Studies on Lao-Chao Culture Filtrate for a Flavoring Agent in a Yogurt-Like Product

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ABSTRACT : Lao-chao is a traditional Chinese fermented rice product with a sweet and fruity flavor, containing high levels of glucose, a little alcohol and milk-clotting characteristics. In order to optimize commercial production of lao-chao, *Rhizopus javanicus* and *Saccharomyces cerevisiae* were selected as the mold and yeast starter, respectively. A commercial mixed starter (chiu-yao) was used as control. Fermentation of the experimental combination revealed a sharp drop in pH (to 4.5) on the fourth day, remaining constant thereafter. Content of reducing sugars gradually decreased throughout the entire fermentation period. Of the free amino acids, higher quantities of alanine, leucine, proline, glutamic acid, glutamine and NH_3 were noted. For sugars, glucose revealed the highest concentration, while organic acid levels, including those for oxalic, lactic, citric and pyroglutamic acid, increased throughout the fermentation period. Twenty-one compounds were identified by gas chromatography from aroma concentrates of the lao-chao culture filtrate, prepared using the headspace method. For the flavor components, higher quantities of ethanol, fusel oil and ester were determined in both culture filtrates. In regard to the evaluation of yogurt-like product, there were significant differences in alcoholic smell, texture and curd firmness. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 4 :602-609)***

Key Words : Lao-Chao, Culture Filtrate, Flavor, Taste, Fermentation, Sensory

INTRODUCTION

In order to develop new yogurt-like dairy products satisfying consumer preferences for a low-acid or non-sour product, culture filtrates from a fermented rice product (lao-chao) had been used as both milk-clotting and flavoring additive (Onyeneho et al., 1987; Kuo et al., 1996; Lin and Chen, 1996). Well-known in China, Lao-chao had traditionally been produced by inoculating steamed glutinous rice with commercial Chinese yeast-ball (chiu-yao), followed by fermentation at room temperature for 2-3 d (Wang and Hesseltine, 1970). Actual fermentation time depended on temperature and personal preferences, with a longer fermentation producing a drink more like wine in flavor. Unlike other fermented foods that were usually salty in flavor, lao-chao had a sweet taste. As the amylopectin content for glutinous rice was relatively high, the amylolytic digestion of starch results in a sweet liquefied pasty product (Fennema, 1985) which could be consumed on its own, or cooked with eggs or other foods as a dessert. It can also be used for other dishes, such as seafood (Wang and Hesseltine, 1970).

It was very difficult to control the microflora of commercial chiu-yao prepared under relatively undefined microbiological conditions. Therefore, it was necessary to screen those appropriate pure cultures for manufacturing lao-chao. According to milk-clotting and flavoring additive characteristics, we had attempted to select a pure culture (Lin and Chen, 1996) for production of the lao-chao, with *R. javanicus* and *S. cerevisiae* chosen as the mold and yeast starter, respectively.

Our objective was to examine the flavor properties of the culture filtrate and to provide the point of reference for a yogurt-like product, using for systematic production through experimental manipulation and evaluation of these properties.

MATERIALS AND METHODS

Preparation of inocula

The mold, *Rhizopus javanicus* (CCRC 30288), and yeast starter, *Saccharomyces cerevisiae* (CCRC 21685), were sourced from the Culture Collection and Research Center (Taiwan) and used to inoculate steamed glutinous rice. Before use, both mold and yeast strain were transferred to slants and incubated at 30±1°C for 6-8 d. Spore suspensions and cell suspensions for inoculation were prepared by adding sterilized distilled water containing Tween 80 (0.1 g/l) to slants and shaking the cultures vigorously for 1 min.

Preparation of fermented rice (lao-chao) and culture filtrate

Both glutinous rice and a commercial starter, chiu-yao, were purchased from a local market in Taipei. Glutinous rice (100 g), which had been washed with distilled water and drained, was soaked with 75 ml distilled water at 20-

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Received September 20, 2001; Accepted December 7, 2001

25°C for 12 h, sterilized at 121°C for 15 min, and then cooled to 35°C. The steamed glutinous rice was inoculated with 0.5 g of chiu-yao powder or with 0.5 ml of the blend of suspensions containing 5×10^6 spores of *R. javanicus* and that containing 5×10^6 cells of *S. cerevisiae*, followed by static incubation at 30±1°C in a 250 ml beaker and covered with aluminium foil up to 4-12 d. After fermentation, the culture filtrate was obtained by filtration through four layers of cheesecloth. The sediment in the culture filtrate was eliminated by centrifuging at 1,480×g for 10 min (Kubota, KR-20000T, Tokyo 113, Japan). The culture filtrate was stored at 4°C for further analysis.

