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Chemical and sensory changes associated Yu-lu fermentation process – A traditional Chinese fish sauce

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Abstract

The production of 'Yu-lu', a Chinese traditional fermented fish sauce, was replicated in the laboratory with little adaption in order to study the chemical, microorganism and sensory changes associated with the process. Yu-lu was made by incubating mixtures of small anchovies and 30% salt (salt/fish, w/w) at 30 ± 5 °C for 180 days, then rising the incubation temperature to 50 °C for 7 days. Changes in total soluble nitrogen (TSN), TCA (trichloroacetic acid) soluble peptides, formaldehyde nitrogen, total acid, total volatile base nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), composition of amino acid, the non-enzymatic browning index and total plate counts of Yu-lu were observed. TSN, formaldehyde nitrogen, TCA soluble peptides, total titratable acid and non-enzymatic browning index increased throughout the fermentation period. Glutamic acid, lysine, leucine and valine were prominent in Yu-lu. The results from QDA test showed that incubation after 180 days for a week can accelerate the flavor formation of Yu-lu.

Keywords: Yu-lu; Traditional fermentation; Fish sauce; Anchovy

1. Introduction

Yu-lu is a traditional fermented fish sauce widely consumed as condiment for cooking in the Southern and Eastern parts of China, especially in Guangdong and Fujian provinces. It is produced by fermenting anchovies (or the other small marine fish) which have been preconditioned with salt to about 1:3 (salt:fish, wet wt). Fermentation results from the action of enzymes in the fish and some halotolerant and halophile microorganisms. Hydrolysis of the fish protein occurs, resulting in free amino acid, peptides and ammonia. In the presence of high concentrations of salt, pathogenic microorganism growth is controlled and the salt results in a desirable taste and aroma (Catharina, Keshun, & Huang, 1999). The clear brown liquid portion of fermented fish is called Yu-lu. Recently, Yu-lu has been rediscovered for its strong and complicated umami taste and nutritive value. Yu-lu contains all essential amino acid and many vitamins and minerals (Lopetcharat, Choi, & Park, 2001). It also can be the main source of protein in the diet in some areas.

Present Yu-lu process, like some traditional processes, lacks quality assessment and quality control. It is anticipated that more people would consume Yu-lu if the production process involved good hygienic techniques and quality control. Although some works have been done on Asian fish sauce (Dissaraphong, Benjakul, & Visessanguan, 2006; Shih, Chen, & Yu, 2003), there has few on the chemical and sensory changes associated with Yu-lu fermentation processing. The present study, therefore, is aimed at investigating the chemical and sensory properties of Yu-lu fermentation process, especially during the first 6 months.

2. Materials and methods

2.1. Raw material

Anchovies (approximate size 18 cm length and 80 g body weight) were caught off Nanhai sea in China, the

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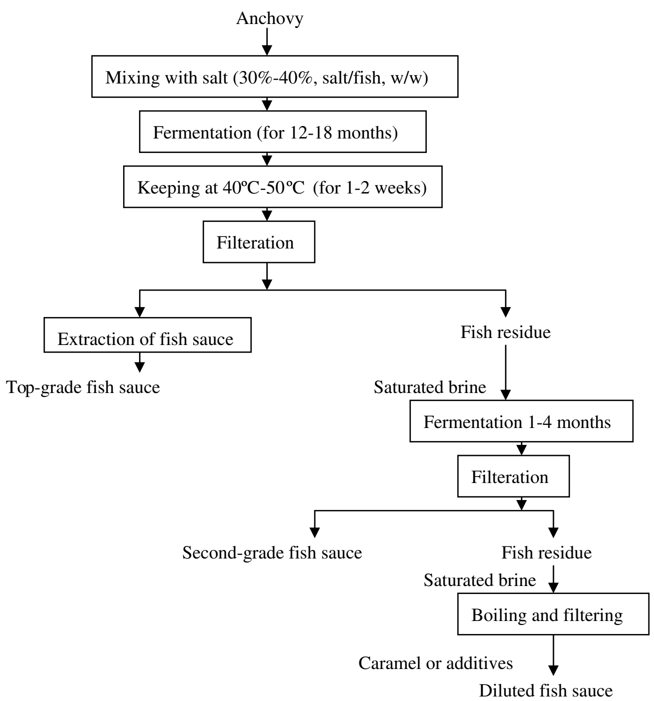

Fig. 1. Traditional Yu-lu process.

whole fish mixed with 30% (w/w) salt immediately, then transported to the laboratory. Fig. 1 is a flow chart of the industrialized Yu-lu process (Adapted from Wilaipan, 1990).

