Screening of Variables Affecting Extraction of Pectin from Unripe Banana (*Musa acuminata × balbisiana* var. Cardaba) Peel using Plackett–Burman Design

Receil Ann B. Tanaid*

### ABSTRACT

Pectin is an important ingredient used as thickener, water binder, and stabilizer in food. Its production could be economically attractive especially if extracted from food processing wastes and by-products. This study screened the different experimental conditions that led to the significant extraction of quality pectin from Cardaba banana. The large production of banana products, which results in discarded banana peels, presents an opportunity for creating alternative processes involving this waste. The Plackett–Burman screening method was used to identify the most important factors early in the experimentation phase when complete knowledge about the factors affecting the process or method were unavailable. Seven selected variables, namely: peel nature, peel size, peel-solvent ratio, pH, extraction temperature, extraction time, and extractant:ethanol ratio, were subjected to the screening experiment. These variables are relevant to the extraction of pectin in unripe banana (*Musa acuminata × balbisiana* var. Cardaba) peel. Variable screening results showed that extraction time, extraction temperature, and peel:solvent ratios had significant effects on the extraction process. These factors could be further optimized to extract the best quality pectin from unripe banana peel.

**Keywords**: banana, Cardaba, pectin, Plackett-Burman

### INTRODUCTION

Cardaba banana (*Musa acuminata × balbisiana* var. Cardaba) is a triploid hybrid (ABB) banana cultivar originating from the Philippines (Dela Cruz et al 2007). It is one of the most important banana varieties in Philippine cuisine, but its biggest potential is for processing into chips (Dela Cruz et al 2008) wherein unripe bananas are used. The edible portion of Cardaba banana is only 60% of the entire fruit, while the rest, specifically the banana peel, is considered waste (Dela Cruz 2008). However, fruit and vegetable wastes are rich sources of carbohydrates, proteins, fats, minerals, and fibers (Gowe 1983). Therefore, they have potential uses in the food industry, one of which is as food additives such as antioxidants, antimicrobials, colorants, flavorings, and thickener agents (Ayala- Zavala & González-Aguilar, 2011). Pectin has also been investigated for its usefulness in the pharmaceutical industry, particularly stabilizing liquid pharmaceutical emulsions and suspensions.

To date, commercial pectin used for the preparation of different banana products, which results in discarded banana peels, presents an opportunity for creating alternative processes involving this waste. The Plackett–Burman screening method was used to identify the most important factors early in the experimentation phase when complete knowledge about the factors affecting the process or method were unavailable. Seven selected variables, namely: peel nature, peel size, peel-solvent ratio, pH, extraction temperature, extraction time, and extractant:ethanol ratio, were subjected to the screening experiment. These variables are relevant to the extraction of pectin in unripe banana (*Musa acuminata × balbisiana* var. Cardaba) peel. Variable screening results showed that extraction time, extraction temperature, and peel:solvent ratios had significant effects on the extraction process. These factors could be further optimized to extract the best quality pectin from unripe banana peel.
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Pectin is the methylated ester of polygalacturonic acid which contains 1, 4-linked α-D-galacturonic acid residues. Pectin is capable of forming gels with sugar and acid. Because of this gelling ability, one of the well-known uses of pectin is in high-sugar jams and confectionery jellies, dating back to at least the 18th century (IPPA 2001). Because it is a natural additive for foods, pectin is being considered for a number of applications beyond the traditional jams and jellies. Pectins are now used as thickeners, water binders, and stabilizers. It is used in yogurts and pastry glazes and as a stabilizer in drinkable yogurts and blends of milk and fruit juices (May 1990). Pectin has also been investigated for its usefulness in the pharmaceutical industry, particularly stabilizing liquid pharmaceutical emulsions and suspensions.

To date, commercial pectin used for the preparation of different confections and gelling products (such as marmalades, jams, preserves, and low calorie gels) are principally produced from two pectin-rich sources, lime and apple pomace. The yield of pectin usually depends on the extraction conditions such as temperature, extraction time, pH, type of extraction solvents (Yeoh et al 2008), and many others.

The aim of this study was to screen the different experimental conditions that lead to the significant extraction of quality pectin from Cardaba banana peels. The results of the study will be subsequently used for further research on extraction optimization. As many factors can influence the pectin extraction yield and quality, Plackett–Burman design was applied to screen important variables affecting extraction of pectin from unripe banana.