Determination of pH value and reducing sugars

The pH value was measured with an Orion Model-520A pH meter (Orion, USA). Reducing sugars were measured according to the method of Lin and Chen (1996), with the absorbance of the solution determined at 540 nm. A calibration curve was prepared using glucose (0.03 to 0.3 g/l).

Determination of sugars and organic acids

The lao-chao extract was analyzed for sugars and organic acids. Analysis of sugars was performed according to the method of Konosu et al. (1978), with slight modification. Briefly, 50 ml extract was added to 50 ml alcohol solution (80%, vol/vol), then the sample was stirred for 45 min and centrifuged at 10,621×g for 10 min to produce a top layer which was removed and filtered through a 0.45 μm membrane filter. The filtered 20 μl sample was injected into a Gilson HPLC system (Gilson Medical Electronics, Inc., WI, USA). The column used for sugar analysis was a Sphereclone NH_2 column (5 μm, 250 mm × 4.6 mm; Phenomenex, USA). The flow rate for the mobile phase (acetonitrile: water, 75:25, vol/vol) was 0.7 ml/min with detection by a Gilson Model 133 refractive index detector.

Analysis of organic acids was performed according to the method of Chen and Chou (1991), with slight modification. A 50 ml volume of lao-chao culture filtrate was added to 50 ml distilled water. The sample was stirred for 45 min and then treated for 20 min with 10 ml perchloric acid (10%, vol/vol) at room temperature. The sample was centrifuged at 10,621×g for 10 min to provide a top layer, which was then removed and filtered through a 0.45 μm membrane filter. A filtered 20 μl sample was injected into a Gilson HPLC system. The column used for organic-acid analysis was a Luna C_{18} (5 μm, 250 mm× 4.6 mm; Phenomenex, USA). The flow rate for the mobile phase (2% KH_2PO_4, pH 2.4, wt/vol) was 0.5 ml/min with a Gilson Model 155 UV/VIS detector operating at 220 nm. Sugars and organic acids were identified by relative retention times and externally quantified using standards obtained from the Sigma Chemical Company (St. Louis, MO, USA) and Merck-Clevenot Corp. (Darmstadt, Germany).

Determination of free amino acids

The Slocum and Cummings (1991) method for pre-treatment of samples was adopted, and a sample volume of 50 μl was analyzed using a Beckman 6300 amino acid analyzer (Beckman, Inc., U.S.A.).

Headspace volatile analysis

Volatile compounds in the headspace of the culture filtrate were collected using a CDS 6000 purge and trap sample concentrator (CDS Analytical, Inc., Oxford, USA). Five milliliters of culture filtrate was added to 5 ml distilled water and a stirrer was used for gentle sample mixing. This solution was then sampled using a 5 ml syringe and n-pentadacane was added as an internal standard. The volatile compounds were purged with helium for 10 min and collected in a Tenax trap, which was then dried before being back-flushed to the gas chromatograph. Volatile sampling was performed in triplicate on days 4, 6, 8, 10 and 12.

Volatile compounds were analyzed using a Dani educational gas chromatograph (Dani Inc., Italy), equipped with flame ionization detector and fitted with a Stabilwax fused-silica capillary column (30 m, 0.25 mm i.d., 0.25 μm df; Hewlett-Packard, USA) with He as carrier gas. The column temperature program was: 43°C for 2 min, then 1.5°C/min to 200°C and held at 200°C for 20 min; with constant injector-port (250°C) and detector temperatures (250°C). Volatile identification was performed by retention times using authenticated compounds, with volatile quantification performed according to the internal standard method (Bangs and Reineccius, 1981).

