency. Therefore, all the tests were performed at all the selected rotational speed.

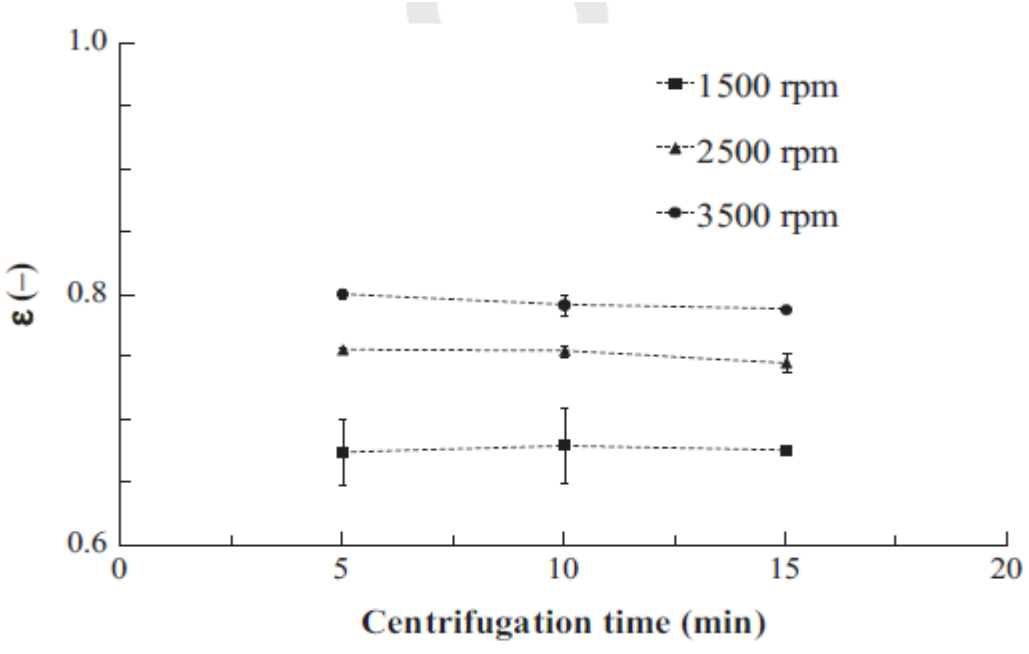

Figure 2 Centrifugation yield as a function of time and rotational speed.

Effect of dilution on supernatant amount and concentration

Figure 3 reports x as a function of the dilution for 60 g amount of hydrolyzed H. It clearly appears that the mass of supernatant increases up to 40% when increasing the dilution fraction and this factor has to be considered in an industrial scale up because of the higher separation cost on increasing the amount of feed liquid.

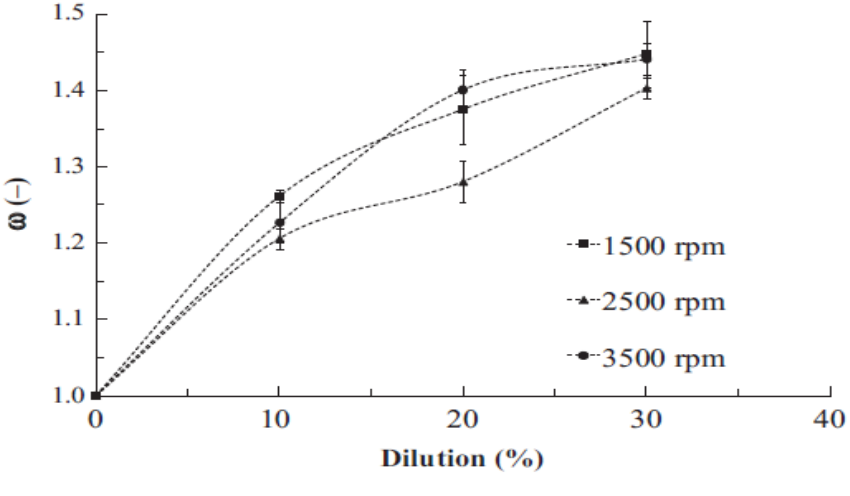

Figure 3 Liquid stream ratio as a function of the dilution.

In addition, ε was revealed to be similar for rotational speed. Figure 4 reports another important parameter to be considered when dealing with separation costs: the concentration of pectin in the supernatant L_{SP}.

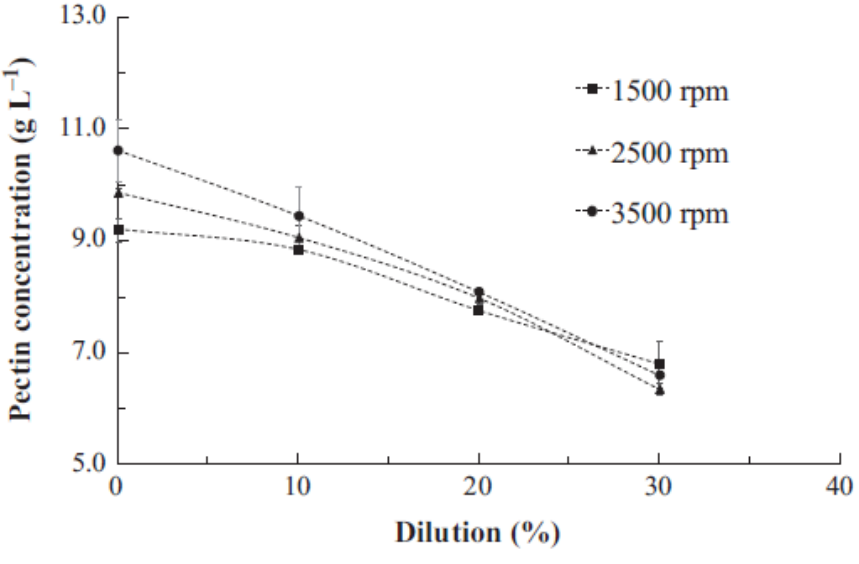

Figure 4 Pectin concentration as a function of the dilution.

Process efficiency evaluation

Table 1 reports the separation efficiency (η_E) at different dilution degree and centrifugation speed.

	Rotational speed (rpm)		
Dilution (%)	1500	2500	3500
0	0.68 ± 0.03	0.75 ± 0.04	0.76 ± 0.04
10	0.78 ± 0.04	0.82 ± 0.04	0.84 ± 0.04
20	0.78 ± 0.04	0.80 ± 0.04	0.88 ± 0.04
30	0.75 ± 0.04	0.81 ± 0.04	0.84 ± 0.04

Table 1 Separation efficiency (η_E) at different dilution and centrifugation speed

η_E increases as the added water amount is augmented and this effect is repeated at the same level when increasing the rotational speed of the centrifuge. Therefore, these data confirm that it is possible to improve the overall process separation efficiency by the dilution level. Moreover, the real objective of the process is to improve the pectin recovery and this is achieved if it improves the amount of liquid LH recovered from the native suspension H. Table 2 shows the value of the liquid recovery efficiency as a function of the dilution.

	Rotational speed (rpm)		
Dilution (%)	1500	2500	3500
10	1.10	1.06	1.08
20	1.06	1.01	1.10
30	1.00	1.00	1.03

Table 2 Liquid recovery efficiency (η_R) at different dilution and centrifugation speed

It is noteworthy that for dilution of 10% and 20% η_R increased by about 10% with respect to the unity value, revealing that it is possible to extract more pectin solution from the hydrolyzed H, in comparison with the undiluted extraction process. In addition, data revealed that this favorable effect becomes negligible if dilution is increased up to 30%. Therefore, the optimum region of dilution up to 20% of the native hydrolyzed material is another relevant evidence of the proposed analysis.

Pectin recovery

From the calculated efficiencies, it clearly appears that the addition of water improves the process performance and this trend should be quantified in terms of pectin overall recovery. Figure 5 reports the mass of recovered pectin normalized with respect to the inlet hydrolyzed H. It clearly appears the increase in pectin recovery, with a maximum located in a dilution percentage lower than 20%, at any

investigated centrifugation speed. For higher dilution values, a drop in process efficiency is found as a consequence of the prevailing effect of pectin concentration dilution to the liquid recovery efficiency; η_R drops back to one at 30% dilution.

Galacturonic acid content was determined and constant value equal to 75.9% was obtained, for any of the investigated centrifugation and dilution conditions, whilst a 57.5% degree of esterification was found. This confirms that different conditions are affecting only process efficiency and not the quality of extracted pectin.

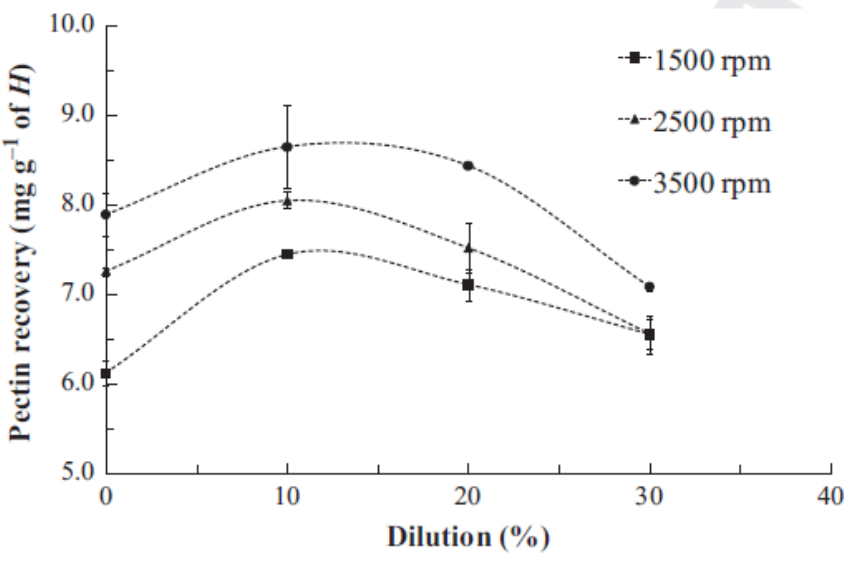

Figure 5 Pectin recovery as a function of the dilution.

The galacturonic acid content, higher than 65%, also respects the requirements for definition of pectin used as food additives (May, 1990; JECFA, 2009).

Effect of dilution-water contact time

To check if the observed g_R increase was a trivial consequence of water addition effect on the hydrolysis/extraction chemical process, water was added to the hydrolyzed slurry and left for 24 h. Over this period of time, samples were collected and separation procedure was performed and extraction efficiency calculated; 80 mL of water was added to the relative amount of hydrolyzed slurry

to give a 10% dilution sample as used in the previous experimental plan. Using the same experimental set-up described in the Materials and Methods section, the suspension was stirred and conditioned at 65 °C for 24 h, and samples were analyzed straightaway after the addition (normal test condition) and then after 2.5, 5, 7.5 and 24 h. To verify the progression of hydrolysis and extraction reactions, pH was measured before proceeding to the separation and pectin recovery procedure (centrifugation time 10 min; rotational speed). Data shown in Fig. 6 clearly indicate that neither pH nor pectin recovery was changed on increasing the contact time with dilution water. This confirms the chemical stability of the used hydrolyzed slurry, excluding that the observed increase in process efficiency is a consequence of chemical reaction's further progression.

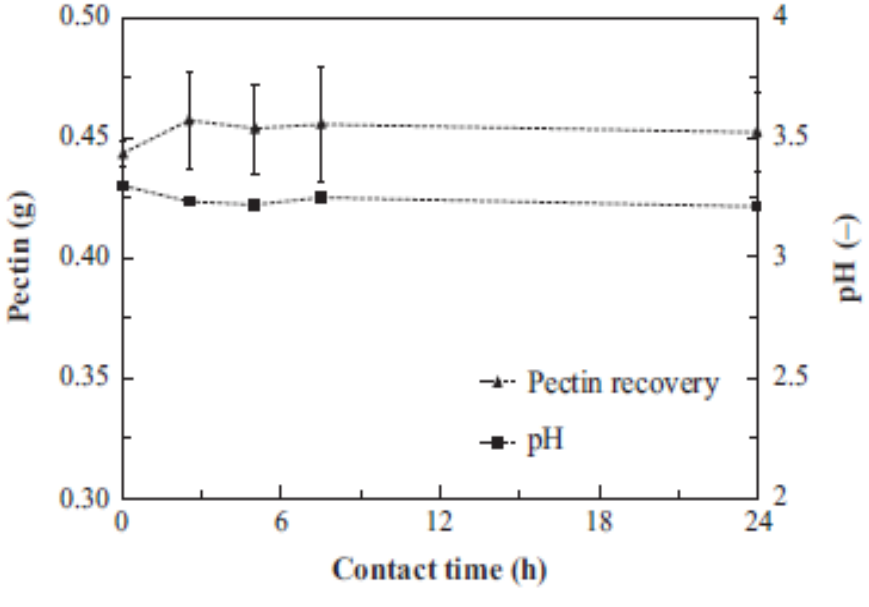

Figure 6 Contact time effect on pH and pectin recovery yield.

CONCLUSIONS

In this paper, the effect of dilution by water addition over the efficiency of the centrifugal separation of pectin solution from hydrolyzed slurry was investigated, to maximize the pectin recovery. The

effect of water addition on recovery of pectin was verified by using a laboratory-scale centrifugal separation process in series with an alcoholic pectin precipitation procedure. Different dilution ratios were used increasing the amount of supernatant liquid extracted from the centrifugation with a lower pectin concentration. As combined result of these opposite factors, the pectin recovery yield (referred to the unity of mass of original citrus peel suspension) improved on addition of water between 10% and 20%. This effect could be attributed to the progress of hydrolysis/extraction reactions, affected by the water addition, but no evidence of process improvement was observed when increasing contact time between dilution water and native pectin suspension. Therefore, it was concluded that the increase in pectin recovery was attributed to the effect of dilution, increasing the amount of extracted polymer.

