Carbohydrate Polymers 79 (2010) 584–589

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Comparing biosorbent ability of modified citrus and durian rind pectin

Wong Weng Wai, Abbas F.M. AlKarkhi, Azhar Mat Easa *

School of Industrial Technology, 11800 USM, Minden, Penang, Malaysia

ARTICLE INFO

Article history:
Received 16 October 2008
Received in revised form 9 September 2009
Accepted 11 September 2009
Available online 18 September 2009

Keywords:
Biosorbents
Heavy metals
Modified durian rind pectin
Modified citrus pectin
MANOVA
Cluster analysis

ABSTRACT

Biosorbent ability of modified durian rind, durian rind, citrus and modified citrus pectin for removals of toxic heavy metals was investigated, and data were analyzed using multivariate analysis of variance (MANOVA) and cluster analysis (CA). Degree of esterification (% DE) of the biosorbents ranged between 22.33% and 60.81%, and was in the order; modified citrus pectin < modified durian rind pectin < durian rind pectin < citrus pectin. In most cases the order of biosorbent ability was; modified citrus pectin > modified durian rind pectin, citrus pectin > durian rind pectin. MANOVA showed a significant difference between samples and concentration of biosorbents, while CA classified the four biosorbent samples (based on biosorbent ability) into three different clusters; (1) citrus pectin and modified durian rind pectin, (2) durian rind pectin and (3) modified citrus pectin. The uptake of heavy metal by biosorbents was dependent on chemical structure of pectin and increased with biosorbent concentration and in most cases in accordance with the reduction in % DE.

1. Introduction

Heavy metals are dangerous because they tend to undergo a process of bioaccumulation. Their potential toxic effects accumulate within sensitive organs and tissues. For humans, poisoning by most of these metals causes severe dysfunction of kidney, reproductive system, liver, brain and central nervous system (Manahan, 1994).

Pectin are the ionic plant polysaccharides, which main structural features are the linear chains containing more than 100 (1–4)-linked α-D-galacturonic acid residue (Schols & Voragen, 1996). Pectin is typically used as a gelling agent in certain food products. As the ability to form gels of pectin depends on the molecular size and degree of esterification (DE), it is expected that pectin of different sources to have different gelling ability. At present commercial pectins are almost exclusively derived from citrus peel or apple pomace, both by-products from juice industries. Apple pomace contains 10–15% of pectin on a dry matter basis, while citrus peel contains 20–30%. One potential use of pectin is as a biosorbent. Heavy metal binding ability of pectin has been reported by Khotimchenko, Kovalev, and Khotimchenko (2007) and Kartel, Kupchik, and Veisov (1999). Modified citrus pectin or MCP is a complex polysaccharide obtained from the peel and pulp of citrus fruits. It is produced from citrus pectin via pH and temperature modification that breaks it into shorter, non-branched, galactose-rich carbohydrate chains. These shorter chains dissolve more readily in water and are better absorbed and utilized by the body than the long-chain pectin. Heavy metal removal ability of modified citrus pectin in human blood stream has been demonstrated by Eliaz and Rode (2003). Other than the heavy metal removal ability, modified citrus pectin also has a binding affinity for galectins on the surface of cancer cells, resulting in an inhibition, or blocking of cancer cell aggregation, adhesion and metastasis (Raz & Loton, 1987). It can be envisaged that a conversion of conventional pectin into its modified counterparts promises greater return in investment.

Durian (*Durio zibethinus*) is one of the famous fruit commodities in Malaysia and some other South East Asia countries. The edible portion of the fruit, known as the aril only accounts for about 15–30% of the mass of the entire fruit (Brown, 1997). During the season of durian, the amounts of the rind disposition as waste could lead to environmental problems. Water soluble polysaccharides that had been extracted from durian rind contain a high amount of pectin (Hokputsa et al., 2004) that could be further utilized for industrial uses. At pH 3 and in the presence of 65% sugar, durian rind pectin formed strong gels (Easa, 2005) suggesting the suitability of the product as a gelling agent. However the use of durian rind pectin and modified durian rind pectin as a biosorbents has never been studied. Such an evaluation is expected to enhance the value of pectin from durian rind waste.