MATERIALS AND METHODS

Extraction Process of Pectin from Banana Peel

Two forms of banana peel were used for pectin extraction: dried and fresh. Dried banana peel was prepared using Castillo-Israel's (2015) method, wherein fresh banana peel was dried in a forced draft oven at 55°C for 24 hours. On the other hand, fresh banana peel was thinly sliced and soaked in water prior to pectin extraction.

The extraction of pectin followed different methods based on the levels and conditions used. Banana peel was added to distilled water, pH 7 (Mohdet al 2012), and 0.50 N citric acid, pH 2 (Virk & Sogi 2004). Peel to solvent (extractant) ratio was set at 1:10 and 1:50. These were heated while
continuously stirring at different extracting temperatures (60°C and 100°C) on a hot plate for 30 and 180 minutes (Koffi et al 2013). The solution was cooled and filtered through an ordinary screen with 1-mm mesh size and two-layer cheesecloth. The filtrate was collected then added with absolute ethanol at 1:1 and 1:3 filtrate:ethanol ratio, then kept at room temperature overnight. The precipitate (ethanol-insoluble fraction) was filtered through Whatman filter paper No. 4. It was washed with 75% ethanol, then with 80% ethanol to remove soluble impurities (Azad et al 2014). The residue was oven dried for 12 hours at 55°C, cooled, and weighed.

**Experimental Design for Screening Experiment**

The screening experiment used the Plackett-Burman design to identify the most important factors that affected pectin extraction. Each parameter studied was explored at two (2) levels: high and low values (Table 1). Seven parameters were investigated in the experiment (Table 2) having eight (8) runs and producing different product responses, which were characterized chemically.

**Table 1. High and low values for screening experiment for banana peel pectin extraction process**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Peel Nature</th>
<th>Peel Form</th>
<th>Peel: Solvent Ratio</th>
<th>pH</th>
<th>Extraction Temperature (°C)</th>
<th>Extraction Time (Min)</th>
<th>Ethanol: Filtrate Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ High level</td>
<td>Fresh</td>
<td>Medium slices</td>
<td>1:50</td>
<td>7</td>
<td>100</td>
<td>180 min</td>
<td>1:3</td>
</tr>
<tr>
<td>- Low level</td>
<td>Dried</td>
<td>Crushed</td>
<td>1:10</td>
<td>2</td>
<td>60</td>
<td>30 min</td>
<td>1:1</td>
</tr>
</tbody>
</table>

**Table 2. Plackett-Burman 8-run design with 7-variables that would affect the extraction of pectin from Cardaba banana peel**

<table>
<thead>
<tr>
<th>Peel Nature</th>
<th>Peel Form</th>
<th>Peel: Solvent Ratio</th>
<th>pH</th>
<th>Extraction Temperature (°C)</th>
<th>Extraction Time (Min)</th>
<th>Ethanol: Filtrate Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Fresh</td>
<td>Sliced</td>
<td>1:50</td>
<td>2</td>
<td>100</td>
<td>30</td>
<td>1:1</td>
</tr>
<tr>
<td>2 Fresh</td>
<td>Sliced</td>
<td>1:10</td>
<td>7</td>
<td>60</td>
<td>30</td>
<td>1:3</td>
</tr>
<tr>
<td>3 Fresh</td>
<td>Crushed</td>
<td>1:50</td>
<td>2</td>
<td>60</td>
<td>180</td>
<td>1:3</td>
</tr>
<tr>
<td>4 Dried</td>
<td>Sliced</td>
<td>1:10</td>
<td>2</td>
<td>100</td>
<td>180</td>
<td>1:3</td>
</tr>
<tr>
<td>5 Fresh</td>
<td>Crushed</td>
<td>1:10</td>
<td>7</td>
<td>100</td>
<td>180</td>
<td>1:1</td>
</tr>
<tr>
<td>6 Dried</td>
<td>Crushed</td>
<td>1:50</td>
<td>7</td>
<td>100</td>
<td>30</td>
<td>1:3</td>
</tr>
<tr>
<td>7 Dried</td>
<td>Sliced</td>
<td>1:50</td>
<td>7</td>
<td>60</td>
<td>180</td>
<td>1:1</td>
</tr>
<tr>
<td>8 Dried</td>
<td>Crushed</td>
<td>1:10</td>
<td>2</td>
<td>60</td>
<td>30</td>
<td>1:1</td>
</tr>
</tbody>
</table>
Screening of Variables Affecting Extraction of Pectin from Unripe Banana Peel

