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Binding Studies of Pectin in Presence of Divalent Cations

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The absorption capacity of low methoxy hydrated and amidated pectins to various metal cations and their effects on rheological properties due to binding of metal cations were investigated. Individual and competitive binding experiments were carried out using low-methoxy hydrated and amidated pectin solutions contained in dialysis bags and using metal cation solutions as the dialyzate. Equilibration took up to 40 h. The metal ions bound were in the order $Mn^{2+} > Fe^{2+} > Cu^{2+} > Ca^{2+} > Zn^{2+}$. Calcium binding experiments were undertaken at various pH levels in order to find out the optimum pH values for maximum binding, that for calcium being pH 5.

Keywords: Pectin, Dialysis, Cation binding, Atomic absorption, Binding curve, Pectin gel, Plateau-region.

INTRODUCTION

Pectins are soluble dietary fibers and are mainly present in the primary cell walls and in the middle lamella of plants [1]. Pectin is a group of complex structural polysaccharides present in higher plant cell walls [2]. The traditional, commercial sources of pectin are citrus peel and apple pomace [3]. Different plant sources produce pectins with differing molecular structures, pectins are widely used as texture modifiers, emulsion stabilizers, gelling and thickening agents in food and processing systems [4]. Pectins are composed by a linear backbone of randomly connected (1,4)-linked a-D-galactosyluronic acid residues partially esterified [5], are smooth regions of pectin structure and these ''smooth'' galacturonic regions are interrupted by ''hairy'' rhamnoga-lacturonic regions in which galacturonic acid residues are inter-spersed with (1,2)-linked a-L-rhamnopyranose residues [6]. Pectins are characterized by their degree of methylation (DM) and degree of esterification (DE) of the carboxyl groups [7]. If the degree of esterification is > 50 % it is referred to as high methoxyl (HM) pectin. Whereas with degree of esterification is < 50 % is referred to as low methoxy (LM) pectin [3,8].

Pectins are able to bind with a range of mono and divalent metal cations, the binding property will be affected by the physical properties like degree of esterification [9-11], also affected by the conditions, ion concentration and pH [12-14]. The binding also is dependent on initial addition of ion concentration [13,15]. The aim of this study was to investigate the binding capacity of pectin towards various divalent cations, influence of pH and the effect of calcium ion concentration on the amount of cation bound to pectin and establishing the competitive binding studies.

EXPERIMENTAL

Low methoxy and low methoxy amidated pectin samples were provided by CP Kelco (Denmark) and their specifications are given in Table-1.

TABLE-1
PECTIN SAMPLE SPECIFICATION

Sample No	Sample name	Specification (as supplied)
1	Pectin-LM H-331	DE-34.2

DE = Degree of esterification; LM = Low methoxy

$FeSO_4 \cdot 7H_2O$ (98 %), $CaCl_2$ (98 %), $ZnCl_2$ (99 %), $MnCl_2 \cdot 7H_2O$ (99 %) and $CuCl_2$ (98 %), were purchased from Aldrich Chemicals. Dialysis tubing with a diameter of 28.6 mm and with a 12-14,000 Dalton molecular weight cut-off was purchased from Medicell International Ltd.

The investigations were carried out using dialysis, atomic absorption spectrometry techniques. Dialysis was used in carrying out all the binding experiments related to binding studies. Atomic absorption spectrometry was used in cation measuring.

The required length of dialysis tubing was boiled in a mixture (50:50) of deionized water and industrial methylated spirit (IMS) for 10-15 min. It was then washed and stored in deionized water prior to use. 1 % pectin solutions were

prepared by slowly adding 1 g of pectin to 99 mL of deionized water with stirring. Pectin solutions (25 mL) were transferred to dialysis bags which were immersed for up to 72 h in bottles fitted with screw caps to prevent evaporation. The dialyzate contained metal ions in solution. For the binding experiments, the cations used were Ca^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+}, all the experiments were carried out in duplicates. All cation solutions prepared according to the equivalent carboxylate [COO^-] concentrations of pectin in distilled water, were of one, two, three and four equivalents (*viz.*, 1X, 2X, 3X and 4X) respectively, all being made up in the same manner. At the end of the experiments, 1 mL of aliquots was collected to analyze the concentrations of the metal ions bound to pectin.