The objective of this study was to compare biosorbent ability of modified durian rind pectin with durian rind pectin, citrus pectin and modified citrus pectin. Modification of durian rind pectin is expected to change the carboxyl residues in pectin and would affect the biosorbent ability of the modified products. The biosorbent abilities of the pectin samples will be statistically analyzed using multivariate statistical analysis.

* Corresponding author. Tel.: +60 4 6533888x2222; fax: +60 4 6573678.
E-mail address: azhar@usm.my (A.M. Easa).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.09.018

2. Materials and methods

2.1. Materials

Durian rinds were obtained from local fruits stall in Penang, Malaysia. Citrus pectin (CP) was purchased from Sigma; modified citrus pectin, MCP (PectaSol®) was obtained from EcoNugenics®; metal cations were used as their salts; $Pb(NO_3)_2$, $NiCl_2$, $CuSO_4$ were purchased from Sigma; $ZnSO_4$ from Riedel-de-Haën, $CdCl_2$ from Fluka. All other chemicals used were of analytical grade.

2.2. Preparation of pectin from durian rind

The extraction method was modified from the combination methods described by Hokputsa et al. (2004) and McCready (1965). The durian rind (solid–liquid ratio; 1:9, w/v) were gently stirred in a mild acid aqueous solution adjusted to pH 2 with 1 M HCl. Then, the solution was extracted at 90 °C for 4 h. The resulting slurries were filtered through cheese cloth and allowed to cool to room temperature (25 °C). Acidified ethanol (4% HCl in 95% EtOH) was added in the ratio 1:4 (v/v) and kept for 1 h. The mixture was centrifuged at 1710*g* for 15 min in a Bench Top Centrifuge (Kubota 5100, Fujioka, Japan).

The gel-like precipitate was collected and re-suspended in distilled water with the ratio 1:4 (w/v). Then, the solution was re-washed twice with 95% ethanol (1:2 v/v) and centrifuged for 15 min (1710*g*). Precipitate was collected and dried in a vacuum oven at 25 °C for 8 h. The pectin was ground and sieved (mesh no. 60) for further experiments. This pectin was designated as durian rind pectin or DRP.

2.3. Modification of durian rind pectin

The pH and temperature modification of DRP was performed according to Nangia-Makker et al. (2002) and Platt and Raz (1992). Initially, DRP was solubilized as a 1.5% solution in distilled water, and its pH was increased to 10.0 with NaOH (3 N), followed by a 1 h incubation at 50–60 °C. Then, it was cooled to room temperature while its pH was adjusted to 3.0 with 3 N HCl and stored overnight. Samples are then precipitated the next day with 95% ethanol and incubated at −20 °C for 2 h, filtered, washed with acetone, and dried in a vacuum oven at 25 °C for 8 h. The pectin was ground and sieved (mesh no. 60) for further experiments. This pectin was designated as modified durian rind pectin or mDRP.

2.4. Determination of the degree of esterification

All pectin samples were dried and desiccated in a vacuum jar prior to Fourier transform infrared (FT-IR) analysis. FT-IR spectra of pectins were obtained using a 2000 FT-IR spectrometer (Perkin–Elmer, Germany). The spectra were recorded at the absorbance mode from 4000 to 400 cm^{-1} (mid infrared region). At least triplicate spectra were recorded for each sample. Because the DE is defined as (number of esterified carboxylic groups/number of total carboxylic groups) × 100, it is inferred that the ratio of the area of the band at 1730 cm^{-1} (corresponding to the number of esterified carboxylic groups) over the sum of the areas of the bands between 1730 and 1600 cm^{-1} (corresponding to the number of total carboxylic groups) should be proportional to the DE, i.e. DE = $A_{1730}/(A_{1730} + A_{1600})$ (Chatjigakis et al., 1998; Manrique & Lajolo, 2002).

