Effect of Loog-pang sources on Job's tears sato production

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Abstract

Sato is an alcoholic beverage produced from the fermentation of rice using Chinese yeast cake or "Loog-pang" in Thai term. Some regions produce Sato using other grains such as sorghum, maize and purple rice. This research aims to compare the effects of Loog-pang on the quality of Job's tears Sato. Therefore, six different sources of Loog-pang were used in this study, namely, Vientiane (VTE), Savannakhet (SVK) and Pakse (PKS), which were obtained from Lao P.D.R., the Loog-pang powder was obtained from Vietnam (VTN) and Loog-pang from Muang district, Phayao (PYT) and Mae Suai district, Chiang Rai (CRT), Thailand. The microbial communities of the Loog-pang were yeasts (6.15-8.99 log cfu/g) and molds (5.07-6.47 log cfu/g), while lactic acid bacteria, which cause sour taste in Sato, were found in CRT, PYT and PKS in the range of 3.85-6.87 log cfu/g. The Job's tears grain was obtained from Lao P.D.R., and the chemical compositions were; moisture content of 10.04±0.12%, crude protein of 12.74±0.14%, ash content of 1.48±0.10%, crude fat of 4.60±0.21, carbohydrates of 81.18±0.30% and starch content of 63.75±1.76%, respectively. The Job's tears Sato production process combined of 2 steps. The first step was the preparation of Koji, which was started by cleaning Job's tears grain with tap water, soaking for 12 h, steaming for 1 h, air cooling and degumming by washing. Then, it was mixed with each Loog-pang at 0.2% (w/w), filled into a container and incubated at ambient temperature (30-35°C) for 5 days. The second step was mashing, performed by adding drinking water at the ratio of 2:1 (w/w) and incubated at ambient temperature for 14 days. The samples were periodically taken every 2 days for chemical analysis. The results showed that the first day of fermentation after mashing, the level of reducing sugar in the VTN batch of Sato was higher than the other sources. The maximum alcohol content of all samples was obtained at the 10th day of fermentation. Furthermore, the Loog-pang from VTN showed the alcohol content of 10.10% (v/v), which was higher than the others (8.75-9.50%), with the total acidity (TA) of 4.37 g/L (as lactic acid), the total soluble solid (TSS) of 4.30, a residual reducing sugar of 1.37 mg/mL and pH of 3.30, respectively.

Keywords: Job's tears, Sato, Loog-pang, Alcoholic beverages

1. Introduction

Like other traditional fermented alcoholic beverages in the East-Asian countries (1), rice wines (Sato) are popular in Thailand and Lao P.D.R. It is a source of income for farming families in rural areas. In Thailand and Lao P.D.R, Sato means wine, specifically, a traditional fermented Thai rice wine, which contains a level of ethanol not higher than 15% by volume (2).

The preparation of Sato is normally carried out by mixing steamed glutinous rice with Chinese yeast cakes, or "Loog-pang" in Thai term, which leads to variation in the

quality and stability of the rice wine products. In the preparation of Loog-pang, rice is ground and thoroughly mixed with certain spices, such as garlic and pepper (3), depending on the location of production. Loog-pang, commonly known as "Chinese yeast cake" among Western people, in Thai term it is known as a "fermentation starter", which is used for the production of traditional fermented products from starchy raw materials, such as Kao-mag (alcoholic sweetened rice), Sato (rice wine) and Num Som Sai Chu (vinegar). These types of fermentation starter have been used in many Asian countries with various local names, such as Banh Men in Vietnam, Bubod in the Philippines, Chu in China, Koji in Japan, Murcha in India and Nuruk in Korea (3, 4).

The making of Sato can be characterized as the conversion of rice (*Oryza sativa* L.) by physical, microbiological and biochemical operations, including steaming, inoculation with a starter, mashing and fermentation. The alcohol content of Sato or rice wine varies and can reach up to 15 mL/100 mL (about 12 g/100 mL). By distillation, the products with approximately 50 mL/100 mL (about 40 g/100 mL) alcohol can be obtained (5).

