International Food Research Journal 23(6): 2519-2524 (December 2016)

Journal homepage: *http://www.ifrj.upm.edu.my*

Optimization of extraction process of plant-based gelatin replacer

[1,2*]Jaswir, I., [1]Alotaibi, A., [1,2]Jamal, P., [3]Octavianti, F., [4]Lestari, W., [5]Hendri, R. and [6]Alkahtani, H.

[1]*Bioprocess and Biomolecular Engineering Research Unit, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak 53100, Kuala Lumpur, Malaysia*

[2]*International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, Jalan Gombak 53100, Kuala Lumpur, Malaysia*

[3]*Faculty of Dentistry, Universiti Sains Islam Malaysia, Pandan Indah, 55100, Kuala Lumpur*

[4]*Faculty of Dentistry, International Islamic University Malaysia, Kuantan Campus, Malaysia*

[5]*Faculty of Fisheries, Riau University, Pekanbaru, Indonesia*

[6]*College of Food and Agriculture Sciences, Food Science and Nutrition Department, PO BOX 2460, King Saud University, Riyadh 11451, Saudi Arabia*

Article history

Received: 4 April 2015
Received in revised form: 7 March 2016
Accepted: 17 March 2016

Keywords

Gelatin replacer
Mango peel
Sensory properties
Viscosity

Abstract

Gelatin is one of the most widely used food ingredients, with wide applications in the food industry. It was reported that 41% of the gelatin produced in the world is sourced from pig skin, 28.5% from bovine hides and 29.5% from bovine bones. However, factors such as the outbreak of BSE (a.k.a. mad cow disease) and increasing demand for non-mammalian gelatin for halal and kosher food markets have revived interest in gelatin replacers from plant sources. In this study, we have successfully extracted valuable pectin—as gelatin replacer--from various types of plant wastes. Pectin is a high value functional food ingredient widely used as a gelling agent and stabilizer. It is also an abundant, ubiquitous and multifunctional component of the cell walls of all land plants. Mango peel was screened as the ideal source for high-yield (36.6%) pectin of satisfactory quality. The results indicate that citric acid was the best solution for recovery of pectin from mango peels. An extraction temperature of 90°C and pH 2 provided the optimum conditions for maximum yield of pectin. The resulting crude mango peel pectin (CMPP) was analyzed for physicochemical parameters. The results indicated values for ash content (0.0412%), moisture content (0.303%), viscosity (45.18%), galacturonic acid content (36.8-37.2-40%) and degree of esterification (38.3-41%). Following analysis of its gelling properties and sensory evaluation, CMPP has good potential to be applied in the food industry as a low-methoxyl pectin and a cheap source of gelatin replacer for jam preparations.

Introduction

Gelatin is regarded as a special and unique hydrocolloid, serving multiple functions with a wide range of applications. Gelatin has long been used as a food ingredient (e.g., gelling and foaming agent), in the preparation of pharmaceutical products (e.g., soft and hard capsule, microspheres), in the biomedical field (wound dressing and three-dimensional tissue regeneration) and in numerous non-food applications (e.g., photography). The main sources of gelatin include pigskin, cattle bones and cattle hide. Gelatin is a product obtained by partial hydrolysis of collagen derived from animal skin, white connective tissue, and bones (Morrison *et al*., 1999). Currently, the main sources for commercial gelatin are limited to pig skins or cow skins and bones. These sources are cheap, leading to a relatively low cost of the final gelatin product.

The issue of gelatin replacement has existed for many years for the vegetarian, halal and kosher markets, but has gained increased interest in the last decade, particularly within Europe with the emergence of bovine spongiform encephalopathy (‘‘mad cow disease’’) in the 1980s (Morrison *et al*., 1999). Since then, there has been much concern about using gelatin derived from possibly infected animal parts. Since most commercial gelatins are obtained from either pigskin or cow hide, there has been considerable interest in finding and using alternative substitutes. As a result, the academia and industry have been trying for several years to develop alternatives to gelatin that possess most or all of the unique functional properties of mammalian gelatin. Driven by the foreseeable demand for halal/kosher gelatin, industries are now striving to develop

*Corresponding author.
Email: *irwandi@iium.edu.my*

gelatin-free products in which mammalian gelatin is no longer used, either as a processing aid or as an ingredient. The search for new gelling agents to replace mammalian gelatin has led to patents for fish gelatin production (Grossman and Bergman, 1992) as well as several published methods for fish gelatin extraction (Go´mez-Guille´n *et al*., 2002; Gudmundsson and Hafsteinsson, 1997).

