Production of *Acetobacter aceti* Starter Powder by Low-Temperature Thermal Drying

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Abstract

Development of Acetobacter aceti TISTR102 starter powder by low-temperature thermal drying is investigated for vinegar production. The powder was prepared by mixing 4 ml of cell coated by 20% mannitol (w/v) with 10 g of rice bran as carrier and dried at 35oC for 12 h. The starter powder showed better residual viability (1.53x1014 CFU/g) when compared with freeze-dried cells (2.73x109 CFU/g). Ability of starter power in vinegar production from palm sap was acceptable as acetic acid content of about 3.99 g/100ml was obtained within 4 days of fermentation. The starter powder was stored at 4oC or room temperature for 1 month, in an aluminum foil bag or a linear low-density polyethylene (LLDPE) bag, with or without vacuum. The cell viability and fermentability remained unchange after storage for 1 week but decreased afterwards. It was found that the starter powder stored in aluminum foil bag, under vacuum for 1 month at 4oC gave higher viability than that stored at the room temperature.

Keywords: *Acetobacter aceti,* vinegar, starter powder, low-temperature thermal drying, storage

Introduction and Objective

Vinegar is produced in both the household level to industrial scale. Production at household level in traditional, it takes quite a long time to produce. The vinegar production by natural starter culture it is impossible to control the quality of vinegar. A pure culture has been popular in the industry but it is not suitable for production in the household because people lack the tools and knowledge to use a pure culture. The use of pure culture, it is easy to use and the quality of vinegar is stable. Nowadays, many methods for the production of starter powder such as freeze drying and spray drying (Ndoye *et al*., 2007) because it preserves the culture of the time and the ability to recover the fine. On the other hand, the starter culture resulted in decreased cell viability and starter culture efficiencies, due to the cell membranes of microorganisms have been damaged during the drying process and from the high-temperature drying. Drying by heating is not suitable for production of starter culture. However, at present the application of low-temperature thermal drying to produce the starter powder. This method is cheaper than freezing and spray drying. Also, the low-temperature thermal drying can reduce the loss of the cell viability. This method, it is one option in the application process for the production of starter powder.

Thus, this study aims to develop a culture of vinegar powder production process by low-temperature thermal drying for use in quality control of vinegar production.

Materials and Methods

2.1 Cell cultivation

The *Acetobacter aceti* TISTR102 was provided by Microbiological Resources Centre, Thailand Institute of Scientific and Technological Research. *A. aceti* TISTR102 was maintained on a GYE agar slant at 4°C and subculture at about 1 month interval (GYE agar contained glucose 100 g/l, yeast extract 10 g/l and agar 20 g/l). One loop of cells from the slant was inoculated into 150 ml GYE broth, incubated for 24 hrs at 30°C on a rotary shaker with agitation of 100 rpm and then employed as the preculture. Then, they were inoculated with 5% w/w the preculture into a 600 ml flask containing 400 ml of aseptic the palm sap was used as the raw material, incubated for 4 days at 30°C on a rotary shaker with agitation of 180 rpm. Cells were then harvested by centrifugation for 10 min at 9600g at 4°C, washed with a phosphate buffer followed by centrifugation.

2.2 The production of starter powder by low-temperature thermal drying.

The cell paste was resuspended into 20% w/w mannitol as protective agent and hold 1 hr at room temperature. Mixed 4 ml the suspension with 10 g of rice bran as carrier and drying at 35°C for 12 h.

2.3 The production of starter powder by freeze drying

The cell paste was resuspended into 20% w/w mannitol as protective agent and frozen overnight at -60°C before freeze-drying for 24 hrs.

2.4 Analytical procedure

To compare low-temperature thermally-dried *A. aceti* TISTR102 with wet cell and freeze-dried cell and with those low-themperature thermally-dried cells stored for 1 week and 1 month. The viable cells were enumerated by with using spread plate technique on GYE agar. The starter powders (1g) were rehydration in peptone water by mixing thoroughly with a vortex mixer for 10 min and spreaded onto the surface of GYE agar. The acetic acid content was determined by titration with 0.1N NaOH using phenolphthalein as indicator (AOAC., 2000). The starter powders (1g) were rehydration in peptone water by mixing thoroughly with a vortex mixer for 10 min and added 5% w/w the suspension into a 250 ml flask containing 150 ml of the palm sap wine (6% ethanol), incubated for 2-4 days at 30°C on a rotary shaker with agitation of 100 rpm.

