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*Full Length Research Paper*

# Effect of ammonium oxalate and acetic acid at several extraction time and pH on some physicochemical properties of pectin from cocoa husks (*Theobroma cacao*)

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**Pectin was extracted from cocoa husk using two solvents (ammonium oxalate and acetic acid), extraction times (60 and 120 min) and pH (1.6, 2.6, 3.6 and 4.6). The pectin characteristics were yield, moisture content, ash content, equivalent weight, methoxyl, anhydrogalacturonic acid and degree of esterification. The result showed that the yield of pectin was 10.44 to 17.30%, the moisture content was 10.46 to 12.35%, ash was 8.45 to 12.93%, equivalent weight was 510.68 to 645.19, methoxyl was 4.62 to 6.01%, anhydrogalacturonic acid of 55.04 to 63.54% and the esterification degree of 45.26 to 55.31%. Ammonium oxalate was effective at pH 4.6, while the acetic acid was effective at pH 2.6 in pectin extraction. The extraction time for 120 min gave a higher yield compared to 60 min. Ammonium oxalate at pH 4.6 for 120 min has given the highest yield of pectin.**

**Key words:** Acetic acid, ammonium oxalate, cocoa husk, extraction, pectin.

## INTRODUCTION

Cocoa husk is a waste product from the cocoa industry, and at present is a serious disposal problem. The increasing process of cocoa beans brings increasing wastes and this is a good reason to come up with a useful outlet for this by-product (Redgwell et al., 2003).

One of the potential researches to increase economic value of cocoa husk is as a source of pectin. Pectin is a polysaccharide mainly consists of a back bone of 1,4-linked α-D-galacturonic acid (GA) units partly methylesterified (Seggiani et al., 2009).

It is commonly found in the cell walls and the middle lamella of higher plants (Voragen et al., 1995). This polysaccharide consists of 300 to 1000 chains of anhydrogalacturonic acid units (Yeoh et al., 2008). Pectin has widely application in food industry as a thickener, an emulsifier, a texturizer and stabilizer. Pectin usually added in jams and jellies as a gelling agent. It has also been used as fat replacers in spread, ice cream and salad dressings.

In term of nutrition, pectin has been considered to lower blood cholesterol levels and low density lipoprotein cholesterol fraction which give benefits to human health (Liu et al., 2006). In addition, pectin fibers may be used for preparation of various dietary products which could be useful in the prevention of hyperlipidemia as well as large bowel cancer (Iglesias and Lozano, 2004).

Moreover, Nikolic and Mojovic (2007) added that some pectin derivatives have been used for preparation of polysaccharide-based vaccines and for drug delivery pharmaceuticals. Pectin has show potential pharmaceutical uses and is presently considered as a carrier material in colon-specific drug delivery system

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(Sriamornsak and Nunthanid, 1998). Two studies by Ashford et al. (1993) and Rubinstein and Radai (1993) was firstly demonstrated the potential of pectin or its salt as a carrier for colonic drug delivery. The justification for this is that pectin and calcium pectinate will be degraded by colonic pectinolytic enzymes (Englyst et al., 1987), but will retard drug release in the upper gastrointestinal tract due to its insolubility and because it is not degraded by gastric or intestinal enzymes (Sandberg et al., 1983).

Monsoor (2005) similar to Unal and Bellur (2009) described that the composition and structural entities of pectin depend on their source and extraction methods, and also caused by the activity of specific cell wall bound enzymes. According to FAO (1969), pectin has administered intravenously on human diet at high levels without acute toxic effects and considered as safe additive. Furthermore, based on the food and drug administration (FDA) database, select committee on GRAS substances (SCOGS) has listed pectin as one of the substances listed under the GRAS (Generally Recognized as Safe) substance (FDA, 1977).

Pectin can be divided into two types based on the degree of esterification (DE) of the pectin. Pectin with DE higher than 50%, called high methoxyl pectin (HMP), will form gel with sucrose at pH lower than 3.5, while low methoxyl pectin (LMP; DE < 50%) will form gel in the presence of calcium ion (Mesbahi et al., 2005; Iglesias and Lozano, 2004; Levigne et al., 2002). Pectin can be obtained from many sources with variation of yield percentage. Citrus peels and apple pomace are the major raw materials used for the production of commercial pectin by using acid extraction method with the yield about 25 and 12% pectin (May, 1990). Other sources such as mango peels, sugar beet and sunflower head residues consist of 10-20% pectin and soy hull pectin about 26 to 28% (Kalapathy and Proctor, 2001). For these reason, we are interested to produce pectin from the cocoa husks as an alternative source for apple and citrus pectin.

Several methods about extraction of pectin had been described by previous scientists, but for cocoa husks still limited. Adomako (1972) had been studied about pectin extraction of cocoa husk using acetic acid, Mollea et al. (2008) extracted the cocoa husks with water (1:25, w:v), acidified or not with HCl, at 95°C, for 1 h or more. The process was optimized based on pH and temperature. The yield of pectin obtained was about 6-9% depending on the extraction condition. Acidic condition was the most suitable for recovery the pectin from cocoa husk. The best yield was obtained at pH 2.5 and 1 h extraction time. Although the yield of pectin from cocoa husk was quite low, but cocoa husk also can be considered as an alternative source for pectin.