Job's-tears, or, "Loog-deuay" in Thai terms (*Coix lacryma-jobi*), coixseed, adlay, or adlai, is a tall grain-bearing tropical plant of the family Poaceae, or grass family (6). Like other cereals, there are many cultivars of Job's tears, including soft-shelled and easily-threshed types with a sweet kernel. In some, the hulled grain is adapted for parching or boiling like rice, while in others it can be milled, ground into flour and baked into bread. A hundred grams of Job's-tears grain contains about 380 kcal, 11.2 g water, 15.4 g protein, 6.2 g fat, 65.3 g total carbohydrates, 0.8 g fiber, 1.9 g ash, and 50-60% starch (7, 8). The grains are utilized in soups, porridge, drinks and pastries. In India, the Nagas used the grain for brewing a beer called "Zhu" or "Dzu". A Japanese Job's tears variety called "Ma-Yuen" is with an auto fat extraction system (2050 Soxtec, Foss Tecator, Skåne län, Sweden). The brewed into a tea and an alcoholic beverage, and roasted seeds were made into a coffee-like drink (8, 9). In Thailand, Job's tears are used as an ingredient in several food recipes and are consumed by people. Two commercial drinking products containing Job's tears, Pro-Fit and P-Life are now available but not popular (8). In Laos P.D.R., Job's tears grains and their products can be an important export good, even with the current low price. Currently, the domestic processing of Job's tears is relatively small. Thus, this research aimed to investigate the use of Job's tears grains for Sato production. The effect of Loog-pang sources on the quality of Job's tears Sato, were also investigated.

2. Materials and Methods

2.1 Preparation of raw materials

Job's tears grains were purchased from the Pakxeuang region, Luang Prabang province, Lao P.D.R. They were peeled of husk and bran and stored at 20°C until used. The selected Loog-pang obtained from 6 sources, 3 sources were from Lao P.D.R (Vientiane capital (VTE), Savannakhet province (SVK) and Pakse district Champasack province (PKS)), 2 sources from Muang district, Phayao (PYT) and Mae Suia district, Chiang Rai (CRT), Thailand, and another from Vietnam (VTN). All of the Loog-pang samples were stored in a refrigerator at 4°C until used.

2.2 Physical-chemical analysis of Job's tears grain

The individual grain sizes and cumulative weight of 1,000 grains were measured by AACC (10) and the color was measured by AOAC (11). The chemical compositions, such as moisture, protein and ash contents were determined according to the AOAC (11). The conversion factor of protein was 5.7. Lipid content was analyzed

starch content was determined by the phenol-sulfuric method (12). All determinations were conducted in triplicate.

2.3 Microbiological community of Loog-pang

The microbial communities were determined by spread plate technique, molds were selected in PDA, yeasts were selected in YM agar, lactic acid bacteria were selected in the MRS agar and acetic acid bacteria were selected in the acetic acid bacteria medium agar (11). Colonies were counted after incubation 35°C for 2 days. All determinations were conducted in triplicate.

2.4 Sato making

Job's tears grains were soaked for 12 h before steaming at 100°C for 1 h and air cooling. The grains were then divided into 500 g and degumming by washing thoroughly with drinking water. Then, they were mixed with 0.2% (w/w) of each Look-pang. They then were contained in a 2 liter flask and stored at ambient temperature (30-35°C) for 5 days. After that was the mashing step, which was performed by adding drinking water two parts per one part of Job's tears (2:1, w/w). At this point they were left to ferment ambient temperature for 14 days. The experiments were carried out for all 6 sources of Loog-pang and made in three replicates.

The data sets were collected every 2 days after mashing step for 14 days. Total soluble solid (TSS), alcohol content, pH, total acidity (TA) and residual reducing sugar were measured. The pH of all samples, were determined by using a pH meter (Denver, USA). Acidity was expressed in terms of g/L as lactic acid (11). Total soluble solid was measured by hand refractometer. Alcohol was determined by Ebulliometer (13), and reducing sugar was determined by DNS method (14).

2.5 Data analysis

All experiments were conducted in replicate and the results were expressed as the mean ± the standard deviation. The SPSS statistical program was used to analyze data. The values that had no common superscript were significantly different ($P<0.05$) according to Duncan's multiple range test.