Preparation of yogurt-like product and determination of curd firmness

The yogurt-like product was prepared by adding culture filtrate to pasteurized (at 65°C for 30 min) skim milk containing 125 g/l non-fat milk powder (Anchor, Wellington, New Zealand) at 1: 10 by volume and then incubating at 38±1°C for 2 h. After cooling at 4°C for 2 h, the curd firmness of product was determined.

Curd firmness, as measured by the breaking force of the milk coagulum, was determined using a rheometer (Fudoh, NRM-2010J-CW, Japan) with a rheoplotter (Rikadenki Kogyo, FR 801, Japan). The yogurt-like product (33 ml, containing 3 ml of culture filtrate and 30 ml of pasteurized skim milk) in a 50 ml beaker was tested at 4°C. The rheometer adaptor No.4 (20 mm dia) was used and the table speed was 50 mm/min.

Sensory evaluation

Culture filtrate and the yogurt-like product were presented to a panel composed of volunteer graduate and undergraduate students from the National Taiwan University, Department of Animal Science. The panelists were fifteen women and fifteen men, aged 21-32 yr. A nine-point hedonic scale was used to evaluate the product's sensory characteristics and scores recorded for aroma, taste and acceptance (one-extreme dislike, three-moderate dislike, five-neither like nor dislike, seven-like moderately and nine -like extremely) (Meiselman, 1984).

Statistical analysis

Results were analyzed using the general linear model procedure from the SAS software package (SAS Institute Inc., 1987), with Duncan's multiple range test for significance (Montgomery, 1991) used to detect differences between treatment means. All experiments were replicated three times.

RESULTS AND DISCUSSION

Volumes of culture filtrate

Traditional lao-chao was produced by inoculating steamed glutinous rice with a commercial starter, chiu-yao, which mainly contained fungal cultures for converting sugars into alcohol, and fermenting at room temperature for 2-3 d (Wei and Jong, 1983). To utilize the lao-chao culture filtrate, suitable fungal cultures were selected from those screened in our previous study (Chen et al., 1998). Of these fungal cultures, culture filtrate from *R. javanicus* combined with *S. cerevisiae* starter produced more ethanol and less reducing sugar than those from chiu-yao, but exhibited similar aroma components. As a result of these characteristics, we made the decision to select *R. javanicus* and *S. cerevisiae* as the mold and yeast starter of choice for flavor-additive development.

During the fermentation period, the rice grains that had been treated with the combination of *R. javanicus* and *S. cerevisiae* became soft and succulent, producing a large volume of clear, effervescent, pale-yellow liquid, similar to that produced with the chiu-yao starter. In addition, the volume of culture filtrate treated with this combination of *R. javanicus* and *S. cerevisiae* was much greater than for the chiu-yao combination ($p<0.05$), with the peak production of lao-chao culture filtrate occurring on or near the eighth day (figure 1).

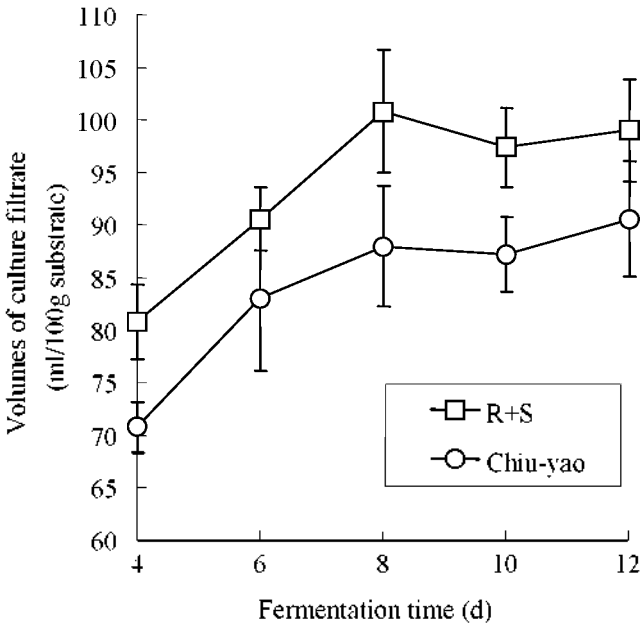

Figure 1. The volumes of lao-chao culture filtrate during fermentation with various inocula