The aim of this study was to evaluate the impact of ammonium oxalate compared to acetic acid at different times and pH of extraction on the physicochemical properties of pectin from cocoa husks.

## MATERIALS AND METHODS

The cocoa fruits were obtained from Ladang Serborne Kuala Lumpur Kepong Bhd, Pahang Malaysia. Variety of cocoa fruits choosed was Chlone Prang Besar No. 123. The cocoa fruits were washed with water to remove any impurities such as sand and cut into two parts, then the cocoa seeds was separated from cocoa husks. Cocoa husk was chopped into pieces of 1 $cm^2$ using a stainless steel knife. The husk was then immersed into sodium metabisulphite to prevent browning reaction. The cocoa husk was dried using vacuum oven for 8 h at 50°C. Next, the dried cocoa husks were ground into powder by using a grinder.

### Extraction procedures

The extraction conditions were applied using ammonium oxalate and acetic acid for 60 and 120 min at pH 1.6, 2.6, 3.6 and 4.6. About 20 g of dried husk was stirred with 200 ml at 85°C of each of the foregoing extracting solutions. After extraction, the slurries were filtered through cheesecloth and squeezed until the liquid came out as much as possible. The residue was extracted again to obtain pectin; although, it is still lagging. The filtrate of both extraction processes were mixed together. Pectin was precipitated by adding 3 volumes (600 mL) of ethanol 95% and let for overnight. The precipitate was filtered with a Whatman No. 1 qualitative filter paper. Pectin was purified by washed three times with ethanol 95% (each time 100 mL ethanol), three times with ethanol 99% (each time 50 mL) again, and finally dried using vacuum oven at 40°C for 8 h.

### Analysis and characterization of pectin

#### Moisture and ash content

Moisture and ash content was determined according to the Association of Analytical Chemists (AOAC) methods (AOAC, 1990). The moisture content was determined by oven drying method. The pre-dry aluminum weighing dish was initially weighed followed by 1 g of pectin plus that aluminum weighing dish. Then, the sample was placed into an oven at 105°C for overnight. Lastly, the sample was allowed to cool down in desiccators before weighed again to get the dry sample weight. For the ash content determination, porcelain crucibles was initially dried in muffle furnace at 600°C for 3 h and then was put into desiccators before weighed. 1 g of sample then was put into that porcelain crucibles and put into the muffle furnace at 600°C for 3 h. Then it was allowed to cool down in desiccators before it was weighed.

#### Equivalent weight

Equivalent weight, methoxyl content and anhydrogalacturonic acid (AGA) were determined by the standard methods of Owens et al. (1952). Equivalent weight was done by weighing 0.5 g pectin (moisture free) in a 250 ml conical flask, moistened with 5 ml ethanol, added 1 g of sodium chloride to sharpen the end point, added 100 ml of carbon dioxide free distilled water and 6 drops of phenol red indicator. The pectin substances were stirred rapidly to dissolve, and then titrated slowly with 0.1 N NaOH until the colour of the indicator changed (pH 7.5) and persisted for at least 30 s. The neutralised solution was saved for methoxyl determination.

#### *Methoxyl content*

Methoxyl (MeO) content was determined by adding 25 ml of 0.25 N

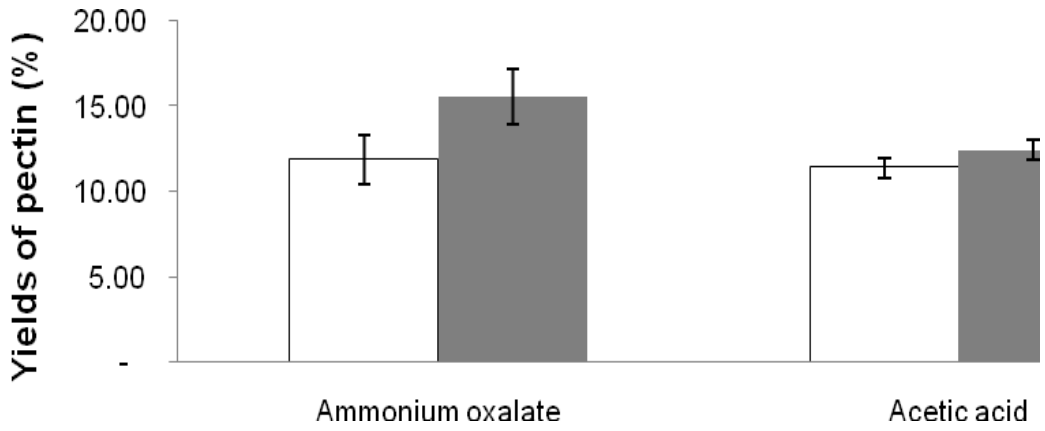


**Figure 1.** Effect of solvents and extraction time on yield of cocoa husks pectin (☐ 60 min; ■ 120 min).

NaOH to the neutral solution, mixing thoroughly, and allowed to stand for 30 min at room temperature in a stopper flask. 25 ml of 0.25 N HCl was then added and titrated with 0.1 N NaOH to the same end point as before.

