# CRAB AS A COCONUT OIL SEPARATING AGENT

by

**Sebastian Margino** *)

**Abstract**

The role of sterilized and nonsterilized crab extract on the separation of coconut oil was examined using grated coconut meat as substrate. Sterilized crab extract was prepared by suspension and centrifugation of crushed crab and then filtrated using Millipore filter. Sterilized crab extract has proteolytic activity but not lipolytic one. It was found that the sterilized crab extract supported the growth of proteolytic microbes, isolated from fermentation process of coconut oil.

Both sterilized and nonsterilized crab extract gave no significant difference in the quantity of coconut oil obtained. However, coconut oil from the sterilized crab extract has better quality. The peroxide value of coconut oil from unsterilized crab extract was higher than that of standard quality of coconut oil according to SII 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 (59%) and protein (19.25%) (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 physic, chemic, 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 or rodents (16). Another example, oil was removed after the protein was denaturated 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 consummed (15).

Proteolytic enzymes were used in enzymatic method. Here, papaya leaves or young papaya fruit which contain papainase were added into grated 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 grated coconut meat or coconut milk. The common microorganisms used are *Lactobacillus plantarum, L., delbrueckii, Candida tropicallis, Torulopsis famata, Hansenula schulgii* and *Trichosporon pullulans* (13, 16).

An interesting method widely used in Kulonprogo is the used of crab (river crab, *Parathelphusa* sp).) as inoculant to separate the coconut oil. Crab was crushed and inoculated to grated coconut meat. It was incubated for 10 — 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 interrested to be solved, especially about the crab inoculant itself. It still not clear either the important 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 grated coconut meat and act as oil separating active agent during incubation. In the later case, there is

*)Educative Staf Fac. Agriculture*GMU.

possibility that certain chemical(s) found in crab inoculant (growth factors or vitamins) the growth and activity of the microorganisms.

In previous research, several bacteria and yeast were isolated from fermentation process of coconut oil using crab inoculum (11). These microorganisms have proteolytic and/or lipolytic activity. However, these activities gave no significant difference in the ability to separate coconut oil (19). Inculation of nonsterilized crab extract enchanced the quantity of separated coconut oil, but it has high rancidity (9).

From these viewpoints, therefore, this study was conducted to investigate the possibility that enzyme(s) from the crab play in direct important role as a separating active agent of coconut oil; or indirect role as a supporting agent for the growth or activity of microbes in separating coconut oil.

## Materials and Methods

### 1. Substrate and Inoculum

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

### 2. Sterilization of crab extract

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

### 3. Examination of sterilized crab extract

*a. Enzymatic activity of crab extract*

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

*b. Influences of crab extract on the growth of isolated microbes*

It was examined using paper-disc method on specific media. The bacterial or yeast was grown separately on the Nutrient Agar or Soybean-Sprout Agar, respectively. At the same time, sterilized paper-discs 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 or 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 as close 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 this grated coconut turned to brown. This brownish coconut meat 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

other volatile matter (6). Volume of separated oil showed the ability of inoculum to separate lipid molecule from the protein.

For comparison study, nonsterilized crab extract was used as inoculum in the same procedure.

### 5. *Physical and chemical analysis of coconut oil*

The resulted coconut oil was examined its physical and chemical characteristics. These included: color and aroma, density, moisture and volatile matter concentration, rancidity, saponification number, Yod number, and concentration of free fatty acids (FFA) (1, 6). An industrial produced coconut oil found in market was analyzed as comparison study. The results were compared with the quality standard for coconut oil of SII and AOCS (American Oil Chemist's Society).

## Results and Discussion

### *Preparation of sterilized crab extract*

At first, the ratio of crab extract and distilled water were 1:1, 1:2, and 1:3 (w/v). However, 1:1 and 1:2 ratios were too high for filtration (pore disturbance). Therefore, it was decided that 1:3 was the appropriate ratio. Centrifugation before filtration was conducted to eliminate the large and hard portions of crushed crab (bones and outer parts of the skin) which disturbed the filtration.