There are some replacements for gelatin from plant sources such as carrageenan (from seaweed), agar (or agar-agar, also from seaweed), pectin, konnyaku (from konnyaku potato) and guar gum. Starches from corn and wheat are also used. Carrageenan comes from algae or seaweed, and can be used as a thickening agent in place of gelatin. It is usually derived from red alga, sometimes called Irish moss. Carrageenan is a common ingredient in many foods, including milk products like yogurt or chocolate milk (Corpet *et al*., 1997). Agar is a gelatinous substance derived from seaweed. The gelling agent is an unbranched polysaccharide obtained from the cell walls of some species of red algae, primarily from the genera Gelidium and Gracilaria, or seaweed *(Sphaerococcus euchema)*. Commercially it is derived primarily from Gelidium amansii. Agar (agar agar) can be used as a laxative, a vegetarian gelatin substitute, a thickener for soups, in jellies, ice cream and Japanese desserts such as anmitsu, as a clarifying agent in brewing, and for paper sizing fabrics (Gerbe and Sathyanarayana, 2001).

Konnyaku is a plant of the genus *Amorphophallus*. It is native to warm subtropical to tropical eastern Asia, from Japan and China to Indonesia. It is also used as a vegan substitute for gelatin. It is typically mottled grey and firmer in consistency than most gelatins. It has very little taste; the common variety tastes vaguely like salt. It is valued more for its texture than flavor (Teramoto and Uchigami, 2000). Guar gum, also called guaran, is a galactomannan. It is primarily the ground endosperm of guar beans. It is typically produced as a free flowing, pale, off-white colored, coarse to fine ground powder. Guar gum is economical because it has almost 8 times the water-thickening potency of cornstarch - only a very small quantity is needed for producing sufficient viscosity. Thus it can be used in various multi-phase formulations: as an emulsifier because it helps to prevent oil droplets from coalescing, and/or as a stabilizer because it helps to prevent solid particles from settling (Eyre and Caswell, 1991).

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described in 1825 by Henri Braconnot. It is produced commercially as a white to light brown powder, mainly extracted from citrus fruits, and is used in food as a gelling agent particularly in jams and jellies. It is also used in fillings, sweets, as a stabilizer in fruit juices and milk drinks and as a source of dietary fiber (Hanke and Northcote, 1975).

Compared to other types of gelatin sources from plant, pectin is the most common and easily extracted from fruits. Due to its physico-chemical characteristics, gelatin increases the physico-function of foods, which adds value to the product. However, only few fruits have been chosen to produce pectin in Malaysia.

With the increase of production of processed fruit products, the amount of fruit waste being generated is increasing enormously. Large amounts of fruit waste pose the problem of disposal without causing environmental pollution. This waste can be effectively recycled by manufacturing useful byproducts from them. A valuable byproduct that can be obtained from fruit wastes is pectin. Pectin is the designation for water-soluble pectinic acid (colloidal polygalacturonic acids) of varying methyl ester content and degree of neutralization, which are capable of forming gels with sugar and acids under suitable conditions (GITCO, 1999).

Materials and Methods

Materials

Local tropical fruit waste was purchased locally (orange, mango, banana, watermelon, guava, and mangosteen). Other materials include blender, hot plate, cheesecloth, grinder, oven, desiccator, Bunsen burner, furnace muffle Spectrophotometer, FT-IR spectrophotometry, viscometer, colourimeter, TA.HD plus Texture Analyzer with ½" Cyl. Delrin, patch No. 11343) from (Halal Products Research Institute, UPM), refrigerator, ethanol, distilled water, Polygalacturonic acid, standard commercial citrus pectin DE (45), citric acid, carbazole reagent, sodium hydroxide solution, sulphuric acid and 0.25 mm sieve.

Fruit waste powder preparation

Fresh fruit waste from local fruit shops were dried in an oven at 80°C for 24 hours, and ground into a powder. The powders were placed in sealed bags and held at refrigerated temperature unless the extraction procedure was performed immediately after peel preparation.