$$\%\,MeO = \frac{meq\ of\ NaOH\ \ x\ 31\ x\ 100}{wt\ of\ sample\ (mg)} \quad (1)$$

**Anhydrogalacturonic acid (AGA)**

The AUA content can be calculated from equivalent weight and methoxyl content of pectin.

$$\%\,AGA = \frac{176\,x100}{Z} \quad (2)$$

Where, 176 is the molecular weight of AGA and

$$Z = \frac{wt\ of\ sample\ (mg)}{meq\ of\ alkali\ free\ acid + meq\ of\ alkali\ for\ methoxyl} \quad (3)$$

**Degree of esterification**

The degree of esterification (DE) of pectin can be determined by methoxyl and AGA content.

$$\%\,DE = \frac{176\,X\,\%\,MeO\,x100}{31\,X\,\%\,AGA} \quad (4)$$

## RESULTS AND DISCUSSION

### Yield

The yield of the cocoa husks pectin expressed as dry weight of the extract varied from 10.44 to 17.30%, depending on the extraction condition used. The highest percentage of pectin (15.59%) obtained was on extraction with ammonium oxalate and with 120 min extraction time (Figure 1).

The study Koubala et al. (2008a) and Koubala et al. (2008b) on the pectin from the skins of ambarella, mangoes and oranges that are extracted using ammonium oxalate also give a higher yield compared with hydrochloric acid and deionised water. Extraction time 120 min was more effective than 60 min either using ammonium oxalate or acetic acid, but no significant differences. Yujaroen et al. (2008) stated that the longer the extraction time, the higher the percentage of pectin derived. Ammonium oxalate was more effective in extracting pectin at pH 4.6 (15.38%) but not significantly different with a pH 3.6, while the acetic acid was more effective to extract pectin at pH 2.6 (12:57%) that is not significantly different with pH 1.6 ($P>0.05$) (Figure 2). In addition, during the extraction process carried out, the problems of liquid pectin extracted at pH 1.6 with acetic acid had a red color and caused pectin was separated red. This happened just as the study conducted by Mollea et al. (2008) on the extraction of pectin from cocoa husks with hydrochloric acid. In this investigation, the red color appeared on the extraction conditions of pH 1.5 and 1.0. Mollea et al. (2008) stated that the red color arise due to the presence of other compounds such as tannins which is one component of cocoa husks extracted together with pectin.

### Moisture content

The moisture content of pectin was 10.46 to 12.15%. This value was quite high compared to soy hull pectin (6 to 7%) (Kalapathy and Proctor, 2001). This might be due to incompletely drying process of the samples. Based on the quality standards of commercial pectin, all of pectin is produced to meet the standards are not far above 12%.

