Influences of Barks Addition on the Growth of Microorganisms and the Ingredients of Basi in Basi Fermentation

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Using a mixture of samac bark and fruit in equal quantity (SBF), which is used in the sugarcane wine—"basi" brewing in the Pangasinan of Philippines, we have prepared basi with and without the addition of barks, and have studied the impact of barks addition on the growth of microorganisms and the ingredients of basi. No impact of SBF addition was seen on the growth of yeasts like *Saccharomyces cerevisiae*, and no difference was found in the alcohol yields between basi with and without SBF addition. The growth of lactic acid bacteria was completely inhibited by SBF, indicating that the growth of lactic acid bacteria—the cause of souring—can be suppressed by the addition of barks in basi fermentation. Besides, the basi after fermentation with the addition of SBF had a strong astringency resulted from the polyphenol components dissolved from SBF. Though basi with SBF addition had a sugar concentration as high as 11.2%, as its sweetness had been moderated by the astringency of polyphenol, it had a mild taste, showing an important significance of barks addition to confer astringency in basi fermentation.

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There is a sugarcane wine called "basi" in the northwest of Luzon in the Philippines from old times¹⁾. Basi is a fermented liquor and has a strong sweetness and astringency. The preparation method of basi is handed down from generation to generation among the people and some barks are added to the sugarcane juice before fermentation. Usually, the barks used in basi preparation are bark, fruit and leaf of samac (*Macharanga grandifolia* Linn.) and bark of duhat (*Syzgium cumini* Skeels).

We have previously investigated the influence of various aqueous extracts of barks added to basi on the growths of lactic acid bacteria, acetic acid bacteria and yeasts, and have found that most of the aqueous extracts intensely inhibit the growths of lactic acid bacteria and acetic acid bacteria, which are the cause of souring during basi fermentation, suggesting that barks addition brings about an opportune control of microorganisms in basi fermentation²⁾. Moreover, we have studied substances inhibiting the growth of lactic acid bacteria in the barks added to basi and have

identified that, the major inhibitory substance is a high molecular weight condensed tannin consisting of catechin and leucoanthocyanin in samac bark, a gallotannin with multiple galloyl radicals and *m*-digalloyl radicals bound to the glucose or xylose of sugar in samac fruit, and a high molecular weight condensed tannin consisting of gallic acid and leucoanthocyanin in duhat bark^{3)~6)}.

In this study, together with the elucidation on basi ingredients by analyzing the basi produced in the Philippines, we have performed the fermentation of basi with and without the addition of barks, compared the transition of microorganisms and the change of ingredients during the fermentation, and investigated the impact of barks addition on the growth of microorganisms and the ingredients of basi.

Materials and Methods

1. Materials

Basi produced in the La Union of Philippines were used as materials to analyze the ingredients.

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Fig.1 Procedures for the fermentation of basi

The bubod made in the La Union was used as starter in basi fermentation, and air-dry samac bark and fruit used in the Pangasinan¹⁾ were used as barks to add to basi.

2. Basi fermentation

The fermentation of basi with and without the addition of barks was carried out according to the procedures shown in Fig.1. To 650ml water, 350 g brown sugar was added and dissolved by heating, then the solution was divided into 400ml each in two Erlenmeyer flasks of 1,000ml volume and sterilized at 120°C for 10 min. After sterilization, to one flask 4 g crushed bubod alone was added and to the other both 4 g crushed bubod and 10 g mixture of samac bark and fruit in equal quantity (SBF) were added, and both were fermented at 25 °C for 12 days.

3. Ingredient analysis

Alcohol was determined by hydrometer method⁷⁾, total sugar by Somogyi-Nelson method⁸⁾ after hydrolysis of sucrose, total acid by alkaline titration method⁹⁾, and polyphenol by Folin-Denis method¹⁰⁾. The hydrolysis of sucrose was carried out at 65°C for 15 min after adding 5ml of 25% HCl to 50ml of material solution. Total sugar was calculated as glucose, total acid as succinic acid, and polyphenol as D-(+)-catechin.

Organic acids were determined individually using an automatic carboxylic acid analyzer (S-500; Seishin Pharmacy, Ltd., Tokyo). Protein has to be

removed beforehand from the material solution for the automatic carboxylic acid analyzer and this was done by adding 2ml 10% HClO₄ to 8ml of the material solution, refrigerating overnight, and removing the protein precipitate by centrifugation¹¹⁾.

4. Measurement of viable cell count

Viable cell count was measured by plate culture method¹²⁾. Yeasts and molds were cultured on YM agar media (yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, glucose 1.0%, agar 1.5%, pH 5.8) at 28°C for 23 days, and their viable cell counts were calculated respectively. For yeasts, those with filamentous rim in a colony were calculated as R yeast and others as S yeast. Lactic acid bacteria were cultured on GYP agar media containing CaCO₃ (glucose 1.0%, yeast extract 0.5%, peptone 0.5%, CaCO₃ 0.5%, agar 1.5%, pH 6.8) at 37°C for 23 days and the viable cell count was calculated as colonies with a clear zone due to the elution of CaCO₃ from the media.