3. Results and Discussion

3.1 The physical and chemical properties of Job's tears

The shape of Job's tears grains was oval and quite wide (Table 1). The cross thick and longitudinal sections were 6.51±0.56, 4.50±0.35 and 6.26±0.54 mm respectively. The weight of 1,000 was 126.5±5.3 g. The chemical composition of whole grain Job's tears is presented in Table 2. The moisture content (MC) was 10.04±0.12%, while crude protein (CP), crude fat, ash content, carbohydrates and starch content (dry basis) were, 12.74±0.14, 4.60±0.21, 1.48±0.10, 81.18±0.30 and 63.75±1.76%, respectively. These results were similar to the Job's tears from Thailand, Vietnam, and Taiwan (15, 16).

3.2 The microbial community of Loog-pang

The number of microorganism communities in each of the Loog-pang source were identified in Table 3 ($P<0.05$), VTN was high in yeast and mold, with no finding of lactic acid bacteria (LAB). The same results were found in VTE and SVK sources. In contrast, LAB was found in CRT, PYT and PKS sources. These results were similar to the reports of Chaijamrus and Mouthung (17) that the Loog-pang from north-eastern region of Thailand had a microbial community dominated by molds and yeasts at cell densities of 6.58 and 6.71 log cfu/g, respectively. In Loog-pang, there were various species of yeasts, molds and acid

Table 1 The Characteristics of Job's tears grains

Physical properties	Value	Color	Value
Weight in 1,000 grains (g)	126.5±5.3	L*	0.08±0.01
Length (mm)	6.26±0.54	a*	0.14±0.02
Width (mm)	6.51±0.56	b*	0.09±0.02
Thickness (mm)	4.50±0.35		

Table 2 The chemical composition of Job's tears grains

Chemical compositions	Wet basis (%)	Dry basis (%)
Moisture content (MC)	10.04±0.12	-
Crude protein (CP)	11.46±0.11	12.74±0.14
Fat	4.14±0.18	4.60±0.21
Ash	1.34±0.09	1.48±0.10
Carbohydrates	73.12±0.36	81.18±0.30
Starch	57.35±1.58	63.75±1.76

producing bacteria. *Saccharomycopsis fibuligera* was found to be the principle yeast species, and it is the main amylase producer in the Loog-pang. Other amylase producers in the Loog-pang were *Rhizopus* sp. and *Amylomyces* sp. (18). However, Mucoraceae, amylolytic molds involved in traditional alcohol fermentations were predominant in Loog-pang (7, 18, 19), Ragi tapé and Sake Koji (20), but not found in Banh men (17, 21).

In addition, the species of acetic acid bacteria can grow in an oxygenated environment (17). Although not verified empirically, the role of LAB (*Pediococcus pentosaceus*) in traditional alcohol fermentation starter is often thought to be an acidification that favors the growth of yeasts and molds while suppressing the growth of food spoiling bacteria (17, 18).

3.3 The fermentation of Sato

The alcohol content of all samples was quite steep during the first 2 days, with the PYT source showing a higher rate of alcohol production compared with the other samples. Then, the alcohol content of all samples was slowly increased with a comparable rate until the end of fermentation (14 days).

Table 3 The microbiology communities in each Loog-pang sources

Loog-pang sources	Number of microorganisms (log cfu/g)			
	Yeasts	Molds	LAB	AAB
VTE	7.70 ± 0.05^{c}	6.34 ± 0.04^{a}	$\_^{nd}$	$\_^{nd}$
SVK	8.32 ± 0.04^{b}	5.90 ± 0.09^{b}	$\_^{nd}$	$\_^{nd}$
PKS	6.15 ± 0.09^{d}	5.07 ± 0.13^{c}	3.85 ± 0.10^{c}	$\_^{nd}$
VTN	8.99 ± 0.05^{a}	6.36 ± 0.09^{a}	$\_^{nd}$	$\_^{nd}$
PYT	8.14 ± 0.23^{b}	5.95 ± 0.10^{b}	6.38 ± 0.15^{b}	$\_^{nd}$
CRT	8.15 ± 0.13^{b}	6.47 ± 0.06^{a}	6.87 ± 0.08^{a}	$\_^{nd}$