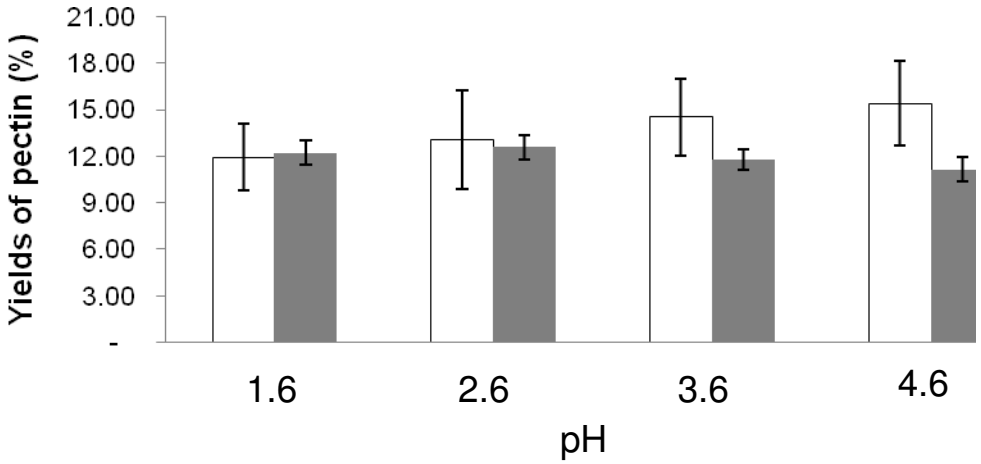


**Figure 2.** Effect of solvents and pH on yield of cocoa husks pectin (☐ ammonium oxalate; ■ acetic acid).

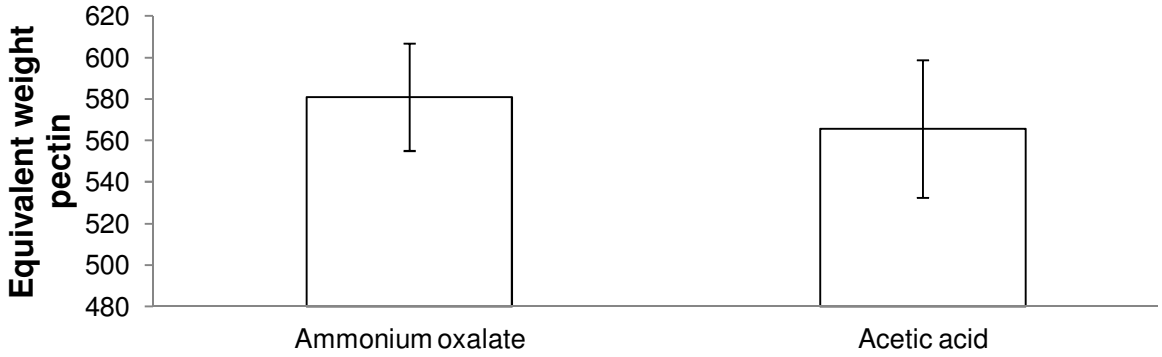


**Figure 3.** Effect of solvents on equivalent weight of cocoa husks pectin.

## Ash content

Ash content of the pectin was 8.15 to 12.93%. The highest percentage of pectin ash content obtained was the extraction with ammonium oxalate for 120 min at low pH. Cocoa husk pectin obtained in this research was categorized as pectin with high ash content. Ranganna (1977) stipulated that the pectin of high ash content contain about 10.69% ash content and the pectin of low ash content contain about 0.76% ash content.

## Equivalent weight

Equivalent weight of pectin in this study was 510.68 to 645.19. The ammonium oxalate produced pectin with higher equivalent weight (580.81) compared with acetic acid (565.49), but the difference was not significant (Figure 3). Equivalent weight of pectin is the total content of free galacturonic acid (not esterified) in the molecular chains of pectin (Ranganna, 1977). Therefore, equivalent weight of pectin associated with the degree of esterification and methoxyl content. According to Rouse (1977), the higher degree of esterification causes the decrease of free acid content of pectin that the equivalent weight also increased.

The extraction time (120 min) gave a lower equivalent weight (549.92) and significantly different compared with 60 min (596.39) (Figure 4). Long extraction time causes the depolymerization reaction and deesterification pectin become pectic acid (Kim et al., 1978).

Extraction of pectin at pH 1.6 produced pectin with high equivalent weight (595.97) and was significantly different from the pectin extracted at pH 3.6 and 4.6 with high equivalent weight of 560.78 and 559.52 respectively, but did not differ significantly from the equivalent weight (576.35) of pectin extracted at pH 2.6 (Figure 5). Low pH produced pectin with higher equivalent weight, because low pH can cause polymerization of pectin into a longer chain, up to the free acid was reduced. Decreased the amount of free acid will increased the equivalent weight of pectin.