**Characterization of Pectin**

**Pectin Yield**

Pectin yield was calculated using the formula:

\[
Pectin \, (\%) = \frac{Weight \, (g) \, of \, pectin}{Weight \, (g) \, of \, peel \, taken \, for \, extraction} \times 100
\]

**Moisture Content Determination**

One gram of pectin sample was weighed, ground, and placed in an aluminum foil. The sample was dried in an oven for 5 hours at 100°C, cooled in a desiccator, and then weighed. This sample was not used for subsequent measurement, as pectin tends to degrade after exposure to the atmosphere. One percent was added to the percent moisture observed to conform to the Fischer method (Johnson 1945 as cited by Castillo-Israel 2015). The moisture content was determined using the formula:

\[
Moisture \, content \, (\%) = \frac{Weight \, (g) \, of \, residue}{Weight \, (g) \, of \, sample} \times 100
\]

**Ash Content Determination**

One to two (1-2) grams of pectin was ground to pass an 80-mesh screen. It was then placed into tared crucibles and was burnt in a furnace for 3-4 hours at 600°C. The ash content was determined using the formula:

\[
Ash \, content \, (\%) = \frac{Weight \, (g) \, of \, ash}{Weight \, (g) \, of \, sample} \times 100
\]

**Determination of Equivalent Weight**

The equivalent weight was determined by Ranganna’s method (1995). About 0.5 g sample was placed in a 250 mL conical flask and added with 5 mL ethanol, 1g of NaCl, and 100 mL of distilled water. Finally, about six (6) drops of phenol red was added and titrated against standardized 0.1 N NaOH.

The end point was indicated by a purple or faint pink color. This neutralized solution was stored for determination of methoxyl content. The equivalent weight was calculated using the formula:

\[
Equivalent \, weight = \frac{Weight \, (g) \, of \, sample \times 1000}{Volume \, of \, alkali \times Normality \, of \, alkali}
\]
RESULTS AND DISCUSSION

Results of Variable Screening

Plackett-Burman was used as the experimental design to screen the important variables affecting pectin extraction from unripe banana peel. Results showed that the variables having more significant results were the peel nature, peel-solvent ratio, extraction temperature, and extraction time. The variables with less significant results were the peel size, pH, and ethanol:pectin extract ratio (Tables 3 & 4).

Controlling the moisture content of the peels was unnecessary since these were stored in an uncontrolled environment, thus contact with the surrounding’s relative humidity must have affected the moisture content. The degree of moisture absorption depends on the type of dried product and the ambient conditions such as temperature, humidity, and contact time (Dauthy 1995). This was also held constant to produce a constant yield percentage in both dry and wet banana peel. The moisture content of peel used was 87.5% wet basis and 7.01% dry basis.

Table 3. Summary of effects for yield, moisture content, ash content, and equivalent weight

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Yield (%)</th>
<th>Moisture Content (%)</th>
<th>Ash (%)</th>
<th>Equivalent Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peel Nature</td>
<td>-</td>
<td>0.22**</td>
<td>-</td>
<td>4.06</td>
</tr>
<tr>
<td>Peel Size</td>
<td>-</td>
<td>0.11**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PS Ratio</td>
<td>-</td>
<td>0.12**</td>
<td>-</td>
<td>4.06</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>0.48**</td>
<td>-</td>
<td>0.23</td>
</tr>
<tr>
<td>E. temp</td>
<td>0.89**</td>
<td>0.26*</td>
<td>-</td>
<td>4.72**</td>
</tr>
<tr>
<td>E. time</td>
<td>0.10**</td>
<td>-</td>
<td>-</td>
<td>25.95**</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean/Interc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>ns</td>
</tr>
<tr>
<td>M</td>
<td>ns</td>
</tr>
<tr>
<td>A</td>
<td>ns</td>
</tr>
<tr>
<td>E</td>
<td>ns</td>
</tr>
<tr>
<td>MeO</td>
<td>8.16</td>
</tr>
<tr>
<td>AUA</td>
<td>68.58</td>
</tr>
<tr>
<td>DE</td>
<td>67.48</td>
</tr>
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</table>

Table 4. Summary of effects for methoxyl content (MeO), anhydrouronic acid content (AUA), and degree of esterification (DE)

<table>
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Determination of Methoxyl Content (MeO)