2.5. Heavy metal biosorption

Adsorption studies were performed according to Kartel et al. (1999) and Senthilkummar, Bharathi, Nithyanandhi, and Subburam (2000) with slight modification. About 0.1, 0.2, 0.5 and 1.0 g/100 mL of different pectin biosorbents were added into 100 ml flasks containing 50 ml of a 10 mMol/L solution of metal salts. The flasks were agitated under constant stirring for 2 h at room temperature. The liquid and solid phases were separated by centrifugation at 1710*g* for 5 min. The heavy metals in the supernatant were estimated using atomic absorption spectroscopy (Perkin–Elmer Analyst 100). The experiments were carried out in triplicate and the results are presented as mean values.

2.6. Statistical analysis

2.6.1. Multivariate analysis of variance (MANOVA)

Multivariate analysis of variance is used where several dependent variables (*P*) are measured on each sampling unit instead of one variable. The objective of MANOVA is to compare the mean vectors of *k* groups for significant difference. Equality of the mean vectors implies that the *k* means are equal for each variable, and if two means differ for just one variable then we conclude that the mean vectors of the *k* groups are different.

2.6.2. Cluster analysis

Cluster analysis (CA) is a multivariate technique, whose primary purpose is to classify the objects of the system into categories or clusters based on their similarities, and the objective is to find an optimal grouping for which the observations or objects within each cluster are similar, but the clusters are dissimilar to each other. Hierarchical clustering is the most common approach in which clusters are formed sequentially. The most similar objects are first grouped, and these initial groups are merged according to their similarities. Eventually as the similarity decreases all subgroups are fused into a single cluster. CA was applied to physical properties of banana flour using a linkage method. In the linkage method, the distances or similarities between two clusters A and B is defined as the minimum distance between a point in A and a point in B:

$$D(A,B) = \min\{d(y_i, y_j), \quad \text{for } y_i \text{in } A \text{ and } y_j \text{ in } B\} \tag{1}$$

where $d(y_i, y_j)$ is the Euclidean distance in (1).

At each step the distance is found for every pair of clusters and the two clusters with smallest distance (largest similarity) are merged. After two clusters are merged the procedure is repeated for the next step: the distances between all pairs of clusters are calculated again, and the pair with minimum distance is merged into a single cluster. The result of a hierarchical clustering procedure can be displayed graphically using a tree diagram, also known as a dendrogram, which shows all the steps in the hierarchical procedure (Alvin, 2002; Richard & Dean, 2002).

3. Results and discussion

The FT-IR spectra of pectin samples are presented in Fig. 1. Stronger bands occurring between 1760–1730, and 1630–1600 cm^{-1} are derived from the ester carbonyl groups and carboxylate ion stretching band, respectively (Chatjigakis et al., 1998; Manrique & Lajolo, 2002). It was observed that the intensity of the absorbance or band area of the ester carbonyl groups increased in unmodified pectin (CP and DRP), in contrast, the absorbance intensity or the band area of the carboxylate stretching band (1730–1760 cm^{-1}) decreased (Fig. 1). In a similar manner, the intensity of the absorbance or band area of the free carboxylate groups (1630–1600 cm^{-1}) increased in the modified pectin (mDRP and MCP). These observations established the basis for quantitative analysis of % DE of pectins by FT-IR: the 1760–1730 cm^{-1} bands