2.2. Fish samples preparation

The mixtures were placed in experimental tanks (pottery, 4 L volume). Then the tanks were covered tightly with triple-layer gauze clothe to maintain a semiaerobic condition and incubated at room temperature (20–25 °C for the first 2 months, and thereafter, in the range between 25 °C and 36 °C). The tanks were incubated at 50 °C for 7 days for sixth months. The tanks were mixed monthly until the fifth month and then left to stand. The liquid formed was taken for analysis on days 30, 60, 90, 120, 150, 180 and 187.

2.3. Collection of liquid

After fermentation and incubation for the designated time, the samples were thoroughly mixed by shaking the tanks. Separate samples were taken and filtrate through a ShuangQuan filtered paper (Model 102). Filtrate was used for analysis.

2.4. Chemical analysis

2.4.1. Total soluble nitrogen (TSN) and TCA soluble peptides content

Total soluble nitrogen content of fish sauce samples were measured using Kjedahl method (AOAC, 1999), total soluble nitrogen content was expressed as g/100 ml. TCA soluble peptides were measured by Morrissey, Wu, and Lin (1993) and expressed as mg/ml. The analyses were all carried out in triplicate.

2.4.2. Formaldehyde nitrogen and total titratable acid

Formaldehyde nitrogen was determined by titration (Beddows, Ismail, & Steinkraus, 1979). Diluted samples (20 ml) were mixed with 60 ml H_2O and titrated to pH 9.6 with 0.05 N NaOH before 10ml formalin solution (37%) was added. The volume of consumed was recorded in order to determine the total *titratable* acid of samples. The samples were finally titrated to pH 9.2 with 0.05 N NaOH. All analyses were carried out in triplicate.

2.4.3. Free amino acid composition

The filtrate samples were also used to determine the amino acid composition using the HPLC method (Hughes & Frutiger, 1990) with High performance liquid chromatography (Waters Ltd., Milford, MA, USA), and PICO.TAG amino acid analysis column. The detect wavelength is 254 nm, temperature was set at 38 °C, the flow rate is 1 ml/min. The concentrations of amino acid in Yu-lu samples were calculated by calibrating with stand amino acids (Amino acid standard solution, type H, Sigma AAS18, St. Louis, MO,USA). All analyses were carried out in triplicate.

2.4.4. Total volatile base nitrogen (TVB-N) and trimethylamine (TMA-N) contents

TVB-N and TMA-N content of each sample were measured using the Conway microdiffusion assay according to the method of Conway and Byrne (1936). The TVB-N and TMA-N extract of fermented fish sauce samples in 10% trichloroacetic acid (TCA, sigma, St. Louis, MO, USA) were absorbed by boric acid. TVB-N and TMA-N were released after addition of saturated K_2CO_3 and then titrated with 0.01 N HCl. In order to determine the TMA-N content, formaldehyde was added to the samples. TVB-N and TMA-N content were calculated and expressed in mg/100 g sample. All analyses were carried out in triplicate.

2.4.5. Histamine

Histamine was analyzed using the colorimetric method of Patange, Mukundan, and Kumar (2005) with minor modifications. 2 ml of *n*-pentanol was added to the glass-stoppered test tube and the tube shaken vigorously for 1 min and allowed to stand for 2 min and then shaken briefly to break the protein gel. All analyses were carried out in triplicate.

2.5. Physical analysis

Non-enzymatic browning was determined using the method of Hendel, Bailey, and Taylor (1950). Prior to analysis, sample (5 ml) was extracted in 50 ml of 50% (w/v) ethanol with a continuous stirring for 1 h. The extracted was filtered using a Shuangquan filter paper (Model 102). The filtrate was then subjected to the absorbance measurement at 420 nm using a spectrophotometer (Spectrumlab 22PC, Shanghai, PRC).