After several trial and error investigations using many kinds of filtration methods followed by sterility examination (Materials & Methods 2), it was found that filtration using Millipore filter (0.22 um) resulted in steril crab extract.

### *Enzymatic examination of crab extract*

The appearance of clear zone on the proteolytic medium and not on the lipolytic mediun (Fig. 3) indicated that the sterilized crab extract has proteolytic activity but not lypolytic. Table 1 showed the detail number of the proteolytic and lypolytic examinations. These data were consistant with those by Sarwanto (1985), 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 isolates*

With the modification of surface-plating 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. Conversally, 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, 16).

The pH value of crab extract was 7.1, within the range of optimal pH for the activity of proteolytic enzymes (6.0 — 9.0). The activity of proteolytic enzymes needs Ca, Co, Zn and Fe as cofactors (12) and these cofactors 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 growth and/or activity of proteolytic microorganisms in separating coconut oil.

Starter inoculation system traditionally applied for 3—5 times during separation process using crab inoculum did not decrease the quantity of separated coconut oil (3). It means that the important agent has increased during the inoculation 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 meat*

Three kinds of inocula, control (uninoculated), sterilized crab extract, and nonsterilized crab extract, were applied in this 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.

### *Phisical 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 255.0 — 265.0 for IIS, or 250.0 — 264.0 for AOCS). This means that molecular weight of lipid in this coconut oil was high, which indicated that there was no or very litlle oxidation of high-molecular weight of lipid which resulted in short carbon chain of lipid (18). This possibility was consistant with the data that this coconut oil has low number of FFA.

Analysis of other coconut oil (steril or nonsteril crab extract and the pristo oil) showed that these oil have a good quality based on the SII and AOCS (Table 4). Except of peroxide value, the coconut oil produced by nonsterilized crab extract inoculum showed higher value than maximum number for IIS and AOCS. This result was consistant with Martani (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 peroxide number was caused by the high concentration of peroxides and hydro-peroxide substances, which was resulted from the oxidation of fatty acids, would be oxidied further to keton. Methyl nonyl keton is ussually found in coconut oil showing high rancidity (10).

This study showed a new problem. The high peroxide value of coconut oil produced by nonsterilized inoculum indicated that this traditionally produced coconut oil can not be stored for long period. This problem may coused by the activity of undesired microbial contamination. However, it is impossible to innovate sterilization procedures to common people. One possibility is by adding an appropriate antioxidant into coconut oil. Some antioxidant ussually added into coconut oil are: propyl gallate (PG), Octyl gallate (OG), dodecyl gallate (DG), butylated hydrocyanisole (BHA) and butylated hydroxytoluene (BHT) (10, 18).

## Conclusion

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

The growth of microorganisms isolated from the fermentation process of separation

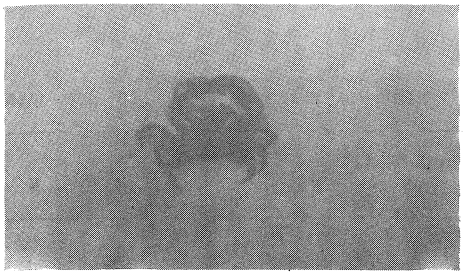

Fig. 1 **Crab (*Parathelphusa* sp.) obtained from Winongo river, used as inoculum in this study.**