Results

1. Basi ingredients

The ingredients of basi produced in the Philippines wine were shown in Table 1 and compared with them of red wine¹³⁾ which contains polyphenol of grape seeds. The alcohol content of basi was similar to that of the red wine. The total sugar content of basi was as high as that of Edufuaule wine having high sugar content. The

Table 1 Comparison of ingredients between basi and red wine

Wine	Alcohol (ml/100ml)	Total sugar (g/100ml)	Total acid (mg/100ml)	Polyphenol (mg/100ml)	pH
Basi	12.5	12.20	283	285	4.21
Red wine*	11.8	0.15	620	132	3.50

* These values were quoted from 'Constituents in Fermented Foods' (1999)¹³⁾

Table 2 Viable cell counts in bubod

Microorganism	Viable cell count (CFU/ g)
Mold	7.0×10^5
Yeast	
R yeast*	3.8×10^8
S yeast**	6.6×10^7
Lactic acid bacteria	8.3×10^5

*Colonies with filamentous rim were counted as R yeast.

**Colonies with smooth surface were counted as S yeast.

total acid content of basi was lower than that of the red wine. The polyphenol content of basi was higher than that of the red wine and the sweetness of sugar was moderated by the strong astringency of polyphenol.

2. Influence of SBF addition on the growth of microorganisms in basi fermentation

The microorganisms included in the bubod used as a starter in basi fermentation were yeasts, molds and lactic acid bacteria, as shown in Table 2. In the bubod, the number of yeasts with filamentous rim in a colony (R yeasts) were 3.8×10^8 CFU/ g and the number of other yeasts (S yeasts) were 6.6×10^7 CFU/ g. The main R yeast was identified *Sacchromycopsis fibuligera* and the main S yeast was identified *Saccharomyces cerevisiae*. And the main mold was *Rhizopus* and the main lactic acid

bacterium was *Pediococcus*^{(14), (15)}

Using the bubod as a starter, we have carried out fermentation of basi with and without SBF addition, and have compared the transition of microorganisms in both during the fermentation process. The results were showed in Fig.2. No significant difference in the growth of S yeasts with *S. cerevisiae* was seen between basi with SBF and that without SBF, regardless of a slight growth inhibition in the early stage of fermentation in basi with SBF. Nearly no growth of R yeasts with *S. fibuligera* was seen in either basi with SBF or that without SBF, and all died out on the 4th day of fermentation when the growth of S yeasts became significant. In lactic acid bacteria, significant growth was seen in the early stage of fermentation in basi without SBF, while no growth at all was observed

Fig. 2 Changes of the viable cell counts of yeasts and lactic acid bacteria during the fermentation of basi with and without the addition of samac bark and fruit

The fermentation of basi was carried out according to the procedures shown in Fig. 1.

○ : S yeasts in basi without SBF, ● : S yeasts in basi with SBF,
△ : R yeasts in basi without SBF, ▲ : R yeasts in basi with SBF,
□ : lactic acid bacteria in basi without SBF.

Fig. 3 Changes of the ingredients during the fermentation of basi with and without the addition of samac bark and fruit

The fermentation of basi was carried out according to the procedures shown in Fig. 1.

○ : total sugar in basi without SBF, ● : total sugar in basi with SBF,
△ : alcohol in basi without SBF, ▲ : alcohol in basi with SBF,
□ : total acid in basi without SBF, ■ : total acid in basi with SBF.

Table 3 Ingredients of basi with and without the addition of samac bark and fruit

Basi	Alcohol (mℓ/100mℓ)	Total sugar (g/100mℓ)	Total acid (mg/100mℓ)	Polyphenol (mg/100mℓ)	pH
Without SBF	12.3	13.5	385	28	3.90
With SBF	12.7	11.2	388	144	4.02

The fermentation of basi was carried out according to the procedures shown in Fig.1.

Table 4 Organic acids in basi with and without the addition of samac bark and fruit(mg/100mℓ)

Organic acid	Basi	
	Without SBF	With SBF
Formic acid	7.6	8.1
Acetic acid	135.2	180.1
Succinic acid	76.0	79.0
Lactic acid	150.8	57.4
Malic acid	13.9	40.5
Citric acid	18.0	22.9
Pyruvic acid	18.9	27.7
α -Ketoglutaric acid	7.5	4.5

The fermentation of basi was carried out according to the procedures shown in Fig.1.

in basi with SBF. This result indicated that the growth of lactic acid bacteria was completely inhibited by the addition of SBF. On the other hand, no growth of molds was found in either basi with or without SBF.

3. Influence of SBF addition on the basi ingredients in basi fermentation

The change of ingredients during fermentation process had been compared between basi with and without SBF, and the result was shown in Fig.3. The production of alcohol was late in basi with SBF in the early days and this is consistent with the result that the growth of S yeasts is late in this basi compared to that without SBF. Nevertheless, no difference was seen in the alcohol yield between two types of basi from the 6th day of fermentation. Similarly, the production of acids was also late in basi with SBF in the early days, but the acid yield was the same in two types of basi from the 8th day of fermentation.

