JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov 2015. Vol. **9**(Spl. Edn. 2), p. 513-518

Microbial Population and Degradation on Rice Granules during Fermentation of *Bedak sejuk*

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(Received: 14 September 2015; accepted: 15 November 2015)

***Bedak sejuk* is a traditional fermented rice based cosmetic used by women in Malaysia. To date, the production of both homemade and commercial *bedak sejuk* depends on natural fermentation without using starter cultures. Here, we monitored the microbial population and evaluate the degradation of rice starch in the initial batch fermentation of *bedak sejuk*. *Bedak sejuk* sample was prepared by natural fermentation of local rice grains (5% broken). The microbial population changes and degradation of rice starch were analysed for two weeks. Within two weeks, the total microbial, anaerobic bacteria, yeast and mould count increased while coliforms count decreased. The total microbial, anaerobic bacteria, yeast, mould and coliform counts were 11.44±0.54, 6.14±0.27, 2.78±0.22, 1.31±0.19 and 1.24±0.13 log CFU/mL, respectively. The liquefaction activity of α-amylase was higher on the second day (42.3±0.6 μg/min/g) but after that it declined. Total carbohydrate decreased from 80.8 to 80.1 % while reducing sugar of the rice granules and in fermented supernatant increased from 0.02 to 0.15 % and 0.03 to 0.92 g/L, respectively. While the degree of hydrolysis shows an increase but the increment was low. From the scanning electron microscope pictures, the rice grains still retained their whole figures but the fermented starch lost their surface smoothness. Taken together, our results suggest that the changes of microbial population affected the rice starch during the natural fermentation.**

Key words: Lactic acid bacteria (LAB), Yeast, Natural Fermentation, Rice Starch, *Bedak sejuk*.

The traditional production of *bedak sejuk* is a somewhat unique fermentation process due to its repeating fermentation process, resulted longer fermentation time taken to complete the production. *Bedak sejuk* is a fermented rice-based cosmetic, normally in the form of water droplet pastilles. These pastilles are natural cosmetic product as they are produced only using rice grains and water. The rice grains will be soaked in tap water until they become white rice paste[1]. Literally, *bedak sejuk* means cool powder which implies when the pastilles are mixed with water and applied on face skin, it will give a cooling effect. Sawaki *et al.*[2] reported that the cosmetics produced by adding the LAB fermented rice have a good feeling when and after used; expansion and smoothness on applying and wet feeling after applying on skin. This implies why the older called these pastilles as cool powder.

The soaking water during the fermentation of *bedak sejuk* is changed intermittently because of its pungent smell. Battock & Azam-Ali[3] reported that during fermentation, spoilage organisms will utilize protein as an energy

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source producing unpleasant odour. This unpleasant odour normally resulted from a long fermentation time. As the traditional process of *bedak sejuk* production that being used is still a traditional method which passes from generation to generation, the changing of the soaking water is not based on scientific knowledge[4]. Therefore the soaking water is frequently changed, leading to a long overall soaking time to complete the production. This portrayed that the process is less effective, timely and costly.

Little information is available about the microbial composition during the soaking of rice grains which can show high variability depending on the rice grain types and fermentation conditions that are difficult to control even in the laboratory experiment. The nature of the natural fermentation with respect to the role played by the microorganisms and the soaking water conditions on the physicochemical changes and on the characteristics of the final product is not known. Therefore, the aim of this work was to study the microbial population dynamics during the initial fermentation and evaluate the degradation of rice starch.

Preparation of *Bedak sejuk*

The preparation of *bedak sejuk* was followed the traditional process of making *bedak sejuk*. The principal ingredients are 5% broken local polished rice grains (*Indica*) and tap water. Based on our previous study, the soaking or fermentation process will take place for 14 days. The conditions for the fermentation were; (1) polished rice grains were not cleaned or washed first, (2) the container used was not sterilized and (3) polished rice grains will ferment undisturbed at ambient temperature. 250 g of local polished rice grains will be soaked in tap water (w/v) and aliquots of the fermented supernatant were collected every days throughout the soaking. Sample of *bedak sejuk* (rice granules) were collected at the end of soaking process.

