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ScienceDirect
Bioresource Technology 99 (2008) 5561–5566
BIORESOURCE TECHNOLOGY

Optimization of extraction of high-ester pectin from passion fruit peel (*Passiflora edulis* flavicarpa) with citric acid by using response surface methodology

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Received 12 December 2006; received in revised form 26 October 2007; accepted 26 October 2007
Available online 20 February 2008

Abstract

A central composite design was employed to optimize the extraction of pectin with citric acid. The independent variables were citric acid concentration (0.086–2.91% w/v) and extraction time (17–102 min). The combined effect of these variables on the degree of esterification was investigated. Results have shown that the generated regression models adequately explained the data variation and significantly represented the actual relationship between the independent variables and the responses. Besides that, the citric acid concentration was the most important factor to affect the degree of esterification, as it exerted a significant influence on the dependent variable. Lower citric acid concentration increased the pectin degree of esterification. The surface response showed the relationships between the independent variables, and thus responses were generated. Through this surface, the satisfactory condition of 0.086% w/v citric acid for 60 min was established for extraction of high-ester yellow passion fruit pectin.

Keywords: Pectin extraction; Passion fruit peel; Degree of esterification; Response surface methodology; Central composite design

1. Introduction

Pectin is a high-value functional food ingredient widely used as gelling agent and stabilizer. It is also an abundant, ubiquitous and multifunctional component of the cell walls of all land plants (Willats et al., 2006).

The main raw materials used to produce commercial pectin are apple pomace and citrus peels (May, 1990). Very little is known about the pectic substances in passion fruit in general. The two main edible species cultivated for commercial purposes are the purple passion fruit (*P. edulis* Sims), typically consumed fresh due to its sweeter taste, and the yellow passion fruit or 'maracuja' (*P. edulis* f. flavicarpa Degener), commonly used for production of juice; either pure or sweetened due to its slightly acidic taste (Yapo and Koffi, 2006). The peel represents about half of the fruit mass. Because of significant juice production, the peels, as a major waste, have become a substantial burden to the environment. Hence it is necessary to find a feasible way to turn the peels into useful products or to adequately dispose of them, seeking a positive environmental impact (Liu et al., 2006).

According to May (1999), only a few source materials have been used for commercial production of pectin as food additive. One of the reasons for this is that most of the pectic materials present in nature do not have any functional properties; in particular, the ability to form sugar acid gel systems, and this property has been the main requirement of commercial pectin until recently.

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doi:10.1016/j.biortech.2007.10.058

Pectin extraction is a multiple-stage physicochemical process in which the hydrolysis and extraction of pectin macromolecules from plant tissue and their solubilisation take place under the influence of different factors, mainly temperature, pH, and time (Pagán et al., 2001). Reports are available on the extraction of pectins with mineral acids, such as sulfuric acid (Yapo et al., 2007; Yapo et al., 2007), hydrochloric acid (Iglesias and Lozano, 2004; Kratchanova et al., 2004; Mesbahi et al., 2005; Fishman et al., 2006; Faravash and Ashtiani, in press), nitric acid (Pagán et al., 2001; Yapo and Koffi, 2006), and tartaric acid (Canteri-Schemin et al., 2005). However, very little is known about the extraction of pectin with citric acid (Virk and Sogi, 2004; Canteri-Schemin et al., 2005; Marcon et al., 2005), that could be better than the other extractors from an economic as from an environmental point of view.

Pectin is a polymer of α-galacturonic acid with a variable number of methyl ester groups (Liu et al., 2006). However, pectin also contains α-L- rhamnopyranosyl residues in the backbone chain and branch chains of arabinan and galactan, and their fine structure vary considerably (Shingthong et al., 2004).

Some of the carboxylic groups of galacturonic acid molecules in the pectin chains are methyl esterified and the percentage of esterified groups is expressed as DE (degree of esterification). Depending on the degree of esterification, pectin is divided into two major groups: high-ester pectin, with DE higher than 50%, and low-ester pectin, with DE lower than 50% (Thakur et al., 1997). In high-ester pectin, the junction zones are formed by the cross-linking of homogalacturan through hydrogen bond and the hydrophobic interaction between methoxyl groups, both of which are promoted by high-sugar concentration and low pH. In low-ester pectin, junction zones are formed by calcium cross-linking between free carboxyl groups (Willats et al., 2006).