Table 3 showed the result of the comparison of ingredients in two types of basi after 12 days of fermentation. The alcohol content was 12.3mℓ/100mℓ in basi without SBF and 12.7mℓ/100mℓ in basi with SBF, being a little higher in the latter. Polyphenol was the ingredient having significantly different contents between basi with SBF and that without SBF; its content was 28mg/100mℓ in basi without SBF and 144mg/100mℓ, about 5-fold higher in basi

with SBF. In addition, organic acids have been compared on the 12th day of fermentation and the result was shown in Table 4. There was no difference in the total acid content between the two types of basi, but difference was seen in individual organic acid. In basi without SBF, lactic acid and acetic acid were numerous. In basi with SBF, acetic acid was numerous but lactic acid was less.

Discussion

The preparation method of basi is different from area to area¹⁾. In some areas, starter is used for fermentation while in other areas, fermentation is performed naturally without starter. In areas where starter is used, bubod is usually used as a starter. Bubod is made according to the following process: To some smashed rice and ginger, water is added and the mixture is kneaded; next some crushed old bubod is added and the mixture is made into a disc; then the disc is left indoors for several days to let microorganisms grow and is finally air-dried completely in the open. In the Philippines, bubod is used as a starter mainly in tapuy, a rice wine¹⁶⁾. In this study, using the bubod as a starter, we have carried out fermentation of basi with and without the addition of SBF, and have compared the transition of microorganisms and the change of ingredients in the two basi. In the fermentation of basi with SBF, SBF was added in 2.5% to the

sugar solution based on the preparation method in the Pangasinan.

In both basi with SBF and that without SBF, significant growth of S yeasts with *S. cerevisiae* which performs alcohol fermentation being the majority is seen from the beginning of fermentation, and no influence of SBF addition is found on the growth of S yeasts. R yeasts with *S. fibuligera* being the majority die out from the 4th day of fermentation when the growth of S yeasts becomes significantly in both basi with and without SBF, and this is thought to be resulted from the alcohol produced by *S. cerevisiae*. In contrast to the significant growth in the early stage of fermentation in basi without SBF, the growth of lactic acid bacteria is not seen at all in basi with SBF, showing a complete inhibition of growth of lactic acid bacteria by SBF addition. In the previous paper²⁾, we have reported that most of the aqueous extracts of barks added to basi inhibit the growth of lactic acid bacteria. In this study, we have confirmed that the growth of lactic acid bacteria—the cause of souring—is inhibited by the addition of barks in basi fermentation. Besides, NISHIYAMA, *et al.*¹⁷⁾ reported that souring to occur by acetic acid bacteria and lactic acid bacteria is prevented by the addition of mangrove bark in the fermentation of tuba, a nipa sap wine in Philippines.

In basi with SBF, the growth of S yeasts is a little late and as a result the production of alcohol is also slightly late but on the 12th day of fermentation, no difference in the alcohol yield is found between basi with SBF and that without SBF. On the 12th day of fermentation, the total acid contents are the same in the two basi, but the lactic acid contents are greatly different, being 150.8mg/100ml in basi without SBF and 57.4mg/100ml in basi with SBF. Since no growth of lactic acid bacteria is found in basi with SBF, the low lactic acid content in this basi is thought to be due to the lack of growth of lactic acid bacteria. However, basi with SBF includes lots of acetic acid and the total acid content is the same as that of basi without SBF. This result suggests that acid production of yeasts is encouraged by addition of SBF. Besides, in basi with SBF, the polyphenol content reaches to 144mg/100ml on the 12th day of fermentation, resulting in a strong astringency in the basi. In basi without SBF, the sugar content is 13.5 g/100ml on the 12th day of fermentation and

the sweetness is too strong to drink as an alcoholic beverage. Whereas in basi with SBF, regardless of its 11.2 g/100ml sugar, it has a mild taste since its sweetness has been moderated by the strong astringency of polyphenol. Therefore the conferring of astringency by adding barks is an important significance for basi and that is similar to the conferring of astringency on red wine by adding grape seeds.

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バシ発酵における樹皮類添加の微生物の生育 ならびにバシ成分への影響

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フィリピンではバシ（甘蔗酒）の発酵の際に樹皮類が添加されることから、発酵過程における樹皮類添加の微生物の生育ならびにバシ成分への影響について検討した。酵母の生育に対しては樹皮類添加の影響はほとんどみられず、発酵後のアルコール生成量に影響はみられなかった。しかし、乳酸菌は樹皮類の添加により完全に生育を抑制され、バシの発酵では樹皮類の添加によって酸敗の原因となる乳酸菌の生育が抑えられることが確認された。また、樹皮類を添加することによりそれらのポリフェノール成分が溶出して渋味を呈し、原料糖の甘味が緩和されて飲みやすい味となったことから、バシの製造では渋味の付与も樹皮類添加の意義の一つであると考えられた。

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