EXPERIMENTAL AND DFT STUDY OF GELLING FACTOR OF PECTIN

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Abstract

Industrial processing of coffee cherries is done to isolate coffee powder by removing shell and mucilagenous parts from coffee pulp. The process produces coffee with less quantity of caffeine, tannins, polyphenols, and pectin. Pectin is a methylated ester of polygalacturonic acid which divides into two groups based on degrees of esterification. In this work, the gelling behavior of pectin was studied. It was found that value of galacturonic acid was 447.24 mg and an equivalent weight was 207.47. In addition, Density Functional Theory (DFT) was applied to study the chemical structure of extracted pectin with pectin molecular variation of 2-8 monomers, using different amounts of methoxyl substitution. It was found that molecular weight could affect to the molecular structure as the longer the molecular structure, the more linearity it presents.

Keywords: Pectin, coffee pulp, density functional theory

Introduction

Coffee is enjoyed by millions of people around the world and its components have been extensively researched. Nowadays, Chiang Rai province is the biggest area of planted coffee in Thailand, especially at Doi Chang. The processing of coffee starts with the conversion of coffee cherries into green coffee beans, and the removal of both the pulp and hull by either a wet or dry method. Pulp is the thick layer, approximately 0.5-2 mm, which encloses the coffee cherries. Coffee pulp represents 28.7% of the weight of the fruit. The highest organic component in coffee pulp is tannins (1.80-8.56% of dry weight) followed by total pectic substances (6.5% of dry weight) (Braham, 1979). Ramirez-Martinez, J.R reported the 0.27% chemical composition of the coffee husks was pectin (Ramirez-Martinez, 2006).

Pectin is a carbohydrate polymer compound found in the middle lamella and

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primary cell walls of plant tissues (Thibault, 1979). In addition, pectin is a substrate of the enzyme pectinase found in the cells of plants between the fibers at the top of the first layer of the cell wall which hold cells together. It is a linear polymers of α-d-galacturonic acid in which the d-galacturonic acid units are linked by 1→4 glycosidic linkages (Rolin, 1990) and also serves as a main component of parenchyma tissue which contains arabinose, galactose, and galacturonic acid (Rathinavelu, 2005). Pectin is a high-molecular weight compound with pectinic acid. When it is mixed with food and water, it becomes thickener and stabilizer and serves as gelling agents in many food products, such as jam and jellies. Pectin gels are formed from dissolved pectins by physical or chemical changes that decrease the solubility of the pectin molecules. The gelation mechanism of pectin is mainly controlled by the degree of esterification (DE) (Rathinavelu, 2005). Commonly, two types of pectin gels are distinguished. The first type, made from high methoxyl (HM) pectin (DE above 50%), forms gels in an acidic environment and in the presence of sucrose. The second type of pectin gel is composed of low methoxyl (LM) pectin (DE below 50%). It forms gels in the presence of alkaline earth elements, especially calcium (Thibault *et al*., 1979). In these gels, the macromolecules are cross-linked by divalent calcium ions (Thibault, 1985a; Thibault, 1985b)

In both cases, gelation and gel properties depend upon many factors, including pH, temperature, DE, sugar content, calcium content and pectin content (Milas, 1996). The poor gelling ability is mainly a result of high acetyl content (Pippen, 1950), low molecular weight (Thibault, 1985) and high proportion of side-chains (Keenan, 1985). Consequently, to improve gelling properties, many research aimed to study structural modifications of pectin molecules.

Generally, there are no exact characteristics of pectin (Mazeau, 2000). D-galacturonic acid residues form most of the molecules, in blocks of 'smooth' and 'hairy' regions. The molecule does not adopt a straight conformation in solution, but is extended and curved due to large amount of flexibility. The carboxylate groups tend to expand the structure of pectins as a result of their charge, unless they interact through divalent cationic bridging. Methylation of these carboxylic acid groups forms their own methyl esters, which take up a similar space but are much more hydrophobic, thus they have different effects on the structure of the surrounding water. The chain length is long as it requires a minimum of 14-20 residues to cooperate (Ralet, 2001). HM-pectins (> 43% esterified, usually ~67%) gel is formed by the formation of hydrogen-bonding and hydrophobic interactions in the presence of acids to reduce electrostatic repulsions and sugars (Tsoga, 2004).