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CHAPTER 3

COMPATIBILITY ANALYSIS OF PECTIN AT DIFFERENT ESTERIFICATION DEGREE FROM INTRINSIC VISCOSITY DATA OF DILUTED TERNARY SOLUTIONS

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Abstract

This paper aims to set-up a compatibility criterion for couple of pectins at different degree of methoxylation (DM) based on specific viscosity data, slightly modifying a criterion already proposed in the field of synthetic polymers. Four different commercial pectins were preliminarily characterized by using dilute solution viscosity measurements and molecular weight was calculated from intrinsic viscosity data. Four ternary dilute solutions of pectin at different DM were also investigated and two compatibility criteria, based on viscosity data, were applied. Data revealed that positive molecular interactions take place when pectins in solution have a different DM (i.e. HM/LM mixtures), whilst an incompatible behavior was found for similar pectin in solution (i.e. both HM or LM). In addition, in the case of incompatible pairs of pectin, also the apparent average molecular weight of mixed pectin, obtained by measurements carried out on ternary solutions, showed an increase with respect to the

additive mixing rule, owing to repulsive forces. On the contrary, owing to attractive forces in case of compatible pectin pairs, the apparent molecular weight is lower than the calculated average.

INTRODUCTION

Pectin is one of the most diffused hydrocolloid used in food industry for its capability to control texture and sensory properties by means of the gel characteristics [1]. In this concern, different papers reported on the mechanical characterization of pectin gel prepared including all the compound used in real applications, such as sugar, pH and [Ca^{2+}] buffer solutions and at different temperatures [2,3]. In addition, increase in applications of these polysaccharides is also favored by the opportunity to use mixtures of pectin giving gel properties tailored for specific applications. In this concern, it has been shown in [4] that the gel strength increases when using mixture of high methoxyl (HM) and low methoxyl (LM) pectin, with respect to the gel formed using the same concentration of single pectin. The observed synergistic effect was also a function of the sugar level, showing that storage modulus strongly depends on this factor as well on the overall pectin concentration. Beside this approach, focused of the pectin performance evaluation by investigating the gel properties, a wide range of experimental techniques have been proposed to study the pectin properties based on its hydrodynamic properties in dilute solutions. It is well known that it is possible to estimate the pectin molecular weight from the hydrodynamic volume of a single molecule, by measuring viscosity data of dilute solutions (intrinsic viscosity) [5]. Three main factors affect the intrinsic viscosity value of linear polyelectrolyte in binary solution: molar mass, charge and surface charge and, in the case of pectin, these factors are correlated. Viscosity measurement was also performed to pectin at different degree of methoxylation (DM): even though the authors did not specify the demethoxylation technology (enzymatic or chemical process), Morris and co-workers [6] showed that intrinsic viscosity is sensitive

to pectin chain flexibility as affected by the degree of esterification. On the contrary other authors [7] compared de-methoxylated pectin obtained from either pectin methylesterase enzyme (PME) or alkaline process. Results clearly indicates that intrinsic viscosity was more sensitive to molecular weight variation (more pronounced in the case of chemical process) than to the degree of methoxylation (DM). Also the effect of salt addition on the viscometric behavior of HM and LM pectins solution was investigated [8]. The authors attribute this effect to different intermolecular forces leading to stronger aggregates for LM pectin binary solutions. Fishman and co-workers [9] investigated the effect of the electrolyte type on molecular properties of pectin, suggesting that aggregation takes place with a major extend when using $NaNO_3$ than NaCl or KCl and that this effect depends on the DM, because smaller ions (like Li^+) can better solvate the few polar carboxyl groups. In addition to molecular weight determination and single polysaccharide properties investigation, viscosity measurements can be used to evaluate the compatibility of polymer–polymer mixture in dilute aqueous solution. This procedure is fairly common for synthetic polymers and different compatibility criteria have been proposed and applied, based on the Huggins plot analysis [10, 11]. In a less extensive way, the approach of ternary mixtures analysis has been recently applied also to biopolymers mixtures in order to investigate biopolymer interactions, even though the application of any compatibility criterion is reported in the open literature. The molecular interactions of xanthan gum and waxy corn starch in a mixed solvent (water/DMSO) was studied [12] as well as the behavior of ternary aqueous solutions of xanthan gum and locust bean gum [13]. Results indicated that, also in the case of biopolymers, the dilute solution viscometry was able to detect the effect of multiple solutes and Huggins parameters can quantitatively estimate the interactions between chains. The analysis of the effect of salt concentration on solute interactions for xanthan/guar system [14] indicates that the salt level can strongly affect the intensity of the synergistic behaviour of polysaccharides in terms of

mixture intrinsic viscosity. Recently a model to calculate the viscosity of ternary dilute solutions of some biopolymers, based on the excess properties, has been proposed [15]. Despite this approach was confirmed as suitable to investigate the interactions between biopolymers, no results have been obtained so far for pectin mixtures. This paper attempts to cover some of this aspects, reporting on the viscometric study of solution of pectin at different DM. Viscosity of ternary solutions of different pectins and for different relative amount, was measured at different concentration and date were used to investigate the polymer/polymer compatibility and interactions, as a function of pectin characteristics.

MATERIALS AND METHODS

Materials

Four different pectins from Silva Extract S.r.L., (Rende, Italy) have been used in this work: two at high (HM-1, HM-2) and two at low degree of esterification (LM-1, LM-2) as reported in Table1. Samples were prepared by thoroughly dispersing pectin in 0.1 M NaCl solution, prepared with water from reverse osmosis [14]. Diluted solutions of ternary systems (indicated as HM-1/LM-1, HM-1/HM-2, LM-1/LM-2, and HM-2/LM-2) were prepared at different pectins molar ratios (1:3-1:1-3:1). In order to measure viscosity as a function of the solution concentration, either binary or ternary solutions were prepared at nominal concentration of solute(s) ranging from 0.08 to 0.16 (g/dl). After preparation, samples were continuously stirred at room temperature for 4 h before measurement.

Densities of solutions were also measured using an analytic balance equipped with a dedicated kit (Crystal 500, Gibertini Italy).

Table 1
Capillary viscometry characteristics for pectins binary mixtures and apparent molecular weight from Huggins (H) and Kraemer (K) equations.

Pectin	DM (%)	Molecular weight (Da)	K_H (–)	K_K (–)
HM-1	54.2 ± 2.7	52,689 ± 12 (H) 51,616 ± 4 (K)	0.112 ± 0.007	−0.289 ± 0.007
HM-2	60.6 ± 2.0	42,389 ± 32 (H) 43,981 ± 3 (K)	0.770 ± 0.016	0.057 ± 0.003
LM-1	39.6 ± 3.0	62,143 ± 74 (H) 61,776 ± 40 (K)	0.275 ± 0.022	−0.193 ± 0.014
LM-2	42.7 ± 2.1	52,729 ± 106 (H) 56,823 ± 12 (K)	0.997 ± 0.027	0.113 ± 0.005

Viscosity measurements

Viscosity measurements were performed with a Cannon Fenske- type capillary viscometer ASTM n.25 (Carlo Erba, Italy) and the flow time was measured with an accuracy of 1 s. Viscometer was immersed in a thermostatic water bath, under precise temperature control, at 30.0 ± 0.1 °C (Julabo, USA). After the sample loading into the viscometer, the solution was allowed to equilibrate at the bath temperature before starting the experiment. Any of the samples was prepared and independently measured three times.

Data analysis

For any concentration, the kinematic viscosity was measured and the dynamic one was obtained by multiplying the value by the appropriate density value. Relative viscosity η_{rel} can be calculated as the ratio of solution and solvent viscosity as [5]:

$$\eta_{rel} = \eta / \eta_s \quad (1)$$

Whilst specific viscosity η_{sp} and intrinsic viscosity $[\eta]$ are defined respectively as:

$$\eta_{sp} = \eta_{rel} - 1 = (\eta - \eta_s) / \eta_s \quad (2)$$

$$[\eta] = \lim_{C \to 0} \frac{\eta_{sp}}{c} \quad (3)$$

where C is the solute concentration (g ml^{-1}). The intrinsic viscosity [g] can be calculated by extrapolating the value of specific viscosity at zero solute concentration. In order to guarantee the dilute solution regime, specific viscosity range has to be defined. Two different ranges for specific viscosity were proposed in the open literature: values between 1.1 and 1.5 [16] or between 1.2 and 2 [17]. In the present work, measurements were performed by keeping viscosity in the range suggested in [16]. If the plot of specific viscosity versus concentration shows a linear trend, the Huggins equation can be used to calculate intrinsic viscosity from the intercept and the Huggins parameter K_H from the line slope [8]:

$$\eta_{sp}/C = [\eta] + K_H[\eta]^2 C \quad (4)$$

The intrinsic viscosity is related to the specific volume of a single macromolecule in the dilute solution and the Huggins constant is an indicator of the interactions between solvent molecules and the macromolecular species in solution [18]. An alternative way to calculate intrinsic viscosity is the so called Kramer plot, where a handled value of relative viscosity is plot versus solution concentration [11]:

$$(\ln \eta_{rel})/c = [\eta] + k_K [\eta]^2 C \quad (5)$$

Also in this plot, in presence of a linear trend, intrinsic viscosity is the intercept and the Kraemer parameter K_K can be calculated from the line slope with the same physical meaning as the Huggins parameter [6, 13, 19].

Molecular weight calculation

As already mentioned, intrinsic viscosity is a characteristic of macromolecules related directly to their ability to affect flow and indirectly to the molecule size and shape. In case of molecular weight distribution, the relation between intrinsic viscosity and molecular weight allows a quick but reliable estimation of the average value the intrinsic viscosity is related to molecular weight (MW) through the Mark-Houwink-Sakurada equation [5]:

$$[\eta] = K\,M^{a} \tag{6}$$

where K and a are temperature-depending parameters, solute and solvent characteristics. For pectin the following values are available [20]: K = $9.55*10^{-4}$ (g/dl) and a = 0.73 and they were used throughout all the paper.

Compatibility criterion

The Huggins equation for a binary mixture of the i-polymer can be rewritten as [10]:

$$\frac{(\eta_{sp})_i}{C_i} = [\eta]_i + b_{ii} C_i \tag{7}$$

Referring to a mixture of polysaccharides, with an overall concentration Cm (g ml^{-1}) Eq. (7) becomes:

$$\frac{(\eta_{sp})_m}{C_m} = [\eta]_m + b_m \cdot C_m \tag{8}$$

Also in case of polysaccharides mixtures, parameters can be calculated from experimental data. If the multi-component solution follows the rules of an ideal mixture, any property referred can also be calculated as weight-average of the same property of the binary mixtures:

$$\frac{(\eta_{sp})_m}{C_m} = \sum_i \frac{(\eta_{sp})_i}{C_i} \cdot \omega_i \tag{9}$$

where $\omega_i = C_i/C_m$ is the weight fraction of i-polymer. By combining Eqs. (8) and (9), it is possible to calculate multi-component system properties from data of binary solutions:

$$\frac{(\eta_{sp})_m}{C_m} = [\eta]_m^{id} + b_m^{id} \cdot C_m \tag{10}$$

where

$$[\eta]_m^{id} = \sum_i [\eta]_i \cdot \omega_i$$
$$b_m^{id} = \sum_i b_{ii} \cdot \omega_i^2 \tag{11}$$

Comparison of calculated parameter of Eq. (11) and data from experimental points in Eq. (8) can give an idea whether multi-component pectin solutions are close to ideal behavior or not. In addition, in the case of ternary solutions (i.e. two polysaccharides in aqueous solvent), the same parameter can be used to set-up a compatibility criterion for the couple of biopolymers [10] by calculating the differences:

$$\Delta\eta_m = [\eta]_m - [\eta]_m^{id} \tag{12}$$

$$\Delta b_m = b_m - b_m^{id} \tag{13}$$

As suggested in the same paper the compatibility of polymers is defined as the occurrence of attractive molecular interactions and when it holds:

$$Compatibility \quad \begin{cases} \Delta\eta_m < 0 \\ \Delta b_m > 0 \end{cases} \tag{14}$$

On the contrary, the situation of incompatible polymers take place in presence of repulsive interactions between molecules and the condition is reported as:

$$Incompatibility \quad \begin{cases} \Delta\eta_m > 0 \\ \Delta b_m < 0 \end{cases} \tag{15}$$

RESULTS AND DISCUSSION

Binary solutions

The viscosity of binary solutions was measured and both the Huggins and Kramer plot were derived. Fig. 1 shows an example of plot for LM-1 pectin. It can be observed that relative viscosity varied in a linear way within the range suggested in [16] therefore Eqs. (4) and (5) were used to calculate intrinsic viscosity by linear regression (Table Curve 2D, Jandel Sci., USA), and Eq. (6) allowed to calculate the pectin molecular weight. Data in Table 1 shows that molecular weight of pectin was in the range 54–62 kDa and 43–52 kDa, for HM and LM pectin respectively. As already mentioned, results of [7] confirm that molecular weight from intrinsic viscosity data is a reliable estimation of the chain length less dependent on the level of side- functionalisation (degree of esterification). It is also noteworthy

that that no differences were found when comparing MW calculated from Huggins and Kraemer plot, therefore it was decided to use only the Huggins analysis in the analysis of remaining data.

Ternary solutions

Ternary mixtures of investigated pectins were prepared in the molar ratios as fixed in Table 2, by assuming the molecular weight of single pectin from intrinsic viscosity of binary solution. Also in the case of ternary mixtures, dilution guaranteed a relative viscosity within the Billmayer range and, according to Eq. (8), mixtures parameters were calculated using linear regression (Table Curve 2D, Jandel Sci., USA). From binary solution data, ‘‘ideal’’ parameters, as defined in Eq. (11), were calculated for all the samples and data reported in Table 2. If the intrinsic viscosity variation in Eq. (12) is considered, data of Fig. 2 shows that pectin compatibility strongly depends on the pectin degree of methoxylation. In fact, when pectin in the mixtures have the same DM (i.e. both HM or both LM) data analysis clearly indicates that polysaccharides behave as incompatible polymers showing in both cases a positive value of $\Delta\eta_m$. On the contrary in case of solution of pectin with different DM (i.e. HM/LM), the criterion suggest a compatibility of the polysaccharides evidencing how attractive interactions take place between biopolymers chains at different DM. Results of compatibility analysis are confirmed when the curve slope difference (Eq. (13)) is calculated. Also this compatibility criterion (Fig. 3) indicates an incompatible system (positive values of Δb_m) in the case of homologous DM of pectin in solution, whilst compatible behavior was found for both HM–LM solutions.

