CRAB AS A COCONUT OIL SEPARATING AGENT

by
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Abstract

The role of stabilized and non-stabilized crab extract on the separation of coconut oil was examined using graded coconut meal as substrate. Stabilized crab extract was prepared by sulphuric acid and cationexchange of crushed crab and then filtered using Milipore filter. Stabilized crab extract had a negative effect on microbiological activity but not on postharvest spoilage. It was found that the standard crab extract inhibited the growth of proteolytic microorganisms isolated from fermentation process of coconut oil. Both stabilized and non-stabilized crab extract gave no significant difference in the quantity of coconut oil recovered. However, coconut oil from the stabilized crab extract had better quality. The peroxide value of coconut oil from untreated crab extract was higher than that of standard quality of coconut oil according to S6 and AOCS. The possible roles of crab inoculum in fermentation process of coconut oil are discussed.

Introduction

Coconut oil is made from coconut meat, in which the main components are lipid (98%) and protein (1.25%) (17). In the coconut meat, lipid molecules are bound with protein molecules (15). To separate the oil, they must be separated from the protein molecules. Several methods were applied, such as physical, chemical, enzymatic and microbiological method (13, 14, 16).

In physical method, coconut oil is produced from copra. Unfortunately, the copra sometimes are contaminated by toxin-producing fungi, insects, and rodents (16). Another example, oil was removed after the protein was denatured by boiling coconut milk for hours. This traditionally produced coconut oil is very tasty and has a good aroma. However, it needs a lot of fuel and time consumed (15).

Proteolytic enzymes were used in an enzymatic method. Here, pigaye leaves or young papaya fruit which contain pepsinogen were added into graded coconut meat (14).

Application of microbiological activities in separating lipid molecules is the principal method of microbiological production of coconut oil. In this method, the microbes were inoculated into graded coconut meat or coconut milk. The common microorganisms used are Lactobacillus plantarum, L. delbrueckii, Candida tropic平, Torulopsis farisina, Hansenula sylvigiflora and Trichosporon pullulans (13, 16).

An interesting method widely used in Pulipropo is the use of crab (live crab, Parathelphus hirsutus) as inoculant to separate the coconut oil. Crab was crushed and incubated to graded coconut meat. It was incubated for 12–14 hours, the coconut meat was sun-dried and pressed to separate the oil (3, 9). This simple method needs no fuel, and can be applied as home industry.

This last method has problems interest ed to be solved, especially about the crab inoculant itself. It still not clear either the impor tant agent for separating the coconut oil is a certain substance(s) contained in the body of the crab itself, such as the proteolytic enzymes; or the activity of synthesized enzymes by microorganisms usually found as symbiont with the crab in river water. These microorganisms grow in graded coconut meat and act as oil separating active agent during incubation. In the later case, there is

Tech Vol. 11, No. 4

Agrotech Vol. 11, No. 4

23
possibility that certain chemicals in crab inoculant (growth factors or vitamins) may affect the growth and activity of the microorganisms involved.

In previous research, several bacteria and yeasts were isolated from the fermentation process of coconut oil using crab inoculum (11). These microorganisms have proteolytic and/or lipolytic activity. However, the specific activities gave no significant difference in the ability to separate coconut oil (19). Incubation of non-sterilized crab extract enhanced the quantity of separated coconut oil, but it has high viscosity (9).

From these viewpoints, therefore, this study was conducted to investigate the possibility that enzymes from the crab play an important role as a separating active agent of coconut oil, or as a supporting agent for the growth or activity of microorganisms in separating coconut oil.

Materials and Methods

1. Substrate and inoculum

The substrate for coconut oil production was mature coconut meat. The inoculant used were river crab (Pachycheles tigrinus (L.)) (Fig. 1), and bacterial and yeast strains which were isolated from the fermentation process of coconut oil separation using crab inoculum (11).

2. Sterilization of crab extract

Crab extract was prepared by suspending crushed crab in distilled water (1:3, w/v). To separate the large and hard portions of crab, suspension was centrifuged (5000 rpm, 2 min). The supernatant was heat-sterilized using Millipore filter (pore size 0.22 μm). Quantity examination was done by spreading the filtrate on Nutrient Agar for bacteria, or Spizog Agar Medium for yeast and fungi examination.

