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Pectin extraction from cocoa pod husk (*Theobroma cacao L.)* by hydrolysis with citric and acetic acid

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Abstract : Cocoa crops development presents challenges concerning the proper disposal of waste generated by this activity, and the obtaining of value-added products. Thus, the aim of this study was to obtain pectin from cocoa pod husk (Theobroma cacao L.). Infrared spectrum results showed peaks of galacturonic acid functional groups, indicating the presence of pectin in the cocoa husk. In extraction stage was carried out acid hydrolysis with citric or acetic acid at different pH (2, 2.5 and 3), 90°C temperature and 90 minutes, and was evaluated its influence on yield, methoxyl and galacturonic acid content. From the established extraction conditions, aqueous citric acid at pH 2.0 provided the highest yield and methoxyl content, 18.12% and 15.5% respectively. While the galacturonic acid content showed better results with acetic acid at pH 3 with an 83.1%. Finally, it was concluded that cocoa pod husks are suitable to obtain pectin, and a marked influence of the type of acid on response variables in the pectin extraction process was observed.
Keywords: Pectin, cocoa pod husk, hydrolysis, citric acid, acetic acid.

Introduction

Currently, Lignocellulosic biomass is considered a promising raw material to biofuels and high value products[1,2,3,4].Cocoa is considered an important investment in agro-industrial production; however, the development of this crop raises challenges concerning the proper disposal of wastes generated by this activity, where due to phytosanitary problems over 50% post-harvest losses are generated[5]. In addition, the chocolate industry only uses 30% of cocoa fruit, which generates a large volume of husks and shells that can be useful to obtain value-added products[6,7,8].

Waste disposal is one of the major problems in the countries[3,9].Regarding usual disposal of cocoa pod husk, it has been used as cattle feed, however theobromine content restricts the portion that can be consumed so their use has been limited. Another usage has been the husk ashes aiming to make soap in Ghana and Nigeria[10], or its conversion into a fertilizer rich in potassium by adding starch and mix granulation[11]. Additionally, it has been proposed as absorbing of pollutants in aqueous solutions[12]. This cocoa waste has been used to obtain activated carbon filters for different industrial applications[13,14.15,16]. Sanchez[17] has reported its use as a filler to make multinutritional blocks, organic fertilizer, raw material for biogas digesters, compost for cocoa seedlings growth, fodder during drought, among others.

Previous studies have shown the possibility of extracting pectin from cocoa pod husk[6,18,19]. Pectin is a raw material used in the production of several food products that require gelling, thickening, texturizing, emulsifying, and stabilizing agents as fat substitutes in foods of low caloric intake, among others[18,20].

Vriesmann*et al*[21]examined variables that have influence in pectin extraction from cocoa pod husk using citric acid, where pH levels were 1.0, 2.0 and 3.0; extraction temperature of 50, 75 and 100 ° C; and duration of extraction of 30, 60 and 90 min. The highest yield (10.6%) was at pH 1.0, 60 min and 100º C, the results indicated a significant relationship of the linear effect of temperature and quadratic effect of time regarding pectin yield, being enhanced with increasing time and temperature extraction. This study tested an organic acid to improve the extraction yield using an extraction procedure with low environmental impacts, besides the benefits demonstrated in other studies in terms of yield and physicochemical properties compared to mineral acids[21].

In 2014, Suarez and Orozco[22]obtained pectin from cacao shell (Theobroma cacao L.) byproduct of chocolate industry. This extraction was done by hydrolysis with citric acid at pH 3.0, varying temperature (70°C or 90°C) and processing time (75 or 95 minutes). They found that at higher temperatures and longer extraction time, yield increased but quality of pectin decreased. They concluded that the best extraction conditions were at T = 70°C and t = 95 min, since, although it was not the highest performance, pectin was of good quality. At those conditions, yield was 8.83%, methoxyl content 3.4%, equivalent weight 2335 mg/meq, total phenols 9.68%, total carbohydrates 42.03% and galacturonic acid content 26.86%[22].