The main objective was to develop an approach that would bring a better understanding of the relationships between the variables (citric acid concentration and time of extraction) and the response (degree of esterification); and to obtain optimum conditions for pectin extraction from passion fruit peel. Thus a response surface methodology (RSM) using central composite design was employed. The advantage of this methodology is the simultaneous investigation of the main factors from a small number of experiments. The investigated region can lead to optimum pectin extraction conditions.

2. Methods

Passion fruits (*Passiflora edulis* flavicarpa) were obtained from the CEASA fruit farm, Florianópolis, Brazil, from March to May 2005. The fruits at the same ripening stage and with similar peel colours were selected. All the chemical reagents used were of analytical grade.

2.1. Preparation and chemical analysis of passion fruit peel flour

The passion fruit was washed and the pulp was separated from the flesh. The pulp was not studied at all. The skin (flavedo) was removed and the peels were dried in a air-circulate oven (Model 171, FABBE, São Paulo, Brazil) at 55 °C until their weight was constant. The dried peels were then milled to a dry 60 mesh size powdered passion fruit peel and the resulting product, referred to as 'passion fruit peel flour', was used as the raw material for all the pectin extraction and characterization assays. The passion fruit peel flour was packaged in a polyethylene bag and stored in a freezer (-18 ± 2 °C) until required.

The passion fruit peel flour was analysed for moisture, lipid, crude protein ($N \times 6.25$), total ash contents (AOAC, 1998), and soluble and insoluble dietary fibre content (AACC, 1999), expressed as g/100 g (dry basis). All the analyses were carried out in triplicate ($n = 3$). Total carbohydrate was calculated by difference.

2.2. Pectin extraction

The extraction procedure was according to the Canteri-Schemin et al. (2005) method, with slight modification. Pectin was extracted with different citric acid concentrations and extraction times, under reflux in a condensation system at 97 °C (solute/solvent 1:50). The hot acid extract was filtered through the ordinary screen with 1 mm mesh size equipped with two-layer cheesecloth, and the filtrate was cooled down to 4 °C. The filtrate (containing pectin) was centrifuged for 30 min at 6000 rpm. The supernatant was precipitate with absolute ethanol (1:2 v/v) and then left to rest for one hour in order to allow pectin flotation (Kalapathy and Proctor, 2001). The floating pectin was separated by filtration (# 40 filter paper) and rinsed with absolute ethanol. The pectin produced was dried in an air-circulate oven at 45 °C for 12 h. The resulting material was milled to a dry 60 mesh size powdered pectin.

2.3. Determination of degree of esterification

The degree of esterification (DE) of pectin samples were determined by the potentiometric titration method by Bochek et al. (2001). Dried pectin (0.2 g) was placed in a weighing bottle for titration and wetted with ethanol. Distilled water, at 40 °C (20 mL), was added by stirring. The polymer was dissolved by stirring for 2 h. The resulting solution was titrated with 0.1 N NaOH in the presence of phenolphthalein and the result was recorded as the initial titre (It). Then, a 0.1 N NaOH solution (10 mL) was added to a neutralized polygalacturonic acid sample after determination of the free carboxy groups. The weighing bottle was plugged with a stopper. The content was stirred at room temperature for 2 h to saponify the esterified carboxy

Table 1
Experimental and coded levels of two variables employed for pectin extraction in the central composite experimental design

Variables	Level				
	$-a$[a]	−1	0	+1	$+a$[a]
Citric acid concentration (% w/v)	0.086	0.500	1.500	2.500	2.914
Extraction time (min)	17.58	30.00	60.00	90.00	102.42

[a] $a = \pm 1.414$ for $k = 2$ (two independent variables).

groups of the polymer. Then 0.1 N HCl (10 mL) was added. Excess HCl was titrated with 0.1 N NaOH. The number of the esterified carboxy groups was calculated from the volume of 0.1 NaOH solution spent for titration (the final titre – Ft).

The DE was calculated by using

$$\%\mathrm{DE} = \left(\frac{\mathrm{Ft}}{\mathrm{It} + \mathrm{Ft}}\right) \cdot 100 \quad (1)$$

2.4. Experimental design and statistical analysis

Response surface methodology (RSM) was used to determine the optimum condition for pectin extraction from passion fruit peel flour. The central composite design (CCD) with two independent variables was employed (Box and Wilson, 1951; Teófilo and Ferreira, 2006). The variables used were: citric acid concentration (Citr.) and extraction time (Et.). The minimum, maximum and mean values for citric acid concentration were set at 0.086%, 2.91% and 1.50% (w/v), respectively, and for the effect of extraction time they were set to 17, 102 and 60 min, respectively. The levels were selected based on previous studies (unpublished results). The complete design consisted of 13 experiments including four factorial experiments (levels − and +1), four axial experiments (levels $\pm a$), and five replicates in centre point (Table 1). Experiments in the centre of the design were performed in order to make the estimation of pure error possible. All the experiments were carried out at random in order to minimize the effect of unexplained variability in the observed responses due to systematic errors. The response function (y) measured was degree of esterification (DE) of the extracted pectin.