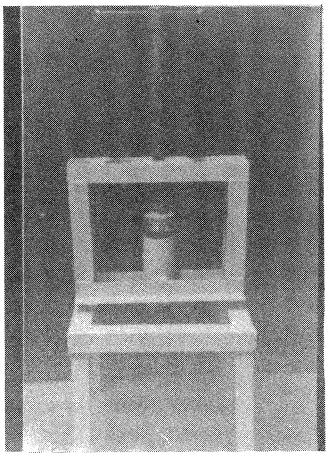

Fig. 2. **Coconut Oil-Presser apparatus (Laboratory scale)**

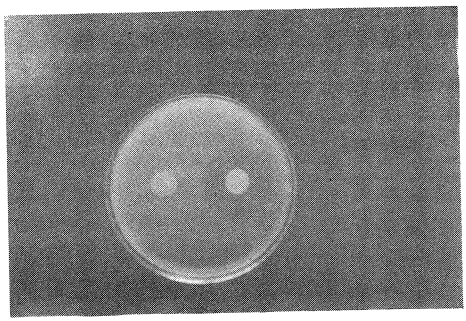

Fig. 3. **Proteolytic activity of sterilized crab extract on specific medium. Clear zone arround the paper-disc indicated the proteolytic activity of sterilized crab extract.**

**Table 1. Proteolytic and lipolytic activities of sterilized crab extract**

| | enzymatic activities[1)] | |
|---|---|---|
| | | average |
| Proteolytic | 20.0/9.0<br>18.0/9.0<br>21.0/9.0<br>20.0/9.0 | 19.7/9.0 |
| Lipolytic | 0.0/9.0[2] | 0.0/9.0 |

[1)]0 of clear zone/0 of peper-disc in mm.

[2)]no clear zone which means no lipolytic activity

coconut oil were influenced by the sterilized crab extract. It was found a tendency that the crab extract supported the growth of proteolytic microorganisms.

These results indicated that sterilized crab extract has an important role directly in the separation of coconut oil because it has proteolytic activity, and also indirectly because its supporting effect on the growth of proteolytic microorganisms.

The quality of coconut oil produced by inoculation of sterilized crab extract has a good quality according to the standard quality of IIS and AOCS. However, inoculation of unsterilized crab extract resulted in coconut oil which has high peroxide number.

The results showed the possible role of crab inoculum in the separation of coconut oil, it was considered as the importance of study for investigating the possible application of antioxidant(s) to obtain a high quantity and quality of coconut oil which has low peroxide number.

Table 2. **Effect of sterilized crab extract on the growth of microbial isolates on agar media**

| Isolates [1] | Enzymatic act. [2] | | Growth react. [2] | | | % | | |
|---|---|---|---|---|---|---|---|---|
| | Proteol | Lipol. | P | Nt | N | P | Nt | N |
| **Bacteria** | | | | | | | | |
| B — 7 | + | + | 1 — 2 | | | | | |
| B — 10 | + | + | 2 — 4 | | | 100 | 0 | 0 |
| B — 2 | + | — | 2 — 3 | | | | | |
| B — 5 | + | — | 5 — 8 | | | | | |
| E — 1 | + | — | 1 — 2 | | | | | |
| E — 4 | + | — | 1 — 2 | | | | | |
| E — 5 | + | — | 2 — 4 | | | | | |
| K — 1 | + | — | 3 — 7 | | | | | |
| K — 6 | + | — | 1 — 3 | | | | | |
| B — 4 | + | — | | Nt | | | | |
| E — 3 | + | — | | Nt | | 77.8 | 22.2 | 0 |
| B — 8 | — | + | 4 — 5 | | | | | |
| E — 2 | — | + | | Nt | | | | |
| K — 5 | — | + | | Nt | | 33.3 | 66.7 | 0 |
| **Yeast** | | | | | | | | |
| A — 6 | + | + | 1 — 4 | | | 100 | 0 | 0 |
| Y — 8 | — | + | 1 — 3 | | | | | |
| Y — 2 | — | + | | Nt | | | | |
| Y — 10 | — | + | | | 1 — 2 | | | |
| Y — 12 | — | + | | | 2 — 4 | 25 | 25 | 50 |
| Y — 1 | — | — | 1 — 3 | | | | | |
| Y — 4 | — | — | 1 — 4 | | | | | |
| Y — 7 | — | — | 2 — 3 | | | | | |
| A — 2 | — | — | 1 — 3 | | | | | |
| Y — 14 | — | — | | Nt | | | | |
| A — 3 | — | — | | Nt | | | | |
| A — 1 | — | — | | | 2 — 10 | | | |
| A — 5 | — | — | | | 1 — 2 | 50 | 25 | 25 |