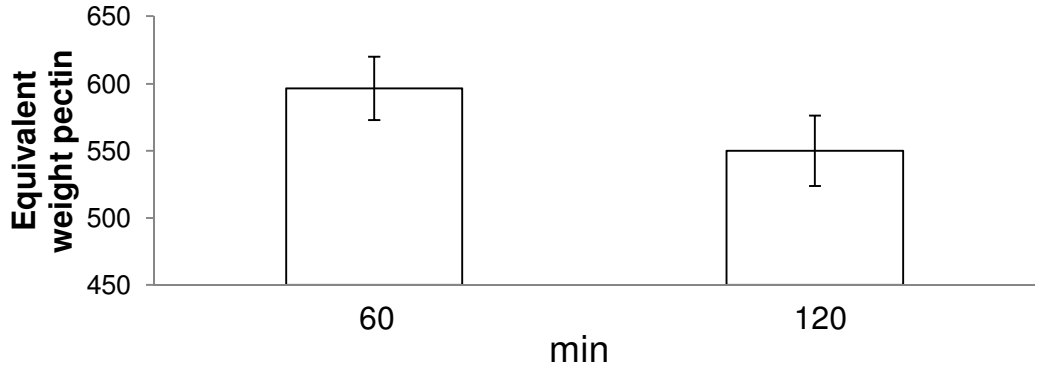


**Figure 4.** Effect of extraction time on equivalent weight of cocoa husks pectin.

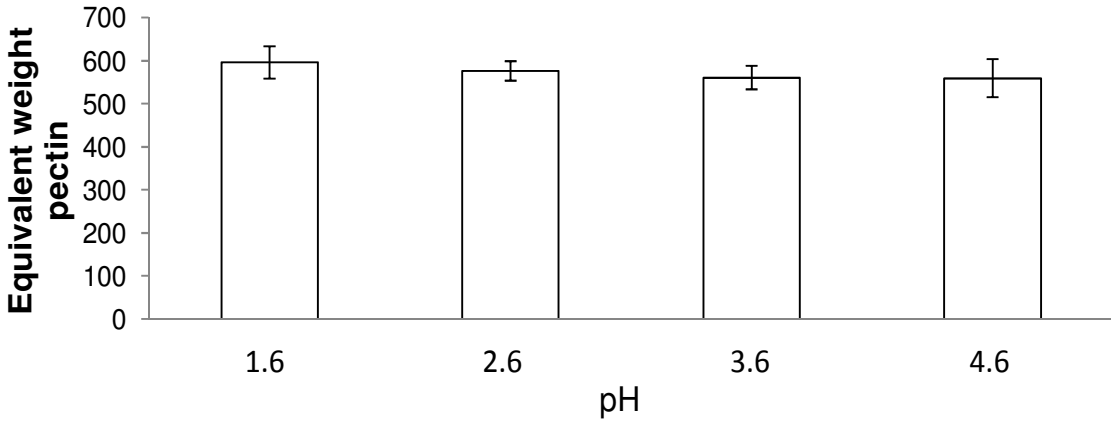


**Figure 5.** Effect of pH on equivalent weight of cocoa husks pectin.

**Table 1.** Methoxyl content of cocoa husks pectin.

| Extraction time | pH | Ammonium oxalate | Acetic acid |
|---|---|---|---|
| 60 min | 1.6 | $5.43 \pm 0.05^{bcd}$ | $5.58 \pm 0.09^{bc}$ |
| | 2.6 | $5.70 \pm 0.20^{ab}$ | $5.21 \pm 0.35^{cde}$ |
| | 3.6 | $5.40 \pm 0.09^{bcde}$ | $5.15 \pm 0.08^{de}$ |
| | 4.6 | $5.20 \pm 0.22^{cde}$ | $4.62 \pm 0.04^{f}$ |
| 120 min | 1.6 | $6.01 \pm 0.26^{a}$ | $5.52 \pm 0.09^{bcd}$ |
| | 2.6 | $5.58 \pm 0.17^{bc}$ | $5.43 \pm 0.13^{bcd}$ |
| | 3.6 | $5.50 \pm 0.21^{bcd}$ | $5.24 \pm 0.04^{cde}$ |
| | 4.6 | $5.39 \pm 0.06^{bcde}$ | $5.02 \pm 0.09^{e}$ |

[a-f] Means of % in the same column with different superscript letter differ significantly ($p < 0.05$). Results are means ±SD of three determinations.

## Methoxyl content

Methoxyl content is defined as the number of moles of methyl alcohol in 100 mol galacturonic acid. Methoxyl content of pectin is important to control the gel strength, the setting time, the sensitivity to metal ions and to determine the functional properties of pectin solutions and pectin gel texture (Constenla and Lozano, 2003). Methoxyl content of cocoa husks pectin was 4.62 to 6.01% (Table 1).

The highest methoxyl content of pectin obtained with ammonium oxalate at pH 1.6 for 120 min, but did not