Determination of MeO was done using Ranganna’s method (1995). The neutral solution was collected from determination of equivalent weight and 25 mL of 0.25 N NaOH was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 minutes. After 30 minutes, 25 mL of 0.25 N HCl was added and the mixture was titrated against standardized 0.1 N NaOH. Methoxyl content was calculated using the formula:

\[
\text{Methoxyl content (\%) = \frac{\text{Volume of alkali (mL)} \times \text{Normality of alkali} \times 3.1}{\text{Weight (g) of sample}}}
\]

Determination of Total Anhydrouronic Acid Content (AUA)

Total AUA of pectin was calculated using the following formula (Mohamed & Hasan 1995):

\[
\% \text{ of AUA} = \frac{176 \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000}
\]

when molecular unit of AUA (1 unit)=176 g
where,
\[
z = \text{mL (titre) of NaOH from Equivalent weight determination}
\]
\[
y = \text{mL (titre) of NaOH from methoxyl content determination}
\]
\[
w = \text{weight of sample}
\]

Determination of Degree of Esterification (DE)

The DE of pectin was measured on the basis of methoxyl and AUA content (Owens et al 1952) and calculated using this formula:

\[
\% \text{ DE} = \frac{176 \times \% \text{MeO}}{31 \times \% \text{AUA}} \times 100
\]

Statistical Analysis and Modelling

The results of physico-chemical properties analysis from the Plackett-Burman design were analyzed for variables which have significant effects using Statistica 8.0 software and Statistical Analytical Software version 9 (SAS 2008).
RESULTS AND DISCUSSION

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<th>Moisture Content (%)</th>
<th>Ash (%)</th>
<th>Equivalent Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean/Interc.</td>
<td>1.26&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>12.64&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>13.23&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>864.42&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peel Nature</td>
<td>-0.22**</td>
<td>-0.16&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>4.06&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>40.84**</td>
</tr>
<tr>
<td>Peel Size</td>
<td>-0.11**</td>
<td>-0.12&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-1.7&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-55.33&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>PS Ratio</td>
<td>-0.12**</td>
<td>-0.34*</td>
<td>-1.93&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-39.21**</td>
</tr>
<tr>
<td>pH</td>
<td>-0.48**</td>
<td>0.13&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-69.13**</td>
</tr>
<tr>
<td>E. temp</td>
<td>0.89**</td>
<td>0.26*</td>
<td>-2.53&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>144.13**</td>
</tr>
<tr>
<td>E. time</td>
<td>0.10**</td>
<td>-0.48**</td>
<td>-0.45&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-25.95**</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.0&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-0.93**</td>
<td>1.04&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>-1.97&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ns</sup>not significant (p>0.05)  *significant (p≤ 0.05) **significant (p≤ 0.01)

Table 4. Summary of effects for methoxyl content (MeO), anhydrouronic acid content (AUA), and degree of esterification (DE)

<table>
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<th>Parameter</th>
<th>MeO (%)</th>
<th>AUA (%)</th>
<th>DE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean/Interc.</td>
<td>8.16&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>68.58&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>67.48&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peel Nature</td>
<td>0.35**</td>
<td>0.93**</td>
<td>1.75**</td>
</tr>
<tr>
<td>Peel Size</td>
<td>0.74**</td>
<td>5.40**</td>
<td>0.82**</td>
</tr>
<tr>
<td>PS Ratio</td>
<td>0.29**</td>
<td>2.64**</td>
<td>-0.10&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>-0.05&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.23**</td>
<td>-1.74**</td>
</tr>
<tr>
<td>E. temp</td>
<td>0.55**</td>
<td>-0.18&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>4.72**</td>
</tr>
<tr>
<td>E. time</td>
<td>0.27**</td>
<td>2.11**</td>
<td>0.10&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.12**</td>
<td>6.63**</td>
<td>2.70**</td>
</tr>
</tbody>
</table>

<sup>ns</sup>not significant (p>0.05)  *significant (p≤ 0.05) **significant (p≤ 0.01)
For the pH variable, 0.5N citric acid with pH 2 was used, since pH 2 was found to give the highest yield of extracted pectin from table banana (Girma & Worku 2016). According to Shaha et al (2013), citric acid is the best agent for the extraction of pectin. This is in agreement with the results reported by Virk and Sogi (2004) who compared the yields of pectin extracted from apple with different acids, namely: hydrochloric acid, nitric acid, and citric acid. It was also reported by Yapo (2009) that citric acid was the least pectin degrading (depolymerizing and deesterifying) extracting agent. Therefore, it leads to pectin isolates with the best gelling properties. However, among the acids used in the experiment, it was observed that there was no significant difference in pectin yield.