pH and reducing sugars

Changes in chemical composition during fermentation were due to the amylolytic activity of the mold and yeast (Wang and Hesseltine, 1970). At the beginning of the fermentation, the glutinous rice starch was cut by α-amylase, which caused a random, endo-type hydrolysis, with β-amylase and glucoamylase continuing the process for the production of sugars, including glucose and maltose (Fennema, 1985). Then these sugars were converted to pyruvate by means of the glycolytic pathway. Pyruvate was partly converted to alcohol by fermentation and accompanied the production of water and was partly metabolized to organic acids in the TCA cycle (Potter, 1973). Further, the acidity was possibly due to the free fatty acids and amino acids from mold hydrolysis and nucleotides from fungus degradation (Shieh and Beuchat, 1982). Therefore, the pH level and reducing sugar content for in the filtrate during fermentation were determined (table 1).

At the beginning of the fermentation, the pH of the steamed rice was 6.4, treated with the selected mold and yeast starter combination it dropped sharply to 4.5 by the fourth day, at which time it was 3.9 for the chiu-yao starter, with both remaining constant thereafter. Shieh and Beuchat (1982) had proposed that pH stability was related to the buffer capacity of the fermented product.

Sugars incorporating free aldehyde or ketone groups were known as reducing sugars. These reducing sugars were particularly important because they reacted with other food constituents, such as protein amino acids, to form compounds that affected color, flavor, and other food properties (Potter, 1973).

Reducing-sugar content gradually decreased through the fermentation process for the *R. javanicus* and *S. cerevisiae*

Table 1. Reducing sugar content and pH level during fermentation for lao-chao culture filtrate with various inocula* during fermentation

Treatment	Fermentation time (d)	pH	Reducing-sugar content (mg/ml)
R+S**	4	4.3±0.2[a]	206.7±13.7[a]
	6	4.3±0.1[a]	176.9±10.4[b]
	8	4.5±0.1[b]	155.3±14.7[c]
	10	4.7±0.2[c]	153.8±7.7[c]
	12	4.8±0.1[c]	149.9±11.3[c]
Chiu-yao***	4	3.8±0.2[a]	193.1±8.9[a]
	6	3.7±0.2[a]	222.6±13.7[b]
	8	3.8±0.2[ab]	243.2±5.7[c]
	10	3.8±0.1[ab]	277.5±6.3[d]
	12	3.9±0.1[b]	316.6±7.6[e]

* Lao-chao culture filtrate with various inocula of chiu-yao and *R. javanicus-S. cerevisiae* combination.
** *R. javanicus* combined with *S. cerevisiae*.
*** Commercial starter.
[a,b,c,d,e] Symbols bearing different letters in the same column are significantly different ($p<0.05$).

treated combinations and the chiu-yao treatment was just the reverse, confirming the observations of Chen and Chou (1991). D'amore and Stewart (1987) demonstrated that *S. cerevisiae* had the ability to take up and ferment a wide range of sugars, e.g. sucrose, glucose, fructose, galactose, maltose, and maltotriose. These results also accounted for the higher ethanol being produced by the *R. javanicus-S. cerevisiae* combination (table 5).

Free amino acids

Amino acids played an important part in the formation of color, flavor and taste. Maillard reaction products typically contributed to color production during the fermentation period (Potter, 1973). Amino acids converted to aldehydes and alcohols via the Ehrlich pathway, further to esters influence on flavor (Lehtonen and Suomalainen, 1977). Further, many flavor aldehydes were produced by the Strecker degradation from the Maillard reaction. The taste of the end product was partly due to the amino acids themselves, as well as the compounds that participated in the reaction which also manifest complex flavors (Law, 1981). As fermentation continues, higher quantities of alanine, leucine, proline, glutamic acid, glutamin, and NH_3, were noted relative to the other free amino acids (table 2). The total levels of amino acids for the *R. javanicus* and *S. cerevisiae* treated culture were much lower than the control ($p<0.05$), indicating that the control exhibits higher capacity for protein degradation. This fewer amino acid content may have been the cause of the characteristic aromas of the two combinations during the fermentation period, with an slightly acrid, fruity and rum-like aroma noted for the selected variant while the control smelled warm, fruity and rum-like.