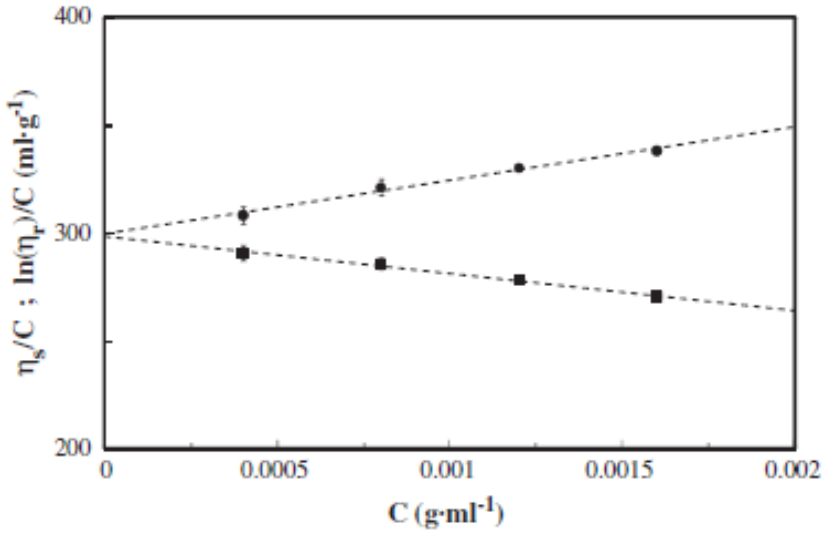

Fig. 1. Huggins (●) and Kraemer (■) equations plot for MW determination (pure pectin LM-1).

Table 2
Huggin's equation parameter for polymer pairs solutions: experimental and data from additive model.

Mixture A/B	Molar ratio	ω_A (-)	$[\eta]_m$ (ml g^{-1})	b_m (ml g^{-1})^2	$[\eta]_m^{id}$ (ml g^{-1})	b_m^{id} (ml g^{-1})^2
HM-1/HM-2	1:3	0.294	249.6	6332	238.5	20,365
	1:1	0.555	257.2	4146	248.9	10,043
	3:1	0.789	266.6	6625	257.6	6695
LM-1/LM-2	1:3	0.282	316.9	16,847	275.5	38,332
	1:1	0.541	336.6	20,620	284.3	22,112
	3:1	0.780	323.3	11,194	292.4	18,479
HM-1/LM-1	1:3	0.220	239.5	56,165	292.3	15,439
	1:1	0.460	207.1	53,816	284.5	8920
	3:1	0.718	236.5	26,196	275.4	6053
HM-2/LM-2	1:3	0.211	223.4	57,820	257.7	45,645
	1:1	0.446	234.3	36,913	248.5	29,540
	3:1	231.1	26,199	238.3	25,842	231.1

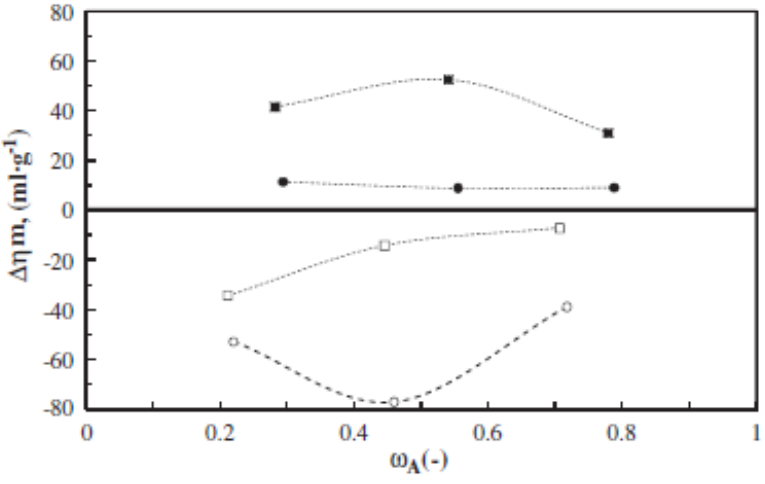

Fig. 2. Plot of $\Delta\eta_m$ for pectin pairs: HM-1/LM-1 (○); HM-2/LM-2 (□); HM-1/HM-2 (●) and LM-1/LM-2 (■).

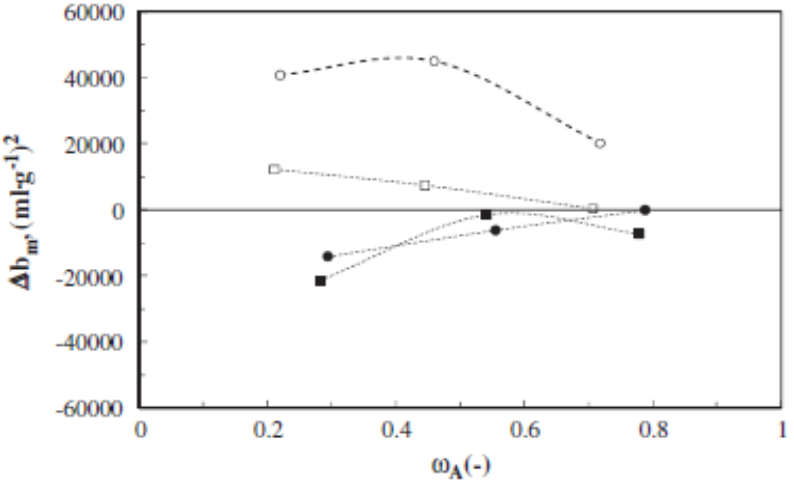

Fig. 3. Plot of Δb_m for compatible polymer pairs: HM-1/LM-1 (○); HM-2/LM-2 (□); HM-1/HM-2 (●) and LM-1/LM-2 (■).

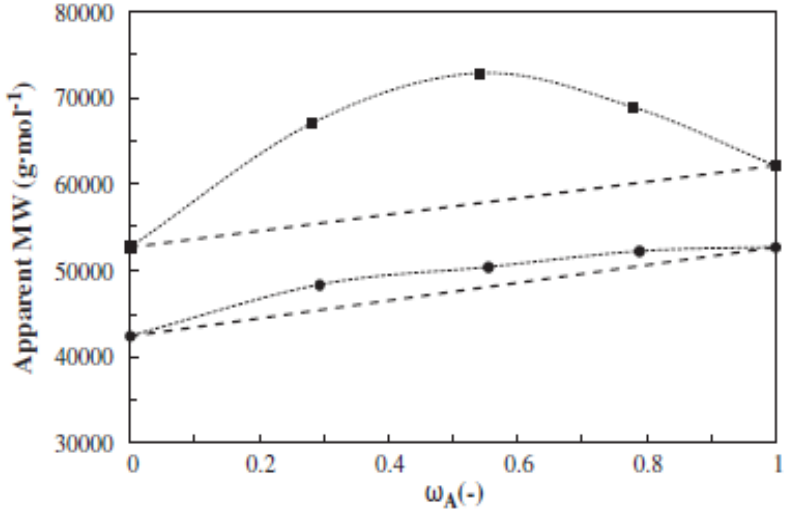

Fig. 4. Plot of apparent molecular weight for incompatible polymer pairs mixtures: HM-1/LM-1 (●) and HM-2/LM-2 (■).

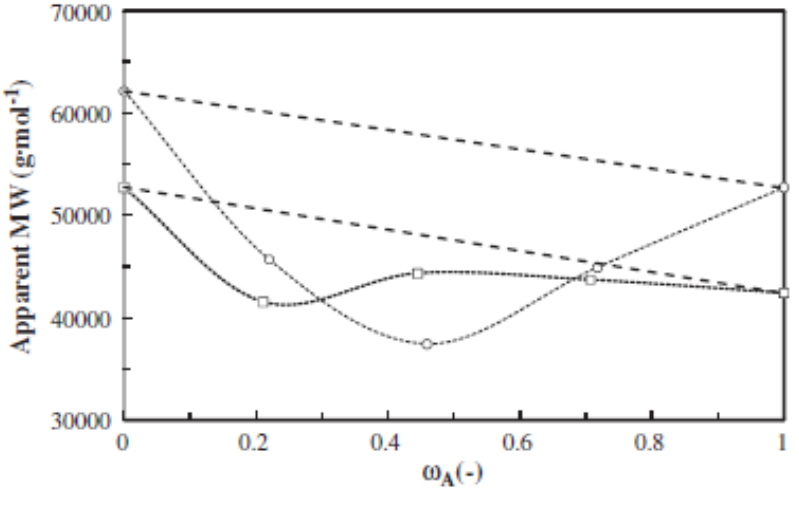

Fig. 5. Plot of apparent molecular weight for compatible polymer pairs mixtures: HM-1/LM-1 (○) and HM-2/LM-2 (□).

It is worthy to notice that results of compatibility criterion do not depend on the molecular weight of the single pectin (Table 1), because in all the investigated ternary systems of Table 2 the difference in the average molecular weight of the two polysaccharides was always around 10 kDa. Despite this constant value the application of both criteria gives different compatibility responses in case of different pairs, according to the rule: compatible if DM is different, incompatible when pectin have a

close DM. Results are also confirmed when calculating the ‘‘apparent’’ molecular weight of the mixtures by using intrinsic viscosity data in Eq. (6). According to literature finding in similar contest [14], molecular weight was calculated from mixtures and data plot versus linear behavior (by assuming no differences in interactions between different chains). Data are divided for homologues mixtures (HM–HM or LM–LM) in Fig. 4 and HL–LM systems in Fig. 5.

It clearly appears that for incompatible pectins, the apparent molecular weight increases with respect to the linear non-interactive behavior. This is in agreement with results of the mentioned authors in case of intermolecular repulsive interactions, causing an increase of the apparent viscosity. On the contrary results confirm that in case of attractive intermolecular forces, such as in Fig. 5, the apparent molecular weight decreases for compatible biopolymer pairs.

CONCLUSIONS

This paper deals with compatibility analysis of pectin based on dilute aqueous solutions viscosity measurements. Simple analysis of specific viscosity was performed for binary and ternary solutions, prepared mixing either homologous (both high or low DM) or different (as DM) pectin pairs. Analysis of intrinsic viscosity for binary solution allowed the calculation of the average pectin molecular weight, that it was confirmed to be fairly independent on the DM [7]. For ternary solutions, for the first time in the case of pectin, different compatibility criteria were applied. Based on the difference between the linear (ideal) mixture parameter and the measured one, it was possible to evaluate the intramolecular interactions between pectin at different DM. All the applied criteria showed that repulsive interactions hold for pectin having close DM, whilst in the case of mixture of pectin at different esterification degree, attractive behavior was found. This result is quite interesting because it indicates that, whilst no dependence on pectin DM can be detected from binary solutions data, ternary mixtures behaviour is strongly dependent on the pectin characteristic as esterification degree.

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CHAPTER 4

COMPATIBILITY CRITERIA COMPARISON FOR SOLUTION OF PECTIN AD DIFFERENT ESTERIFICATION DEGREE

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Abstract

This paper deals to the compatibility of pectin at different degree of methoxylation (DM) in aqueous solutions. Different criteria are available, derived for synthetic polymers and based on specific viscosity data. Differences between criteria come from the type of used solutions. Two types of diluted solutions are used: ternary solutions, including solvent and two pectins and the "polymer-solvent" method. This considers the effect of the addition of a second polymer on the viscosity of an aqueous binary solution of another polymer. Both the methods are tested in order to predict the bio-polymer compatibility. Solution of pectin at different DM (HM/LM mixture), were prepared by measuring dynamic viscosity and the compatibility criteria where applied. Results of both criteria agreed in finding positive interaction as the pectin in solution have a different DM. In addition also the strength of the interaction was calculated.

Keywords: Compatibility criterion, intrinsic viscosity, pectin, "polymer-solvent" method.

INTRODUCTION

In literature, the compatibility of two polymers is evaluated considering the presence and the strength between chains of different polymers of specific interactions, mainly due to hydrogen bonds, Van der Waals forces and electrostatic interactions. Referring to pectin as a particular class of bio-polymers, it is possible to mix different pectin to obtain gel properties tailored for specific applications. It has been shown (Löfgren & Hermansson, 2007) that the gel strength increases when using mixture of high methoxyl (HM) and low methoxyl (LM) pectin, with respect to the gel formed using the same concentration of a single pectin. The observed synergistic effect was also a function of the sugar level, showing that storage modulus strongly depends on this factor as well on the overall pectin concentration. Beside this approach, focused of the pectin performance evaluation by investigating the gel properties, it is useful to set-up experimental techniques to predict the pectin interactions on the base of hydrodynamic properties in dilute solutions. In polymer interaction evaluation, the viscosity of binary and ternary diluted solution is a relevant physical properties in polymer research and engineering (Novak., 2003). In dilute solutions the volume associated with each polymer coil is referred to a single molecule of polymer surrounded by a large amount of solvent. This hydrodynamic volume depends upon the polymer molecular weight and its thermodynamic interaction with the solvent. Favourable polymer-solvent interactions increase the polymer coil hydrodynamic volume whilst the polymer coil volume decreases as the polymer-solvent interactions are unfavourable. Polymer-solvent interactions may depend upon several factors, such as polymer molecular structure, the solution concentration and temperature and on the solvent characteristics (Rushing et al., 2003).