[1] Cit. from Martani (1986)

[2] Indicated 0 zone reaction (in mm)

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

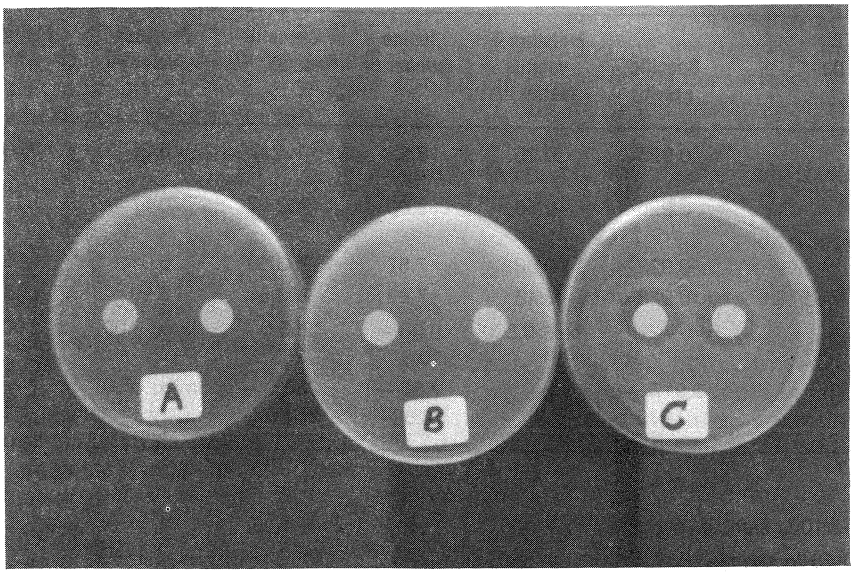


Fig. 4. **Effect of sterilized crab extract on the growth of microbial isolates growing on specific media.**
**A. Crab extract supported the growth of isolate**
**B. Crab extract gave no effect**
**C. Crab extract inhibited the growth of isolate**
**V. Paper-disc with crab extract**
**X. Paper-disc with sterilized aquadest**

Table 3. **Production of coconut oil**

| Inoculum | Oil volume [1)] |
|---|---|
| Control | 34.7 |
| Sterilized crab extract | 35.1 |
| Nonsterilized crab extract | 29.2 |

[1)] In ml/100g (DW) of grated coconut meat

Table 4. **Phisical and chemical analysis of coconut oil**

| Oil | Specific gravity | Moisture & volatile matter [1] | Iodine number [2] | Saponific number [3] | Peroxide Volue [3] | FFA [5] |
|---|---|---|---|---|---|---|
| Control | 0.925 | 0.25 | 8.62 | 102.5 | 0.26 | 0.59 |
| Sterilized crab extract | 0.920 | 0.56 | 8.00 | 252.97 | 0.57 | 1.12 |
| Nonsterilized crab extract | 0.922 | 0.32 | 7.87 | 253.50 | 6.01 | 1.08 |
| "Pristo" fried oil | 0.928 | 0.43 | 7.87 | 253.50 | 3.59 | 1.17 |
| IIS [6] | — | max. 0.500 | 8.00 — 10.0 | 255.0 — 265.0 | Max. 5.00 | 5.00 |
| AOCS [7] | 0.917 — 0.919 | — | 7.50 — 10.50 | 250.0 — 264.0 | max. 0.50 | — |

[1] in %
[2] in g Jod/100g sample
[3] in mg KOH/g sample
[4] in mg oxygen/g sample
[5] as lauric acid in %
[6] Indonesian Industrial Standard (1)
[7] American Oil Chemist's Society

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