After binding experiments had been completed, 1 mL aliquots were collected for subsequent analysis by atomic absorption. The use of 1000 fold diluted samples brought the concentrations within the detection limit of the spectrophotometer. The results obtained were then compared with the standard curves of respective metal ion. At the termination of the binding experiment, 1 mL of analyte was collected in a screw cap bottle and diluted 1000 times using distilled water. The absorbance of the sample was then measured along with that of the standards. Calculation of the number of mol of metal ion bound to one gram of dry pectin was then undertaken and a graph was then plot of the bound metal ion concentration against the initial concentration, thus yielding binding curves. This procedure was followed for all the metal ions used in the binding studies, all the binding experiment measurements were carried out in duplicates.

Binding isotherms (also termed binding curves which are parabolic in shape) were plotted to determine the metal cation bound concentrations (mol/g of dry pectin) and the actual concentrations of metal cations (mol/L). Both the bound and initial concentrations are expressed in moles – the bound concentrations being calculated from atomic absorption and standard curve data.

RESULTS AND DISCUSSION

Binding of cations to pectin: The binding isotherms of Fe^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} respectively with low methoxy pectin are shown in Fig. 1. It was observed that all the cations were exhibited binding ability with pectin with varying strengths which depends on ion specificity. Quantative binding results were 1.7×10^{-3} mol of Fe^{2+}, 1.5×10^{-3} mol of Ca^{2+} 2.5×10^{-3} mol of Mn^{2+}, 1×10^{-3} mole of Zn^{2+} and 1.6×10^{-3} mol of Cu^{2+} were bound at the plateau region of the binding curves.

The amount of Mn^{2+} ion bound was higher among the three ions with LMH pectin; it could be because of higher ion selectivity than the remaining ions. The binding order for the above three cations with LMH pectin was found to be: $Mn^{2+} > Fe^{2+} > Cu^{2+} > Ca^{2+} > Zn^{2+}$. The amount of cations bound by pectin increased as cation concentration increased, the relationship was linear at lower concentrations and reached a plateau region at higher concentrations, where at plateau region/saturation, no more binding was observed. The plateau observed at the highest bound and saturation region, could be due to the carboxylic groups which are fully saturated, there are no more free groups to bind to pectin. Torre *et al.* [13] investigated

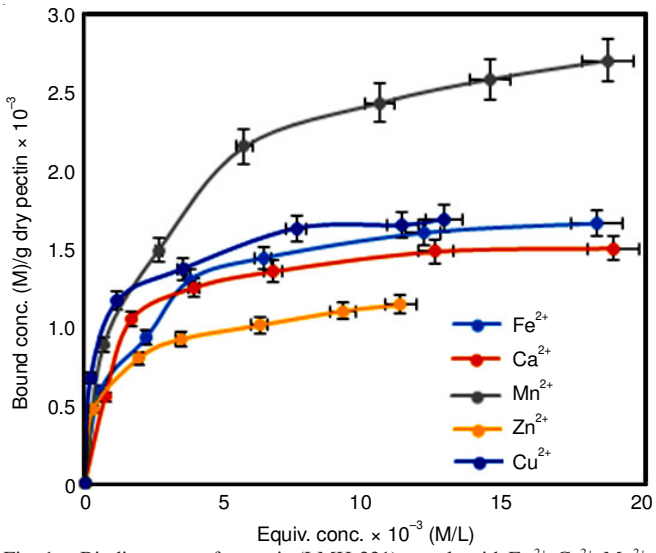

Fig. 1. Binding curves for pectin (LMH-331) sample with Fe^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}

interactions of Fe^{2+}, Fe^{3+} and Ca^{2+} with five food pomace material. They found that the majority of the samples exhibited higher capacity to bind Fe^{2+} than the remaining Fe^{3+} and Ca^{2+} ions and also concluded that the binding was increased with increasing cation concentration and reached the plateau region at higher concentrations when the concentration of pomace materials became saturated with mineral ions. The results of binding of Fe^{2+}, Fe^{3+} and Ca^{2+} ions with pectin in this current study are in good agreement with what Torre *et al.* [13]. It has been found (Table-2), that in terms of M^{2+}/COO^- ratios there is approximately 1 Fe^{2+} ion for every 2.1 carboxylate ions; 1 Ca^{2+} ion for every 2.4 carboxylate ions, there is approximately 1 Mn^{2+} ion for every 1.5 carboxylate ions; 1 Zn^{2+} ions for every 3.67 carboxylate ions and 1 Cu^{2+} ion for every 2.3 carboxylate ions; it is observed that the Zn^{2+} has poor binding ability to pectin.