Screening

A modified method for extraction of pectin from fruit waste was conducted for all the six selected tropical fruit wastes (Berardini *et al*., 2005; Francis and Bell (1975); Norziah *et al.*, 2000; Bravermann, 1949; Rouse and Crandall, 1976; Sakai, 1989). Screening for the best plant waste was done by applying analytical methods.

Design of experiment for optimization

Response surface methodology (RSM) was used to determine optimal conditions for extraction of pectin from fruit waste. RSM was performed using Design Expert software (Version 6.0.8) program. Face entered central composite design (FCCCD) was used to investigate the effects of two independent variables (A: extraction temperature, and B: pH) on the dependent variable (pectin yield). The complete design consisted of 13 experimental points including three replications of the center point.

Results and Discussion

A modified acid extraction method was used for the six selected tropical fruit waste. Orange waste, banana waste, mango waste, mangosteen waste, watermelon waste, and guava waste were used in the preliminary stage to identify fruit waste with the highest yield of pectin. Fresh fruit waste from each fruit was dried and blended into powder form. Pectin yield percentage and the viscosity were determined from dried powders of the fruit waste. Results for all the six fruit wastes are listed in Table 1.

The highest yield of pectin was obtained from mango peel (10.22%) whereas the lowest yield of pectin was obtained from mangosteen peel (1.48%). Gelling properties (gel grade) were analyzed for all the extracted pectin by adding 1% of the pectin into hot water and measuring for viscosity using a viscometer. Results were graded as good, moderate and poor. Pectin from mangosteen had the poorest gelling property (2 cP), together with pectin from guava, and pectin from banana. However, watermelon and mango pectins had moderate gelling properties (13 cP, 18 cP respectively). Even though mango yielded the highest percentage (%) of pectin, pectin from orange waste exhibited very good gelling properties. Previous studies mention that time, temperature, type of acid used and pH play an important role in the extraction of pectin.

Among the six fruit wastes, mango peel was chosen as the ideal fruit waste for further study since it yielded the highest quantity of pectin. Although orange peel also showed interesting yields, pectin from citrus fruits (orange) is very common in the market and several research on pectin from citrus fruits have been published. The gelling property for all the 6 fruit waste pectin was tested manually, since the amount of pectin extracted was very little. The gelling property analysis for mango pectin is discussed in the upcoming sections. Other general characteristics of pectin obtained in this study are as follows: Moisture content (4.42 %), ash content (0.46%), degree of ssterification (41.0), GA (40%), and viscosity (45.18cP).

Optimization of acid extraction of pectin by FCCCD

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw materials (Monroe and Polk, 2000). A total of 13 runs were needed for optimizing the two parameters in the current study. Maximum yield of pectin (36.60%) was recorded under experimental conditions of extraction temperature 90°C, extraction time 2.5 hr, and pH 2. In order to optimize pectin extraction from mango peel, the significant independent variables (pH, T) were further explored, using FCCCD with face-centred star points, i.e. a ± 1 to investigate the desired design level. Thus, no dislocating or axial points with a > 1 were carried out.

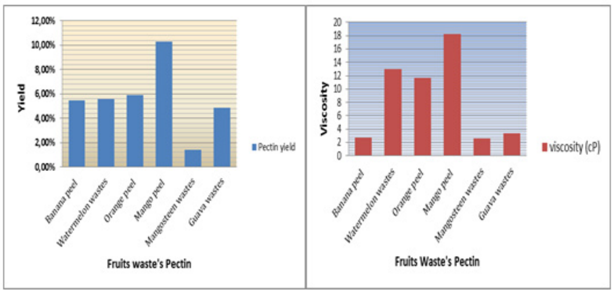
The 3D response surface and 2D contour plots are graphical representations of the regression equation, which is used to determine the optimum value of the parameters. In this study, the main goal of response surface was to efficiently achieve optimum values for concentration, temperature and pH of the extraction process to maximize the response, i.e. pectin yield.