Martin A.K. Williams *et.al* reported that DFT calculations of monomeric-, dimeric-, and trimeric-pectic compounds, in various states of partial methyl esterification showed good experimental results. This extensive calculation does not only confirm the identity of the proposed methyl-band and illustrate its scaling with DM, but also demonstrates the success of the theoretical approach. Thus, DFT calculations are expected to be a valuable tool for interpretation of IR spectra obtained from more complex systems, such as polysaccharide conjugates (Fellah, 2009).

The present work was dedicated to the extraction process for the coffee pulp. Our study concerns the extraction of products containing pectin macromolecules. The properties of the extracted products were established. Finally, the effect of gelling factors, such as molecular weight and methylester substitution of pectin, was investigated through DFT calculations. ept of the cooperative mode . Finally, Section V concludes the paper.

Materials and Methods

Pectins Extraction

Pectin was extracted from the ground coffee pulp with water (Ration 25:1) mainly to remove the pigments. The material was filtered and the solids were treated with 6% w/w solution of sodium hexamethaphosphate (SHMP) at 80°C for 90 min. The solid:liquid ratio was 25:1 and a pH 3.0 was obtained by adding HCl (1 N). After extraction, the pectin solution was filtered through a white filter cloth. The filtrate was precipitated with 95% acidified ethanol alternated with acetone 5 times until it was colorless. The formed gel was allowed to

precipitate for 24 h at 25 °C. After that, the pectin was dried at 35°C for 24 h. The dry pectin was ground to pass through 60 mesh sieves for further study.

Equivalent weight determination

Weigh 0.5 g pectin sample was weighed into a 250 mL conical flask and moistened with 5 mL ethanol. 1.0 g sodium chloride was added to the mixture followed by 100 mL distilled water and few drops of phenol red indicator. Care was taken at this point to ensure that all pectin dissolved and no clumping occurred at the sides of the flask before the titration process (to avoid possible de-esterification) with 0.1 M NaOH to produce a pink solution at the endpoint. Equivalent weight was calculated using the equation below:

$$\text{Equivalent Weight} = (\text{Weight of Pectin} / \text{Volume of Alkali } (cm^3) \times \text{Molarity of Alkali}) \times 100\%.$$

The Galacturonic Acid Determination

Weigh 5.0 g of the sample to the nearest 0.1 mg, and transfer to a appropriate beaker. Stir for 10 min with a mixture of 5 ml of hydrochloric acid and 100 ml of 60% ethanol. Transfer the solution to a fritted-glass filter tube (30 to 60 ml capacity) and wash with six 15 ml portions of the HCl 60% ethanol mixture, followed by 60% ethanol until the filtrate is free of chlorides. Finally, wash with 20 ml of ethanol, dry for 2.5 h in an oven at 105°, cool and measure the weight. Transfer exact one-tenth of the total net weight of the dried sample remained from the 0.5 g original unwashed sample to a 250 ml conical flask, and moistens the sample with 2 ml of ethanol. Add 100 ml of boiled and cooled distilled water, swirl occasionally until a complete solution is formed. Add 5 drops of phenolphthalein, titrate with 0.1 mol/l sodium hydroxide and record the results of the initial titre (V1). Add exactly 20 ml of 0.5 mol/l sodium hydroxide, stopper, shake vigorously and let stand for 15 min. Add exactly 20 ml of 0.5 mol/l hydrochloric acid and shake until the pink colour disappears. Titrate with 0.1 mol/l sodium hydroxide to a light pink colour which persists after vigorous shaking. Record this value as the saponification titre (V2).

Calculate mg of galacturonic acid by the formula:

$$\text{Galacturonic acid (mg)} = 19.41 \times [V1 + V2]$$

Theoretical Methodology

The initial structures of pectins were built. Complete Hessian calculations were performed on a monomer, dimer, tetramer, hexamer, and octamer of α-D-galacturonic acid and decamer with varied methylesterified (0, 50%, and 100%) analogues, using DFT calculations implemented in the Gaussian 09 program package (Frisch, 2004). These ground-states were then subsequently subjected to a complete optimization and Hessian calculation using the B3LYP/3-21G basis set. All simulations were performed at 0 K and in vacuum with charge and spin multiplicity of 0, 1.