3. Examination of sterilized crab extract

a. Enzymatic activity of crab extract

Sterilized crab extract was examined for proteolytic and lipolytic activities semi-quantitatively using paper-disc methods on a specific media explained in previous article (9). The appearance of clear zone around the paper-disc indicated proteolytic or lipolytic activity of the crab extract. A drop of sterile distilled water was used as a control.

b. Influence of crab extract on the growth of aquatic microbes

It was examined using paper-disc method on specific media. The bacteria or yeast was grown separately on the Nutrient Agar or Soybean-Slurry Agar, respectively. At the same time, sterilized paper-disc were placed on the agar surface and dropped with 0.01 ml of crab extract. Control (sterilized distilled water) was also conducted as above. The appearance of clear zone on the heavy population of the microbial growth around the paper-disc indicated the inhibitory or supporting effect on the crab extract on the microbial growth, respectively.

4. Fermentation of grated coconut meat using sterilized crab extract inoculum

100 g of grated coconut meat was inoculated with 3 ml of sterilized crab extract. This ratio was considered to provide as to the condition traditionally used (2). Fermentation was done in polyethylene bags with small holes (2 - 3 cm between holes) and incubated at room temperature for 10 - 14 h. At the end of incubation, the color of the grated coconut turned to brown. This brownish coconut meal was sun-dried for 2 - 5 h. A presser apparatus (Fig. 2) was used to separate the coconut oil. Finally, the oil was removed from emulsified moisture and

Agric Biol J Vol. II, No. 4
of crab extract

It was examined its activated semi-quantitative method on 5 in previous article for zone around the proteolytic or lipolytic zone. A drop of sterile water was added as a control.

Effect on the growth paper-disc method. The bacteria or mold on the Nutrient Agar, respectively, 10 paper discs were placed and dropped at. Control (sterile) also conducted in oil clear zone of the antibiotic growth indicated the in

Effect on sterilized crab extract

zontal was used in sterilized crab extract. The disc was close to the test (2). From the ethylene bags with sterile and in

reaction medium (Fig. 3) indicated that the sterilized crab extract has proteolytic activity but not lipolytic. Table 1 showed the detail number of the proteolytic and lipolytic examinations. These data were consistent with those by Saito (1989), indicating that proteolytic activity of inoculum has an important role in the separation of coconut oil.

Effect of sterilized crab extract on the growth of isolate

In the modification of surface-plate method and paper-disc methods, within 48 h appeared the effect of sterilized crab extract on the growth of bacteria or yeast isolates. If the crab extract has supporting active agent, it will show a more heavy population of microbial growth around the paper-disc. Conversely, inhibition effect will give a clear zone. Neutral effect (no supporting or inhibition) gave no alteration on growth around the paper-disc (Fig. 4 and Table 2).

Results from the agar media showed that sterilized crab extract has supporting active agent on the growth of isolated microbes (especially bacterial isolates) which have proteolytic with or without lipolytic activities. However, its effect showed variation on the lipolytic isolates. These data indicated the possibility that supporting active agent(s) found in crab extract was appropriate only for the proteolytic isolates. These data were supported by the data that microorganisms commonly used as inoculant for coconut separating inoculants have proteolytic activity which found to be an important role in separation of coconut oil (14, 15).

The pH value of crab extract was 7.1, within the range of optimal pH for the activity of proteolytic enzymes (5.0 — 9.0). The activity of proteolytic enzymes needs Ca, Co, Zn and Fe as co-factors (12) and these co-factors can be obtained in crab extract from the bones and outer skin.
The proteolytic activity of crab extract indicated that crab extract has an important role in the separation of coconut oil. Other data also supported and showed that the crab extract gave a specific condition for the grown and/or activity of proteolytic microorganisms in separating coconut oil.

Sterilization system, traditionally applied for 3–5 times during separation process using crab inoculum did not increase the quantity of separated coconut oil (3). It means that the important agent has increased during the sterilization process. These results may indicate that the important agent in this process is not only accounted by proteolytic enzymes from the crab, but also the microbial activities, because without any role of microorganisms, there would be no increase in the number of proteolytic enzymes.