Because of its simplicity and reliability, the viscometric method has been extensively used to study the compatibility of polymers, as it makes possible to further characterise the mixtures of macromolecules in the disperse state. Based on the fact that within liquid medium the attractive interactions causes

expansion of polymer chains while the repulsive interactions lead to the shrinkage of polymer coils, several models were proposed to investigate the polymer–polymer compatibility. (Krigbaum et al 1950, Cragg L.H. et al 1955, Chee K.K. 1990, Garcia R. et al. 1999). Recently this method has been applied to pectin, revealing that the compatibility depends on the different different degree of methoxylation (Migliori, et al., 2010).

Apart from the classic dilution method, the so called "*polymer-solvent"* method was also proposed to study the compatibility of polymers (Lewandowska 2005). This characteristic depends on the polymer interactions and it can be affected the thermodynamic parameter (such as polymer-solvent interaction parameter) and hydrodynamic parameters (such as expansion factor and intrinsic viscosity).

In the classical method of dilution, the measurements are carried out in polymer A/polymer B/solvent ternary mixtures. The intrinsic viscosity and Huggins coefficient (Huggins M.L. 1942) give useful information about the chain hydrodynamic volume and the intrachain interactions, respectively. From a theoretical point of view, the increase of the value of Huggins coefficient was related to the increase in intramolecular interactions which induces shrinkage of polymer chains and reducing the system homogeneity. Whereas, the decrease of the Huggins coefficient value was considered as a sign of the intermolecular interactions increase that promotes the polymer compatibility.

In the polymer-solvent method a mixture of solvent and polymer A at concentration $C_{A,}$ (the solvent-polymer) that is kept constant during an experiment and the viscosity is determined when changing the concentration of polymer B (Danait A et al 1995, Papanagopoulos D. et al 1996). The changes in viscosity can be attributed to both the polymer-polymer interaction (repulsive or attractive intermolecular interaction between polymer A and B) and the concentration-dependent intermolecular excluded volume effect of polymers in solution. By comparing the property of the ternary mixture to those of the binary one it is possible to establish a criterion to evaluate the polymer compatibility

between. Furthermore the mathematical handling of viscosity data can also provide information about the strength of polymer-polymer and polymer-solvent interactions in solution (Haiyang et al 1999). In this paper a comparison between classic ternary compatibility criterion and “solvent polymer” method is presented when applied to two pectins at different degree of methoxylation.

THEORETICAL BACKGROUND

For solutions, relative viscosity η_{rel} is defined as the ratio of solution and solvent viscosity (Lapasin & Pricl, 1995):

$$\eta_{rel} = \frac{\eta_{Solution}}{\eta_{solvent}} \tag{1}$$

For a dilute solution, from this parameter some other relevant ones can be calculated: specific viscosity η_{sp} and intrinsic viscosity $[\eta]$:

$$\eta_{sp} = \eta_{rel} - 1 \tag{2}$$

$$[\eta] = \lim_{C \to 0} \frac{\eta_{sp}}{c} \tag{3}$$

Where C is the solute concentration ($g \cdot dl^{-1}$) and the quantity η_{sp}/C is called reduced viscosity- The intrinsic viscosity $[\eta]$ can be extrapolated from the reduced viscosity value at zero solute concentration. In diluted regime, the plot of reduced viscosity versus concentration shows a linear trend and by using the Huggins equation (Kar & Arslan, 1999):

$$\frac{\eta_{sp}}{C} = [\eta] + [\eta]^2 K_H C \tag{4}$$

the intercept supplies the intrinsic viscosity value, whilst the Huggins parameter K_H can be easily derived from the slope. The intrinsic viscosity is related to hydrodynamic volume of a single macromolecule in the dilute solution and the Huggins constant is an indicator of the interactions between solvent molecules and the macromolecular species in solution (Ma & Pawlik, 2007). This leads to the well known Mark-Houwink-Sakurada relationship (Lapasin & Pricl, 1995), through which the polymer molecular weight (M_W) can be calculated from the intrinsic viscosity value:

$$[\eta] = K(M_W)^a \tag{5}$$

where K and a are material parameters and for pectin the following values are available (Iglesias & Lozano, 2004): $K = 9.55 \cdot 10^{-4}$ (g/dl) and $a = 0.73$.

Compatibility criterion from ternary mixtures data

This compatibility criterion applies when properly considering ternary solutions (i.e two polysaccharides in aqueous solvent). For a generic binary aqueous solution of the *i*-polymer, the Huggins equation becomes (Garcia, Melad, Gómez, Figueruelo & Campos, 1999):

$$\frac{(\eta_{sp})_i}{C_i} = [\eta]_i + b_{ii} C_i \tag{6}$$

Were the following relationship involves the Huggins parameter $b_{ii} = K \cdot [\eta]_i^2$. For a multi-component aqueous solution with a total concentration of polysaccharides C_m ($g \cdot ml^{-1}$) eq. 6 can be rewritten as:

$$\frac{(\eta_{sp})_m}{C_m} = [\eta]_m + b_m C_m \tag{7}$$

and Huggins parameters can be calculated from experimental data. On the other hand, if it is assumed an ideal mixture behaviour of the multi-component solution follows, any material property can be calculated as weight-average of the same quantity of the binary mixture of any component:

$$\frac{(\eta_{sp})_m}{C_m} = \sum_i \frac{(\eta_{sp})_i}{C_i} \cdot \omega_i \qquad (8)$$

Where $\omega_i = c_i/c_m$ is the weight fraction of *i*-polymer. The combination of eq. 8 and 9, allows to estimate the parameters of the Huggins equation for multi-component system binary solutions data:

$$\frac{(\eta_{sp})_m}{C_m} = [\eta]_m^{id} + b_m^{id} \cdot C_m \qquad (9)$$

Where:

$$[\eta]_m^{id} = \sum_i [\eta]_i \cdot \omega_i$$
$$b_m^{id} = \sum_i b_{ii} \cdot \omega_i^2 \qquad (10)$$

The calculated parameters can be compared to those estimated from experimental data in eq.7 to verify if multi-component pectin solution follows the ideal mixture behaviour. This technique have been proposed by Garcia, Melad, Gomez, Figueruelo & Campos, 1999 as compatibility criterion for solutions of polymers pairs, by calculating the differences:

$$\Delta\eta_m = [\eta]_m - [\eta]_m^{id} \qquad (11)$$

$$\Delta b_m = b_m - b_m^{id} \qquad (12)$$

As suggested in the same paper the compatibility of polymers is defined as the occurrence of attractive molecular interactions and when it holds:

$$Compatibility \quad \begin{cases} \Delta\eta_m < 0 \\ \Delta b_m > 0 \end{cases} \tag{13}$$

On the contrary, the situation of incompatible polymers take place in presence of repulsive interactions between molecules and the condition is reported as:

$$Incompatibility \quad \begin{cases} \Delta\eta_m > 0 \\ \Delta b_m < 0 \end{cases} \tag{14}$$

The same criterion have been applied to bio-polymers mixtures, mainly xantan gum and locus beam (Wang, Sun & Wang, 2001; Higiro, Herald & Alavi, 2006, Chenlo, Moreira, Pereira & Silva, 2009) and recently to pectin with very promising results (Migliori, Gabriele, Checchetti & Battipede, 2010).

The polymer solvent compatibility criterion

The interaction between two polymers in dilute solution can be also investigated by using the so called "polymer-solvent" method, i.e by considering the effect of the addition of a second polymer B on the viscosity of an aqueous solution of the polymer *A* (Danait & Deshpandre, 1995). The mixture is assumed to be composed by a "pseudo-solvent" (the proper solvent and the polymer A at a fixed concentration) and the solute polymer B. In this case the intrinsic viscosity of the polymer *B* $[\eta_B]_A$ it can be determined by modifying eq.3:

$$[\eta_B]_A = \lim_{C_B \to 0} \frac{\eta_{sp}(C_A, C_B)}{c_B} \tag{15}$$

And it results as a function of the fixed concentration of the polymer C_A. This value can be calculated from the Huggins equation modified for taking into account the Polymer solvent as follow:

$$\frac{\eta_{sp}(C_A, C_B)}{c_B} = [\eta_B]_A + b_{A,B} C_B \qquad (16)$$

where also the *b* parameter results as a function of the *A* concentration.

Also this method can be used as compatibility criterion when comparing the intrinsic viscosity $[\eta_B]$ of the binary solution of polymer *B* in the pure solvent with those obtained in the solution ternary mixtures at any fixed C_A. Because the intrinsic viscosity is related to the hydrodynamic effect of a single solute molecule, the difference between the two terms $\Delta[\eta]$ can be attributed to the addition of the polymer *A*:

$$\Delta[\eta] = [\eta_B]_A - [\eta_B] \qquad (17)$$

Depending on the strength interaction this difference may varies as a consequence. As proposed by Danait & Deshpandre (1995), $|\Delta[\eta]| < 0.1 dl \cdot g^{-1}$ indicates that the presence of polymer A does not cause any change in the hydrodynamic behaviour of the polymer B, i.e. no change in interaction strength is observed. On the contrary, negative values of the intrinsic viscosity difference (below $-0.1 dl \cdot g^{-1}$) indicates decrease of the hydrodynamic volume of *B* as a consequence of the repulsive effect between the two polymers resulting in the contraction of the molecule coil. On the contrary attractive effect between the two polymers is observed when $\Delta[\eta]$ positive above $0.1 dl \cdot g^{-1}$, because the intrinsic viscosity increases as result of the hydrodynamic volume increase of the *B* polymer when in presence of the polymer *A*. This method has been extensively applied to synthetic polymers (Garcìa, Melad,

Gòmez, Figueruelo, & Campos, 1999; Melad & Mark, 2005) and in some cases a non monotone trend of the intrinsic viscosity difference was found as indication of a change in the interaction strength when varying the amount of "polymer solvent" (Haiyang, Pingping, Guofeng, Peng & Feng, 2000). Nevertheless no applications of this criterion are proposed for bio-polymers in the open literature.

Huggins parameter and polymers interaction strength

The polymer solvent method can be also used to estimate also the intensity of the interaction between polymers when considering the Huggins parameter. Jiang & Han (1998) proposed the so called "cross Huggins" parameter defined as follows:

$$k_{AB} = \left[\frac{[\eta_B]_A}{[\eta_A]} \cdot \left(1 + [\eta_B] \cdot C_B + k_B [\eta_B]^2 \cdot {C_B}^2\right) - 1 \right] \cdot \frac{1}{2[\eta_B] \cdot C_B} \qquad (18)$$

This parameter can be used to estimate the intensity of the interaction between polymers A and B (Duan, Fang, Guo & Zhang, 2009).

MATERIALS AND METHODS

Materials

Pectins used in this work were extracted from dry citrus peel obtained from Tucuman (North Argentina). In order to extract the pectin from the solid, raw material was immersed in aqueous solution of hydrochloric acid at pH ranging from 1.1 to 1.5, for a time ranging between 3 and 6 hours. The temperature during the process was between 60 and 80 °C. The exact values of temperature, pH and time were set according to the characteristics of the processed peel. The liquid was separated from the slurry and the pectin was precipitated by aluminium salts increasing the solution pH between 3.5 and 4.4 with additions of sodium carbonate. The precipitate contains the pectin at the higher methoxylation degree and, in order to decrease this chemical parameter, the solution was stirred in

acidic alcoholic at 30°C. When increasing the contact time the DM decreases and table 1 reports either the time or the final measured degree of esterification. At the end of teh process the pectin were separated and dryied before to be used for the experiments.

Dilute aqueous solutions of the pectins were prepared on % w/v with water obtained for inverse osmosis in presence of NaCl 0.1M (Khouryieh et al., 2007). All systems (binary and ternary) were prepared by throroughly dispersing the required amount of pectin in water with a total solid concentration ranging from 0.08 to 0.16 % w/v.

In order to perform compatibility essay, ternary solutions where prepared by using the pectin at higher (68) and lower (36) methoxylation degree. According to the different criteria to be compared, two types of ternary aqueous mixtures were prepared:

- *The proper "ternary solution" where the two pectins were added at three different molar ratios (1:3; 1:1; 3:1).*
- *The "polymer solvent"solutions where one of the pectin was added in a fixed parametric amount, whilst the other pectin was changed, acting as "polymer solute".*

Solutions used with the last technique, referred to the method of "polymer solvent", were prepared in the following way: the pectin, to be used "polymer solvent", was dissolved to give a series of solutions having concentration from 0.2 to 0.8 g/l. The pectin used as solute was then added to the previous solutions up to final concentrations ranging 0.2 and 0.8 g/l. After preparation, all the samples were continuously stirred at room temperature for 4 h before measurement. Densities of solutions were also measured using an analytic balance equipped with a dedicated kit (Crystal 500, Gibertini Italy).

Viscosity measurements

Viscosity measurements were performed with a Cannon Fenske-type capillary viscometer ASTM n.25 (Carlo Erba, Italy) and the flow time was measured with an accuracy of 1 s. Viscometer was immersed

in a thermostatic water bath, under precise temperature control, at 30.0 ± 0.1 °C (Jualbo, USA). After the sample loading into the viscometer, the solution was allowed to equilibrate at the bath temperature before starting the experiment. Any of the samples was prepared and independently measured three times.

RESULTS AND DISCUSSION

Binary solutions

The viscosity of binary solutions of pectin at different DM were measured and the Huggins equation was used to calculate intrinsic viscosity by linear regression (Table Curve 2D, Jandel Sci., USA). Eq.5 was then applied to calculate the pectin molecular and data are reported in Table 1, revealing a slight decrease (about 10%) of the molecular weight when decreasing the pectin DM. This demonstrated that, in this case, the demethoxylation treatment did not significantly change the length of the pectin main chain. This trend is different from the evidence showed by Hotchkiss, Savary, Cameron, Chau, Bruillette, Luzio & Fishman (2002) where a more pronounced effect of the demethoxylation process was found on the pectin molecular weight: basic process lead to a decrease of the calculated molecular weight, whilst enzymatic treatment did marginally affect this parameter. In addition, as reported in the same paper, the molecular weight form intrinsic viscosity data is a reliable estimation of the chain length less dependent on the level of side-functionalisation (degree of esterification). This is an important evidence for the reliability of the work proposed in the rest of the paper, because the different behaviours of ternary mixtures of pectin at different DM can be explained as result of variation in polymer-polymer interactions, as all the molecules have the same molecular weight.