2.6. Microbiological analysis

Microorganisms in Yu-lu are important to the quality of fish sauce. An aliquot of the fermented Yu-lu were taken for microbial counts. Samples were taken aseptically from the Yu-lu tanks and homogenized. Serial dilutions of homogenates were made and total viable counts were determined by the pour plate method, using Plate Count Agar (PCA, Oxoid, CM463) containing 0.5% NaCl as the medium. Plates were incubated at 35 °C for 48 h (Harrigan & Mccanee, 1976).

2.7. Sensory evaluation

Quantitative descriptive analysis (QDA) was performed to determine the flavor characteristics of samples. Analysis was carried out with an internal panel of nine members (five man and four women, age 20–35), Training was based on trial sessions (a total of 6 and 1 h of review for each subsequent month during the study), and the ability of panelists to discriminate and reproduce results was tested in replicate tests on Yu-lu. Evaluation instructions were given orally during training sessions according to Yu-lu prepared at different times. The panelists were trained to differentiate among these samples according to each attribute. The panelists agreed to methods (compression with tongue on roof of mouth, time to wait before noting aftertaste) that would be used to determine levels for given attributes. Commercially available Yu-lu made in China (30% NaCl, from Anchovy) was received by the trained panelists as the very first (reference) sample at the beginning of each session for panelist acclimation every test day. The panelists used the reference to recalibrate as necessary at the start of each panel, but the sample was removed from the booth prior to evaluation of subsequent samples. All samples were coded with random three-digit numbers, and served to the panelists in a randomized complete block design. Each sample (about 1 mL) poured into a small cup was evaluated in the mouth and by sniffing. After evaluation of one sample, the panel member washed his/her mouth with tap water. The samples were scored along seven scales (−1.5, −1, −0.5,0, +0.5, +1, +1.5) for six kinds of tastes or four kinds of odors. Significant difference for each taste and odor among the four samples was analyzed by analysis of variance. Members were asked to compare flavor attribute of each sample (meaty, sour, umami, salty, ammonia, cheesy, fecal, fishy, rancid and roasty) and '−1.5' accounts for a not perceivable intensity while '1.5' accounts for an extreme intensity of an attribute.

2.8. Statistical analysis

Analysis of variance (ANOVA) was used to search for significant differences between mean values of the different results. The results are presented as means ± SD. The parallels $N = 3$ were used for all analyses.

3. Results and discussion

3.1. Total soluble nitrogen, TCA soluble peptides, total titratable acid and formaldehyde nitrogen content

Changes in total soluble nitrogen (TSN), TCA soluble peptides, total titratable acid and formaldehyde nitrogen of all fish sauce samples of different ferment time were shown in Fig. 2. The total soluble nitrogen, TCA soluble peptides, total titratable acid and formaldehyde nitrogen content of all the filtrate increased continuously with increasing fermentation time ($p < 0.05$). The changes of formaldehyde nitrogen were similar to the changes in TSN. A similar observation was made during a study on 'Bekasang', a Indonesian traditional fermented fish sauce (Ijong & Ohta, 1996). The increases of both TSN and formaldehyde nitrogen content during processing of Yu-lu could be attributed to the combined effects of autolysis and microbial degradation of the fish muscle. The evidence for autolysis in the experiment was provided by the changes in the total amino acid concentration during fermentation (Table 1). It was reported that 60–80% amino compounds in fish sauce are amino acid (Park, Fukumoto, & Fujita, 2001). The total nitrogen content is an objective index used to classify the quality of Yu-lu, It is mainly from protein nitrogen and non-protein nitrogen compounds, such as free amino acids, nucleotide, peptides, ammonia, urea and TMAO. These components contribute to the specific aroma and flavor (Shahidi, Sikorski, & Pan, 1994). Formaldehyde nitrogen content is used as a convenient index of the degree of protein hydrolysis (Chaveesuk, Smith, & Simpson, 1993).