Microbial enumeration

The quantities of the dominant microbial were enumerated on the basis of colony-forming unis (CFU) in selective media. 1 mL of the fermented supernatant were homogenized in 9 mL sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, 1000 mL distilled water, pH 7.0 ± 0.2). Total anaerobic bacteria were enumerated using MRS agar (Merck) and incubated (in an anaerobic jar) at 37 °C for 48 h. Yeast and moulds were counted by surface plating on Dichloran Rose Bengal Chloramphenicol agar (DRBC) (Oxoid, Unipath, Basingstoke, UK) and incubated aerobically at 25 °C for 3-6 days. While Coliforms were plated on Petri-Film (3M, St. Paul, MN, USA) specific for coliform determinations and incubated at 37 °C.

All samples were measured in triplicate. The basic statistical method was used to calculate the means and standard deviation of the triplicate results of each sample.

Liquefying (α-amylase) activity

The activity of a-amylase was determined by slightly modifying the method described by Maity *et al*.[5]. Briefly, 0.1 mL of supernatant was incubated with 0.5 mL of soluble starch (1%, w/v), and 0.4 mL of buffer (0.1 M phosphate buffer for pH 7.0) and incubated at 40 °C for 10 min. The reaction was terminated by the addition of 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent and the liberated reducing sugars were estimated colorimetrically according to the method of Miller[6]. One unit of amylase activity was defined as the amount of enzyme releasing 1 µmol of reducing sugars (glucose equivalents) per minute at pH 7.0 at 40 °C.

Determination total carbohydrate, reducing sugar and degree of hydrolysis

For the rice granules sample, total carbohydrate was estimated by an ethanol extraction by the phenol-sulphuric acid method[7]. The ethanol extraction method was altered from the work done by Anthony & Chandra[8]; 10 g of milled rice granules were extracted with 5 ml of hot 80% ethanol twice, then centrifuged at 8000 x g for 10 min and the supernatant was collected. Total reducing sugar was estimated by the 3,5-dinitrosalicylic acid method[6] with the ethanol extraction prepared as above for rice granules while no extraction needed for fermented supernatant. Reducing sugar in fermented supernatant was calculated on the basis of total volume of soaking water.

The degree of hydrolysis was evaluated by monitoring the total carbohydrate of supernatant which measured by phenol sulphuric method[6]. Degree of hydrolysis was calculated using following equation:

$$\text{Degree of hydrolysis}(\%) = \frac{\text{Total carbohydrate of supernatant}(g)}{\text{Amount of starch}(g)} \times 100$$

Scanning electron microscopy (SEM)

The association of microbes onto the rice grains was examined under scanning electron microscope. *Bedak sejuk* was observed using a scanning electron microscope (High Resolution Fesem Supra 55VP), under 2-12 KX magnification and a constant acceleration voltage of 10 kV.

RESULTS AND DISCUSSION

Microbiological analysis

Figure 1 shows the distribution of microbial in the fermented supernatant during fermentation. The anaerobic bacteria counts increased from 3.02 to 6.14 log CFU/mL at the end of fermentation time. Yeasts count increased from 1.68 to 2.78 log CFU/mL, moulds count also increased from 0.73 to 1.31 log CFU/mL while coliforms count decreased from 3.88 to 1.21 log CFU/mL. A high microbial load was observed in the fermented supernatant as high initial counts of 9.31 log CFU/mL was recorded. This initial count could be attributed to the raw material.

The total microbial enumeration showed almost the same pattern of a growth profile of batch process. These pattern was observed too during the previous study[4] but it was a growth profile of cell density of the supernatant. This shows that 14 days of fermentation days are the best fermentation as the growth cycle of the microbial in the soaking water is almost completed.

From the microbial count, it can be suggested that the initial stages of fermentation is dominated by anaerobic bacteria and coliforms but in the later stages by anaerobic bacteria and yeasts. Nche *et al.*,[9] stated that yeasts provide growth factors like vitamin amino acids for bacteria while bacteria will create an acidic environment which is a conducive to the yeast growth. Initially LAB will dominated the fermentation[10] due to their higher growth rate followed later by yeasts in substrates that rich in fermentable sugars.