Results and Discussion

The ability of pectins to form gels is affected by intrinsic factors, including molecular weight, DE, DA and extrinsic factors, such as concentration of the extracted products, calcium content, sequestrant content, hydration temperature. Some factors were discussed as followed.

Extracted Pectin Analysis

The extracted pectins from coffee pulp under the condition above have light brown color as shown in Figure 1. Equivalent weight indicated amounts of methoxyl and carboxyl contents in the molecular structure. The higher the equivalent weight, the higher the methoxyl group presents in the structure. However, from Table 1, the equivalent weight of the pectin, extracted from coffee pulp, was high (207.47 mg/mol). It showed that the pectin from these sources were suitable for an industrial use. The ratio of galacturonic acid to rhamnose or the purification of pectin was showed in Galacturonic acid value which was 447.24 mg in this study. This value implies to the higher DE of the extracted pectin due to its carboxyl

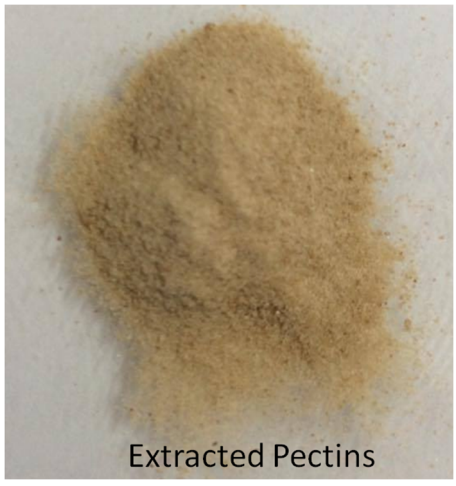

Figure 1. The characteristic of coffee pulp pecti

groups methylesterified. In addition, the yield of pectin from coffee pulp was quite high at 5.28%. These results would tend to go along with our previous study (Rakitikul, 2016) which shown the higher DE of coffee pulp's pectin at 93.75%.

In order to validate the extracted product, the pectin was extracted from coffee pulp three times and three pectin samples were subjected to FT-IR analysis (Thermo Fisher, Nicolet iS5). The bands relevant for the structural organization are as shown in Figure 2. The spectra had one intense pectin band at 1641 cm^{-1} assigned to the asymmetric stretching vibration of carboxylate anion $\nu_{as(COO-)}$, due respectively to the infrared absorption of the carboxylic ester and the any protonated carboxylic acid groups. In the region of 1250

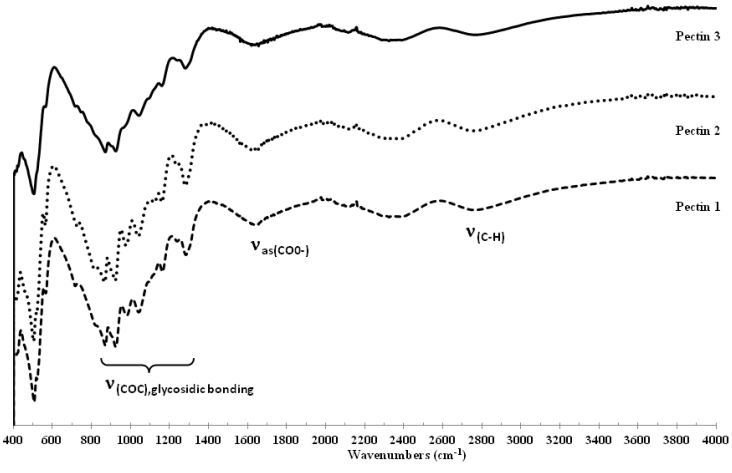

Figure 2. FTIR spectra of pectin from coffee pulp

Table 1. Extracted pectin characterized for coffee pulp

Parameter	Pectin of Coffee pulp*	Standard (Sungpud, 2005)	Lab & pharmaceutical (Sungpud, 2005)
color	Light Brown	-	-
Moisture (%)	4±0.61	-	4.81
Equivalent Weight	207.47±0.82	-	-
Galacturonic Acid (mg)	447.24±0.96	>65	78.54
Yield (%)	5.28±0.40	-	-

Note: * All measurements were carried out in triplicate for each of the sample. The value in this column showing the mean±SD.