Organic acids

As a group, the organic acids impart a sour taste to a flavoring composition. Our results indicate that the organic acids in the fermented product included oxalic, lactic, citric and pyroglutamic acid (table 3). Pyroglutamic acid content gradually increased throughout the fermentation process ($p<0.05$), oxalic and lactic acid only had slight increments. In addition to those generated by the TCA cycle, most of the organic acids were produced by the yeasts, molds and bacteria during alcoholic fermentation (Kunkee and Amerine, 1970). Further, amino acids also participated in the formation of organic acids. For example, as the fermentation process became more advanced, glutamic acid and glutamine levels increased, gradually converting to pyroglutamic acid (Chen and Chou, 1991). In this study, the treatments of chiu-yao and *R. javanicus-S. cerevisiae* combination gave similar results, and the former chiu-yao amount was higher than the latter. Moreover, organic acids also influenced the color of the culture filtrate, and many of the pigments natural were pH indicators (Potter, 1973).

Sugars

Glucose, maltose, lactose and maltotrios contents were determined, and that of glucose was the highest (table 4). Sugars contributed to the sweetness of the culture filtrate, and contribute body and oral texture to the final product (Potter, 1973). Glucose content for both the *R. javanicus* and *S. cerevisiae* combination and the control increased throughout the fermentation period. The increase in sugar content was due to the amylolytic activity of the yeasts and molds (Lin et al., 1990), with the source of sweetness in the lao-chao culture filtrate was mainly due to glucose, maltose and lactose. Further, maybe glycerol contributed sweet flavor characteristics by way of lipid hydrolysis or glucose metabolism.

Volatile components

As the aroma and flavor of foods are due largely to volatile organic compounds, headspace analysis is a very popular method of characterization. Using dynamic headspace techniques, twenty-one compounds were identified by gas chromatography in aroma concentrates from lao-chao culture filtrate (table 5). Aroma components included acetaldehyde, ethyl formate, ethyl acetate, ethanol, ethyl butyrate, iso-butanol, n-butanol, iso-amyl alcohol, ethyl caproate, acetoin, ethyl lactate, hexanol, ethyl caprylate, acetic acid, propionic acid, butyric acid, iso-valeric acid, diethyl succinate, 2-phenyl ethyl acetate, 2-phenyl ethanol and octanoic acid. The culture filtrate from

Table 2. Free amino acid content during fermentation of lao-chao culture filtrate with various inocula*

Amino acids	Content (mg/l)									
	R+S** (d)					Chiu-yao*** (d)				
	4	6	8	10	12	4	6	8	10	12
Asp	2.24	2.96	4.35	5.86	7.87	3.59	5.87	7.39	9.28	10.52
Thr	1.21	1.56	2.32	3.18	4.43	2.52	3.92	4.70	5.71	6.45
Ser	1.57	2.17	4.44	6.40	8.96	3.54	5.86	7.18	8.87	10.32
Asn	1.39	1.79	2.77	3.29	4.31	0.84	1.57	2.39	2.96	3.15
Glu	6.90	9.41	12.13	15.23	18.09	3.98	10.63	13.81	17.69	20.03
Gln	4.35	6.12	7.66	9.62	12.02	6.75	6.43	9.39	11.68	18.69
Pro	13.83	14.78	14.86	15.46	15.92	4.68	6.52	8.25	9.59	9.87
Gly	4.62	5.65	7.52	9.40	11.66	3.97	5.95	7.93	9.88	10.61
Ala	11.52	12.58	16.93	19.49	25.57	24.95	32.80	38.42	45.22	49.42
Val	2.92	3.85	5.97	7.13	9.93	2.63	7.05	9.50	11.56	13.14
Met	0.39	0.56	1.13	1.59	2.54	0.70	1.92	2.09	2.42	2.75
Ile	1.17	1.71	2.69	3.26	4.65	1.98	3.60	4.67	5.70	6.39
Leu	4.85	7.66	11.40	13.25	18.27	7.14	12.22	15.73	19.01	22.13
Tyr	3.08	3.85	4.90	5.47	7.55	4.66	6.76	8.47	10.02	11.10
Phe	3.03	4.41	6.36	7.47	10.38	4.56	7.33	9.58	11.49	13.07
GABA	2.02	3.78	2.71	2.67	3.37	3.57	4.66	5.95	5.92	5.17
Lys	0.05	0.14	0.49	0.98	1.37	2.09	3.46	4.14	5.58	6.26
His	0.36	0.39	0.61	0.71	0.78	1.35	1.75	1.71	2.00	2.07
Arg	1.77	1.70	1.29	2.52	3.94	3.60	3.31	4.22	5.19	5.56
Total	67.26^{a}	85.06^{b}	110.54^{c}	132.98^{d}	171.60^{e}	87.10^{a}	131.63^{b}	165.52^{c}	199.75^{d}	218.73^{e}
NH_3	4.79^{a}	6.75^{b}	9.86^{c}	10.38^{d}	11.73^{e}	24.13^{a}	34.88^{b}	38.05^{c}	42.13^{d}	46.56^{e}