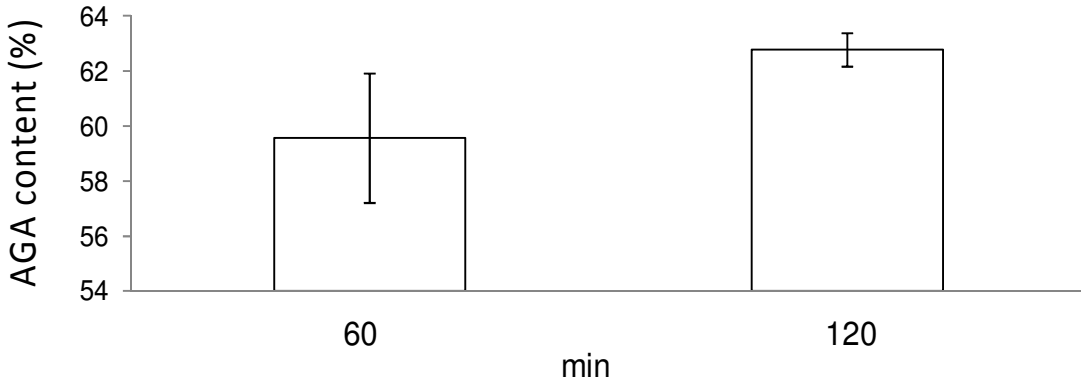


**Figure 6.** Effect of extraction time on AGA content of cocoa husks pectin.

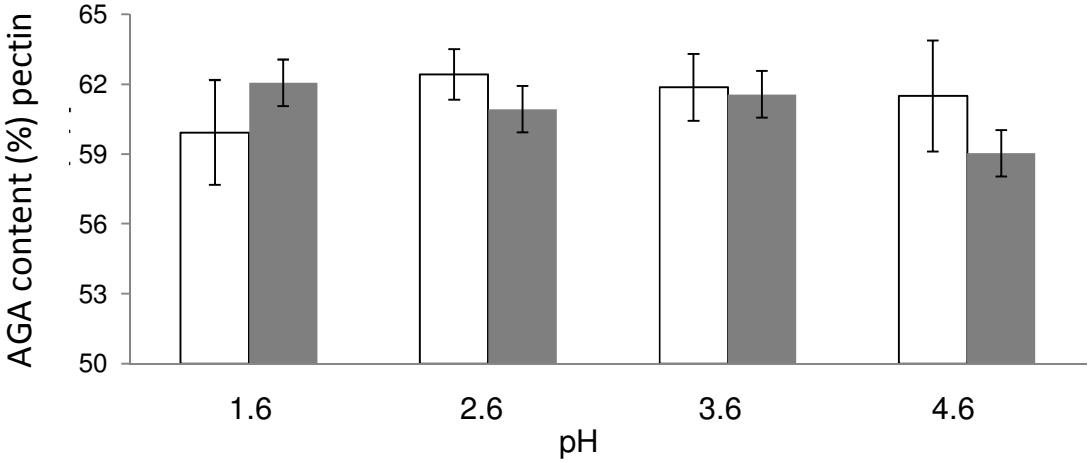


**Figure 7.** Effect of pH on AGA content of cocoa husks pectin (□ ammonium oxalate; ■ acetic acid).

differ significantly from the pectin that extracted with ammonium oxalate at pH 2.6 for 60 min. Based on the number of ester groups, the type of pectin in this study was categoried as low methoxyl pectin because the ester groups were less than 50% (< 7%). It was more profitable because it can be produced directly without going through the deesterification process.

## Anhydrogalacturonic acid (AGA) content

The anhydrogalacturonic acid (AGA) content of pectin was 58.08 to 63.54%. AGA content of pectin quite low at extraction time 60 min (59.56%) and significantly different with the AGA content of pectin at extraction time of 120 min (62.77%) (Figure 6). The AGA content will be higher by increasing time of extraction, may be caused by the hydrolysis reaction of protopectin became D-anhydrogalacturonic acid that a basic component of pectin.

AGA content of pectin extracted by ammonium oxalate and acetic acid did not differ significantly. Similarly, AGA pectin extracted at pH 1.6 did not vary significantly with pH 2.6, 3.6 and 4.6 (Figure 7). Madhav and Pushpalatha (2002) reported that the AGA content of cocoa husks is 52.84% with a gel grade 129, while according Adomako (1972), AGA content of cocoa husks pectin is 60%.

## Degree of esterification (DE)

The degree of esterification (DE) of pectin in this study was 45.26 to 55.31%. The ammonium oxalate produced pectin with a degree of esterification was higher (51.07%) and significantly different than acetic acid (48.68%) (Figure 8). The extraction time of 60 min produced pectin with a degree of esterification higher (50.33%) than 120 min (49.41%) (Figure 9), but the difference was not significant. Longer extraction time might cause degradation process of methyl ester groups in pectin into carboxyl acid.