The regression coefficients for linear, quadratic and interaction terms were determined by using multiple linear regression (MLR). The significance of each regression coefficient was judged statistically by computing the t-value from pure error obtained from the replicates in the central point. The analysis of variance (ANOVA) was applied to validate the model. The regression coefficients were then used to generate response surfaces. All calculations and graphics were performed using electronic worksheets from Microsoft® Excel 2003 in accordance with Teófilo and Ferreira (2006).

3. Results and discussion

3.1. Chemical composition of passion fruit peel flour

The chemical composition of passion fruit peel flour is shown in Table 2. The gravimetric quantification of insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) content obtained from the passion fruit peel flour is presented in Table 3. In agreement with all previously published data for other fruit flours (Pagán and Ibarz, 1999; Virk and Sogi, 2004), low-lipid content and high-ash, soluble and insoluble dietary fibre contents have been found in passion fruit peel flour. The total dietary fibre content in the passion fruit peel flour was 57.36% w/w dry matter (the sum of IDF and SDF). Thus, the passion fruit by-product has proven to be a good source of fibre and it could be used as a raw material for pectin extraction. By comparing the crude protein content of the passion fruit peel flour (Table 2) with their corresponding residual protein content in both IDF and SDF rich materials (Table 3), it clearly indicated that the removal of proteins by using the bacterial protease normally applied in the AACC official methods was not efficient and the a larger amount of the proteins remained in their fractions. This could be due to the protein coprecipitation during the ethanol precipitation step in the AACC method and the inaccessibility of protein by the protease due to the presence of the plant fibre. It has been shown in plant foods that high-fibre content can lead to low-enzyme digestibility of protein (Mongeau et al., 1989; Baer et al., 1997; Cheung and Lee, 1998; Wong et al., 2003).

3.2. Experimental design

Experimental values obtained for the degree of esterification at the designed points are shown in Table 4, in experimental order. Multiple regression coefficients are shown in Table 5. From the results in Table 5, only the quadratic coefficient of the extraction time variable was not significant by t-test at a significance level of 0.05. The Citr. variable has shown to be the most important variable in this system. The results show that lower citric acid concentrations, within the levels studied, contribute to a larger percentage for the degree of esterification. The Citr. × Et. interaction has shown to be the second most important coefficient of this model. Once again, this result suggests a great influence of the citric acid concentration (within the levels studied), because a higher degree of esterification is obtained with low-citric acid concentrations, even with long extraction time. However, with high-citric acid concentrations, a shorter extraction time is necessary in order to obtain a higher degree of esterification.

Table 6 shows the results obtained from the Analysis of Variance (ANOVA). The regression was significant, but the lack of fit was not, and the pure error was low. Due to the high precision of the experimental degree of esterification, the pure error was small and accordingly, the

Table 2
Chemical composition of passion fruit peel flour (g/100 g dry sample, except for moisture)

Components	Passion fruit peel flour[a]
Moisture	9.93 ± 0.12
Ash	7.52 ± 0.02
Protein	4.05 ± 0.61
Lipid	Less than 0.10
Soluble fiber	19.20 ± 0.02
Insoluble fiber	38.05 ± 0.02
Carbohydrate	21.28 ± 0.44

[a] Means ± SD ($n = 3$).

Table 3
Chemical composition of the fibre material (g/100 g dry sample) obtained by AACC method from passion fruit peel flour[a]

Fibre fraction	Dietary fibre rich materials	Residual protein[b]	Ash	Dietary fibre (DF)[c]
Insoluble dietary fibre (IDF)	42.40 ± 0.02	1.35 ± 0.01	3.00 ± 0.01	38.05 ± 0.02
Soluble dietary fibre (SDF)	23.96 ± 0.04	1.49 ± 0.01	3.27 ± 0.01	19.20 ± 0.02

[a] Data are mean values of three determinations ± SD.
[b] Residual protein = $N \times 6.25$.
[c] DF = DF rich materials − residual protein − ash.