* Lao-chao culture filtrate with various inocula of chiu-yao and *R. javanicus-S. cerevisiae* combination.
** *R. javanicus* combined with *S. cerevisiae*.
*** Commercial starter.
^{a,b,c,d,e} Symbols bearing different letters in the same row are significantly different ($p<0.05$).

Table 3. Organic acid content lao-chao culture filtrate during fermentation with various inocula*

Treatment	Organic acids	Storage times (d)/content (mg/ml)				
		4	6	8	10	12
R+S**	Oxalic acid	1.4±0.1	1.4±0.1	1.4±0.2	1.4±0.1	1.4±0.3
	Lactic acid	0.4±0.1	0.4±0.9	0.4±0.2	0.5±0.1	0.5±0.2
	Citric acid	1.6±0.2	1.8±0.1	1.8±0.1	1.9±0.3	1.9±0.5
	Pyroglutamic acid	$14.0±0.2^{a}$	$16.5±1.5^{b}$	$19.4±2.3^{c}$	$20.9±1.9^{d}$	$21.5±2.8^{e}$
Chiu-yao***	Oxalic acid	1.6±0.2	1.6±0.2	1.6±0.3	1.7±0.4	1.8±0.4
	Lactic acid	0.7±0.2	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.3
	Citric acid	2.0±0.3	2.4±0.5	3.5±0.8	3.5±0.4	4.0±0.4
	Pyroglutamic acid	$19.9±1.1^{a}$	$23.9±2.1^{b}$	$26.8±2.4^{c}$	$27.6±2.5^{d}$	$27.9±3.3^{e}$

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the *R. javanicus-S. cerevisiae* combination produced more ethanol (the largest group of aroma compounds) than the control ($p<0.05$), confirming the observations of Chen and Chou (1993). In addition to ethanol, fusel oils were the major group of aroma compounds, which included iso-butanol and iso-amyl alcohol. Both of them are generally considered undesirable in choking odor when concentrated; however, they tend to contribute favorably to the aroma of a fermented beverage in appropriate concentration (Cronk et al., 1979). Chen et al. (1998) reported that fusel oils contributed to the main flavor characteristics with each fusel oil adding a distinct sensory quality. Besides, 2-phenyl ethanol levels of both treatments gradually increased throughout the fermentation period, which generally

Table 4. Sugars levels for lao-chao culture filtrate during fermentation with various inocula*

Treatment	Sugars	Storage times (d)/content (mg/ml)				
		4	6	8	10	12
R+S*	Glucose	194.4±13.7[a]	213.4±12.9[b]	240.4±15.3[c]	285.6±23.4[d]	314.5±21.6[e]
	Maltose	7.3±1.1	9.5±1.7	10.4±1.3	13.3±1.3	15.4±1.5
	Lactose	3.6±1.1	4.8±1.6	4.5±1.9	4.8±1.5	4.9±1.7
	Maltotriose	1.6±0.5	2.1±1.0	2.9±1.0	2.9±1.1	3.1±1.1
Chiu-yao***	Glucose	210.5±15.6[a]	228.4±20.1[b]	241.1±21.4[c]	257.1±28.6[d]	288.7±30.1[e]
	Maltose	9.6±1.0	12.9±2.1	13.8±1.8	14.6±2.1	16.1±3.0
	Lactose	3.7±0.6	4.4±0.8	5.5±1.1	7.0±1.1	7.2±1.6
	Maltotriose	2.0±0.6	2.8±0.6	3.6±0.8	3.9±0.7	3.8±0.8

* Lao-chao culture filtrate with various inocula of chiu-yao and *R. javanicus-S. cerevisiae* combination.
** *R. javanicus* combined with *S. cerevisiae*.
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[a,b,c,d,e] Symbols bearing different letters in the same row are significantly different ($p<0.05$).