Variables such as peel size and pH had negative effects on all parameters. Even though a low pH is necessary to improve the yield, the use of a strong acid solution such as hydrochloric acid could lead to smaller pectin particles owing to partial hydrolysis. Consequently, pectin solubility would increase to the point that no precipitate was formed by the addition of alcohol (Canteri-Schemin et al 2005). The peel size affects the extraction process since the crushed form (low level) contributes to the viscosity of the solution causing inefficient filtration, thus reducing the yield and compromising pectin quality. Using medium-sized peels (high level) also reduced the surface area, thus reducing the penetration rate of the solvent. For ethanol:pectin ratio, the effect on the parameters was positive, thus the high level 1:3 ratio was used.

From these results, the variables considered for possible optimization of pectin extraction were peel-solvent ratio, extraction time, and extraction temperature. Majority of the physico-chemical properties showed a positive effect to extraction temperature and time.

If the effect is positive, the closest value, either lower or higher than the indicated high level (+) concentration, is required during further optimization studies. On the other hand, if the effect is negative, the closest value, either lower or higher than the indicated low level (-) concentration, is used. This applies to the peel:solvent ratio which negatively affected majority of the parameters. These three factors were important in the extraction process since the rate of vaporization during extraction was affected by the amount of solvent, extraction temperature, and time. As the extraction process progressed, the concentration of the pectin in the solution increased causing the extraction rate to progressively decrease.

Increase in viscosity of the solution (due to vaporization) led to the reduction of pectin extract, resulting in a lower yield when precipitated with alcohol. High temperature and longer boiling time encourage energy and water (or solvent) loss through vaporization (Girma & Worku 2016). The peel:solvent ratio must be optimized to attain a pectin extract that, if precipitated with alcohol, will produce the highest yield possible.
Screening of Variables Affecting Extraction of Pectin from Unripe Banana Peel

CONCLUSION AND RECOMMENDATIONS

The Plackett-Burman variable screening was used to identify the variables that would significantly affect pectin extraction. A 7-variable, 8-run screening experiment was conducted with the following variables: peel nature, peel size, peel:solvent ratio, pH, extraction temperature, extraction time, and extractant:ethanol ratio. Values for extraction temperature and time showed significant positive effect, whereas peel:solvent ratio showed significant negative effect on majority of the physico-chemical tests. The results identified extraction time, extraction temperature, and peel:solvent ratio as important factors in pectin extraction. However, these have to be optimized to produce pectin of the best quality. Optimization of extraction temperature, extraction time, and peel:solvent ratio is suggested to produce the best quality of pectin from Cardaba banana peel.

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Changes in Soil Nitrogen, Phosphorus, and Carbon Stocks in a Forest Ecosystem at Different Successional Stages in Leyte, Philippines

ABSTRACT

Forests play a vital role in the global carbon cycle since these are sources and sinks of carbon. This study was conducted to evaluate the changes in soil carbon stocks and some essential nutrients of different succession stages in two different soil types in Leyte Province. A space-for-time substitution approach was done in this study. Measurements of the physical, chemical, and biological properties of the soils were done following standard methods. The sites were characterized as Ultisol (Site 1 – Baybay, Leyte) and Andisol (Site 2 – Ormoc City). Results showed no significant differences among all the soil properties in the different forest succession stages in each site. However, variation in soil properties between sites was clearly observed. Site 2 had higher soil porosity and water holding capacity, but had lower bulk density than Site 1. Soils in Site 2 were more acidic, had higher total organic carbon, total N, and CEC but had lower exchangeable bases and CEC than in Site 1. Both sites had low available P. The C:N ratios in all forest successions were significantly lower in Site 1 than in Site 2. This conforms to the results of substrate-induced respiration, where Site 1 was more active in CO\textsubscript{2} evolution than Site 2.

Moreover, the soils in Site 2 significantly contained more SOC stocks (108-180 Mg C ha\textsuperscript{-1}) than in Site 1 (49-76 Mg C ha\textsuperscript{-1}). However, SOC stocks did not vary significantly in both sites. This result implies that the determination of soil physico-chemical properties is important in evaluating the changes of C:N ratios as well as of SOC stocks. In this study, Andisols had higher potential in storing organic C than Ultisols.

Keywords: successional stages, soil physical and chemical properties, C:N, C stocks


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