Effect of pH on the extraction degree of esterification

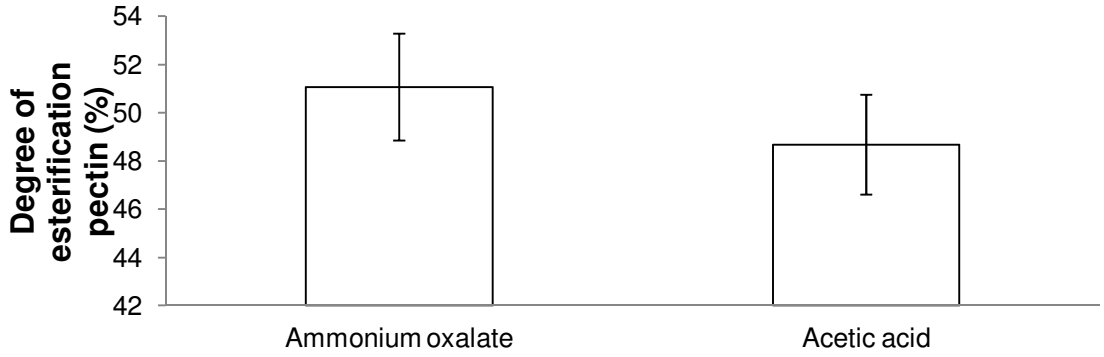


**Figure 8.** Effect of solvents on degree of esterification of cocoa husks pectin.

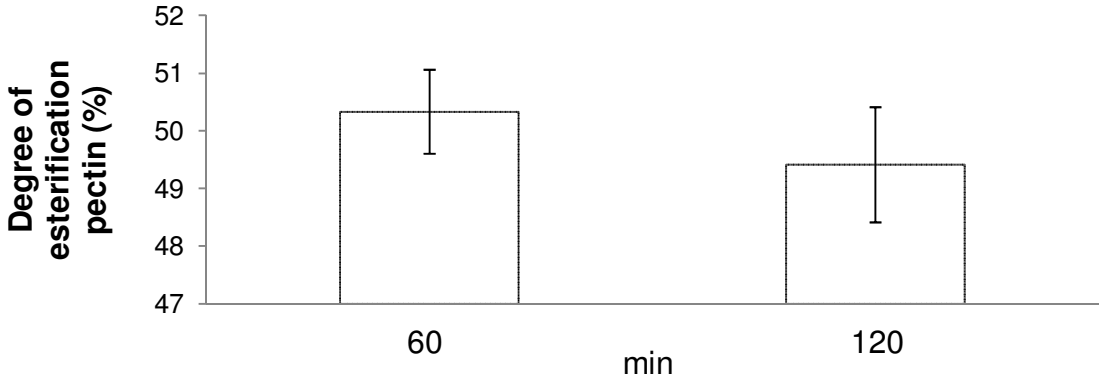


**Figure 9.** Effect of extraction time on degree of esterification of cocoa husks pectin.

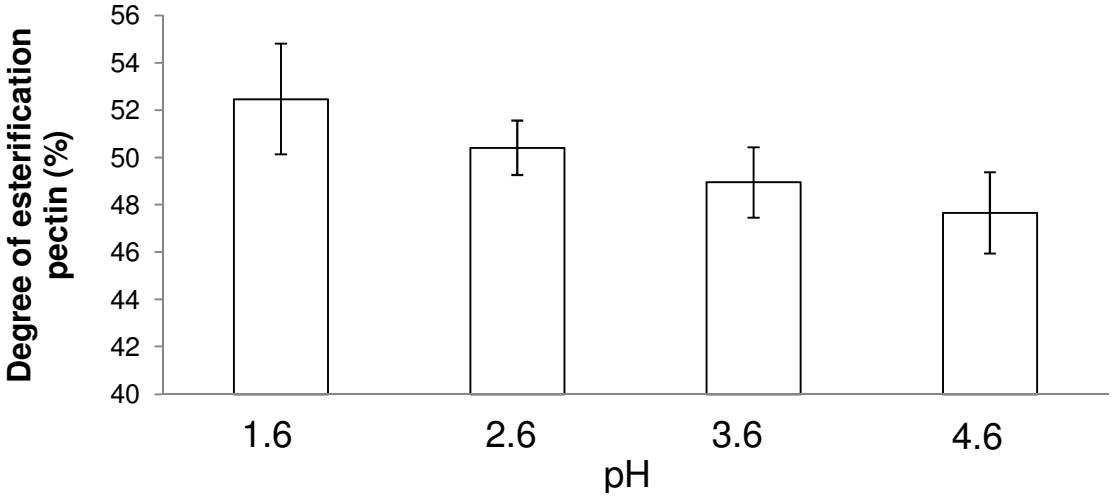


**Figure 10.** Effect of pH on degree of esterification of cocoa husks pectin.

was displayed in Figure 10. Pectin extracted at pH 1.6 had the highest degree of esterification (52.47%) and significantly different than pectin extracted at pH 2.6, 3.6 and 4.6. Low pH led to a higher degree of esterification of cocoa husk pectin. This was due to the degradation of methyl ester groups from pectin into carboxyl acid, which was more effective at acidic condition (low pH). The degree of esterification was the percentage of the amount

of D-galacturonic acid in which the carboxyl group passed through the process of esterification with ethyl alcohol. Ptichkina et al. (2008) stated that pectin with DE> 60% is suitable for use in the food industry. The chemical characteristics of the pectin influencing the quality of the gel are DE which is related to the rate of gel formation in food industry (Pagan et al., 1999). High DE means high gelation temperature when preparing gels by the usual procedure of mixing the hot ingredients and then solidifying by cooling. When the gel batch is cooled below the gelling temperature, gelation occurs after a delay, which is short with pectin of high DE and longer with pectin of lower DE (Daas et al., 2001).

## Conclusion

In the study, different extraction conditions were used to extract the pectin from cocoa husks. Cocoa husks considered rich in pectin, as shown in yield obtained. But, pectin from cocoa husks was quite low quality as compared to commercial pectin. The extraction conditions had major impact on the extraction yields of pectin but did not have significant difference in others physicochemical properties. Ammonium oxalate led to higher extraction yields for cocoa husks compared to acetic acid. Further investigations need to be directed at the characterization of the extracted pectin in order to determine the quality of pectin from cocoa husks as food additive.