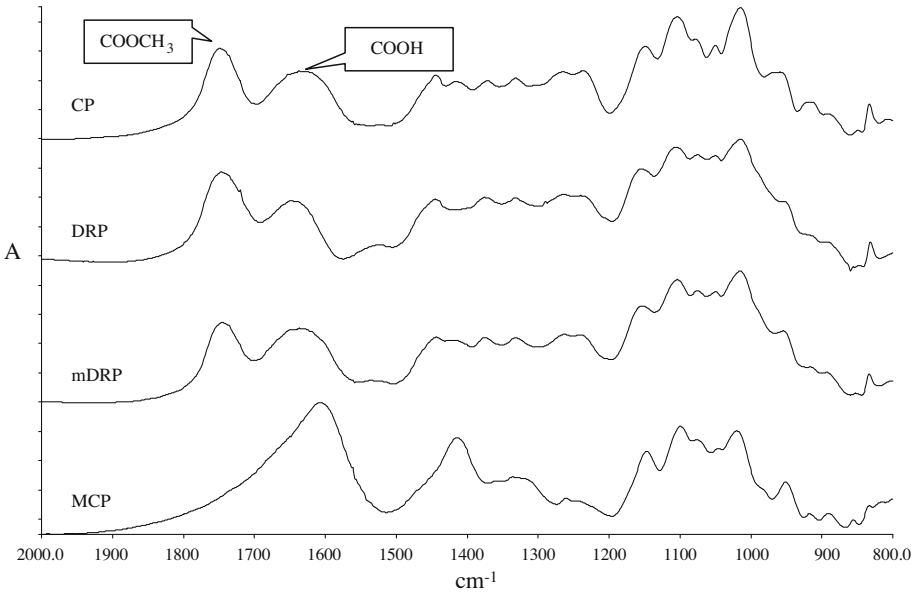

Fig. 1. Fourier transforms infrared spectra of pectin biosorbents.

represent ester carbonyl groups while the 1630–1600 cm^{-1} band represents the free carboxylate groups.

The carboxylate groups also showed two bands, an asymmetrical stretching band near 1650–1550 cm^{-1}, and a weaker symmetric stretching band near 1400 cm^{-1}. In pectin samples, the weaker symmetric (COO^-) stretching is followed by moderately intense absorption patterns between 1300 and 800 cm^{-1}; these collectively are referred to as the fingerprint region for pectin. Other bands of lesser importance in pectin samples are C–H bending, occurring at 1380 cm^{-1}, and C=O stretching occurring at 1300–1000 cm^{-1} (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998; Gnanasambandam & Proctor, 2000).

In order to quantify the % DE of pectins, a calibration curve was constructed based on pectin standards of known % DE. The calibration curve was established from the ratio of $A_{1730}/(A_{1730} + A_{1600})$, as presented in Fig. 2. Using this calibration curve, % DE of pectin was calculated and the results are presented in Table 1. Pectin samples revealed % DE of 60.81, 22.33, 52.55 and 42.07 for CP, MCP, DRP and mDRP, respectively. This results indicated differences in % DE of the biosorbents used, and that modification of DRP had successfully altered the % DE. All pectin samples used were soluble in water at the pH of application. Upon treatment of pectin with heavy metals, gels were formed at the bottom of reaction bottles.

Table 1
Degree of esterification of durian rind and citrus pectin.

Sample	% DE[a]
Citrus pectin (CP)	60.81 ± 0.48
Modified citrus pectin (MCP)	22.33 ± 1.29
Durian rind pectin (DRP)	52.55 ± 0.30
Modified durian rind pectin (mDRP)	42.07 ± 0.84

[a] Mean of triplicate samples ± standard deviation.

These were filtered after the end of process for heavy metals analysis.

Descriptive statistics for different biosorbent samples and five heavy metals including the maximum, minimum, mean and standard deviations are presented in Table 2. The minimum and maximum values are the results of low (0.1 g/100 mL) and high level (1 g/100 mL) of biosorbent concentrations. The mean values represent the average response of four levels repeated three times. It can be seen that MCP yielded the highest heavy metal removal for all parameters compared with other samples at a high level of 1 g/100 mL biosorbent application, while DRP exhibited the lowest removal of heavy metals, except for Pb (II) where mDRP showed the lowest removal at 0.1 g/100 mL concentration. The

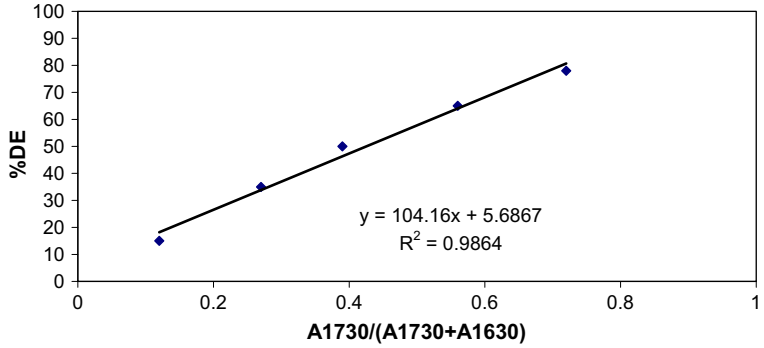

Fig. 2. Calibration curve of absorbance from the FT-IR spectra of pectin standards: ratio of the peak area at 1730 cm^{-1} over the sum of the peak areas at 1730 and 1600 cm^{-1} versus degree of esterification of pectins (%).