Table 5. Volatile components for lao-chao culture filtrate during fermentation with various inocula*

Peak No.	Volatile components	Content** (mg/l)									
		R+S*** (d)					Chiu-yao**** (d)				
		4	6	8	10	12	4	6	8	10	12
1	Acetaldehyde	5.2	5.7	6.9	8.1	9.5	6.6	8.4	9.6	11.2	10.6
2	Ethyl formate	3.3	3.9	7.8	10.3	11.5	4.7	6.4	8.8	10.9	13.2
3	Ethyl acetate	11.1	11.7	12.6	13.5	15.1	10.8	14.1	15.8	17.5	18.0
4	Ethanol	56,384.6[a]	57,443.2[b]	59,074.1[c]	61,125.4[d]	62,033.1[e]	4,4481.8[a]	46.631.5[a]	47.326.6[b]	48.342.3[c]	47,756.1[bc]
5	Ethyl butyrate	14.9	13.9	15.7	15.9	16.8	63.4	65.1	69.7	68.8	70.4
6	Iso- butanol	41.3	47.1	46.8	44.8	44.2	22.9	28.4	30.2	28.9	28.6
7	n-butanol	–	–	–	trace	trace	–	–	5.9	3.3	3.5
8	Iso-amyl alcohol	3,312.0	3,392.5	3,413.8	3,345.2	3,326.6	3,173.3	3,321.4	3,375.1	3,268.3	3,117.1
9	Ethyl caproate	–	–	trace	trace	–	trace	trace	trace	3.9	5.7
10	Acetoin	–	trace	–	–	trace	12.7	13.3	13.5	14.8	14.1
11	Ethyl lactate	–	–	–	trace	trace	63.7	64.9	66.1	66.7	61.3
12	Hexanol	–	trace	trace	3.8	4.3	trace	trace	5.7	6.7	6.5
13	Ethyl caprylate	–	–	trace	–	–	trace	trace	trace	trace	trace
14	Acetic acid	–	trace	4.7	5.6	7.2	535.1	644.3	586.6	603.5	651.1
15	Propionic acid	trace	4.5	6.6	8.9	14.7	trace	14.0	19.3	23.3	21.2
16	Butyric acid	10.6	13.9	18.3	14.4	16.3	34.2	35.5	37.6	35.8	37.0
17	Iso-valeric acid	–	–	–	–	–	–	trace	trace	5.0	7.7
18	Diethyl succinate	–	trace	trace	6.5	8.3	4.6	9.0	9.7	12.5	13.3
19	2-phenyl ethyl acetate	203.0	233.7	284.6	306.3	317.1	273.8	303.4	355.2	361.1	364.9
20	2-phenyl ethanol	374.1	513.4	672.4	697.7	742.1	1,063.5	1.101.4	1,225.7	1.273.2	1,314.6
21	Octanoic acid	trace	trace	5.3	8.1	7.7	18.4	18.9	14.7	16.5	17.1

* Lao-chao culture filtrate with various inocula of chiu-yao and *R. javanicus-S. cerevisiae* combination.
** Calculated using *n*-pentadecane as internal standard.
*** *R. javanicus* combined with *S. cerevisiae*.
**** Commercial starter.
[a,b,c,d,e] Symbols bearing different letters in the same row are significantly different ($p<0.05$).

contributed a rose-like flavor (Heath, 1978).