## ACKNOWLEDGEMENTS

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### REFERENCES

Adomako D (1972). Cocoa pod husk pectin. Phytochemistry, 11: 1145-1148.
AOAC (1990). Official Methods of Analysis. Ed. Ke-15. United States of America: Association of Analytical Chemist. Inc.
Ashford M, Fell JT, Attwood D, Sharma H, Woodhead PJ (1993). An evaluation of pectin as a carrier for drug targeting to the colon. J. Controlled Release, 26: 213-220.
Constenla D, Lozano JE (2003). Kinetic model of pektin demethylation. Latin American Appl. Res., 33: 91-96.
Daas PJH, Boxma B, Hopman AMCP, Voragen AGJ, Schols HA (2001). Nonesterified galacturonic acid sequence homology of pectins. Biopolymers, 58: 1.
Englyst HN, Hay S, Macfarlane GT (1987). Polysaccharide breakdown by mixed populations of human faecal bacteria. Microbiol. Ecol., 95: 163-171.
FAO (1969). Nutrition meetings of the FAO, p. 133.
FDA (1977). Database of Select Committee on GRAS Substances (SCOGS) Reviews Pectin, Retrieved from http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogsListing&id=229
Iglesias MI, Lozano JE (2004). Extraction and characterisation of sunflower pectin. J. Food Engin., 62: 215-223.
Kalapathy U, Proctor A (2001). Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. Food Chem., 73: 393-396.
Kim WJ, Sosulski F, Lee SCK (1978). Chemical and gelation characteristics of ammonia-demethylated sunflower pectins. J. Food Sci., 43: 1436-1439.
Koubala BB, Kansci G, Mbome LI, Mbiapo FT, Crepeau MJ, Thibault JF, Ralet MC (2008a). Effect of extraction conditions on some physicochemical characteristics of pectin from “Amelioree” and “Mango” mango peel. Food Hydrocolloids, 22: 1345-1351.
Koubala BB, Mbome LI, Kansci G, Mbiapo FT, Crepeau MJ, Thibault JF, Ralet MC (2008b). Physicochemical properties of pectin from ambarella peels (*Spondias cytherea*) obtained using different extraction conditions. Food Chem., 106: 1202-1207.
Levigne S, Ralet MC, Thibault JF (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. Carbohydr. Polymers. 49: 145-153.
Liu Y, Shi J, Langrish TAG (2006). Water-based extraction of pectin from flavedo and albedo of orange peels. Chemical Engin. J., 120: 203-209.
Madhav A, Pushpalatha PB (2002). Characterization of pectin extracted from different fruit wastes. J. Trop. Agric., 40: 53-55.
May CD (1990). Industrial pectins: sources, production and applications. Carbohydr. Polymers, 12: 79–99.
Mesbahi G, Jamaliana J, Farahnaky A (2005). A comparative study on functional properties of beet and citrus pectins in food systems. Food Hydrocolloids, 19: 731-738.
Mollea C, Chiampo F, Conti R (2008). Extraction and characterization of pectins from cocoa husks: A preliminary study. Food Chem., 107: 1353-1356.
Monsoor MA (2005). Effect of drying methods on the functional properties of soy hull pectin. Carbohydr. Polymers, 61: 362-367.
Nikolic MV, Mojovic L (2007). Hydrolysis of apple pectin by the coordinated activity of pectic enzymes. Food Chem., 101: 1-9.
Owens HS, McCready RM, Shepard AD, Schultz TH, Pippen EL, Swenson HA, Miers JC, Erlandsen RF, Maclay WD (1952). Methods used at Western Regional Research Laboratory for extraction of pectic materials.. USDA Bur. Agric. Ind. Chem., p. 9.
Pagan J, Ibarz A, Llorca M, Coll L (1999). Quality of industrial pectin extracted from peach pomace at different pH and temperatures. J. Sci. Food Agric., 79: 1038-1042.
Ptichkina NM, Markina OA, Rumyanseva GN (2008). Pectin extraction from pumpkin with the aid of microbial enzymes. J. Food Hydrocolloids, 22: 192-195.
Ranganna S (1977). Manual of analysis of fruit and vegetable products. McGraw Hill, New Delhi.
Redgwell R, Trovato V, Merinat S, Curti D, Hediger S, Manez A (2003). Dietary fibre in cocoa shell: Caharacterisation of component polysaccharides. Food Chem., 81: 103-112.
Rouse AH (1977). Pectin: distribution, significance. Dalam Nagy SP, Shaw E, Veldhuis MK (eds). Citrus Science and Technol. (1). The AVI Publishing Company Inc.
Rubinstein A, Radai R (1995). *In vitro* and *in vivo* analysis of colon specificity of calcium pectinate formulations. Eur. J. Pharmaceut. Biopharmaceutics, 41: 291-295.
Sandberg AS, Ahderinne R, Andersson H, Hallgren B, Hulten L (1983). The effect of citrus pectin on the absorption of nutrients in the small intestine. Human Nutrition – Clinical Nutr., 37: 171-183.
Seggiani M, Puccini M, Pierini M, Giovando S, Forneris C (2009). Effect of different extraction and precipitation methods on yield and quality of pectin. Int. J. Food Sci. Technol., 44: 574-580.
Sriamornsak P, Nunthanid J (1998). Calcium pectinate gel beads for controlled release drug delivery: I. Preparation and *in vitro* release studies. Int. J. Pharma., 160: 207-212.
Unal MU, Bellur E (2009). Extraction and characterisation of pectin methylesterase from black carrot (*Daneus carota L.*). Food Chem., 116: 836-840.
Voragen AGJ, Pilnik W, Thibault JF, Axelos MAV, Renart CMGC (1995). Pectins. In A. M. Stephen (Ed.), Food polysaccharides and

their applications. New York: Marcel Dekker, pp. 287-369.
Yeoh S, Shi J, Langrish TAG (2008). Comparisons between different techniques for water-based extraction of pectin from orange peels. Desalination, 218: 229-237.
Yujaroen P, Supjaroenkul U, Rungrodnimitchai S (2008). Extraction of pectin from sugar palm meat. Thammasat. Int. J. Sci. Technol., 13: 44-47.