Table 1 Binary solutions parameters from Huggins equation for pectin at different DM and calculated molecular weight

Demethoxylation Time (h)	*DM (%)*	*[η] ($g \cdot dl^{-1}$)*	*b ($(g \cdot dl^{-1})^2$)*	*k (-)*	*MW (kDa)*
0	68	4.24± 0.07	17.39± 0.61	0.97± 0.07	99249 ± 378
10	57	4.15 ± 0.01	9.75 ± 0.09	0.56 ± 0.01	96508 ± 27
28	42	4.04 ± 0.01	9.88 ± 0.07	0.60 ± 0.01	92935 ± 20
42	36	3.96 ± 0.06	15.64 ± 0.51	0.99 ± 0.04	90487 ± 290

Ternary solutions

Ternary solution viscosity was measured for mixtures at different composition. The molar ratios of HM and LM pectin were varied, by assuming the molecular weight of single pectin from intrinsic viscosity of binary solution. Also for ternary mixtures, dilution guaranteed a relative viscosity value compatible with the Billmayer range and parameters of eq. 7 were calculated using linear regression (Table Curve 2D, Jandel Sci., USA). From binary solution data, additive ("ideal") parameters, as defined in eq.10, were also calculated and data reported in Table 2.

The compatibility criterion as defined in eq.s 11 – 14 was applied and data of $\Delta\eta_m$ and Δb_m (fig. 1) shows both the compatibility of the investigated pectin. This confirm the recent findings of compatibility between pectin at different methoxylation degree as result the attractive interactions between bio-polymers chains at different DM (Migliori, Gabriele, Checchetti & Battipede, 2010).

Table 2 Huggin's equation parameter for ternary solutions: experimental and data from additive model.

Molar ratio HM: LM	ω_{HM} (-)	$[\eta]_m$ $(dl\cdot g^{-1})$	b_m $(dl\cdot g^{-1})^2$	$[\eta]_m^{id}$ $(dl\cdot g^{-1})$	b_m^{id} $(dl\cdot g^{-1})^2$
1:3	0.686	2.75 ± 0.24	42.2 ± 2.93	4.16	16.84
1:1	0.523	3.74 ± 0.36	30.3 ± 3.3	4.11	16.55
3:1	0.354	2.64 ± 0.09	45.8 ± 1.1	4.06	16.26

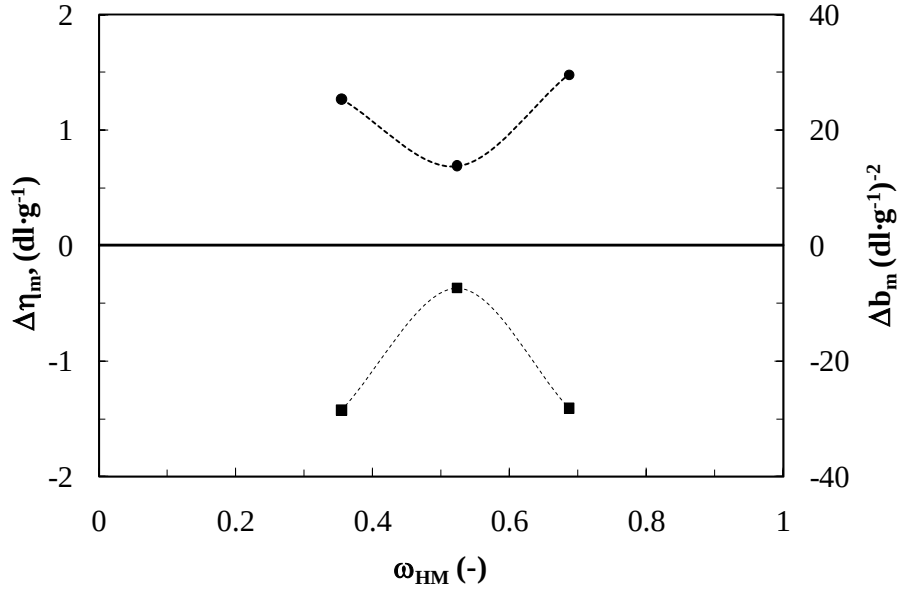

Figure 1 Plot of $\Delta\eta_m$ (■) and Δb_m (●) for ternary solutions of pectin pair HM / LM.

Apparent molecular weight, from ternary solutions intrinsic viscosity was calculated, according to Khouryieh, Herlad, Aramouni & Alavi (2007) in similar contest, and data are plot in fig.2 together with the linear behaviour (i.e. no differences in interactions between different chains). Results confirm

that, when in presence of attractive intermolecular forces, the apparent molecular weight decreases for compatible bio-polymer pairs.

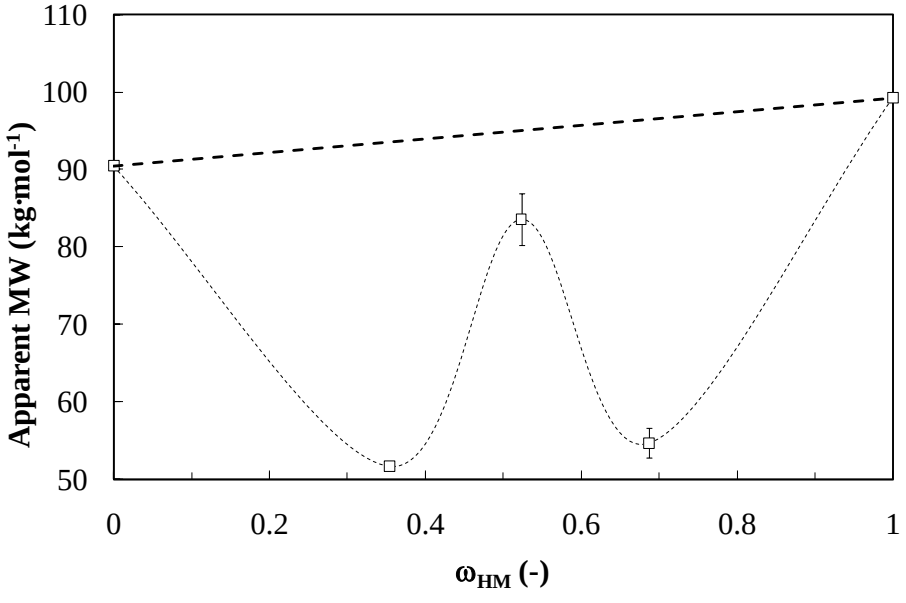

Figure 2 Apparent Molecular Weight for ternary solutions of pectin pair HM / LM.

Polymer solvent method

Reduced viscosity value for solution at constant LM and HM content are reported in Fig. 3 and Fig.4 respectively. All the experimental points follow a linear trend within the investigated concentration range and the reduced viscosity value increases as the concentration of "solvent polymer" is augmented. On the contrary, the line slope decreases as the "polymer-solvent" concentration increases and a negative slope is observed for both systems at the higher investigated concentration value. This behaviour can be explained because, at lower polymer concentration, the increase in specific viscosity is predominant to the polymer concentration increase. As a consequence, at low polymer

concentration their ratio (i.e. the reduced viscosity) increases more and the slope turns from positive to negative.

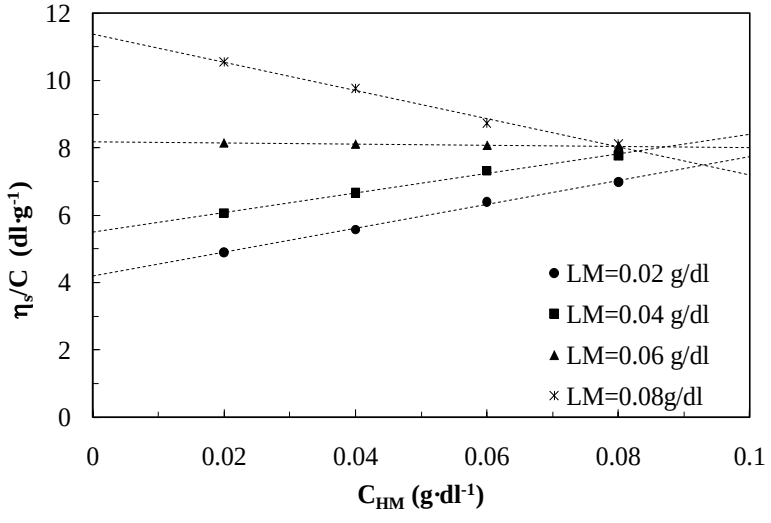

Figure 3 Reduced viscosity of solutions of HM pectin in water + LM pectin as solvent.

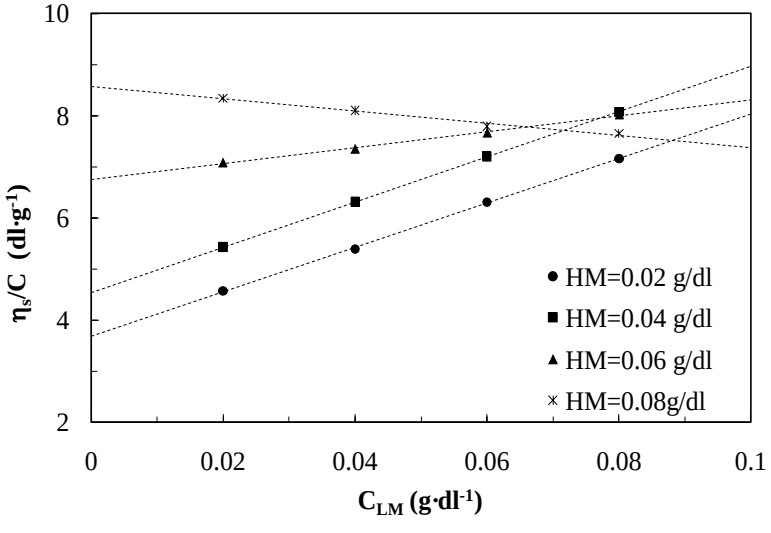

Figure 4 Reduced viscosity of solutions of LM pectin in water + HM pectin as solvent.

Compatibility criterion

In figure 5 are presented the data of intrinsic viscosity from eq. 15-16, as a function of the solvent polymer concentration.

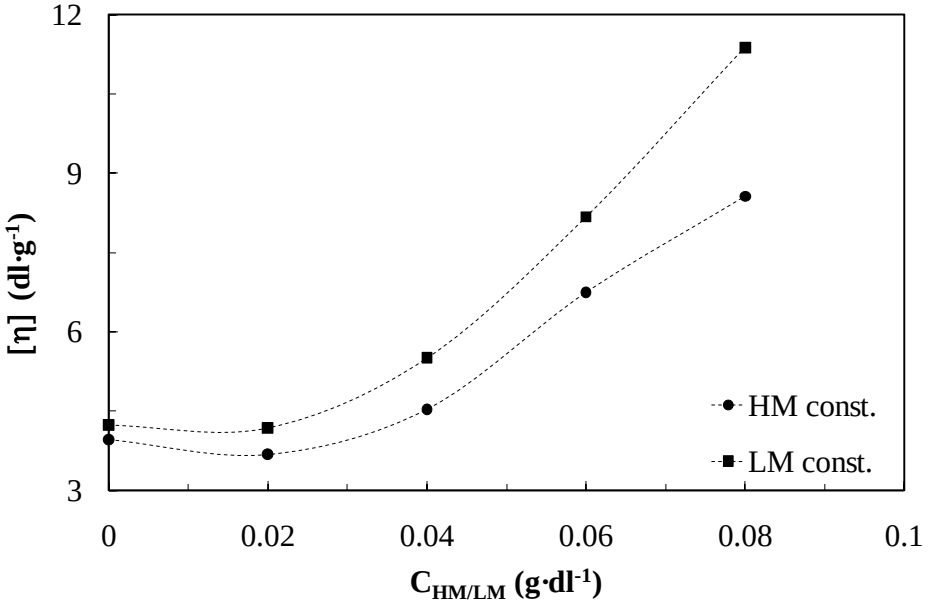

Figure 5 Intrinsic viscosity from polymer solvent method of solution containing water + LM pectin (■) and water + HM pectin (●) as solvent

It clearly appears that the apparent hydrodynamic volume (directly related to the intrinsic viscosity) does not significantly change when adding small quantity of "solvent-polymer" (below 0.02 $g \cdot dl^{-1}$). On the contrary, the hydrodynamic volume increases significantly, if compared to the binary solution, when increasing the solvent amount. This indicates the presence of positive interactions between the two biopolymers. In addition the effect of volume increase is greater for the mixture with constant LM as its curve increases with a slope greater than the curve at constant HM pectin. The application of

compatibility criterion is also reported in table 3 and $\Delta[\eta]$ values (form eq.17) are positive for any polymer concentration except for the lower value.

Table 3 Intrinsic viscosity and compatibility criterion from polymer solvent method of solution containing water + LM pectin (■) and water + HM pectin (●) as solvent.

	C_{HM}	$[\eta_{LM}]_{HM}$	$\Delta[\eta]$
	$(g \cdot dl^{-1})$	$(dl \cdot g^{-1})$	$(dl \cdot g^{-1})$
	0.02	3.69 ± 0.04	-0.281
Solvent Polymer	0.04	4.54 ± 0.03	0.574
HM	0.06	6.75 ± 0.04	2.78
	0.08	8.56 ± 0.07	4.60
	C_{LM}	$[\eta_{HM}]_{LM}$	$\Delta[\eta]$
	$(g \cdot dl^{-1})$	$(dl \cdot g^{-1})$	$(dl \cdot g^{-1})$
	0.02	4.19 ± 0.09	-0.052
Solvent Polymer	0.04	5.51 ±0.08	1.27
LM	0.06	8.17 ± 0.01	3.93
	0.08	11.38 ± 0.15	7.13

According to Danait & Deshpandre (1995) this reveals a weak repulsive effect between molecules at low concentration whilst the increasing positive value indicates a significant attractive effect when

increasing the polymer concentration. It is also noteworthy that the effect does not change when changing the "solvent" role from the HM to the LM pectin.