Table 2
Descriptive statistics of heavy metal removals (% removal) of four types of pectin.

Sample[a]	Parameter	Minimum	Maximum	Mean[b]	Standard deviation
CP	Cd (II)	7.64	40.82	20.30	12.00
	Cu (II)	22.22	81.48	47.50	23.15
	Zn (II)	5.88	44.19	21.54	14.80
	Pb (II)	7.02	68.75	32.79	23.92
	Ni (II)	7.14	54.55	27.07	18.34
MCP	Cd (II)	15.97	94.31	50.84	32.08
	Cu (II)	18.52	98.59	61.16	32.43
	Zn (II)	13.37	95.51	47.01	34.56
	Pb (II)	13.51	98.07	51.11	35.87
	Ni (II)	8.93	92.95	45.87	33.56
DRP	Cd (II)	3.42	10.96	7.32	2.85
	Cu (II)	7.41	59.26	28.42	18.87
	Zn (II)	2.94	9.30	6.25	2.29
	Pb (II)	4.46	39.47	18.47	13.93
	Ni (II)	6.56	27.87	17.13	7.58
mDRP	Cd (II)	8.22	41.10	23.51	14.21
	Cu (II)	22.22	81.48	49.67	23.15
	Zn (II)	5.06	44.77	19.08	14.86
	Pb (II)	1.75	58.77	25.30	22.26
	Ni (II)	10.91	43.64	27.46	12.52

[a] CP, citrus pectin; MCP, modified citrus pectin; DRP, durian rind pectin; mDRP, modified durian rind pectin.
[B] The average of response for all concentrations.

Table 3
Results of MANOVA for heavy metal removal.

Effect	Test	*F*	*P*-value
Sample	Pillai's Trace	62.37	<0.001
	Wilks' Lambda	294.52	<0.001
	Hotelling's Trace	1479.44	<0.001
	Roy's Largest Root	4863.08	<0.001
Concentration	Pillai's Trace	13.19	<0.001
	Wilks' Lambda	119.25	<0.001
	Hotelling's Trace	2342.20	<0.001
	Roy's Largest Root	7889.23	<0.001
Sample*concentration	Pillai's Trace	11.57	<0.001
	Wilks' Lambda	38.86	<0.001
	Hotelling's Trace	240.54	<0.001
	Roy's Largest Root	1380.59	<0.001

spread around the mean was high for all heavy metals and pectin samples, and this is due to the difference in response of heavy metal removal at different levels. Cu (II) showed the highest % removals as compared to other metals. Therefore, the influence of biosorbent concentration (0.1–1 g/100 mL) on % removals of Cu (II) metal ions is shown in Fig 3 as example of the effect of biosorbent concentration on % removal. It can be seen that % removals increased with levels of biosorbents used. Except for 0.1 g/100 mL concentration, the order of biosorbent ability for Cu (II) removal was MCP > mDRP, CP > DRP.

Multivariate analysis of variance (MANOVA) was applied to study the effect of four pectin samples and four different concentrations on the removals of heavy metals (Cu (II), Cd (II), Zn (II), Pb (II), and Ni (II)). The results of MANOVA (Table 3) showed that the interaction between samples and concentrations was statistically significant ($P < 0.001$). This indicates that the effect of pectin samples depends on the level of the concentration and vice versa, in another word the difference in response between the levels of concentration is not the same for all samples. Tukey's test was performed to find the differences between four different samples. Tukey's results showed that the pectin samples were statistically different ($P < 0.05$) in terms of heavy metal removals, except for the heavy metals Cu (II) and Ni (II) where CP and mDRP did not show significant difference. The results of Tukey's test for different concentration of pectin samples exhibited a strong significant difference ($P < 0.05$).