Response surface methodology plays a key role in identifying the optimum values of the independent variables efficiently, under which dependent variables could achieve a maximum response. In the response surface plot and contour plot, optimal pectin yield (%) was obtained along with two continuous variables, while extraction time was fixed to 2.50 hours. In the figures, the maximum predicted value indicated by the surface was confined to the smallest ellipse in the contour diagram. Elliptical contours are obtained when a perfect interaction is present between the independent variables (Eloff, 1998). The independent variables and maximum predicted values from the figures corresponded with the optimum values of the dependent variables (responses) obtained by the equations (Monroe and Polk, 2000). In this study an optimal pectin yield of 36.60% is predicted at 90.00°C and pH 2.00 of extraction solution.

Based on the DE results obtained from FTIR spectroscopy, titrimetric analysis of CMPP and values for galacturonic acid content of CMPP, CMPP was classified as a low-methoxyl pectin (LMP). The

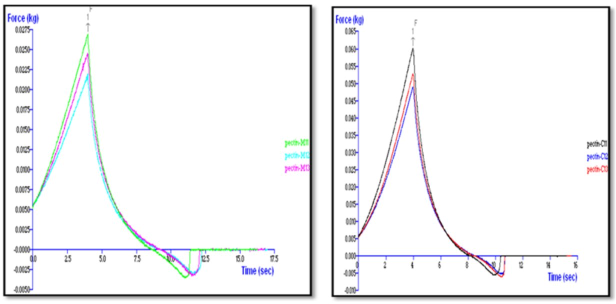
Table 1. Comparison of yields for pectin extracted from different tropical local fruit wastes

Fruit wastes	Drying loss %	Weight of dry peel (g)	Pectin weight (g) Mean ± SD	Pectin yield (%) Mean ± SD
Mango peel	84.91%	60.00 g	6.17±0.04	10.28±0.08
Banana peel	87.33%	60.00 g	3.31±0.03	5.49±0.17
Watermelon rind and peel	85.34%	60.00 g	3.35±0.02	5.56±0.21
Orange peel	76.67%	60.00 g	3.56±0.03	5.91±0.28
Mangosteen rind and peel	88.21%	60.00 g	0.85±0.03	1.39±0.09
Guava seeds and peel	88.03%	60.00 g	2.93±0.03	4.88±0.34



(a) (b)

Figure 1. Comparison of the pectin yield (a) and viscosity (b) of pectin extracted from six different fruit wastes



(a) (b)

Figure 2. (a) Gel strength graph of bake-resistant jam produced from commercial pectin (b) gel strength graph of bake-resistant jam produced from CMPP

values obtained for degree of esterification of CMPP were 38.3% and 41%, both lower than 50%, which is indicative of LMPs (Thibault and Ralet, 2001).

Low methoxyl pectin–gelling behaviour

The gel formation property of low- methyl ester pectins is as follows: when small amounts of calcium ions are added, the pectin chains start to bond over calcium bridges. With increasing calcium ion concentrations, gelation sets in. In case of an exceeding dosage of calcium ions, calcium pectinate will precipitate under the given gel forming conditions, which is referred to as ”pre-gelling”. The gel texture will then lose its elasticity, becomes pastier and shows a lower breaking strength.

The precipitation of calcium pectinate is reversible if the gel is heated again above the setting temperature and cooled down in a destruction-free process. The amount of calcium ions required for proper gelation largely depends on the concentration of soluble solids, the sugar, the pH-value and the buffer substances of the product.

Application of the FWP in food products as a gelling agent and quality determination of food products

The application of LM pectin is directly based on its gelling properties. As only a low quantity of sugar is needed for the gelation of LM pectin, it is widely used in “low-calorie gelled products”. The large pH zone for gelation makes it useful in food products which require non-acidic conditions for gelation. LM pectin and its amidated version are well adapted for use in gelled milk products whereby interactions with caseins may take place. Additionally, this pectin also finds applications in the production of fruit- based jams (Hoefler, 1991; Pilgrim, 1991; Thakur, 1997).

Jam is a fruit processed product which has an extended consumption shelf life. Fresh fruits and processed fruits can be used for jam processing. Dehydrated fruits in particular are used when raw material is lacking, as it can be continuously fed into processing during out-of-harvest seasons and keeps the quality of jam at a regular consistency.