VTE: Vientiane; SVK: Savannakhet; PKS: Pakse; VTN: Vietnam; PYT: Phayao; CRT: Chiang Rai; LAB: lactic acid bacteria; AAB: acetic lactic acid bacteria; $\_^{nd}$: not detected

$^{a, b, c,}$ Different letters within the same column indicate a significant difference ($P<0.05$)

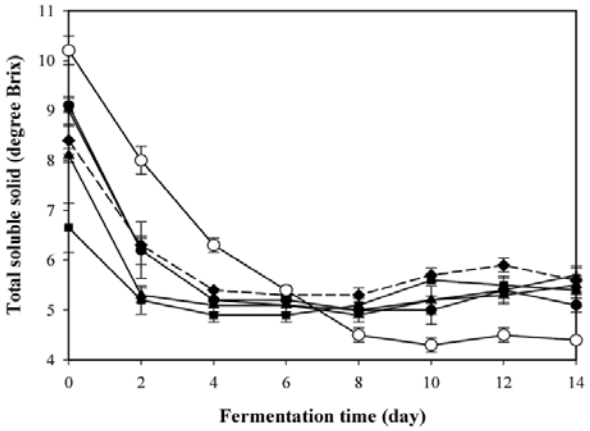

Figure 1 Changes of total soluble solid during the Sato fermentation period; VTE (●), SVK (■), PKS (◆), VTN (○), PYT (▲), CRT (✚)

From Figure 1, on the first day after mashing, the total soluble solid in VTN was higher than the others and remained constant on the 10th day, corresponding with the other sources on reducing sugar content and alcoholic production (Figure 2 and 3).

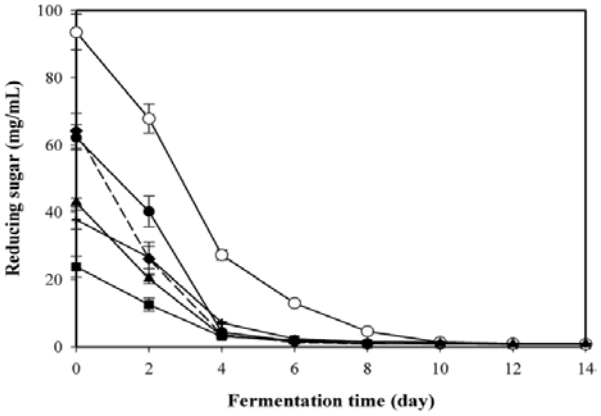

Figure 2 Changes of reducing sugar during the Sato fermentation period; VTE (●), SVK (■), PKS (◆), VTN (○), PYT (▲), CRT (✚)

The reducing sugar concentration of VTN on the first day was the highest (93.56±5.29 mg/mL) while other Loog-pang sources were 24-64 mg/mL. On the 10th day, the alcohol concentration level of VTN, PYT, PKS, SVK, VTE and CRT were 10.10±0.28, 9.50±0.14, 9.20±0.14, 9.00±0.14, 8.85±0.35 and 8.75±0.21%v/v, respectively ($P<0.05$).

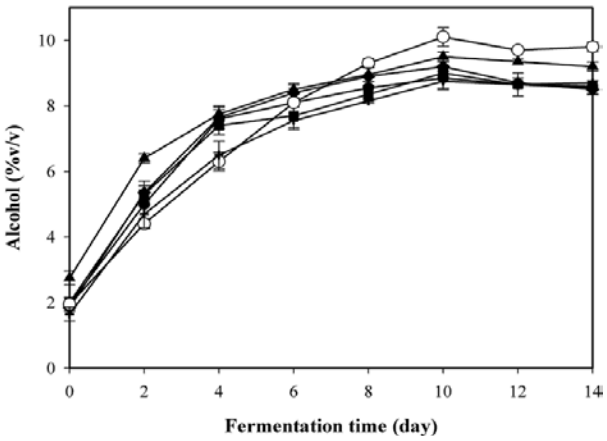

Figure 3 Changes of ethanol content during the Sato fermentation period; VTE (●), SVK (■), PKS (◆), VTN (○), PYT (▲), CRT (✚)

During Sato fermentation, the pH value of each sample remained low before a slow increase started after the 6th day. Their pH level on the first day was between 3.72-3.22, and the 10th day had levels between 3.23-3.65 (Figure 4).