Results and Discussion

3.1 Effect of the drying process

Low-temperature thermal drying and freeze drying were used to investigate the effect of dehydration on cell viability and stability. The use of 20% w/w mannitol as protective agent, added to the fresh paste before drying. The results are presented in table 1.

Table 1. Comparison of efficiency by wet, low-temperature thermally-dried and freeze-dried cells of A. aceti TISTR102.

Sample	Cell viability (CFU/g)	Acetic acid (g/100 ml)	
		2 days	4 days
Wet cells	$2.44x10^{14}$	0.45	4.40
Low-temperature thermally-dried cells	$1.53x10^{14}$	0.45	3.99
Freeze-dried cells	$2.73x10^{9}$	0.23	2.79

Low-temperature thermally-dried cells showed the cell viability $1.53x10^{14}$ CFU/g and the water activity (a_w) 0.408. While, freeze-dried cells showed the cell viability $2.73x10^{9}$ CFU/g and a_w 0.413. Both low-temperature thermally-dried cells and freeze-dried cells had the water activity is lower than 0.6, the amount of bacteria, yeast and mold can not grow (Zapsalis and Beck, 1985).

The result showed low-temperature thermally-dried and wet cells exhibited similar the cell viability and acetic acid content. While, freeze-dried cells is efficiency lower may be caused the cell viability in freeze-dried cells below due to dehydration, the cell viability were decreased to occur during the drying or freezing. when the ice crystals and the concentration of salt increased both inside and outside the cell can cause dehydration which the cell walls were destroyed.

3.2 Stability of low-temperature thermally-dried cells

Ten gram of starter powder *A. aceti* TISTR 102 was placed in a plastic bag or aluminum foil laminate pouch sealing by either vacuum or atmospheric condition (Fig. 1 and 2) and then stored at room temperature (30 ± 1°C) or 4°C for 1 month. The result showed that duration of storage a month to keep the culture of starter powder at room temperature conditions contained the cell viability at a room temperature below 4°C during storage. While packaged in aluminum foil laminate pouch and vacuum sealed plastic bag and store at 4 °C. The starter powder has the highest amount of the cell viability for 1 week of conservation. Vinegar fermentation for 4 days showed that starter

powder is stored at 4°C with acetic acid production was higher than storage at room temperature. In addition, the starter powder in a aluminium foil laminate pouch and sealed in a vacuum has the highest amount of the cell viability and acetic acid production.

Stability of starter powder stored at room temperature decreased quickly in 1 month of conservation, while stability of starter powder stored at 4°C decreased slightly. Storage temperature affect the survival of the samples. In addition, they were maintained in an oxygen atmosphere. Survival rates are low because the oxygen can penetrate into cells that react with cell dry during storage. Which is oxygen free radicals can accumulate, but can not be metabolizing light or be removed from the cells. The reaction is irreversible damage to cells .

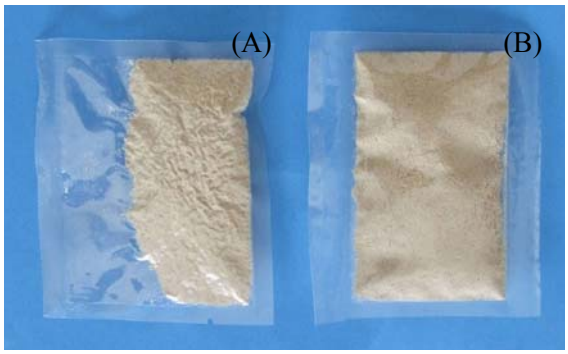

Figure 1. Starter powder of A. aceti TISTR 102 in a plastic bag, sealing the two conditions: vacuum (A) and atmosphere (B).

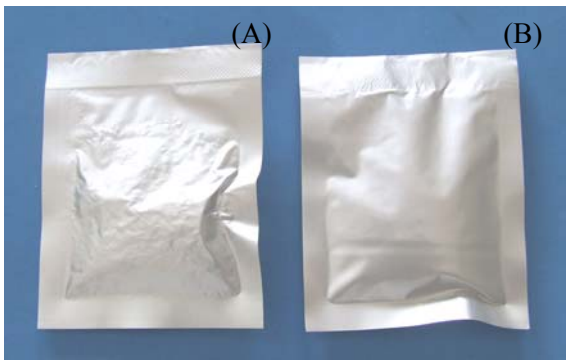

Figure 2. Starter powder of A. aceti TISTR 102 in a aluminum foil laminate pouch, sealing the two conditions: vacuum (A) and atmosphere (B).

Acknowledgements

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