Huggins parameter evaluation

The "cross Huggins parameter" K_{AB}, as defined in eq. 17, has been calculated for both series of solutions and data are reported in fig. 6.

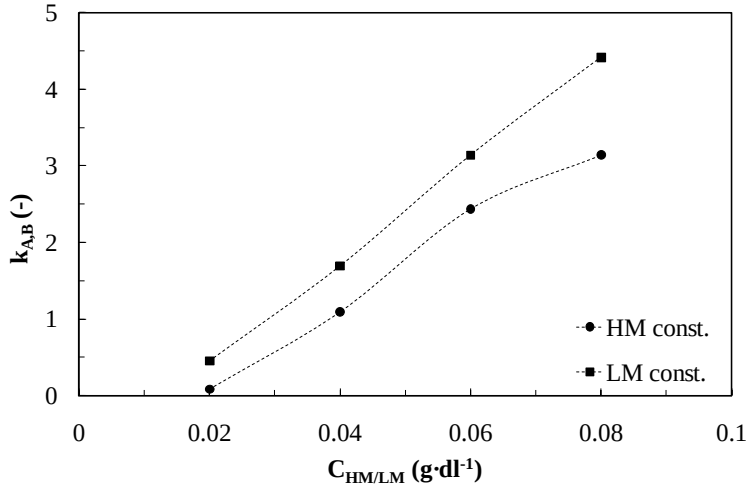

Figure 6 Interaction parameter k_{AB} from polymer solvent method of solution containing water + LM pectin (■) and water + HM pectin (●) as solvent

Also the application of this parameter, related to the interaction strength, confirms that at low "solute-polymer" concentration interaction modification is quite weak. On the contrary, at higher pectin concentrations, the parameter indicates an increase in interaction strength as the K_{AB} increases. In addition the parameters values are different when changing the "polymer-solvent" form the HM to the LM pectin. In fact, the lower K_{AB} values indicates that the interaction strengthening effect is lower when quantity of LM pectin is added to a binary mixture containing HM pectin.

Therefore, from the “polymer-solvent” method application it can be concluded that in presence of pectin at different molecular weight, in ternary solutions attractive interaction take place and their intensity increases as the overall biopolymer concentration is improved.

CONCLUSIONS

In this paper a comparison of two different compatibility criteria for mixtures of pectin at different DM has been proposed. Based on synthetic polymers findings, diluted solutions of pectin having different DM, where prepared and, according to previous finding of the same authors, it was confirmed that a large difference in esterification degree lead to a compatibility between the two polymers. By using the same pectin, the so-called “polymer-solvent” method was used to verify the bio-polymer compatibility. In this method the concentration of one of the polymer is kept constant and the concentration of the second pectin is varied. All the series, keeping constant both polymers respectively, were investigated and the application of the criteria confirmed the compatibly results. In addition the analysis of the “cross Huggins” interaction parameters K_{AB} revealed that for very diluted solutions no difference of interaction can be measured when adding a type of pectin into a “polymer-solvent” diluted solution. On the contrary, when increasing the concentration of the added polymer the interaction strength show a marked increase and, as a valuable result, this parameter appear to be sensitive also to the characteristics of the “polymer-solvent”. In other words, for pectin ad very different DM, this method show differences in the interaction force if pectin A is added to a solution of pectin B in comparison to the addition of pectin B to a solution of pectin A.

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CHAPTER 5

VISCOMETRIC STUDY ON THE INTERMOLECULAR INTERACTIONS BETWEEN PECTIN-PECTIN MIXTURES IN SOLUTION

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Abstract

The viscosity behavior of mixtures formed by pairs of pectin viz., (i) HM68 and HM57, (ii) HM68 and LM36; (iii) LM42 and LM36, in aqueous dilute solution has been studied . The intrinsic viscosity and the viscometric interaction parameters have been experimentally measured for the ternary system by using two methods: 1) measurements of a mixture of two biopolymers A and B as a solute in a single solvent [$(A+B)_{solute}$/solvent]; 2) measurements of a mixture of polymer A as a solute in solution containing a constant concentration of polymer B [A_{solute}/(B+solvent)], (the polymer solvent method, Dondos A. & Benoit H, 1975). The estimation of the compatibility degree of the above pectin pairs has been done by means of three criteria: a) the sign of Δb_m e $[\Delta\eta]_m$ according to the formalism developed by Garcia et. al. (1999; b) the sign of $\Delta[\eta]=[\eta_B]_A-[\eta_B]$ according to the method developed by Danait et al., (1995). The data obtained from the viscometry studies showed that the examined mixtures were compatible if the pectins used have a different degree of esterification (HM/LM).

Keywords: compatibility, intrinsic viscosity, viscometric coefficients

INTRODUCTION

Pectin is widely used as gelling agent and stabilizer and for these properties is an interesting candidate in the development of low sugar applications (Löfgren & Hermansson , 2007). Various factors determine gelling properties including temperature, pectin type, degree of methyl-esterification (DE), degree of acetylation (DA), pH, sugar and other solutes, and calcium. To alter their functional properties, pectin can be chemically de-eesterified with acid, alkali or ammonia (May, 1990). Chemical de-esterification of pectin is a random process that can result in decrease molecular weight due to depolymerization of the pectin backbone α-(1→4)-galacturonosyl glycosidic bonds by β-elimination (Hotchkiss et al., 2002). Mixtures of pectins are often used in food applications to obtain products with certain functionalities. Several studies have demonstrated that not only the structure of high methoxyl (HM), low methoxyl (LM) and mixed pectin gels differed greatly but also large variations in rheological behavior was observed during the gelation of pectin (Löfgren, Walkenström & Hermansson, 2002; Löfgren et al., 2005). As one aspect of the physical gelation is due to a microscopic degree of heterogeneity or phase incompatibility, some authors have suggested that there is a link between the formation of the gel and the microscopic incompatibility (Lapasin R, Pricl, S.(1995). In recent years, considerable attention has been devoted to use of the dilute-solution viscosity (DSV) measurement as a technique to predict polymer-polymer compatibility (Chee, 1990; Sun et al., 1992; Danait & Deshpande, 1995, Garcia et al 1997, 1999; Jiang, W.J. & Han, S.J., 1998, Haiyang et al., 1998, 1999, 2000). Dilute solution viscometry method provide information about both the polymer-polymer interactions and polymer-solvent interaction in solution. The effectiveness of dilute solution viscometry method is based on the assumption that the mutual interactions of

macromolecules in solution have a great influence on the viscosity in the ternary systems. As the two polymers are dissolved in the common solvent their hydrodynamic volume and configuration are greatly affected by the solvent selected, so polymer-solvent interactions also play an important role in characterizing the viscosity behavior in ternary systems. In general the viscosity measurement of ternary system can be performed by two techniques, i.e., the viscosity measurement of ternary system can be determined either by putting the polymers A and B with certain weight ratio in a pure solvent or by putting the polymer A (referred to a guest or probe polymer) in solution in which the polymer B (referred to as host or matrix polymer) is found at a constant concentration. The last, referred to as the method of polymer solvent, (Tewari & Srivastava, 1992), has proved to be peculiarly interesting and useful for two reasons. First, the Huggins mutual interaction parameter K_{AB} can be determined and it is well known that its value indicates directly the intermolecular interaction between polymers A and B in solution (Haiyang et al., 2000). Second, the intrinsic viscosity of polymer A in solution containing a constant concentration of polymer B, $[\eta_A]_B$, should reflect the change in polymer dimensions of polymer A in presence of polymer B or said more directly, due to the intermolecular interactions between polymer A and B in solution, (Lewandowska, 2005). In this article, the viscosity measurements of dilute solutions of pectin have been performed on the basis of two methods: the classical dilution of a ternary system (polymers and solvent) and the so-called polymer-solvent method (polymer in a polymer solution). Compatibility and polymer–polymer interaction has been investigated. Attraction between two component molecules may cause swell of macromolecular coils resulting in an increase in viscosity, or otherwise, repulsion may cause shrinkage of the macromolecular coils giving a decrease in viscosity. In a ternary system (polymer A - polymer B - solvent) the thermodynamic interactions are the most important, beside the hydrodynamic interactions. The thermodynamic interactions include interaction between segments of the same polymer,

interaction between each component polymer and solvent, and interaction between two component polymers. All these interactions are the contributing factors in the change of viscosity, but differing in extent, which might depend on the nature of solvent. The thermodynamic interactions include the intramolecular excluded volume effect, resulting in an expansion of the coil in solution and the intermolecular excluded volume effect, resulting in a contraction of the coil in solution. In the present work were measured the intrinsic viscosity and Huggins coefficient to have useful information about polymer-polymer compatibility. Theoretically the increase of the value of Huggins coefficient was interpreted as the result of the increase in intramolecular interactions which induces shrinkage of polymer chains and reducing the system homogeneity. Whereas, the decrease of the Huggins coefficient value was considered as a sign of the intermolecular interactions that favours the polymer compatibility. We focus on polymer pectin-pectin interactions HM-HM, LM-LM, HM-LM and viscosity data are examined in terms of Huggins equation. This analysis leads to suitable parameters that can be used to characterize the behavior of pectin mixtures. The main objective is the valutation of the compatibility or incompatibility in mixtures of HM and/or LM in terms of these parameters.

MATERIAL AND METHOD

De-esterification of pectin

The samples studied in this work were prepared by using dry citrus peel (Silva Extract, Italy). In order to extract pectin, the citrus peel was immersed in aqueous solution of hydrochloric acid up to reach a pH value of 1,1-1,5, for a time ranging between 3 and 6 hours, maintaining an temperature between 60 and 80 °C. The exact values of temperature, pH and time were set according to the characteristics of the processed peel. The liquid was recovered from the slurry and the pectin was precipitated by aluminum salts to a pH between 3.5 and 4.4 with additions of sodium carbonate. The precipitate was stirred in acidic alcoholic solution for increasing times to obtain the pectin to different degrees of

esterification. The de-esterification process was occurred at 30°C. DM values and times are reported in Table 1. (DM 68: *time 0*; DM 57: *+10 h*; DM 42: *+28 h***;** DM 36: *42 h***)**

Solutions

Aqueous solutions of the pectins were prepared on % g/l with water obtained from reverse osmosis in presence of NaCl 0.1M (Khouryieh et al., 2007). All systems (binary and ternary) in dilute range were prepared, at nominal concentration from 0.08 to 0.16 % g/l, by thoroughly dispersing the required amount of pectin in water. According to the different compatibility criteria to be used, two types of aqueous mixtures of the pectins were prepared: the real "ternary solutions" at three different pectin molar ratios (0.25:0.75; 0.5:0.5; 0.75:0.25) and were prepared mixing the corresponding weight mass of pectin. The diluted solutions of ternary systems investigated are indicated as HM68/LM36, HM68/HM57, and LM42/LM36. Solutions based on the method of "polymer solvent", were prepared in the following way: the pectin, whose solution was used as polymer solvent, was dissolved to give a series of solutions having concentration from 0.2 to 0.8 g/l. The pectin used as solute was dissolved in the previous solutions up to final concentrations ranging 0.2 and 0.8 g/l. The following series of solutions were prepared

1. *[LM36 in (HM68+ H_2O)] (A)*
2. *[HM68 in (LM36+ H_2O)] (B)*
3. *[LM36 in (LM42+ H_2O)] (C)*
4. *[LM42 in (LM36+ H_2O)] (D)*
5. *[HM68 in (HM57+ H_2O)] (E)*
6. *[HM68 in (HM57+ H_2O)] (F)*

After preparation, all samples were continuously stirred with magnetic stir bar at room temperature for 4 h before measurement. Densities of solutions were also measured using an analytic balance equipped with a dedicated kit (Crystal 500, Gibertini Italy).

Viscometry measurements

Viscosity measurements were performed with a Cannon Fenske-type capillary viscometer ASTM n.25 (Carlo Erba, Italy) and the flow time was measured with an accuracy of 1 s. Viscometer was immersed in a thermostatic water bath, under precise temperature control, at 30.0 ± 0.1 °C (Jualbo, USA). After the sample loading into the viscometer, the solution was allowed to equilibrate at the bath temperature before starting the experiment for 10 m. Each of the samples was prepared and independently measured three times.

Data analysis

For each concentration the intrinsic viscosity was determined experimentally from measurements of the viscosity at very low concentration (C). Denoting solution and solvent viscosity as, respectively, $_{solution}$ and $_{solvent}$, [ ] is defined by the following relationships:

Relative viscosity: $_{rel} = _{solution}/_{solvent}$ (1)

Specific viscosity: $_{sp} = _{rel} - 1$ (2)

Reduced viscosity: $_{red} = _{sp}/$ (3)

Intrinsic viscosity: [ ] = — (4)

The intrinsic viscosity can be obtained by measuring specific viscosities at different concentrations and extrapolating $_{sp}$/C at zero solute concentration. The extrapolations are usually done for relative

viscosities values between 1.1-1.5, according to Billmeyer procedure (Iglesias et al., 2004), or between 1.2-2, according to Rao procedure (Iglesias et al., 2004). In the present work, pectin solutions were therefore diluted to be within the Billmeyer range.