Table 4
Variables, levels and responses of degree of esterification based on citric acid concentration and extraction time

Exp. order	Variable levels[a]		Response
	Citr. (% w/v)	Et. (min)	DE (%)
12	1.5 (0)	60.0 (0)	47.62
9	1.5 (0)	60.0 (0)	46.51
11	1.5 (0)	60.0 (0)	49.71
4	2.5 (+1)	90.0 (+1)	27.52
13	1.5 (0)	60.0 (0)	49.13
1	0.5 (−1)	30.0 (−1)	67.04
8	1.5 (0)	102.4 (+a)	44.31
7	1.5 (0)	17.6 (−a)	46.27
10	1.5 (0)	60.0 (0)	50.00
6	2.914 (+a)	60.0 (0)	27.69
2	2.5 (+1)	30.0 (−1)	40.70
5	0.086 (−a)	60.0 (0)	78.59
3	0.5 (−1)	90.0 (+1)	69.44

[a] Coded levels are with brackets.

Table 5
Coded regression coefficients, standard error and p values for the model built

	Coeff.	Std. err.	t	p
Mean	48.59[a]	0.66	73.25	2.08E−07
$b_{\text{Citr.}}$	−17.53[a]	0.52	−33.43	5.00E−06
$b_{\text{Et.}}$	−1.69[a]	0.52	−3.23	3.20E−02
$b^2_{\text{Citr.}}$	2.76[a]	0.56	4.91	7.97E−03
$b^2_{\text{Et.}}$	−1.16	0.56	−2.07	1.08E−01
$b_{\text{Citr.}} \times b_{\text{Et.}}$	−3.90[a]	0.74	−5.25	6.29E−03

[a] Significant level for t-test: 0.05, degree of freedom: 4.

Table 6
ANOVA for regression model built for DE

Variation	SS	DF	MS	F	p
Regression*	2611.60	5	522.32	138.9	7.61E-07
Residues	26.32	7	3.76		
Lack of fit	17.52	3	5.84	2.6	0.18
Pure error	8.80	4	2.20		
Total SS	2637.90	12			

* Significative at significance level of 0.05.

standard error was calculated by the mean squares residual (MS residual). The t-test was accomplished with 12 degrees of freedom and a significance level of 0.05.

A graphical representation of the models' quality can be seen in Fig. 1A and B. The predicted ($\hat{y}$) versus measured (y) values for the degree of pectin esterification shows that the quadratic model fits very well the data, with a correlation coefficient of 0.99 (Fig. 1A). The residuals ($y - \hat{y}$) plot (Fig. 1B) shows that their values are low and does not appear to show any regular trend. This is confirmed by the correlation coefficients for linear and polynomial fit of residuals versus measured data that were of 0.099 and 0.025, respectively. Thus, it can be assumed that normality, independence and randomness of the residuals were satisfied (see Teófilo and Ferreira (2006) for further discussion on the use of residuals).

The model built for the degree of esterification is in Eq. (2), and Fig. 2 shows the response surface elaborated from this quadratic regression model. Both, the coefficients in the Eq. (2) and the response surface in the Fig. 2 were obtained from coded levels as shown in Tables 1 and 4.

$$\text{DE}\ (\%) = 48.589 - 17.56\,\text{Citr.} - 1.698\,\text{Et.} + 2.7809\,\text{Citr.}^2 - 3.895\,\text{Citr.} - \text{Et.} \quad (2)$$

This surface shows that an increase in the degree of esterification of pectin is obtained when the variables Citr. and Et. are used at the levels −1.414 (Citr. 0.086% w/v) and

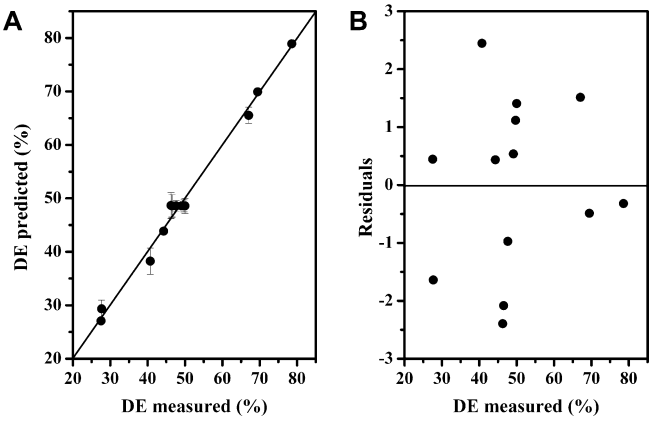

Fig. 1. (A) Plot of the predicted versus measured degree of esterification. (B) Plot of residual versus measured degree of esterification of the pectin samples.