Yeast secreted different hydrolytic enzymes such as α-amylase, β-amylase, glucoamylase and proteases[11,12] that facilitated a rapid degradation of available starch of the rice granules. It has been reported that the bacterial presence during rice fermentation was dominated by LAB that can create conditions that inhibited growth of the pathogen[13]. This is probably why the coliform count decrease during the soaking process.

Table 1. Chemical components of rice grains and fermented supernatants of *bedak sejuk*

Soaking	Polished rice	After soaking
Fermentation time (day)	0	14
Rice grains		
Total carbohydrate (%)	80.8±0.17	80.1±0.17
Reducing sugar (%)	0.02±0.13	0.15±0.17
Reducing sugar of fermented supernatant (mg/mL)	0.03±0.14	0.92±0.11

Results given as means and standard deviation of triplicate samples

α-Amylase activity

The liquefaction activity of α-amylase was higher on the second day (42.3±0.6 µg/min/g) but after that it declined. This hydrolytic enzyme were produced by yeast and mould during the fermentation. α-amylase will contribute to the saccharification and liquefaction of the rice. Normally, starch was first degrade into limit dextrins by α-amylase whereas glucose was specifically produced from dextrins by glucoamylase[14].

Variation of total carbohydrates, reducing sugar during fermentation and degree of hydrolysis

The total carbohydrate decreased while reducing sugars from the rice granules samples and reducing sugars in the fermented supernatant increased throughout the soaking process (Table 1). Total carbohydrate percentage in polished rice grains slightly decreased after the soaking from 80.8 to 80.1 %. The reducing sugar of the polished rice grains was increased rapidly after the first fermentation, from 0.02 to 0.15 %. The reducing sugar in the fermented supernatant also shows the same trend as it increased rapidly from 0.03 to 0.92 mg/mL at the end of soaking process.

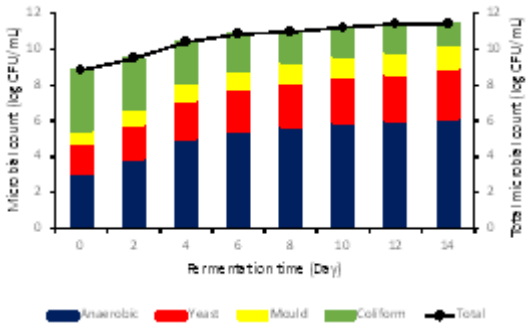

Fig. 1. The distribution of microbial in the fermented supernatant during fermentation

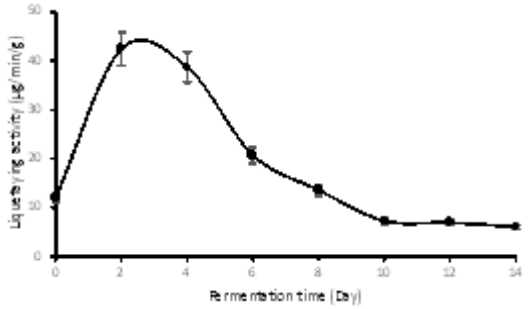

Fig. 2. Changes of liquefying activity during *bedak sejuk* production

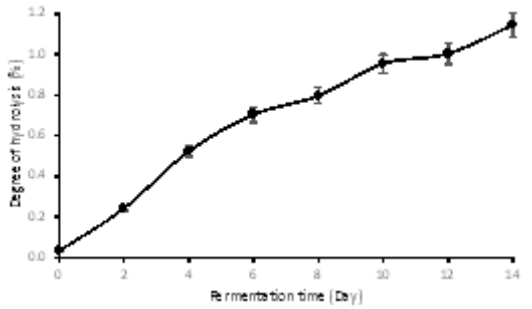

Fig. 3. Degree of hydrolysis of rice during the *bedak sejuk* production

The polished rice grains were soaked without washing in this work. Thus, carbon source from polishing step is available for the microorganisms. This supported by Lu *et al*.[15] which reported that rich amount of damaged starch that occurred during the polishing process of the rice served as the carbon source for the LAB or yeast at the first stage. But in this work, the total carbohydrate was found slightly decreased during the fermentation which suggests that the damaged starch was first used as the carbon source instead of the reducing sugars. This results are not in agreement to those obtained by other cereals fermentation[16,17].