Compatibility criteria from ternary systems

The intrinsic viscosity $[\eta]$ can be extrapolated from the reduced viscosity value at zero solute concentration. In diluted regime, the plot of reduced viscosity versus concentration gives a straight line and Huggins proposed the below equation (Huggins, 1942):

$$\frac{\eta_{sp}}{C} = [\eta] + [\eta]^2 K_H C \qquad (5)$$

the intercept yields the intrinsic viscosity value whilst from the slope the Huggins parameter K_H can be easily derived. The intrinsic viscosity is a measure of the hydrodynamic volume occupied by a macromolecule in the dilute solution and the Huggins constant characterizes the overall interaction (hydrodynamic as well as thermodynamic) between solvent molecules and the macromolecular species in solution (Ma & Pawlik, 2007). C is the solute concentration ($g \cdot dl^{-1}$). For a generic binary aqueous solution of the *i*-polymer, the Huggins equation can be written as (Garcia, Melad, Gómez, Figueruelo & Campos, 1999):

$$\frac{(\eta_{sp})_i}{C_i} = [\eta]_i + b_{ii} C_i \qquad (6)$$

Where the following relationship involves the Huggins parameter $b_{ii} = K \cdot [\eta]_i^2$. For a multi-component aqueous solution with a total concentration of polysaccharides C_m ($g \cdot ml^{-1}$) eq. 6 can be rewritten as:

$$\frac{(\eta_{sp})_m}{C_m} = [\eta]_m + b_m C_m \tag{7}$$

and Huggins parameters can be calculated from experimental data. On the other hand, if it is assumed an ideal behaviour of the multi-component solution follows, each material property can be calculated as a weighted-average of the same quantity of the binary mixture of any component:

$$\frac{(\eta_{sp})_m}{C_m} = \sum_i \frac{(\eta_{sp})_i}{C_i} \cdot \omega_i \tag{8}$$

Where $\omega_i = C_i/C_m$ is the weight fraction of *i*-polymer. The combination of eq. 8 and 9, allows to estimate the parameters of the Huggins equation for ideal multi-component systems from binary solutions data:

$$\frac{(\eta_{sp})_m}{C_m} = [\eta]_m^{id} + b_m^{id} \cdot C_m \tag{9}$$

where:

$$\begin{aligned} [\eta]_m^{id} &= \sum_i [\eta]_i \cdot \omega_i \\ b_m^{id} &= \sum_i b_{ii} \cdot \omega_i^2 \end{aligned} \tag{10}$$

The calculated parameters can be compared to those estimated from experimental data in eq.7 to verify if multi-component pectin solution follows the ideal behaviour. This technique have been proposed by Garcia, Melad, Gomez, Figueruelo & Campos, (1999) as compatibility criterion for solutions of polymers pairs, by calculating the differences:

$$\Delta\eta_m = [\eta]_m - [\eta]_m^{id} \tag{10}$$

$$\Delta b_m = b_m - b_m^{id} \tag{11}$$

As suggested in the same paper the compatibility of polymers is defined as the occurrence of attractive molecular interactions and when it holds:

$$Compatibility \quad \begin{cases} \Delta\eta_m < 0 \\ \Delta b_m > 0 \end{cases} \tag{12}$$

On the contrary, the situation of incompatible polymers take place in presence of repulsive interactions between molecules and the condition is reported as:

$$Incompatibility \quad \begin{cases} \Delta\eta_m > 0 \\ \Delta b_m < 0 \end{cases} \tag{13}$$

The polymer solvent compatibility criterion

The mixture is assumed to be composed by a “pseudo-solvent” (the proper solvent and the polymer B at a fixed concentration) and the solute polymer A. In this case the intrinsic viscosity of the polymer *B* $[\eta_B]_A$ it can be determined by modifying eq.3:

$$[\eta_A]_B = \lim_{C_A \to 0} \frac{\eta_{sp}(C_A, C_B)}{c_A} \tag{14}$$

and it results as a function of the fixed concentration of the polymer B. This value can be calculated from the Huggins equation modified for taking into account the Polymer solvent as follow:

$$\frac{\eta_{sp}(C_A, C_B)}{c_A} = [\eta_A]_B + b_{A,B} C_A \qquad (15)$$

where also the *b* parameter results as a function of the *B* concentration.

Also this method can be used as compatibility criterion when comparing the intrinsic viscosity $[\eta_A]$ of the binary solution of polymer *A* in the pure solvent with those obtained in the solution ternary mixtures at any fixed C_A. Because the intrinsic viscosity is related to the hydrodynamic effect of a single solute molecule, the difference between the two terms $\Delta[\eta]$ can be attributed to the addition of the polymer *B* (Melad, 2003; Melad & Mark, 2005):

$$\Delta[\eta] = [\eta_A]_B - [\eta_A] \qquad (16)$$

Huggins parameter and polymers interaction strength

The polymer solvent method can be also used to estimate the intensity of the interaction between polymers when considering the Huggins parameter. Jiang & Han (1998) proposed the so called "cross Huggins" parameter defined as follows:

$$k_{AB} = \left[\frac{[\eta_A]_B}{[\eta_A]} \cdot \left(1 + [\eta_B] \cdot C_B + k_B [\eta_B]^2 \cdot C_B{}^2\right) - 1 \right] \cdot \frac{1}{2[\eta_B] \cdot C_B} \qquad (17)$$

where $[\eta_A]_B$ is the intrinsic viscosity of polymer A using polymer B as a solvent at the concentration c_B; $[\eta_A]$ and $[\eta_B]$ are the intrinsic viscosities of polymers A and B in a pure solvent, respectively; and k_B is the Huggins coefficient of polymer B in a pure solvent. K_{AB} can be applied to estimate the intensity of interaction between polymer A and B (Duan, Fang, Guo & Zhang, 2009).

RESULT AND DISCUSSION

The intrinsic viscosity and Huggins associative coefficient of binary systems were determined and were reported in a previous study (Migliori et al., 2010), in view, to understand the behavior of every pectin chains in aqueous solution and to anticipate the nature of interactions in ternary systems. In table 1 are shown the values of Δb and Δη, for ternary of mixtures, as application of criteria of Garcia et al. (Garcia et al., 1999).

Table 1 Viscometric data for ternary mixtures (Pectin A/Pectin B/H_2O) at 30°C.

System	*Mixture*	*Δb*	*Δη*	*Compatibility*
HM68-LM36	*1: 2*	*36,93*	*-1,42*	*yes*
HM68-LM36	*1: 1*	*22,06*	*-0,36*	*yes*
HM68-LM36	*2:1*	*32,69*	*-1,40*	*yes*
HM68-HM57	*1:2*	*-0,05*	*0,52*	*no*
HM68-HM57	*1:1*	*-0,04*	*0,61*	*no*
HM68-HM57	*2:1*	*-0,07*	*0,49*	*no*
LM36-LM42	*1:2*	*-6,10*	*1,46*	*no*
LM36-LM42	*1:1*	*-0,16*	*1,27*	*no*
LM36-LM42	*2:1*	*-4,27*	*1,41*	*no*

The criteria for compatibility in polymer mixtures are based on the comparison between experimental and theoretical or ideal value. In the case of Δb Garcia et al. (1999) proposed a new viscometric interaction parameter, $b^{(id)}$ (see eq.11), instead of that derived from the formalism of Krigbaum-Wall (1950) and Cragg-Bigelow (1955) and considered mathematically erroneous. The second parameter, Δη, is based on the assumption that the intrinsic viscosity can be treated as an excess property by similarity with those of real solutions. As reported in a previous work (Migliori et al., 2010) the

compatibility between pectin with a different degree of methoxylation (HM-LM) or attractive molecular interactions is observed, while systems with only HM or LM pectins show incompatibility or repulsive molecular interactions. These results confirm that the degree of methoxylation is a important parameter for the preparation of compatible systems. From the data reported in table 1, for pairs of pectins in H_2O as common solvent, quantitative differences, mostly for the Δb values, depending on degree of methoxylation of pectin, can be observed. In particular it is noticed that, the LM pectin are more incompatible than HM pectin (negative values higher). From the data the comparison of the compatibility trend for examined mixtures is the following: HM-LM>>HM-HM>LM-LM. The use of $\Delta[\eta_A]_B = ([\eta_A]_B - [\eta_A])$ as a criterion to predict polymer-polymer compatibility (Danait & Deshpande, 1995, and Haiyang et al., 1999) permits to evaluate the interaction between polymers A and B. In tab.2 $\Delta[\eta_A]_B > 0$ for (A) and (B) suggests that strong interactions are stabilized with increasing concentration of polymer solvent, implying that the HM68 and LM 36 pectin are compatible. This attractive interaction between HM68 e LM36 will decrease the intermolecular excluded volume effect. By the acting of the intramolecular excluded volume effect a positive value of $\Delta[\eta_A]_B$ suggests that the intrinsic viscosity of polymer A increases due to the expansion of macromolecules coils in polymer solvent. In tab. 3, for (C) and (D), and in tab. 4, for (E) and (F), $\Delta[\eta_A]_B > 0$ is in contrast with the results of ternary systems. However $\Delta[\eta_A]_B$ diminishes as the polymer solvent concentration increases to reach values close to zero. The trend is opposite to what is observed for (A) and (B) and it demonstrated that the behavior could not be directly connected with expansion of macromolecules. If the intramolecular excluded volume effect decreases this means that the intermolecular exclude effect increases, resulting in contraction of the coil in solution. These

results would indicate an incompatibility between the pectins as demonstrated by the application of criteria of Garcia.

Table 2 Viscometric data of LM36 in the HM68 solution and HM68 in the LM36 solution.

$C_{HM68cost}$ [g/l]	[η] [d/g]	$\Delta([\eta_{LM36}]_{HM68}-[\eta]_{LM36})$
0	*3,96*	*0*
0,2	*3,99*	*0,03*
0,4	*4,54*	*0,58*
0,6	*6,75*	*2,80*
0,8	*8,56*	*4,60*

$C_{LM36cost}$ [g/l]	[η] [l/g]	$\Delta([\eta_{HM68}]_{LM36}-[\eta]_{HM68})$
0	*4,24*	*0*
0,2	*4,16*	*-0,08*
0,4	*5,82*	*1,57*
0,6	*8,15*	*3,91*
0,8	*11,3*	*7,14*

Fig.1 shows the plots of $[\eta_A]_B$ at different concentration C_B for systems with HM/LM (A and B). As can be observed, $[\eta_A]_B$ increases with the increase of the concentration of C_B and this confirms the existence of attractive interactions in solution.

Table 3 Viscometric data of HM57 in the HM68 solution and HM68 in the HM57 solution.

$C_{HM68\ cost}$ [g/l]	[η] l/g]	$\Delta([\eta_{HM57}]_{HM68} - [\eta]_{HM57})$
0	4,15	0
0,2	5,42	1,27
0,4	4,95	0,80
0,6	4,51	0,36
0,8	4,16	0,01

$C_{HM57\ cost}$ [g/l]	[η] l/g]	$\Delta([\eta_{HM68}]_{HM57} - [\eta]_{HM68})$
0	4,24	0
0,2	5,42	1,18
0,4	5.06	0,82
0,6	4.81	0,57
0,8	4,43	0,19

Table 4 Viscometric data of LM36 in the LM42 solution and LM42 in the LM36 solution

$C_{LM36cost}$ [g/dl]	[η] [l/g]	$\Delta([\eta_{LM36}]_{LM42} - [\eta]_{LM36})$
0	4,04	0
0,2	6,57	2,61
0,4	7,02	3.06
0,6	6,45	2,49
0,8	4,78	0,82

$C_{LM42cost}$ [g/dl]	[η] [l/g]	$\Delta([\eta_{LM42}]_{LM36} - [\eta]_{LM36})$
0	3,96	0
0,2	6,47	2,43
0,4	6,70	2,66
0,6	5,69	1.65
0,8	3,71	-0.33

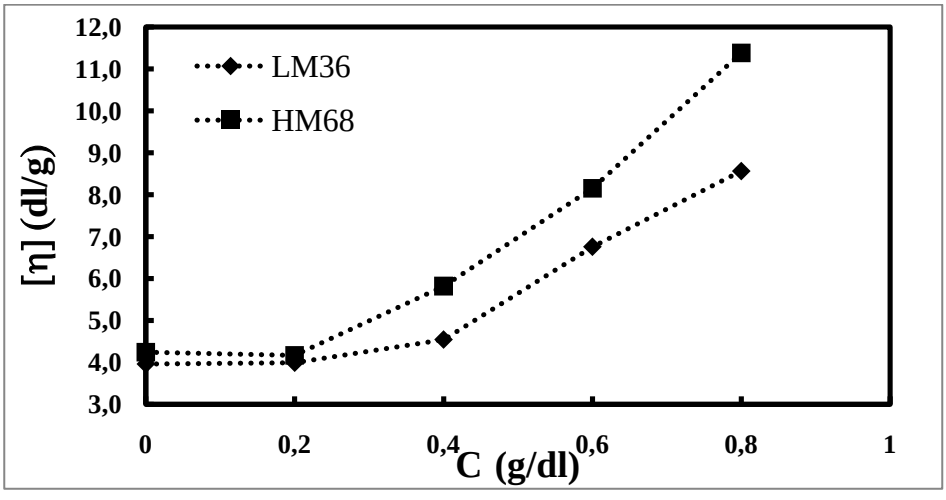

Figure 1 Intrinsic viscosity of HM68 in (water + LM36) and LM36 in (water+HM68) as solvent

Fig.s 2 and 3 show the plots of $[\eta_A]_B$ at different concentration C_B for systems with HM/HM (C and D) and LM/LM (E and F). $[\eta_A]_B$ of series LM in LM (C and D) increase until to 0,4 g/dl and then decrease with the increase of the concentration of the polymer solvent, while $[\eta_A]_B$ of series HM in HM (curves E and F) increase until to 0,2 g/dl and then decrease with the increase of the concentration of the polymer solvent. These results mean that repulsive interactions in solution start to become significant with increasing concentration of C_B.