Previous studies in our laboratory had revealed the main flavor components from treatment with chiu-yao. and treatment with the *R. javanicus-S. cerevisiae* combination exhibiting the most similar results (Chen et al.. 1998). Nevertheless. amounts of these compounds in lao-chao culture filtrate products varied according to the variety of starter used. Wang and Hesseltine (1970) had indicated that

the lipids of rice decomposed to fatty acids, reacted with alcohol to form a mixture of esters and produced a pleasant aroma. In our current study, ethyl formate, ethyl lactate, and ethyl caprylate were generally considered fruity in aroma while concentrated, butyric acid and propionic acid were acrid. Therefore, the aroma of lao-chao culture filtrate was a composite of all the aroma components.

Sensory evaluation

Our previous work had shown that a considerable yield of culture filtrate and suitable as a milk-clotting agent for yogurt-like product were obtained after 8 days fermentation (Lin and Chen, 1996). For the development of yogurt-like dairy products, the sensory evaluations of culture filtrate and yogurt-like product were conducted at the eighth day.

After fermentation, the glutinous rice became very soft, juicy and sweet, with a slightly alcoholic aroma. The culture filtrate was extracted by filtration and centrifugation, and then stored at 4°C until required for evaluation and sensory evaluation was conducted at 25±1°C. Evaluation of the lao-chao culture filtrate was the result of assessment by 30 judges. A sensory profile of acceptive attributes clearly demonstrates in table 6, including overall acceptance, aroma, and taste were assessed similarly by the panellists (except alcoholic smell). As these results demonstrate, the culture filtrate from the *R. javanicus-S. cerevisiae* combination produced more ethanol than the chiu-yao starter, and smelled accordingly, possibly resulting in lower consumer acceptance. A mixture of esters, furthermore, possibly produced the fruity aroma of the culture filtrate (Wang and Hesseltine, 1970).

Table 6. Sensory scores* and curd firmness of yogurt-like product coagulated with culture filtrate

Characteristics	Culture filtrate		Yogurt-like product	
	R+S**	Chiu-yao***	R+S	Chiu-yao
Overall acceptance	7.2±0.9	7.4±1.1	6.1±0.8	6.3±1.0
Aroma acceptance	7.0±1.3	7.1±1.1	6.4±1.1	6.4±1.3
Alcoholic smell	5.1±1.4[a]	7.2±2.1[b]	5.0±1.3[a]	5.9±1.6[b]
Fruity smell	5.6±1.1	5.7±0.7	5.2±0.9	5.8±1.0
Taste acceptance	6.8±1.4	7.0±1.2	5.4±1.1	5.8±1.0
Sweet	5.6±1.2	5.8±1.3	5.3±0.9	5.6±1.1
Alcoholic taste	7.6±1.1	7.5±0.9	6.5±1.5	6.3±1.8
Texture	7.0±1.0	7.2±1.2	5.1±0.7[a]	6.1±1.6[b]
Curd firmness (g)	-	-	30.2±1.8[a]	54.9±3.1[b]

* Nine-point hedonic scale test.

** *R. javanicus* combined with *S. cerevisiae*.

*** Commercial starter.

[a,b] Symbols bearing different letters in the same row are significantly different ($p<0.05$).

In regard to the evaluation of yogurt-like product, a kind of milk curd with a smooth surface and fruity flavor was obtained by adding culture filtrate. There were significant differences ($p<0.05$) in alcoholic smell, texture, and curd firmness. The chiu-yao exhibited the higher breaking force than *R. javanicus-S. cerevisiae* combination. It affected that *R. javanicus-S. cerevisiae* combination did not form a firm milk curd (texture score 5.1). Weng (2001) reported that addition of stabilizers (edible gum) could improve the texture and acceptability. Besides, there was no difference ($p>0.05$) in the other attributes.

CONCLUSION

Lao-chao was a fermented product, more attractive flavor and aroma developed in the final product and the nutritional value was also improved. As the results of this study had demonstrated, yields, reducing sugars, organic acids, sugars and aroma components could be manipulated to vary lao-chao culture-filtrate production and explore prospects for development in yogurt-like products. For example, the culture filtrate from the *R. javanicus-S. cerevisiae* combination produced more ethanol and volume, that will contribute alcoholic aroma and use value to flavor additive development. Our aim for this study was to manipulate the properties of the culture filtrate to consistently produce a highly acceptable fermented-milk product. Determining the flavor components of the culture filtrate will allow the development of flavor additive for simple in-home preparation of fermented-milk products.

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