Strawberry bake-resistant jam and fruit-flavored confectionery jelly were the chosen models for testing the gelling function of CMPP against commercial citrus pectin. Strawberry bake-resistant jam and fruit-flavored confectionery jelly were successfully prepared from CMPP and standard commercial pectin. Jam produced from CMPP was well accepted in terms of aroma, texture, taste, spreadability and overall acceptability. However, instrumental analysis of the jam prepared from CMPP indicated different values compared to jams prepared from commercial citrus pectin and commercial products, as shown in Table 2. The gel strength of jam prepared from CMPP

Table 2. Gel strength of bake-resistant strawberry jam produced from CMPP compared to jam produced from commercial citrus pectin and other commercial brands

Jam Type	Gel strength (g) Mean± SD	C.V%
Jam prepared by mango peel pectin	24.60±2.00	8.20
Jam prepared by commercial citrus pectin	54.10±4.70	8.46
Imperial®	48.77±8.30	17.1
Empire®	48.48±4.50	9.35
Best food®	64.08±12.5	19.5

was much lower ($p \leq 0.05$) than that of commercial products.

The texture profile in Figure 3 shows that jam prepared from commercial citrus pectin had a better texture profile compare to the jam prepared from CMPP. The jam produced from commercial pectin had a firmer texture with better texture readings, whereas the jam produced from CMPP was much softer, with a lower texture profile. However, when the two jams were presented for sensory analysis, the jam prepared from CMPP was more acceptable compared to the jam prepared from commercial citrus pectin. Table 4 indicates the viscosity, spreadability and overall acceptability for both jams.

Although the jam prepared using commercial pectin had a better texture profile, its use depends on industry applications, which depends on consumers' acceptance. Jam prepared using CMPP had better acceptance among the consumers due to its texture, which indicates that CMPP has a good commercial prospect for use in jams and the confectionery industry.

Sensory analysis

Table 3 shows the means and standard deviations for acceptance tests on bake-resistant jams prepared from CMPP and standard commercial citrus pectin. 25 panelists were used for the sensory evaluation of each jam product. Several attributes were evaluated for each product formulation, such as aroma, taste, texture (viscosity), spreadability and overall acceptability.

Commercialization potential

With the increase of processed fruit production, the amount of fruit waste generated is increasing proportionally. Large amounts of this waste pose a disposal problem. This waste can be effectively disposed of through recycling for the manufacture of useful byproducts. With the world production of mangoes reaching 10.2 million metric tons in 1995, approximately 863 thousand tonnes of mango peels are produced as waste (Torres and Bobet, 2001). The utilization of such waste to produce gelatin replacesr in the food industry not only addresses the issue of waste disposal, but may also be very important to the halal market and to add value to the overall production process. Therefore, there is great potential for mango peel pectin to be commercialized in the food market.

Table 3. Sensory acceptability of strawberry bake-resistant jams produced

Characteristics	Scores of sensory evaluation Mean± SD: Jam produced from commercial citrus pectin	Jam produced from CMPP
Aroma	7.12±1.05	7.72±1.17
Texture (viscosity)	6.72±1.59	7.84±1.18
Taste	7.20±1.50	8.00±1.32
Spreadability	6.92±1.26	8.04±1.14
Overall acceptability	6.96±1.51	7.88±1.13

with commercial citrus pectin and CMPP as a gelling agent

Conclusion

Pectin was successfully extracted from six selected tropical fruit waste in Malaysia; the highest yield was obtained from mango peels. Pectin yields for extraction methods were increased through the optimization of extraction parameters.

Acid extraction using citric acid and precipitation using ethanol was found to be the best acid/ alcohol combination. No differences in pectin yield due to the extraction solid to liquid ratio were observed. Additionally, no significant differences in pectin yield were recorded with increased extraction time. However, higher yields were observed with increased extraction temperature. Extraction temperature of 90°C and pH 2 were recorded as the optimum conditions for maximum yield of pectin.

Physicochemical parameters such as, color, ash content and moisture content were studied for CMPP. An acceptable level of color was obtained, and low ash (0.455%) and moisture content (4.42%) were recorded. CMPP showed increased viscosity with increased concentration. Results from analysis of degree of esterification (41%) and galacturonic acid content (40%) classified pectin extracted from mango peel (CMPP) as a low-methoxyl pectin (LMP). CMPP has the potential to be applied in the food industry as a cheap source of gelatin replacer in jam preparations to act as a gelling agent, as indicated and accepted by the analytical method and through sensory evaluation.