Fermentation of grated coconut meal

Three kinds of inocula, control (uninoculated), sterilized crab extract, and nonsterilized crab extract, were applied in the experiment. The quantity of separated coconut oil from each of the above treatments were shown in Table 3. Although the sterilized crab inoculum resulted in the highest quantity of coconut oil, there was no significant difference among the treatments.

Physical and chemical analysis of coconut oil

Table 4 showed the saponification number of produced coconut oil without any inoculum showed much lower than standard (102.5 compared with 265.0 for IIS. or 255.0 for ACS). This means that molecular weight of lipids in this coconut oil was high, which indicated that there was no or very little oxidation of high-molecule weight oil acid which resulted in short-cation chain of lipid (F). This possibility was confirmed with the data that the coconut oil had a very high number of FFA.

Analysis of other coconut oil (starch or nonsterilized crab extract and the peels oil) showed that these oils have a good quality based on the SII and DCCS (Table 4). Except of p-value, the coconut oil produced by nonsterilized crab extract inoculum showed higher value than maximum number for SII and DCCS. This result was consistent with Mariani (1986), which means that coconut oil produced by nonsterilized crab extract could not be stored for a long period because it will be rancid during storage. The high p-value number was caused by the high concentration of peroxides and hydroperoxide substances, which was resulted from the oxidation of fatty acids, could be oxidized further into ketone, methyl nonyl ketone is usually found in coconut oil showing high rancidity (10).

This study showed a new problem that the high peroxide value of coconut oil produced by nonsterilized inoculum indicated that the normally produced coconut oil can not be stored for long period. This problem may be caused by the activity of undesired microbial contamination. One possibility is by adding an appropriate antioxidant into coconut oil. Some antioxidant usually added into coconut oil are propyl galate (PG), Oxyl galate (OG), dodecyl galate (DG), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (10, 18).

Conclusion

The sterilized extract of crab (Parathelphusa schmitti) has proteolytic activity that can be used for the separation of coconut oil.

The growth of microorganisms isolated from the fermentation process of separated...
coconut oil (later referred as the prist oil) showed a good quality based on Table 4. Except for one sample, all samples of coconut oil produced by the inoculum showed high quality number for both the method and the method of testing. The high quality number indicates that the oil has desirable physical properties and is suitable for use as a source of fat or oil. This is supported by the fact that the oil is free from any visible impurities or defects.

To determine the stability of the coconut oil, the oil was stored at ambient temperature for a period of one month. The results showed that the oil remained stable, with no visible changes in the color, odor, or texture. The oil also passed all the standard tests for quality as prescribed by the International Coconut Council.

Table 1. Proteolytic and lipolytic activities of sterilized crab extract

<table>
<thead>
<tr>
<th>Enzymatic Activity</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteolytic</td>
<td>0.096</td>
</tr>
<tr>
<td>Lipolytic</td>
<td>0.009</td>
</tr>
</tbody>
</table>

The results of the proteolytic and lipolytic activities of the sterilized crab extract are shown in Table 1. The proteolytic activity was measured using the caseinase method, while the lipolytic activity was measured using the free fatty acid method. The results show that the enzyme activity is higher in the proteolytic activity than in the lipolytic activity.

The effect of sterilization on the proteolytic activity of the crab extract was also measured and it was found that there was no significant difference in the proteolytic activity of the sterilized and unsterilized crab extract. This indicates that the sterilization process did not affect the quality of the enzyme activity.

The qualitative and quantitative analysis of the sterilized crab extract showed that it is suitable for use as a source of amino acids and essential fatty acids. The extract also showed a good stability over a period of one month at ambient temperature.

The results of the analysis of the sterilized crab extract are shown in Table 1. The results indicate that the enzyme activity is higher in the proteolytic activity than in the lipolytic activity.

The quality of the coconut oil produced by inoculation of sterilized crab extract has a good quality according to the standard quality of the International Coconut Council. However, inoculation of unsterilized crab extract resulted in coconut oil which has a low peroxide number.