Cluster analysis (CA) was used to identify the similarity groups between the pectin samples using different concentrations based on five heavy metals included in this study. CA rendered a dendrogram as shown in Fig. 4, grouping all four pectin samples into three statistically significant clusters (the average of response for all concentrations). Cluster 1 represents CP and mDRP, cluster 2 represents DRP, while cluster 3 represents MCP. Similar results of dendograms were obtained when biosorbent ability of the four pectin samples were analyzed at different concentrations (0.1, 0.2, 0.5 and 1 g/100 mL), signifying that the differences in biosorbent ability were dependent on biosorbent concentration (results not shown). The similarity in biosorbent ability between CP and mDRP increased at 1 g/100 mL concentration compared with other

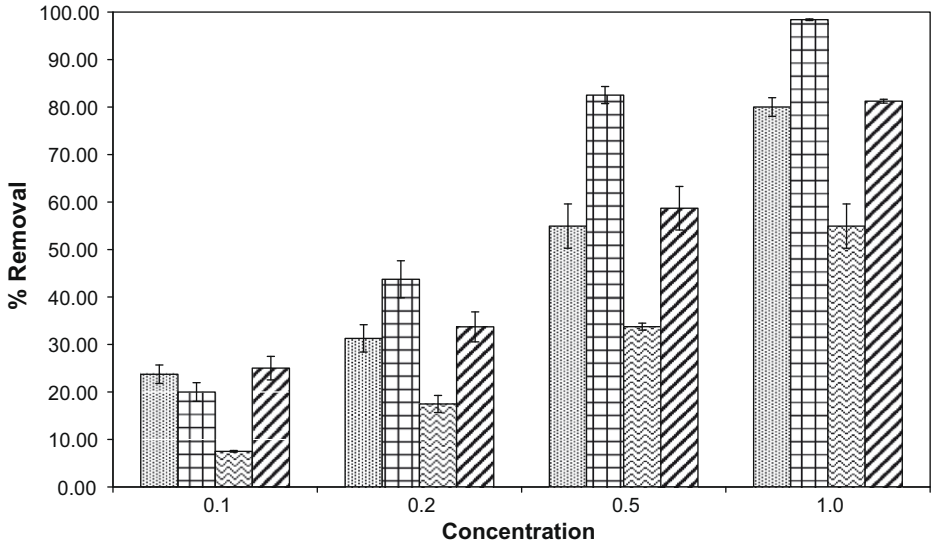

Fig. 3. Percent removal of Cu^{2+} ion by (▨) CP, (▦) MCP, (▩) DRP and (▨) mDRP as a function of biosorbent concentration. Error bars indicate standard deviations of triplicate samples.

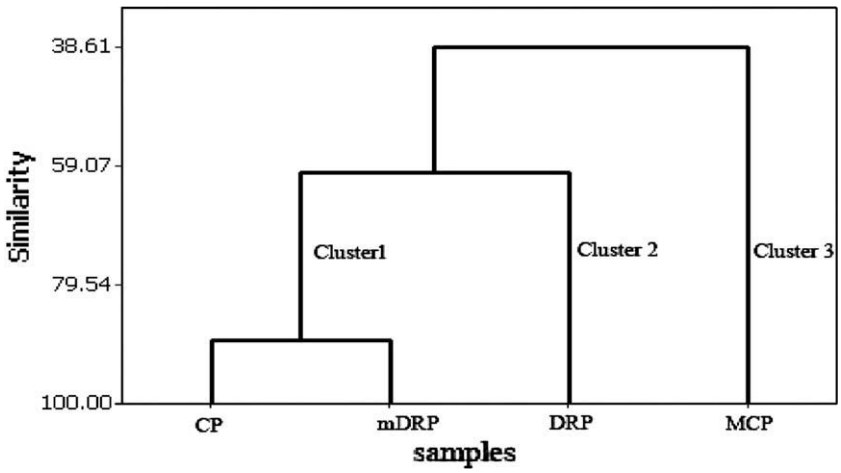

Fig. 4. Dendrogram showing clustering of samples based on biosorbent ability.

levels. This indicates that application at high concentration will make CP and mDRP samples closer in their biosorbent performance than at low concentrations. The similarities between DRP and other biosorbents fluctuates with concentration, for instance at low level of 0.1 g/100 mL the similarity was very small, however the similarity increased at 0.2 g/100 mL level, decreased back at 0.5 g/100 mL and then increased at 1 g/100 mL concentration.