This research provides information related to agro-industrial waste disposal, by the extraction and characterization of pectin from cocoa pod husk proposing more favorable and efficient conditions in the production process. Two organic acids (acetic and citric acid) were chose considering food application of this material. No evidence regarding comparative studies using these two acids in the pectin extraction from cocoa pod husk was found. Similarly, the characteristics of pectins obtained, as moisture and esterification degree with data found in the literature of pectins extracted by different researchers are compared.

Materials and Methods

Experimental design

For experimental design was considered the properties that indicate quality of pectin (yield, galacturonic acid and methoxyl content), i.e. factors that ensure an optimum extraction, such as pH and acid type (Table 1).

Table 1. Factor and levels

Factor name	Symbol	pH Levels			Number of experiments
		Low level	Medium level	High level	
Acid 1 (Citric)	K_1	2.00	2.50	3.00	3.00
Acid 2 (Acetic)	K_2	2.00	2.50	3.00	3.00
Total experiments					6.00
Total experiments with 3 replica					18.00

Treatment of raw material

FundacionCorIntegral (Corporation for regional integral development, San Jacinto-Bolivar) supplied cocoa pods. Raw material was pretreated to prevent deterioration in order to facilitate the extraction process and improve the quality of pectin[23]. First, it was subjected to a washing with a sodium hypochlorite solution (5 mL/L of water) at room temperature for 15 minutes[24], which allowed the disinfection and removal of impurities[23]. Afterward, a washing with distilled water was performed to remove heavy metals or other debris that were not eliminated in the previous procedure[25].

Pods were cut in half, the pulp and cocoa seeds were removed, and the mucilage adhered to the husk walls was scraped[26]. Husks were cut into small pieces and placed in trays to dry in an oven at 55° C for 17h, thus most of material moisture was eliminated, enabling the growth of microorganisms that damage the fruit, and block the activity of pectinolytic enzymes that degrade pectin[18]. Dried husks were milled and sieved, particle size less than 2 mm was selected, and storage in re-sealable zipper bags covered with foil to prevent light degrades the sample[26].

Characterization of the cocoa pod husk

Technique of high-performance liquid chromatography (HPLC) was used to determinate hemicellulose, cellulose and lignin content in cocoa pod husk, and infrared spectrophotometry technique (FTIR) for the identification of galacturonic acid functional groups[27,28], which were made in the laboratory services unit of Pharmaceutical Sciences Faculty at University of Cartagena. The husk's moisture was obtained by weight difference after oven drying at 130°C until constant weight. The ash percentage was the relationship between weights of husk before and after being incinerated in a muffle at 600 ° C during four hours[2930].

Pectin extraction from cocoa pod husk

A specific ratio of substrate-extract 1:10 (w/v) was used for each sample. 20 g of dried and milled cocoa husk was used with 200 mL of acidulated water with the corresponding acid (acetic or citric) to achieve the pH levels (2, 2.5, and 3) according to experimental design. The hydrolysis process was performed placing the sample into a three-necked flask coupled to a Liebig condenser, a mechanical stirrer, and a thermometer. A heating mantle was used to reach the system temperature to 90° C and held steady state during 90 minutes, by manipulating water flow in the condenser and/or heat flux given by the heating mantle; continuous stirring guaranteed the temperature uniformity within the system and prevented solid material were deposited at the flask bottom. Once completed the extraction time, the mixture is indirectly cooled with cold water up to 30°C to stop hydrolysis process to avoid exceeding the set time[18 27].