Crude mango peel pectin as a plant-based low methoxyl pectin would be more suitable in fruit spreads (low sugar jams) and milk dairy products than in confectionery.

Acknowledgment

The authors thank International Islamic University Malaysia for supporting this study.

References

Berardini, N., Knödler, M., Schieber, A. and Carle, R., 2005. Utilization of mango peels as a source of pectin and polyphenolics. Innovative Food Science and Emerging Technologies 6(4): 442-452.

Bravermann, J. B. S. 1949. Citrus products, p. 77. New York: Interscience.

Corpet, D. E., Taché, S. and Préclaire, M., 1997. Carrageenan given as a jelly, does not initiate, but promotes the growth of aberrant crypt foci in the rat colon. Cancer letters 114(1): 53-55.

Eloff, J.N. 1998. A sensitive and quick method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64: 711-713.

Eyre, M. J. and Caswell, S. C. 1991. Sterile culture of Rotylenchulus reniformis on tomato root with gellan gum as a supporting medium. Journal of Nematology 23: 229–231.

Francis, B.J. and Bell, J.M.K., 1975. Commercial pectin: a review. Tropical Science 17 1): 25-44.

Gerbe E. and Sathyanarayana B.N. 2001. Cassava- A new and cheaper alternative to agar or direct in vitro regeneration and microtuber production from nodal cultures of potato. Ethiopian Agricultural research organisation (EARO); African Crop Science Journal 9: 1-8.

Grossman S. and Bergman M., 1992. Process for the production of gelatin from fish skins. U.S. patent 5,093, 474.

GITCO. 1992. Twenty-five Prospective Food Processing Projects. Gujarat Industrial and Technical Consultancy Organization Limited, p. 52. Ahmedabad: GITCO House.

Go´mez-Guille´n, M. C., Turnay, J., Fernandez-Diaz, M. D., Ulmo, N., Lizarbe, M. A., and Montero, P. 2002. Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocolloids 16: 25-34.

Gudmundsson, M. and Hafsteinsson, H. 1997. Gelatin from cod skins as affected by chemical treatments. Journal of Food Science 62: 37-47.

Hanke, D. E., and Northcote, D. H. 1975. Molecular visualization of pectin and DNA by ruthenium red. Biopolymers 14: 1-17.

Hoefler, A. L. 1991. Other pectin food products. In Walter, R. H. (Ed.). The Chemistry and Technology of Pectin, p. 51. San diego: Academic Press.

Monroe, S. and Polk, R. 2000. Antimicrobial use and bacterial resistance. Current Opinions in Microbiology 3: 496-501.

Morrison, N. A., Clark, R. C., Chen, Y. L., Talashek, T. and Sworn, G. 1999. Gelatin alternatives for the food industry. In Nishinari, K., Kremer, F. and Lagaly, G. (Eds.), Physical chemistry and industrial application of gellan gum, p. 127-131. Eidelberg: Springer-Verlag.

Norziah, M. H., Fang, E. O. and Karim, A.A., 2000. Extraction and characterisation of pectin from pomelo fruit peels. Special Publication-Royal Society of Chemistry 251: 27-36.

Pilgrim, G.W., Walter, R.H. and Oakenfull, D.G. 1991. Jams, Jellies and Preserves. In Walter, R. H. (Ed). The Chemistry and Technology of Pectin, p. 23-50. New York: Academic Press.

Rouse, A.H. and Crandall, P.G., 1976. Nitric acid extraction of pectin from citrus peel. Proceedings of the Florida State Horticulture Society 89: 166-168.

Sakai, T. 1989. Process for preparing pectin. U.S. Patent 4,835,262.

Teramoto, A. and Fuchigami, M. 2000. Changes in temperature, texture, and structure of Konnyaku (Konjac Glucomannan Gel) during high-pressure-freezing. Journal of Food Science 65(3): 491-497.

Thakur, B., Singh, R. and Handa, A. 1997. Chemistry and uses of pectin, a review. Critical Review in Food Science and Nutrition 37(1): 47-73.

Thibault, J. F. and Ralet, M. C. 2001. Pectins, their origin, structure and functions. In McCleary, B. V. and Prosky, L. (Eds.), Advanced dietary fiber technology, p. 369–379. Oxford: Blackwell Science.