These experimental results show almost a similar trend to the results of Gardea-Torresdey and coworkers (Gardea-Torresdey, Becker-Hapak, & Darnall, 1990). They investigated the effect of esterification of carboxyl groups of five different algal biomass for metal ions binding, and reported that uptake of copper by algal biomass decreased dramatically by esterification of carboxyl groups. The differing biosorption behaviors of pectin samples may be explained by the "egg-box" model (Axelos & Thibault, 1991). The esterified residues on the galacturonic acid are not active, whereas the negatively charged free carboxyl groups in pectin molecules form the covalent bonds with metal ions. It may be expected that pectin with low degree of esterification would exert considerable higher sorption activity (Khotimchenko et al., 2007). Therefore, MCP that contains the lowest degree of esterification exerted considerably the highest sorption activity (Manunza, Deiana, Pintore, & Gessa, 1998). This also confirms the suggestion that chemical modification of pectin could enhance the adsorption capacity of heavy metals, and the process of modification of DRP had increased the binding of metal ions with carboxyl residues of the pectin. As a result of modification, the biosorbent ability of mDRP was improved and this was almost similar to that of CP. In this study however, the lowest biosorbent ability was shown by DRP. This suggests that the process of pectin extraction from the rind of durian may need optimization to produce higher quality and yield of pectin.

It will be more challenging to assess biosorbent ability of mDRP in human blood stream, even though this kind of experiments has been conducted for MCP (Eliaz & Rode, 2003). A demonstration of such potential will promise a greater value of mDRP as alternative to MCP. The outstanding biosorbent ability of MCP was expected since MCP is a commercial product that has been optimized and properly processed to perform biosorbent functions. As CP was designed as a gelling agent for food products its biosorbent ability was moderate. Nevertheless CP has been shown to be effective in removing lead and mercury from the gastrointestinal tract and respiratory organs (Kohn, 1982) and lead in rats (Khotimchenko, Serguschenko, & Khotimchenko, 2006). A more recent study also showed that pectin compounds are favorable sorbers (Khotimchenko et al., 2007). However, a more exciting areas of research involving pectin and its modified products are those involving the use of modified pectin for protection against cancer (Gunning, Bongaerts, & Morris, 2008). Alkaline and acid treatment changes the structure of pectin and lead to the release of modified "hairy regions" and removals of arabinose residues. These modified fragments may protect against cancer by binding to a protein that plays a role in all stages of cancer progression (Gunning et al., 2008).

4. Conclusion

Based on the cluster analysis the order of effectiveness of heavy metal removal of the biosorbents was MCP > mDRP, CP > DRP. The order of % DE of all pectin samples was MCP < mDRP < DRP < CP. This results support the suggestion that modification of pectin improves the metal uptake that could be due to the differing in chemical structure. The highest sorption ability was shown by MCP, the lowest-esterified pectin. The level of esterification of mDRP may still be improved via optimized modification processes of DRP.

Acknowledgements

A short term grant from USM (304/PTEKIND/636071) is gratefully acknowledged. The authors would like to acknowledge Phuah Eng Tong for his assistance in AAS analyses.

References

Alvin, C. R. (2002). *Methods of multivariate analysis*. USA: John Wiley and Sons Inc..

Axelos, M. A. V., & Thibault, J. F. (1991). The chemistry of low-methoxyl pectin gelation. In R. H. Walter (Ed.), *Chemistry and technology of pectin*. New York: Academic Press.

Brown, M. J. (1997). Durio – a bibliographic review. In R. K. Arora, V. Ramanatha Rao, & A. N. Rao (Eds.), *International Plant Genetic Resources Institute (IPGRI)*.

Chatjigakis, A. K., Pappas, C., Proxenia, N., Kalantzi, O., Rodis, P., & Polissiou, M. (1998). FT-IR spectroscopic determination of the degree of esterification of cell wall pectins from stored peaches and correlation to textural changes. *Carbohydrate Polymers, 37*, 395–408.