Centrifugation of the mixture was held at 4000 rpm for 10 min, obtaining two phases: a supernatant mainly composed of pectin and water, and a wet solid whose constitution was unreacted pulp, precipitate was discarded due to its disposal was not contemplate in this study[27]. To avoid supernatant were contaminated with traces of precipitate the mixture was filtered using Erlenmeyer, funnel, and grade 2 qualitative filter paper. In order to precipitate pectin was added 96% ethanol to the filtrate[31]; the amount of alcohol added corresponded to a 2:1 (v/v) filtrate-ethanol ratio. The pectin was recovered by centrifugation at the same conditions mentioned before (4000 rpm for 10 minutes); in this case, the wet solid corresponded to pectin with gel texture, and the supernatant contained pectin traces, water, and ethanol. Same filtrate-ethanol ratio was added to the supernatant to recover the remaining pectin. The pectin was poured into a petri dish, then began oven drying at 40°C until constant weight, and finally stored in a hermetic bag covered with foil [62627].

Pectin characterization

Pectin yield

A sample of 100 mg of dry pectin was taken, and dried in an oven at 130°C for 1 hour. Then was cooled in a desiccator and weighed to determine moisture content[2930]. After moisture determination, pectin yield was established and corresponded to the amount of pure obtained pectin, i.e. free from moisture, regarding the initial amount of dry cocoa pod husk[32].

Determination of galacturonic acid content

When pectin is hydrolysed in acidic medium releases uronic acid monomers, which get dehydrated in furfuryl derivatives (univalent radical derived from aromatic aldehyde furfural) and form a pink complex by adding Metahydroxydiphenyl (MHDP), having maximum absorption at 520 nm[33]. By this method, the galacturonic acid content in pectin was determined, which is an indicator of purity. Pectins should have a minimum percentage of galacturonic acid between 65% and 74% to guarantee the quality of pectin[34].

5 mg of pectin was weighed and 0.15 mL of sulfuric acid 72% was added, the medium was homogenized for 3 hours at 25°C. Then 1.55 mL of distilled water was added, the solution was heated in an oven to 100°C for 2 hours, once finished this step the mixture was cooled at room temperature and filtered on grade 2 filter paper. Simultaneously, 2.4 mL of 0.0125 M borax solution was prepared, and subjected to ice bath for 10 minutes. Subsequently, 0.4 mL of the acidified pectin solution was added, it was stirred manually and then heated in bain-marie at 100 ° C for 10 minutes. Immediately was cooled in an ice bath, 40 µL of MHDP

was added and the solution was stirred, after exactly 10 minutes the absorbance at 520 nm was measured. Blank and standard samples were prepared the same way but 40 µL of 0.5% sodium hydroxide solution was added[33 3536].

Determination of methoxyl content

1 g of pectin was weighed; 25 mL of 0.25 N NaOH solution was added, and stirred magnetically for 30 minutes. Then, 25 mL of 0.25 N HCl solution was added and titrated with 0.1 N NaOH solution and phenolphthalein was used as an indicator[37].

Results and Discussion

For the run of the experimental design, cocoa pods were received with an average weight of 470.25 g, in accordance with literature (400-477 g)[26 38]. The average weight of pulp and cocoa seeds was 117.56 g. Therefore, the average weight of cocoa husks was 352.69 g, i.e. each cocoa pod contained about 75% of fresh cocoa husk (m/m), similar value to found in literature (74%) [26]. Moisture of cocoa husks was approximately 84% (m/m) similar to results obtained by other authors for cocoa pod husk (82%) [18].

Characterization of cocoa pod husk

The chemical composition of cocoa pod husk is shown in Table 2. This study was assisted by laboratory services unit of Pharmaceutical Sciences and Food Engineering, at University of Cartagena.

Table 2. Chemical composition of cocoa pod husk.

Components	Content [a] (g/100g)
Moisture[b]	84.00
Ashes[b]	18.38
Protein[c]	1.46
Lipids[c]	1.03
Carbohydrates[c]	18.72
Lignin[b]	12.06
Cellulose[b]	18.42
Hemicellulose[b]	10.04
C[c]	18.72
N[c]	0.23

[a]Dry basis except moisture.
[b]Determined according to TAPPIstandards.
[c]Determined according to AOAC methods.