3.2. Amino acid compositions

As shown in Table 1, glutamic acid, lysine, leucine, valine and alanine were prominent in Yu-lu, but the proline

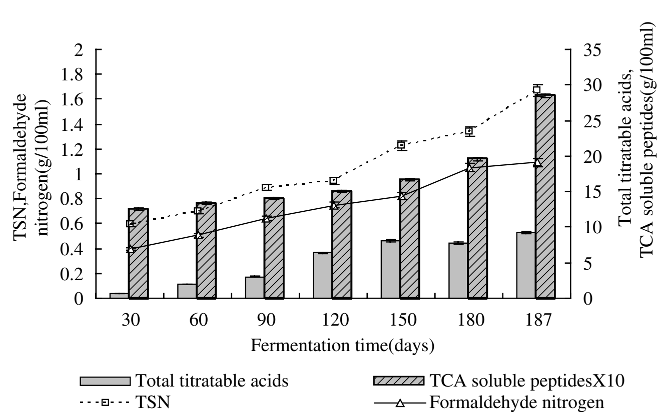

Fig. 2. Total soluble nitrogen, formaldehyde nitrogen, TCA soluble peptides and total titratable acid.

Table 1
The amino acid composition of Yu-lu samples

Amino acid	Ferment 30 days (mg/100 g)	Ferment 60 days (mg/100 g)	Ferment 90 days (mg/100 g)	Ferment 120 days (mg/100 g)	Ferment 150 days (mg/100 g)	Ferment 180 days (mg/100 g)	Incubation at 50 °C for 7 days (mg/100 g)
Aspartate	39.46	33.81	19.68	17.92	71.08	74.60	128.85
Glutamate	177.68	180.55	232.00	245.71	409.29	472.61	582.91
Serine	102.98	64.85	68.9	71.37	276.81	317.98	410.86
Glycine	77.78	54.97	61.58	59.14	82.50	121.73	137.67
Histidine	154.51	138.59	150.98	157.5	277.20	291.37	407.60
Arginine	222.06	236.42	255.04	274.93	239.29	275.94	281.10
Threonine	129.81	123.99	156.82	157.66	185.42	219.97	298.32
Alanine	157.20	161.08	218.18	274.93	355.83	400.62	426.65
Tyrosine	77.82	58.47	45.6	110.85	57.55	78.33	98.36
Valine	149.68	162.83	230.86	297.94	354.26	422.94	430.21
Methionine	81.38	80.08	132.69	152.04	168.77	206.04	234.67
Cysteine	5.09	14.76	21.79	21.84	4.61	8.84	7.81
Isoleucine	102.6	122.30	164.52	218.32	271.84	309.01	359.6
Leucine	200.94	228.48	318.91	397.48	444.45	530.48	481.18
Tryptophan	191.99	414.95	745.49	912.2	350.78	469.51	285.44
Phenylalanine	89.23	112.30	156.73	189.17	200.75	247.19	239.50
Lysine	226.86	329.67	366.28	553.64	476.01	569.50	587.46
Total	2187.10	2518.07	3346.07	4077.9	4226.46	5016.68	5344.56

content was trace. The difference in the amount of each free amino acid among the samples seemed to be attributable to differences in the balance of free amino acids produced by autolysis and microbial action, respectively. Amino acids contribute significantly to the taste of Yu-lu. For example, the typical aroma of glutamic acid is meaty. Glycine, alanine, serine and threonine taste sweet while valine, phenylalanine and histidine give a bitter taste (Liu, 1989). The contribution of amino acids to the aroma of fish sauce has also been reported by Lopetcharat et al. (2001).