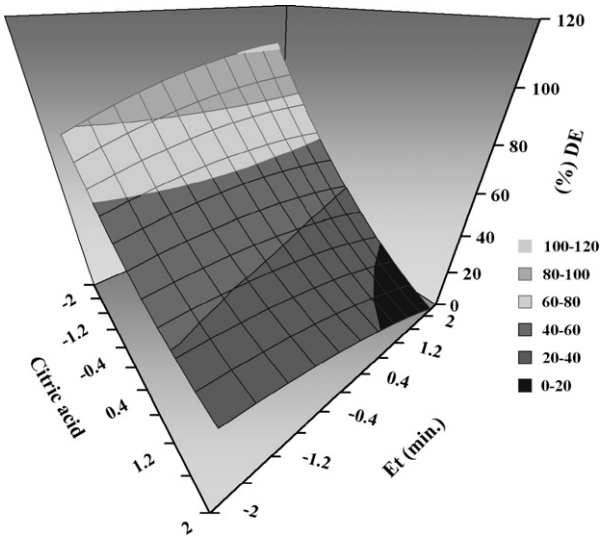

Fig. 2. Response Surface showing the effect of extraction time (coded values) and citric acid concentration (coded values) on degree of esterification.

0 (60 min), respectively. This satisfactory condition yielded pectin with 78.59% of degree of esterification.

Compared to the data found in literature, this degree of esterification of passion fruit peel pectin was close to those of Corona et al. (1996) (71.6%), D'Addosio et al. (2005) (69.7%) and Matsumoto and Otagaki (1990) (73.2%), under different extraction conditions, using hydrochloric acid as extractor. Canteri-Schemin et al. (2005) also obtained high-ester pectin (68.8%), extracted from apple pomace, with citric acid as extractor.

These results demonstrate the successful extraction of pectin with citric acid, providing potential benefits to industrial extraction of pectin, from an economic as well as an environmental point of view.

4. Conclusions

The different conditions (citric acid concentration, extraction time) for high-ester pectin extraction revealed that these variables markedly affect the degree of esterification of the yellow passion fruit pectin. These variables can be related to the extraction conditions by second order polynomials. By using the response surface, the satisfactory condition of the operating variables is obtained graphically in order to reach a high of the degree of esterification of yellow passion fruit pectin.

References

AACC, 1999. Approved Methods, 9th ed. American Association of Cereal Chemists, Saint Paul, Minnesota.

AOAC, 1998. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Washington, DC.

Baer, D.J., Rumpler, W.V., Miles, C.W., Fahey Jr., G.C., 1997. Dietary fiber decreases the metabolizable energy content and nutrient digestibility to mixed diets fed to humans. J. Nutr. 127, 579–586.

Bochek, A.M., Zabivalova, N.M., Petropavlovskii, G.A., 2001. Determination of the esterification degree of polygalacturonic acid. Russ. J. Appl. Chem. 75, 796–799.

Box, G.E.P., Wilson, K.B., 1951. On the experimental attainment of optimal conditions. J. Roy. Stat. Soc. B 13, 1–45.

Canteri-Schemin, M.H., Fertonani, H.C.R., Waszczynskyj, N., Wosiacki, G., 2005. Extraction of pectin from Apple pomace. Braz. Arch. Biol. Technol. 48, 259–266.

Cheung, P.C.K., Lee, M.Y., 1998. Comparative chemical analysis of fiber material prepared by enzymatic and chemical methods from two mushrooms (*Pleurotus sajor-caju* and *Pleurotus tuber-regium*). J. Agric. Food Chem. 46, 4854–4857.

Corona, M., Díaz, A., Páez, G., Ferrer, J., Mármol, Z., Ramones, E., 1996. Extracción y caracterización de pectina de la corteza de parchita. Rev. Fac. Agron – LUZ 13, 785–791.

D'Addosio, R., Páez, G., Marín, M., Mármol, Z., Ferrer, J., 2005. Obtención y caracterización de pectina a partir de la cáscara de parchita (*Passiflora edulis f.flavicarpa* Degener). Rev. Fac. Agron – LUZ 22, 240–249.