Cocoa pod husk showed a low lipid content (1.03%), similar to 1.5% obtained for the same fruit by Vriesmann[26], and other byproducts from agricultural and food industries, such as orange peel 1.55%[31] and peach bagasse <3%[47].

The protein content (1.46%) had resemblance to the obtained from cocona fruits (0.78%), and was near to values that often have fruits, less than 1% of fresh weight[36].

Insoluble fiber content in cocoa pod husk, lignin (12.06%) and hemicellulose (10.04%) is similar to founded in literature for the same fruit, 14 - 21.4% and 8.73 - 11% respectively[39404142]. On the other hand, values obtained for cellulose (18.42%) distance themselves from what other authors reported 35 - 41.9%[39 40 434445].

The knowledge about cellulose, hemicellulose and lignin content is relevant because when processing, fragments of cell wall components are also responsible of rheological properties and hence the importance of material composition[36].

By observing the IR spectrum of cocoa pod husk (Fig. 1) and its representative bands (Table 3), one peak is given at 1735 cm-1, corresponding to an ester group, thus cocoa pod husks shown the presence of unhydrolyzed pectin, i.e. methylated esters of galacturonic acid groups were identified, those composing the polymer chain inside pectin[27].

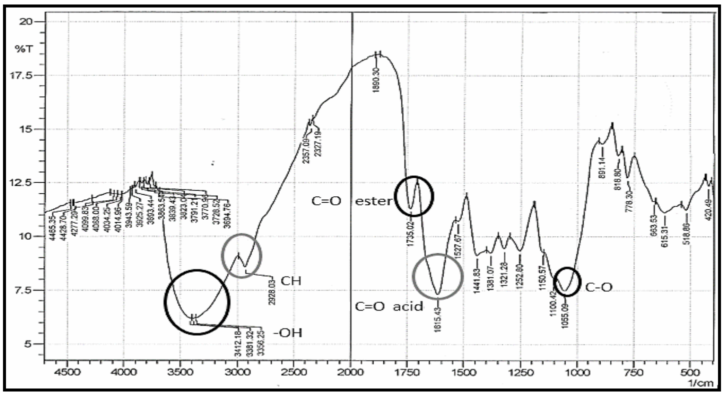

Figure 1. Infrared spectra of cocoa pod husk.

Table 3. IR absorptions for representative functional groups of cocoa pod husk.

Wavelength cm^{-1}	Functional group
3412-3356	O-H
2928	C-H
2357-2327	C≡C
1735	C=O from ester
1615	C=O from acid
1527-1441	C=N / NO_2
1381	C-H
1055	C-O-C

Extraction and characterization of pectin from cocoa pod husk

Is important to highlight both pH and acid type of the mixture influenced the color and tonality of the obtained pectin. In the case of citric acid, although pectins were obtained with a darker tone at higher pH, pH effects may be considered negligible due to the slight variation; whilst acetic acid, marked red and darker hues were noted as pH decreased. The reddish coloration was also perceived by Ramli and Asmawati[37], using acetic acid at pH 1.6 to obtain pectin from cocoa pod husk, they justified the dark red tone based on Mollea*et al*[46] study where pectin was extracted at pH 1.5 - 1.0, and red color was attributed to the presence of other compounds as tannins.

It is noteworthy that other variables influence on the coloration of obtained pectin, are temperature and time of extraction, which for this experimental test remained constant at 90°C and 90 min, respectively. According to Cabarcas*et al*[29], high temperatures and contact time tend to give a darker coloration due to hot extraction, temperature affects in terms of decomposition and stability generating the presence of polyphenols, which interfer in the purity of obtained pectin,in case of a good washing with ethanol is not performed[46].