Fig. 3 shows the degree of hydrolysis. The degree of hydrolysis shows an increase but the increment was low. The degree of hydrolysis at the end of the soaking was only 1.14±1.1%. This suggested that the degradation rate of rice are slow. 0.92±0.11 mg/mL of reducing sugar was produced in supernatant after fermentation. Even though the amount of the reducing sugar was not high but this suggests that the rice starch was degraded during the fermentation. But the degradation is somewhat a partial digestion at a slow degree of hydrolysis.

From all those results, generally, these decreases in the starch content with increased fermentation time are due to the breakdown of starch molecules into sugars by microorganisms during the fermentation process. It is suggests that starch in the rice is hydrolysed into simple sugars then these sugars are metabolised by microorganisms to organic acids or other metabolites. After 14 days of soaking, it is observed that the rice grains was degrading but they were still in granules form but a bit smaller than their original size. This shows that the rice starch underwent somewhat degradation either because of the hydrolysis or acidification.

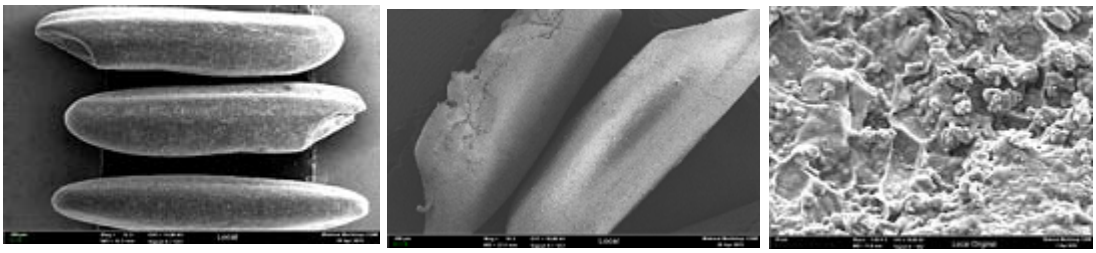

Fig. 4. Scanning electron micrographs of unfermented rice grains (a), natural fermented rice grains (b) and association of microbes with rice grains at the end of fermentation (c)

Scanning electron microscopy (SEM)

The granular structure of native and natural fermentation of rice starches exhibited significant variations in their shape when viewed with scanning electron microscopy. Representative scanning electron micrographs of the residual starch granules are provided in Figure 4. The rice grains still retained their whole figures but their surface changed (Fig. 4a). Typically, the fermented starch lost their surface smoothness (Fig. 4b). SEM analysis also revealed a layer of microbes on the rice grain at the end of fermentation, they formed a thick biofilm consisting a rod shaped bacteria (Fig. 4c).

CONCLUSION

The results presented in this study have been intended as a contribution to understanding of the basis of the natural fermentation process underlying the production of *bedak sejuk*, a traditional Malaysia cosmetic product. It also has given an insight into the role of microorganisms within the natural fermentation process. During the initial stage of fermentation, anaerobic bacteria provide a favourable environment for the later stage of fermentations by yeasts. Natural fermentation on polished rice grains allowing the growth of anaerobic bacteria, yeasts and moulds while decreased of coliform. The increasing of microbial population can alter the structure of rice starch. From the degree of hydrolysis analysis, the degradation occurred is slow but the degradation managed to alter the surface of the rice grains. However, further studies have to be performed to understand the microbial population dynamics and its relation to the final product.

ACKNOWLEDGEMENTS

The authors thank to the Universiti Kebangsaan Malaysia for Dana Lonjakan Penerbitan (DLP-2012-022) for financial support.

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