Faravash, R.S., Ashtiani, F.Z., in press. The effect of pH, ethanol volume and acid washing time on the yield of pectin extraction from peach pomace. Int. J. Food Sci. Technol., doi:10.1111/j.1365-2621.2006.01324.x.

Fishman, M.L., Chau, H.K., Hoagland, P.D., Hotchkiss, A.T., 2006. Microwave-assisted extraction of lime pectin. Food Hydrocolloid. 20, 1170–1177.

Iglesias, M.T., Lozano, J.E., 2004. Extraction and characterization of sunflower pectin. J. Food Eng. 62, 215–223.

Kalapathy, U., Proctor, A., 2001. Effect of acid extraction and alcohol precipitation conditions on yield and purity of soy hull pectin. Food Chem. 73, 393–396.

Kratchanova, M., Pavlova, E., Panchev, I., 2004. The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. Carbohyd. Polym. 56, 181–185.

Liu, Y., Shi, J., Langrish, T.A.G., 2006. Water-based extraction of pectin from flavedo and albedo of orange peels. Chem. Eng. J. 120, 203–209.

Marcon, M.V., Vriesmann, L.C., Wosiacki, G., Beleski-Carneiro, E., 2005. Pectins from apple pomace. Polímeros: Ciência e Tecnologia 15, 127–129.

Matsumoto, L., Otagaki, M., 1990. Pectin content in dried peel of passion fruit. J. Food Sci. 18, 132–137.

May, C.D., 1990. Industrial pectins: sources, production and applications. Carbohyd. Polym. 12, 79–99.

May, C.D., 1999. Pectins. In: Imeson, A. (Ed.), Thickening and Gelling Agents for Foods, second ed. Aspen Publishers, Maryland, pp. 231–261.

Mesbahi, G., Jamalian, J., Farahnaky, A., 2005. A comparative study on functional properties of beet and citrus pectins in foods systems. Food Hydrocolloid. 19, 731–738.

Mongeau, R., Sarwar, G., Peace, R.W., 1989. Relationship between dietary fiber levels and protein digestibility in selected foods as determine in rats. Plant Food. Hum. Nutr. 39, 45–51.

Pagán, J., Ibarz, A., 1999. Extraction and rheological properties of pectin from fresh peach pomace. J. Food Eng. 39, 193–201.

Pagán, J., Ibarz, A., Llorca, M., Pagán, A., Barbosa-Cánovas, G.V., 2001. Extraction and characterization of pectin from stored peach pomace. Food Res. Int. 34, 605–612.

Shingthong, J., Cui, S.W., Ningsanond, S., Goff, H.D., 2004. Structural characterization, degree of esterification and some gelling properties of Krueo Ma Noy (*Cissampelos pareira*) pectin. Carbohyd. Polym. 58, 391–400.

Teófilo, R.F., Ferreira, M.M.C., 2006. Quimiometria II: planilhas eletrônicas para cálculos de planejamentos experimentais, um tutorial. Quim. Nova 29, 338–350.

Thakur, B.R., Singh, R.K., Handa, A.K., 1997. Chemistry and uses of pectin – a reviews. Crit. Rev. Food Sci. 37, 47–73.

Virk, B.S., Sogi, D.S., 2004. Extraction and characterization of pectin from apple pomace (*Malus Pumila* Cv Amri) peel waste. Int. J. Food Prop. 7, 1–11.

Willats, W.G.T., Knox, J.P., Mikkelsen, J.D., 2006. Pectin: new insights into an old polymer are starting to gel. Food Sci. Technol. 17, 97–104.

Wong, K.H., Cheung, P.C.K., Wu, J.Z., 2003. Biochemical and microstructural characteristics of insoluble and soluble dietary fiber prepared from mushroom Sclerotia of *Pleurotus tuber-regium*, *Polyporus rhinoceros*, and *Wolfiporia cocos*. J. Agric. Food Chem. 51, 7197–7202.

Yapo, B.M., Koffi, K.L., 2006. Yelow passion fruit rind – a potential source of low-methoxyl pectin. J. Agric. Food Chem. 54, 2738–2744.

Yapo, B.M., Robert, C., Etienne, I., Wathelet, B., Paquot, M., 2007. Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. Food Chem. 100, 1356–1364.

Yapo, B.M., Wathelet, B., Paquot, M., 2007. Comparison of alcohol precipitation and membrane filtration effects on sugar beet pulp pectin chemical features and surface properties. Food Hydrocolloid. 21, 245–255.