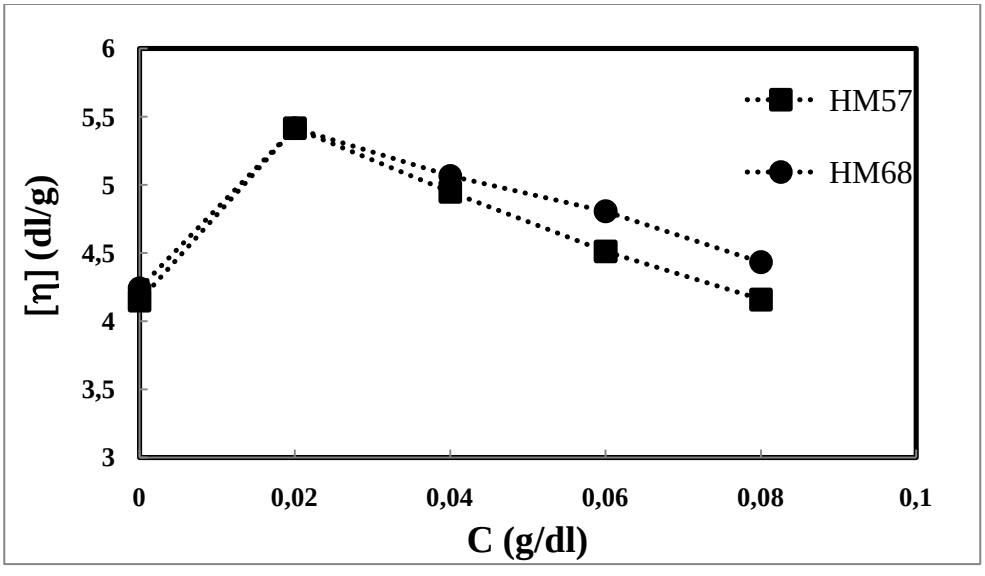

Figure 2 Intrintsic viscosity of HM68 in (water + HM57 pectin) and HM57 in (water + HM68) as solvent

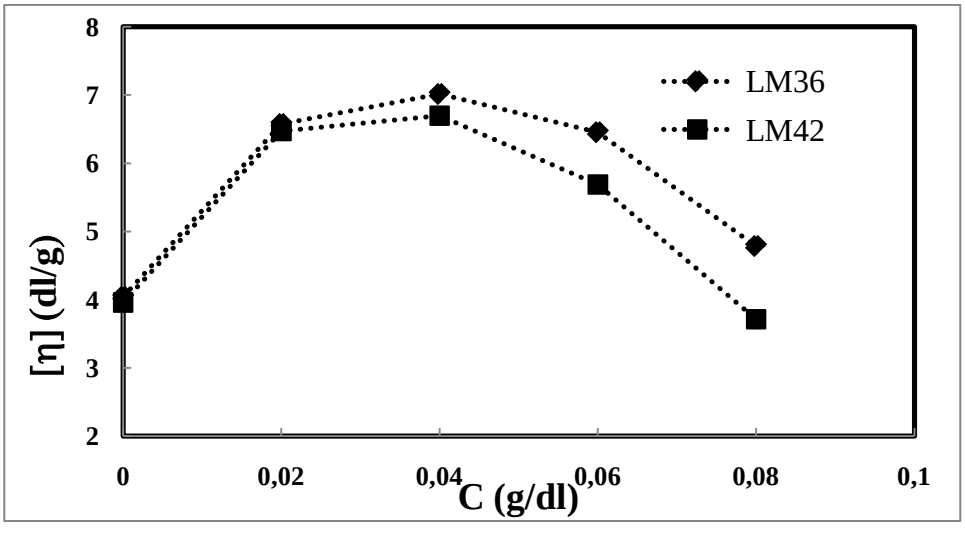

Figure 3 Intrintsic viscosity of LM36 in (water + LM42 pectin) and LM42 in (water + LM36) as solvent

Since in H_2O and in (H_2O+pectin B) the polymer-solvent (pectin A-H_2O) interaction is the same, the change of the intrinsic viscosity of pectin A in solution can be attributed to the polymer-polymer interaction (pectin A-pectin B). The Huggins mutual interaction parameter K_{AB} was obtained from

Eq.17 (Duan et al, 2009). As shown in fig.4, for the systems (A) and (B) K_{AB} increase with increasing $C_{solvent}$, while for the systems (C, D) (figures 5 and 6 and (E, F) K_{AB} decrease with increasing $C_{solvent}$. This behavior can explain the different compatibility, which is found applying the criteria of Garcia et al.. The interactions between compatible pectins (HM/LM) are opposite to the interactions between incompatible pectin (HM/HM and LM/LM). The values relative to compatible pectin pairs indicate that the mutual interaction, K_{AB} between HM68 and LM36 is strong and increase as the concentration of polymer solvent increases, demonstrating that there is an increase in the dimension of the molecules of HM68 and LM36 in presence of LM36 and HM68 respectively. A decrease in K_{AB} for pectin pairs (HM/HM and LM/LM) indicates a reduction of the compatibility between the two pectin because repulsive interactions increase with increasing $C_{solvent}$ That is coherent with incompatibility detected in the ternary systems.

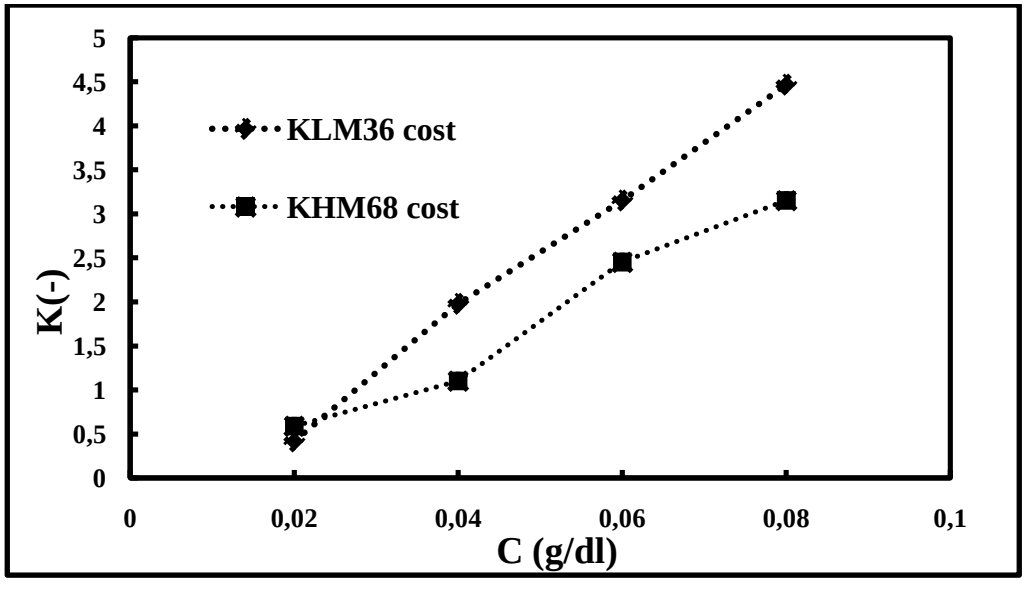

Figure 4 Interaction parameter $K_{HM68\text{-}LM36}$ and $K_{LM36\text{-}HM68}$

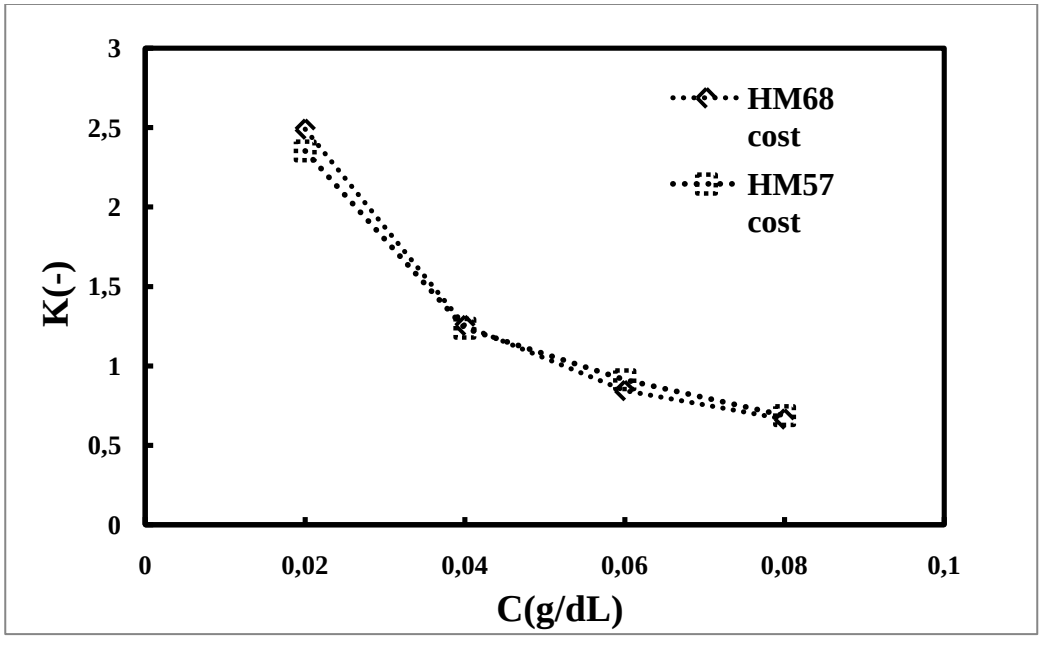

Figure 5 Interaction parameter $K_{HM68\text{-}HM57}$ and $K_{HM57\text{-}HM68}$

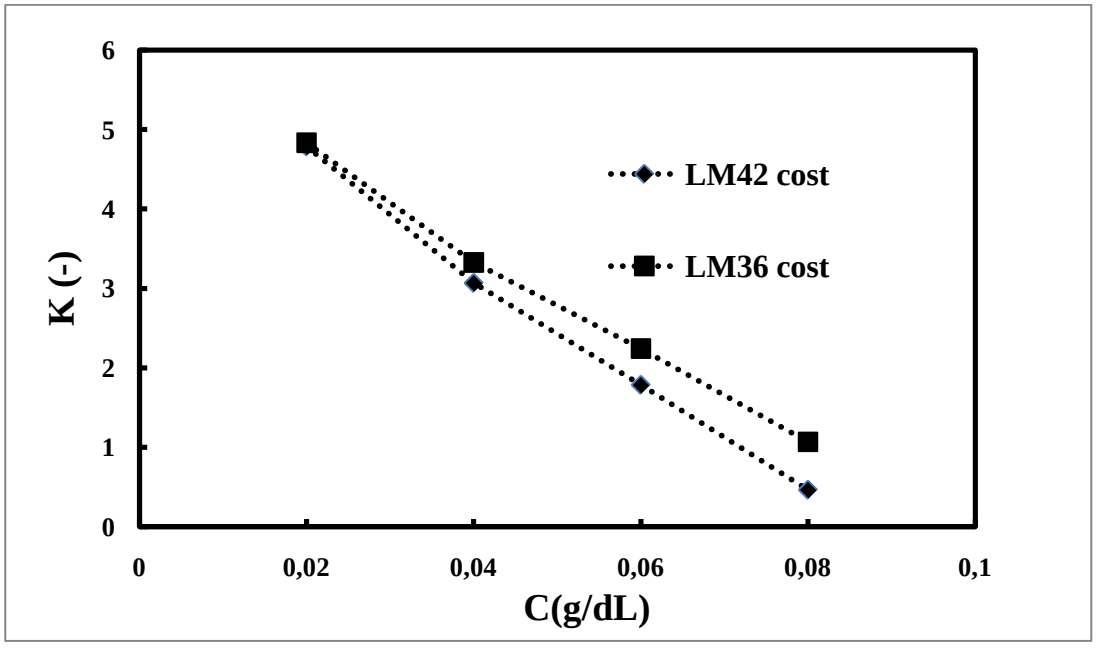

Figure 6 Interaction parameter $K_{LM36\text{-}LM42}$ and $K_{LM42\text{-}LM36}$

CONCLUSION

In summary, three ternary systems were investigated. The studies of hydrodynamic properties of the solutions of HM/LM, HM/HM and LM/LM mixtures measured by the classical dilution method indicate that, applying the criteria of compatibility of Garcia et al. (Garcia et al., 1999), the system HM/LM is compatible while the systems HM/HM and LM/LM are incompatible. The compatibility and the hydrodynamic properties of dilute mixtures depend on the degree of methoxylation of pectin. The obtained results for the method of the polymer solvent suggest that the criterion $\Delta[\eta_A]_B$ in the case of systems with homologous pectins (HM/HM or LM/LM) is in contrast with the criteria of Garcia et al. (1999) applied to the pectin mixtures. The behavior of the intrinsic viscosity, $[\eta_A]_B$, and mutual interaction constant, K_{AB}, in the case of compatible pectins (HM/LM) is opposite from the case of incompatible pectins (HM/HM and LM/LM).

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CONCLUSIONS

The present Ph.D. thesis is composed by two separate analysis: the first one regards the evaluation of the effect of dilution water on the recovery of pectin from pectic sauce by centrifugation, the second one, conducted by capillary viscometry, concerns the determination of the pectin molecular weights and the compatibility among the different pectins.

The first chapter is devoted to a general introduction and presentation of the problems addressed in the thesis. The answers to these questions are written in the form of scientific papers, some already published, and others close to publication.

The first analysis was discussed in chapter 2. The analysis was conducted on the efficiency of separation and the efficiency of extraction and the results are reported on the recovery of pectin with the degree of dilution of pectic sauce.

The second analysis was reported in chapters 3, 4, 5. The third chapter focuses on the characterization of dilute solutions of commercial pectins with different degrees of methoxylation. Intrinsic viscosities and molecular weights of all pectin utilized were determined. The identification of viscometric parameters of binary systems allowed subsequently studying the compatibility of the ternary mixtures on the basis of the comparison between experimental values and those ideals. The fourth chapter deals with the comparison of results obtained from aqueous solutions of different degree of methoxyl pectins (DM) , using two different techniques of viscometry: the classic dilution viscometry on ternary systems and the method of “polymer solvent”. The fifth chapter concluding the research carried out involving different systems of pectins with different degrees of methoxyl (HM / LM) or similar (HM / HM / LM / LM). The all pectins involved were originated from unique HM pectin. In addition to the

compatibility of the systems were calculated and measured viscometric parameters for polymer-solvent and polymer-polymer interactions.

Notes