Yield

If pectin has high moisture, it can promote growth of microorganisms, and is considered a pectin with poor stability and tendency to agglomerate; however, if it is too low, presents a dark color and high resistance to grinding[29]. As seen in Table 4, the samples had low moisture values.

Table 4. Moisture and yield values obtained at different conditions of pH and acid type.

Acid type	pH	Moisture (%)	Yield (%)
Citric	2.0	0.08	15.97
	2.5	0.06	11.94
	3.0	0.03	10.54
Acetic	2.0	0.03	3.57
	2.5	0.03	1.91
	3.0	2.07E-03	1.34

Pareto chart is a graphical representation of analysis of variance where significant effects of variables are observed, Fig. 2 shows the performance of pH (A) and acid (B) factors. It is notable the higher influence of acid type on pectinyield. Gray (-) and white (+) effects of Pareto chart show inverse and direct proportionality, respectively, to the response variable. Thus, the effect of pH tends to decrease yield response as basicity is increased, and a pronounced negative influence on pectin extraction with acetic acid.

The increasing yield with pH decrease may be associated with increased hydrolysis in protopectin bonds, changingto soluble pectin[47], this is also in line as established by Baltazar*et al*[48], at lower pH higher yield is achieved but it needs higher reagents use. Besides is the possibility of extracting different biomolecules existing in the pod husk such as hemicellulose, cellulose, and others during the hydrolysis process[49].

The impact of the type of acid in the amount of pectin obtained, could be related to acid dissociation degree, being both weak organic acids, their difference was the constant value, which was higher for citric favoring pectin hydrolyzing in ionized water.

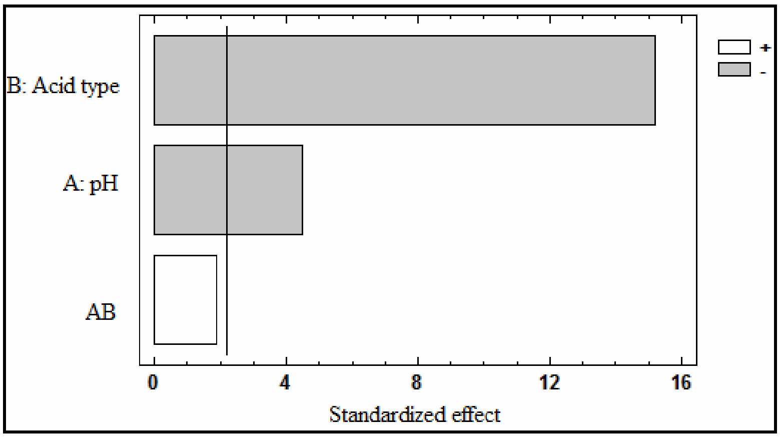

Figure 21. Pareto chart standardized for yield variable

Other authors used citric acid to extract pectin from cocoa pod husk, such as Chan *et al*[32], who obtained the highest yield 7.62% at pH 2.5 with time and extraction temperature 3.0 h and 95 ° C, respectively. Likewise, Vriesmann*et al*[21]obtained 10.6% as higher yield whenextraction conditions were pH 1.0 / 60 min / 100 ° C. Earlier in 2011 they had obtained higher yield (11.2%) at pH 3 but with nitric acid (40).

If the maximum yield of obtained pectin (15.97%) with cocoa pod husk from another Colombia region is compared, as in the case of Betancourt *et al*[18]who used from San Jeronimo (Antioquia) obtaining 4.3% yieldat pH 2.In this study were obtained better results although the conditions were different, they used hydrochloric acid at 80°C and 30 min in hydrolysis stage.

Regarding to cocoa shell, a higher percentage of pectin was extracted from cocoa pod huskwith citric acid at pH 3 (10.54%) comparing to results obtained by Suarez and Orozco[22]at same dependent variables conditions (8.83%), but at 70° C for 95 min.