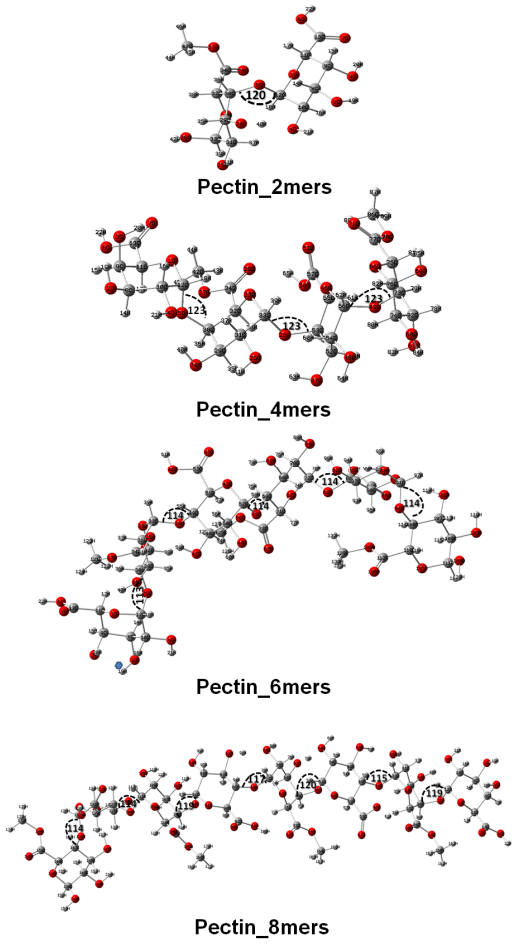

Figure 3. Pectin Conformer with varying from dimers to octamers

cm^{-1} – 950 cm^{-1} contributed to glycosidic bonding ($\nu_{(coc),glycosidic\ bonding}$). Moreover, the spectra of pectin at 2756 cm^{-1} was assign to $\nu_{(C-H)}$.

Molecular Structure Investigation

In order to look at difference in molecular weight effect to gelling properties, 4 different simplest molecules were modeled as shown in Figure 3. The angle between each pectin monomeric tends to decrease from dimeric-pectic compound structure to octameric-pectic compound; from 120°, 123°, 113-114°, and 114-119° respectively. Nonetheless, an octameric-pectic compound has one angle bigger than others (120°) which makes the structure more linear. These could be implied that the longer the molecular structure, the more linearity it presents. In additional, an effect of methylester was studied through adding methyl ($-CH_3$) group into decameric-pectic compound in ratio of 0%, 50%, and 100% as shown in Figure 4. The result showed that 50%methylester has smaller angle affect, when compared to the more linearity of pectin chain performed by 0%methylester. Beyond that, the angle size of a 100%methylester has no significant difference from others 2 structures; however it bent at the center of the structure. This may result from the effect of methylester groups and lead to molecular curving. Moreover, the