TABLE-2
M^{2+}:COO^- BINDING RATIOS FOR LMH-331 PECTIN SAMPLE

Pectin sample	Fe^{2+}: COO^-	Ca^{2+}: COO^-	Mn^{2+}: COO^-	Cu^{2+}: COO^-	Zn^{2+}: COO^-
LMH-331	1:2.16	1:2.46	1:1.47	1:2.3	1:3.67

The plateau region observed at the highest bound and saturation region could be due to the fact that the carboxylic groups are fully saturated and there are no more free groups to bind with. According to Nair *et al.* [10], the binding of Cu^{2+}, Zn^{2+}, Cd^{2+} ions to various gel forming dietary fibers (guar gum, low and high methoxy pectin, sterculia gum) reported that the amount of metal cation bound was a function of the initial concentration of cation added. This is concluded in our individual binding experimental results. The studies on Cu^{2+}, Zn^{2+} binding experiments also showed that the binding order of metal ions was $Cu^{2+} > Zn^{2+}$, Cu^{2+} ion showed that the higher binding ability with pectin which is in agreement to present results of Cu^{2+}, Zn^{2+} individual binding experiments with LMH pectin, observed that $Cu^{2+} > Zn^{2+}$ in the experiments of Cu^{2+} and Zn^{2+} ions binding with LMH pectin, Cu^{2+} ion showed the higher binding ability. Kartel *et al.* [9] worked on evaluation

of adsorption of various industrial pectins from different sources (apple, sugar beet, citrus) towards some heavy metals, all the pectins showed similar binding affinity towards Cu^{2+} and Zn^{2+}, as $Cu^{2+} > Zn^{2+}$, which is similar to present results.

Effect of pH and Ca concentration: Fig. 2 shows the binding curves for Ca^{2+} with pectin LMH-331 sample at various pH values ranging from 2 to 6. It was observed that the LMH-331 pectin sample (at pH 2) had a minimal binding capacity of 1.9×10^{-3} mol whereas at pH 5, it registered the highest binding capacity of 2.68×10^{-3} mol. The binding capacity increased until pH 5 was reached, subsequently decreasing at pH 6 - binding capacity fell as the pH rose above 5. The optimal pH range for Ca^{2+} was found to be at pH 5.

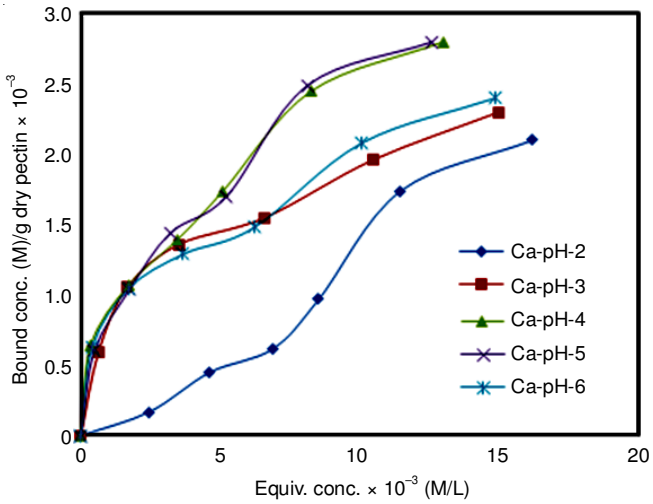

Fig. 2. Binding curves for Ca^{2+} using LMH Pectin at pH 2-6

As reported by Schlemmer [14], the maximum binding was observed for Ca^{2+} ion between pH 5-6 than decreasing, with pectin, which is in consistent to present studies of Ca^{2+} ion binding with LMH pectin at various pH values. In both above comparisons the final measurements of ions were by atomic absorption method. According to Torre *et al.* [13] the binding of Fe^{2+} and Ca^{2+} ions with used natural food pomace materials of apple, olive and lemon pomace which are rich in pectins and concluded that the calcium binding increased with pH and it is maximum between the pH values of 5-6 than started decreasing with pH, which is found same in the Ca^{2+} binding experiments at different pH values. According to Kyomugasho *et al.* [12], calcium binding to pectin increase with increasing pH and showing maxima at pH 5 which is same in this study. Due to the reason at higher pH values an increase in the no of ionized carboxyl groups and also as degree of methylation decreases the number of shared non methylated carboxyl ions in galucturonic acid residues are long enough for formation of egg-boxes increased, so does Ca^{2+} binding affinity [12].