Yujaroen*et al*[51]obtained more effectiveness in extracting pectin with acetic acid at pH 2.6 with 12.57% yield but was not significantly different from obtained at pH 1.6 (P> 0.05).

Methoxyl content

Degree of esterification is defined as the percentage of uronide carboxyl groups esterified with methyl alcohol, on total uronide in pectincontent, there is a corresponding relationship between the percentage of methylation and esterification defined by several authors[34], and this correspondence is reflected in Table 5.

Table 5. Methoxyl content and degree of esterification at different conditions of pH and acid type.

Acid type	pH	Methoxyl (%)	DE (%)
Citric	2.0	13.71	83.99
	2.5	11.30	69.25
	3.0	7.96	48.84
Acetic	2.0	2.48	15.26
	2.5	2.99	18.43
	3.0	3.31	20.22

It was observed that pectins obtained with citric acid are predominantly high methoxylpectins due to its degree of esterification is above 50%, while those obtained with acetic acid are all classified as low methoxylpectins because its degree of esterification is lower than 50%.

The standardized Pareto chart (Fig. 3) for methoxyl content evidence significant effects of pH (A) and acid (B) factors and their interaction (AB), particularly the higher impact by acid type on pectin methoxyl content.

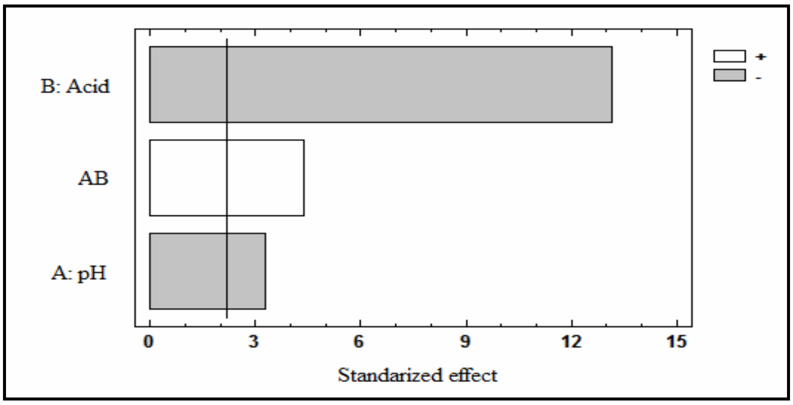

Figure 3. Standardized Pareto chart for methoxyl content.

The high methoxyl classification of pectin with citric acid could be affected by the esterification of polymer carboxyl group with other groups than the methoxyl, probably ethoxyl or the acid group is formed by another group of compounds such as amides[29]. However, the high degree of methoxylationis positive, as it is an indication of easy gelation[47].

The increase in methoxyl content as the pH is increased is consistent as established by Pagan[47], there is a direct relationship between pH and degree of methoxylation caused by increased hydrolysis of esters in the methoxylated carboxyl groups, as was the case of acetic acid.

Ramli and Asmawati[37]also obtained low methoxyl pectins by extracting with acetic acid, esters groups were less than 50% (<7%), the highest value was 5.58% at pH 1.6 for 120 min hydrolysis. Arellanes*et al*[52]extracted pectin from "Manzano" banana peels (Musa AAB) using citric acid at pH 2 obtaining a methoxyl content of 3.23. Betancourt *et al*[18]obtained 93% degree of esterification by extracting pectin with hydrochloric acid at pH 2, 80° C and 30 min of hydrolysis using cocoa pod husk from San Jeronimo (Antioquia), in this study the highest degree of esterification at pH 2 was 94.93%.

Galacturonic acid content

There is variability in the galacturonic acidcontent that presents pectin according to acid types and pH used, as shown in Table 6.

Table 6. Galacturonic acid content at different pH and acid type conditions.

Acid type	pH	Galacturonic acid content (%)
Citric	2.0	27.67
	2.5	39.81
	3.0	40.70
Acetic	2.0	63.05
	2.5	51.01
	3.0	55.83

The Pareto chart in Fig. 4 shows the effect of the acid factor (B); however, it exceeds the reference line mildly indicating its little influence.