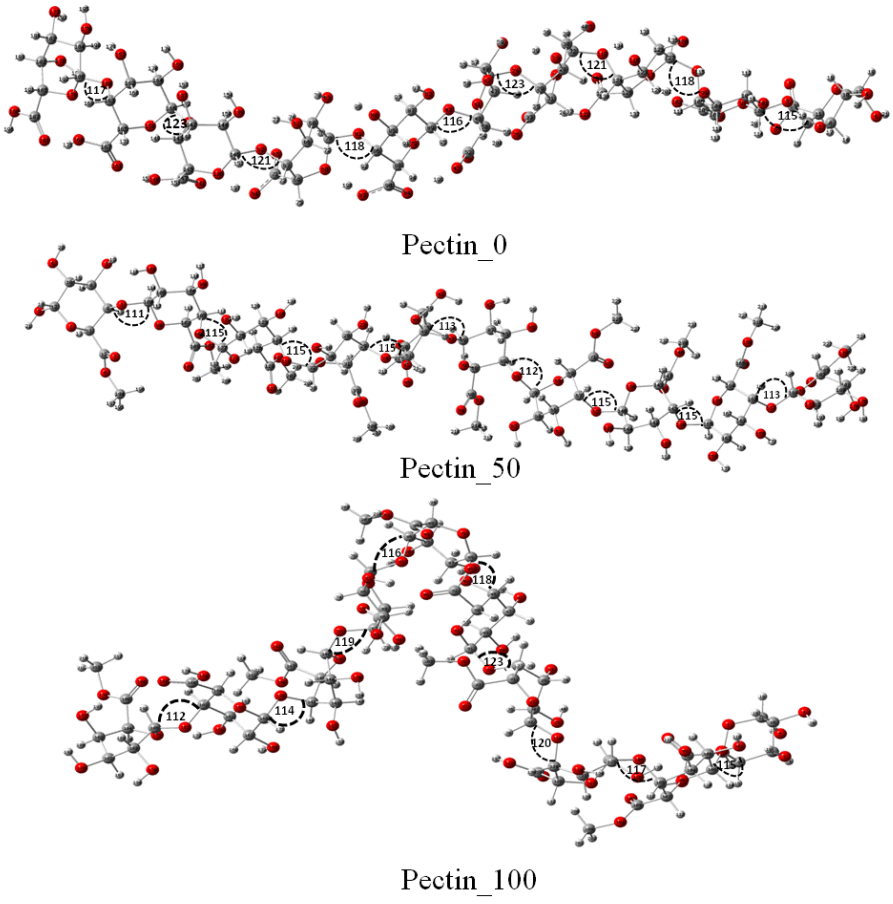

Figure 4. Decamers of pectin structure with varying methyl ester group

vibration spectrum of pectin structure were done at the same level. The results show a good agreement with FTIR spectrum as shown in Figure 5. The highly coupled and conformational specific region between 1100 and 800 cm^{-1} belongs to non-localised, highly coupled vibrations of polysaccharide backbones (Egnelsen and Norgaard, 1996). Two intense bands around 1660 and 1766 cm^{-1} are assigned to $\nu_{C=O,}$ which indicates its stretching vibration in ester and carboxylic acid. Additionally, the presence of $\nu_{C-H, -CH3}$ shown up around 2800-3000 cm^{-1} also indicates an existence of methyl ester group. Peaks around 3054-3420 cm^{-1} suggest that there are stretching vibrations of O-H at carboxylic groups.

Conclusions

As these preliminary results, the waste from coffee processing: coffee pulp could be an alternative source of pectin as it shows the high equivalent weight, galacturonic acid amount. The gelling properties of pectin could be affected from its molecular weight and the degree of esterification as the calculation results. According to the results mentioned above, we would like to probe and predict gelling factors such as pH and temperature of coffee pulp's pectin in our furthered work.

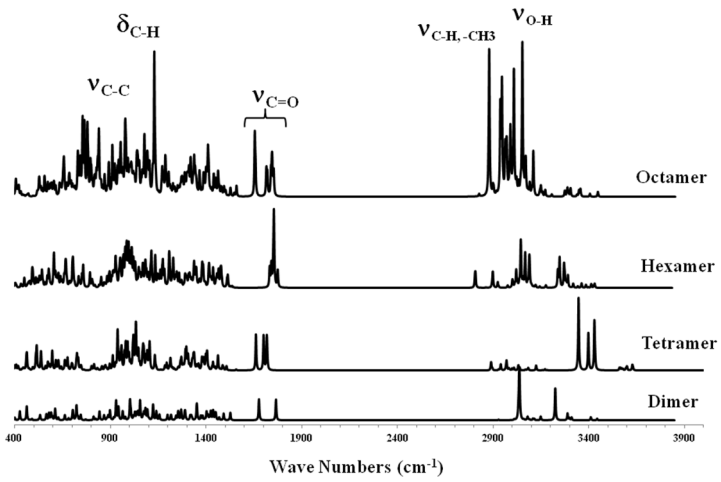

Figure 5. Vibrational Spectrum of pectin with varying from dimers to octamers

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