Competitive binding: Fig. 3 shows the effects of competitive binding studies, for mixtures of Fe^{2+} and Ca^{2+} (mixture 1) and for Fe^{3+} and Ca^{2+} (mixture 2) with low methoxy pectin (LMH-331). It was found that from the experimental results, 1.31×10^{-3} mol of Ca^{2+} and 0.85×10^{-3} mol of Fe^{2+} with mixture 1 and 1.19×10^{-3} mol of Ca^{2+} and 0.98×10^{-3} mol of Fe^{3+} with mixture 2 bound to LMH pectin. It is observed that the amount

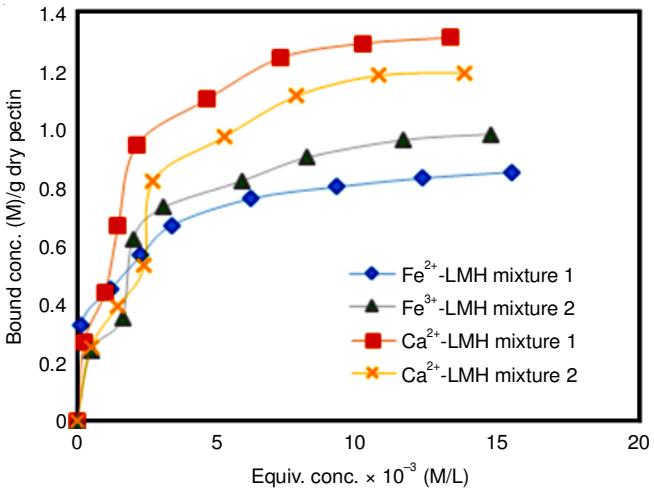

Fig. 3. Competitive binding curve for LMH pectin with mixture 1 and mixture 2

of Ca^{2+} ion bound to pectin was greater than the Fe^{2+} and Fe^{3+}. The Ca^{2+} ion dominated in competitive binding in both the mixtures and in both the pectin samples, may be because the pH was in favour of the binding of Ca^{2+} ion. During the experiment the natural pH value was in the range of 4.5-5.5, this is the favouring condition for binding of Ca^{2+} ions to pectin from the binding experiments at various pH values and also evidence comes from the studies of Schlemmer [14] and Torre *et al.* [13]. They found that the pH around 5 is the favourable condition for the optimum Ca^{2+} ion binding to pectin.

The binding ratios of the number of metal ions to the number of carboxylate ions for the two pectin types are given in Table-3, which show that there are approximately one Ca^{2+} and one Fe^{3+} ion for every four carboxylate ions for the Ca^{2+}/Fe^{3+} mixture. This approximates would be expected from a consideration of the stoichiometric ratios. In the case of the Ca^{2+}/Fe^{2+} mixture, the amount of Ca^{2+} bound is greater than expected and this is believed to be due to the fact that some of the Ca^{2+} ions precipitate as calcium sulphate since the Fe^{2+} ions was present as ferrous sulphate. Precipitation was not evident in the case of the Ca^{2+}/Fe^{3+} mixture since ferric chloride was used.

TABLE-3
M^{2+}:COO^- COMPETITIVE BINDING RATIOS FOR LMH

Pectin sample	Fe^{2+} and Ca^{2+} mixture		Fe^{3+} & Ca^{2+} mixture	
	Fe^{2+}:COO^-	Ca^{2+}:COO^-	Fe^{3+}:COO^-	Ca^{2+}:COO^-
LMH-331	1:4.35	1:2.75	1:3.70	1:3.07

Conclusion

The metal cations interact with anionic polymers like pectin and have the ability to bind carboxylic group sites on the pectin structure through chemical binding. It was observed from individual binding studies that the Mn^{2+} and Fe^{2+} cations (in comparison with those of Cu^{2+}, Ca^{2+} and Zn^{2+}) displayed the highest binding capacity towards LMH-pectin at natural pH conditions, with the binding order $Mn^{2+} > Fe^{2+} > Cu^{2+} > Ca^{2+} > Zn^{2}$. The optimum conditions for divalent calcium (Ca^{2+}) cation binding with LMH pectin occur between pH 5-6. A reduction in binding begins at pH 7, possibly due to complex

formation with counter ions. In competitive binding (between Fe^{2+} and Ca^{2+}) with LMH-pectin, Ca^{2+} was found to have dominated the binding. This phenomenon may have many applications including food processing, heavy metal removal in waste water treatment and in drug delivery.

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