The results obtained in this study could have been affected by the presence of impurities such as neutral sugars, latex, tannins, gums and other compounds present in the structure of the material that could have hydrolyzed along with extracted pectin[49].

Arellanes*et al*[52]when extracting pectin from "Manzano" banana peels (Musa AAB) using citric acid at pH 2 obtained 94.38% of galacturonic acid. Madhav and Pushpalatha[53] reported galacturonic acid content in cocoa pod husk of 52.84%, while Ramli and Asmawati[37] in a range of 58.08 to 63.54%. In this study, a 46.33% average galacturonic acid content was obtained.

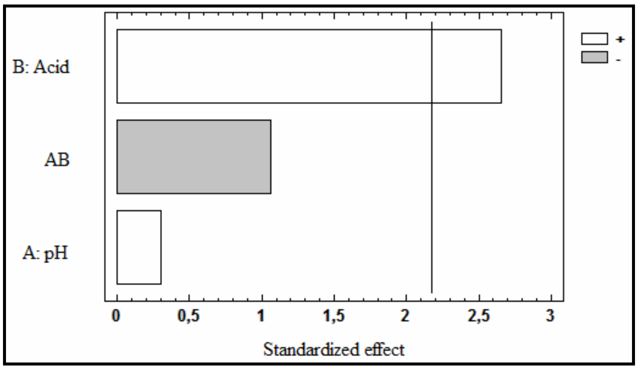

Figure 4. Standardized Pareto chart for galacturonic acid content.

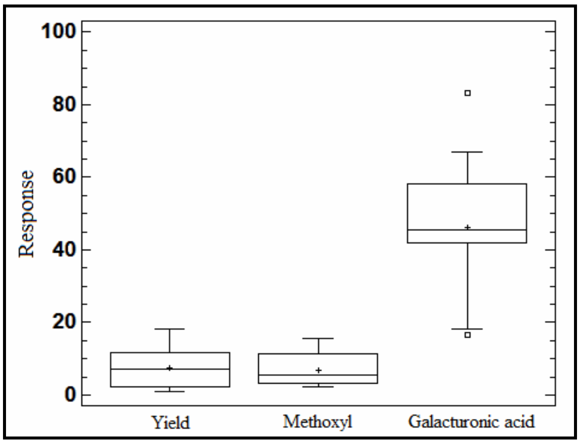

Figure 5. Box-and-Whisker plots of response variables

Regarding to box and whiskers plots of response variables (Fig. 5), the yield box is positive asymmetrical or biased downward, i.e. yield values tend to concentrate toward the bottom of distribution, which corresponds to low obtaining percentage of pectin.

The top of the box is taller than the bottomfor methoxyl and galacturonic acidvariables; this means that percentages between 50% and 75% of samples are more dispersed than between 25% and 50%. In the box of galacturonic acid, two atypical values (upper and lower) are observed. In addition, its distribution is asymmetric negative or biased upward, that is there is a higher content of this variable in the extraction of pectin.

Conclusions

Cocoa pod husk from Bolivar, Colombia, is suitable for its use in pectin extraction, evidenced by IR spectrum of cocoa pod husk, due to a 1735 cm^{-1} band related to un-hydrolyzed pectins. In addition, the fruit contains 12.06% lignin, 10.04% hemicellulose, and 18.42% cellulose.

When performing the process of extracting pectin from cocoa pod husk, the effect of varying pH and acid type acid on the yield and composition of the pectin obtained is observed, highlighting the strong influence of acid type for response variables. The highest yield (15.97%) and methoxyl content (13.71%) using citric acid at pH 2 was obtained; conditions to which Arellanes et al (42) (2011) also performed well. However, higher galacturonic acid content (63.05%) was obtained with acetic acid at pH2.

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