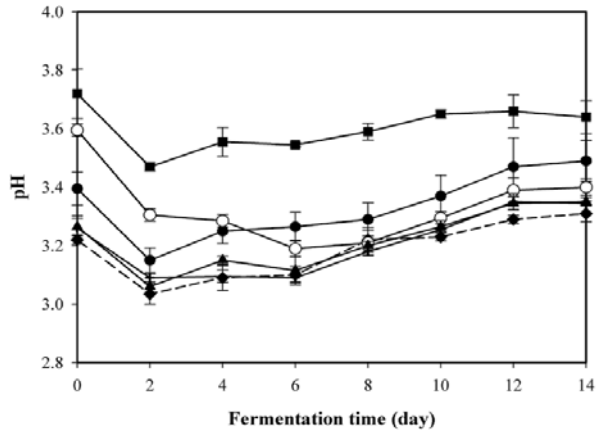

Figure 4 Changes of pH during the Sato fermentation period; VTE (●), SVK (■), PKS (◆), VTN (○), PYT (▲), CRT (✚)

Total acidity (TA) of each sample increased during fermentation, on the 10th and 14th days, TA of CRT, PKS, SVK, VTE, PYT and VTN were 9.45, 9.36, 7.92, 7.65, 7.74 and 4.37, and then 10.08, 9.77, 9.50, 8.33, 8.15 and 4.64 g/L as lactic acid, respectively (Figure 5).

From the mentioned above data Sato made with Loog-pang from Vietnam (VTN)

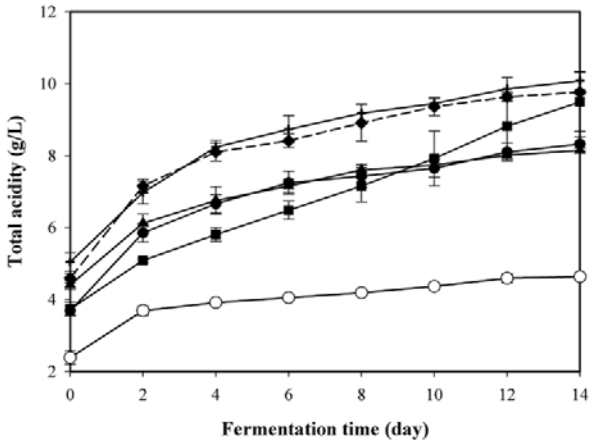

Figure 5 Changes of acid content during the fermentation period; VTE (●), SVK (■), PKS (◆), VTN (○), PYT (▲), CRT (+)

was quite better than the other sources, with a high alcohol content, medium level of pH and low total acidity. The Sato fermented from VTN had high alcohol content, which may be the high number of yeasts and molds, which produce amylase for starch degradation into monosaccharide, especially glucose, after that, the yeast could ferment that glucose and produce ethanol. In this research revealed that the alcohol content in Job's tear Sato are similar to various sources in rice Sato, 9.08-10.48% (2), 10.2-12.0% (5) and 10.8-12.7% by volume (22).

4. Conclusions

In conclusion, regarding the chemical composition of the Job's tears grain, the high carbohydrate and starch content was conducive for Sato production. Regarding the microbial communities of the Loog-pang sources, yeasts and molds held the highest levels, with LAB being found in the CRT, PYT and PKS sources. The optimum fermentation time was 10 days after the mashing step, the VTN source showed the highest level of reducing sugar in Sato, with a higher alcohol content (10.10%, v/v) than other sources. Thus, it can be suggested that a careful choice of Job's tears grain coupled with the correct Loog-pang source could produce a high quality and good tasting Sato. Lastly, if this study were to be conducted further, we would find out more about the chemical compositions and active compounds content, as well as the sensory evaluation.

5. Acknowledgment

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