Coimbra, M. A., Barros, A., Barros, M., Rutledge, D. N., & Delgadillo, I. (1998). Multivariate analysis of uronic acid and neutral sugars in whole pectic samples by FT-IR spectroscopy. *Carbohydrate Polymers, 37*, 241–248.

Easa, A. M. (2005). Functional foods and ingredients from local natural resources. In *Canadian USM joint meeting on biotechnology 2005*, USains Holding, USM, Penang, March 2005.

Eliaz, I., & Rode, D. (2003). The effects of modified citrus pectin on the urinary excretion of toxic elements. In *Fifth annual conference of environmental health scientist: Nutritional toxicology and metabolomics*, August 2003, University of California Davis.

Gardea-Torresdey, J. L., Becker-Hapak, M. K., & Darnall, D. W. (1990). Effect of chemical modification of algal carboxyl groups on metal ion binding. *Environment Science Technology, 24*(9), 1372–1378.

Gnanasambandam, R., & Proctor, A. (2000). Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry, 68*, 327–332.

Gunning, A. P., Bongaerts, R. J. M., & Morris, V. J. (2008). Recognition of galactan components of pectin by galectin-3. *FASEB Journal*. doi: 10.1096/fj.08-106617.

Hokputsa, S., Gerddit, W., Pongsamart, S., Inngjerdingen, K., Heinze, T., Koschella, A., et al. (2004). Water-soluble polysaccharides with pharmaceutical importance from Durian Rinds (*Durio zibethinus* Murr.): Isolation, fractionation, characterisation and bioactivity. *Carbohydrate Polymer, 56*(4), 471–481.

Kartel, M. T., Kupchik, L. A., & Veisov, B. K. (1999). Evaluation of pectin binding of heavy metal ions in aqueous solutions. *Chemosphere, 38*(11), 2591–2596.

Khotimchenko, M., Kovalev, V., & Khotimchenko, Y. (2007). Equilibrium studies of sorption of lead (II) ions by different pectin compounds. *Journal of Hazardous Materials, 149*(3), 693–699.

Khotimchenko, M., Serguschenko, I., & Khotimchenko, Y. (2006). Lead absorption and excretion in rats given insoluble salts of pectin and alginate. *International Journal of Toxicology, 25*, 195–203.

Kohn, R. (1982). Binding of toxic cations to pectin, its oligomeric fragments and plant tissues. *Carbohydrate Polymers, 2*, 273–275.

Manahan, S. E. (1994). *Environmental chemistry*. Boca Raton, FL, USA: Lewis.

Manrique, G. D., & Lajolo, F. M. (2002). FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biology and Technology, 25*, 99–107.

Manunza, B., Deiana, S., Pintore, M., & Gessa, C. (1998). Interaction of Ca^{2+} and Na^{+} ions with polygalacturonate chains: A molecular dynamics study. *Journal of Glycoconjugate, 297*, 300.

McCready, R. M. (1965). Extraction of pectin from citrus peels and conversion of pectin to pectic acid. *Methods in Carbohydrate Chemistry, 5*, 167–170.

Nangia-Makker, P., Hogan, V., Honjo, Y., Baccarini, S., Tait, L., Bresalier, R., et al. (2002). Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin. *Journal of the National Cancer Institute, 94*(24), 1854–1862.

Platt, D., & Raz, A. (1992). Modulation of the lung colonization of B16-F1 melanoma cells by citrus pectin. *Journal of the National Cancer Institute, 84*(6), 438–442.

Raz, A., & Loton, R. (1987). Endogeneous galactoside-binding lectins: A new class of functional cell surface molecules related to metastasis. *Cancer Metastasis Review, 6*, 433–452.

Richard, A. J., & Dean, W. W. (2002). *Applied multivariate statistical analysis*. London: Prentice-Hall.

Schols, H. A., & Voragen, A. G. J. (1996). Complex pectins: Structure elucidation using enzymes. In J. Visser & A. G. J. Voragen (Eds.), *Pectins and pectinases* (pp. 3–19). Amsterdam: Elsevier Science.

Senthilkummar, S., Bharathi, S., Nithyanandhi, D., & Subburam, V. (2000). Biosorption of toxic heavy metals from aqueous solutions. *Bioresource Technology, 75*, 163–165.