The results showed that the possible role of crab inoculum in the separation of coconut oil is high in quality and of quality of coconut oil which has low peroxide number.
<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Enzymatic act. 2)</th>
<th>Protein</th>
<th>Lipid</th>
<th>Growth react. 2)</th>
<th>%</th>
<th>P</th>
<th>N</th>
<th>( R )</th>
</tr>
</thead>
<tbody>
<tr>
<td>B - 7</td>
<td>+</td>
<td>+</td>
<td></td>
<td>1 - 2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B - 10</td>
<td>+</td>
<td>+</td>
<td></td>
<td>2 - 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B - 2</td>
<td>+</td>
<td>-</td>
<td></td>
<td>2 - 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B - 5</td>
<td>+</td>
<td>+</td>
<td></td>
<td>3 - 8</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>E - 1</td>
<td>+</td>
<td>-</td>
<td></td>
<td>1 - 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>E - 4</td>
<td>+</td>
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<tr>
<td>E - 5</td>
<td>+</td>
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<td>2 - 4</td>
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<td></td>
</tr>
<tr>
<td>K - 1</td>
<td>+</td>
<td>-</td>
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<tr>
<td>K - 6</td>
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</tr>
<tr>
<td>E - 3</td>
<td>+</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td>B - 8</td>
<td>-</td>
<td>+</td>
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<td>4 - 5</td>
<td>77.6</td>
<td>22.2</td>
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<tr>
<td>E - 2</td>
<td>+</td>
<td>-</td>
<td></td>
<td>Ni</td>
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</tr>
<tr>
<td>K - 5</td>
<td>-</td>
<td>+</td>
<td></td>
<td>Ni</td>
<td>33.3</td>
<td>66.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

1) Cit. from Mantini (1966)
2) Indicated 0 zone reaction (in mm)

P: Positive, heavy population
N: Neutral, no alteration
Ni: Negative, growth inhibition

Agritech Vol. II, No. 4

28
Fig. 4. Effect of standardized crab extract on the growth of microfloral isolates growing on specific media.

A. Crab extract supported the growth of isolates
B. Crab extract gave no effect
C. Crab extract inhibited the growth of isolate
D. Paper-disc with crab extract
E. Paper-disc with standardized extract

Table 3. Production of coconut oil

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Oil volume $^1$</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>54.7</td>
</tr>
<tr>
<td>Sterilized crab extract</td>
<td>26.1</td>
</tr>
<tr>
<td>Nonsterilized crab extract</td>
<td>29.2</td>
</tr>
</tbody>
</table>

$^1$in ml/100g (DW) of grinded coconut meal.
<table>
<thead>
<tr>
<th></th>
<th>Spécific gravity</th>
<th>Moisture A volatile matter</th>
<th>Insoluble number</th>
<th>Solubility number</th>
<th>Paraffine C。paraffine</th>
<th>PFA (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.920</td>
<td>0.26</td>
<td>8.67</td>
<td>102.6</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>Sterilized crab</td>
<td>0.920</td>
<td>0.66</td>
<td>5.00</td>
<td>256.97</td>
<td>0.57</td>
<td>1.12</td>
</tr>
<tr>
<td>Workers crab</td>
<td>0.922</td>
<td>0.32</td>
<td>7.87</td>
<td>253 50</td>
<td>6.01</td>
<td>1.08</td>
</tr>
<tr>
<td>Nonsterilized crab</td>
<td>0.928</td>
<td>0.43</td>
<td>7.87</td>
<td>253 50</td>
<td>3.59</td>
<td>1.17</td>
</tr>
<tr>
<td>PABO fried oil</td>
<td>0.917</td>
<td>max 0.50</td>
<td>12.0</td>
<td>260.0</td>
<td>max 5.00</td>
<td>5.06</td>
</tr>
<tr>
<td>(5%)</td>
<td>—</td>
<td>—</td>
<td>7.50</td>
<td>256.0</td>
<td>max 0.50</td>
<td>—</td>
</tr>
<tr>
<td>AOCS 7)</td>
<td>0.918</td>
<td>—</td>
<td>—</td>
<td>264.0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1) In %
2) In 1 g of 100g sample
3) In mg of 100g sample
4) In mg of dry weight sample
5) In mg of ashes content in %
6) Indonesian Industrial Standard (17)
7) American Oil Chemist’s Society

References

30
<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Author(s)</th>
<th>Year</th>
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