3.3. Total volatile basic nitrogen, trimethylamine nitrogen and histamine

Changes in TVB-N, TMA-N and histamine contents of Yu-lu during fermentation are presented in Fig. 3. These values show the effect of fermentation on volatile base. TVB-N value increased during the initial fermentation steps demonstrating that at the early stage, fish spoilage microorganism still existed and increased, after 3 months, they were restrained by the high concentration of salt and TVB-N value show a little decrease during the fourth month, then increased after that. High TMA-N concentration in fermented fish products had been observed (Achinewhus & Obob, 2002). In this experiment, TMA-N levels began to decrease to day 120 ($p < 0.05$), thereafter there was a slight increase. It was the results of bacterial reduction of trimethylamine oxide (TMAO), a non-volatile and non-odouriferous compound, to volatile trimethylamine (TMA), which has an ammoniacal smell and the formation of dimethylamine (DMA). The histamine content of Yu-lu increased at the first two month of the fermentation, then high salt content inhibits both the proteolytic enzymes in the fish as well as growth of microorganisms. The content of histamine decreased from around the third month of fermentation and continued to decrease as fermentation progressed ($p < 0.05$). The L-histidine decarboxylation activity of halophilic histamine-forming bacteria was highest at the beginning of the stationary phase of the growth and gradually decreased as the stationary phase proceeded. This fact might explain the very low content of histamine in the commercial fish sauces (Sanceda, Suzuki, & Ohashi, 1999).

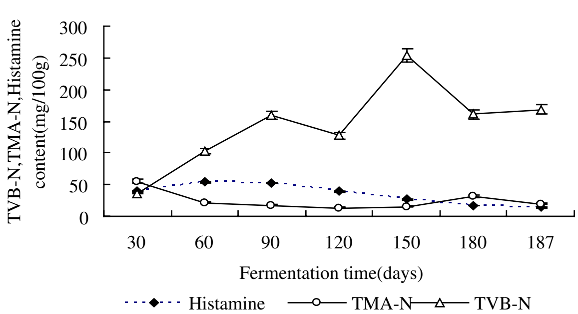

Fig. 3. The total volatile basic nitrogen, trimethylamine nitrogen and histamine content.

3.4. Non-enzymatic browning index and microbiological counts

Non-enzymatic browning index (A_{420}) and microbiological counts were showed in Fig. 4. An increase in browning was observed during fermentation suggesting that brown pigment formed during the extended fermentation time. Greater browning was found in the sample produced by rising incubation temperature to 50 °C for 7 days. The browning contributes to the color of fish sauce and the brown color in Yu-lu was caused by Maillard reaction (Lopetcharat et al., 2001). The increase in absorbance at 420 nm was used as an indicator of brown-

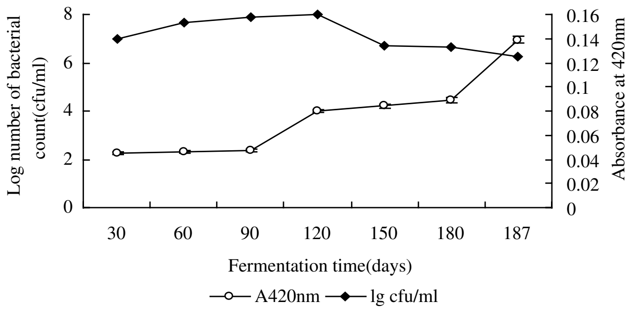

Fig. 4. Non-enzymatic browning index and microbiological counts of Yu-lu.

ing development in the final stage of the browning reaction (Ajandou, Tchiakpe, Ore, Benajiba, & Puigserver, 2001). Soy sauce and fish sauces became darker with melanoidin produced by the Maillard reaction during storage. The reducing sugar content in Yu-lu is trace in this experiment, so the lipid oxidation products and degradation products, such as aldehyde and other carbonyl compounds could react with free amino acid, which could be released to a higher extent with increasing fermentation time. Rising incubation temperature accelerated this reaction.

A continued increase in bacterial counts occurred during the first 120 days and then decreased gradually thereafter in Yu-lu ($p < 0.05$). The bacterial count in the early stages of fermentation was high, it should be explained that, in the initial steps of fermentation, the salt has not yet completely dissolved and penetrated into the fish bodies. Non-halotolerant bacteria contaminated on the fish bodies, some of which may be fish spoilage microorganism. It could grow and have action on fish degradation, but after several months, this group of microorganisms disappeared, leaving only the halotolerant and halophiles groups playing a major role in the fermentation. The gradual change of fish components caused by degrading enzymes, played a role in determining the group of dominant bacteria present (Berna, Sukran, & Sebnem, 2005). It has been reported that these halophiles and halotolerant bacteria mostly were LAB and yeasts (Paludan-Müller, Madsen, & Sophanodora, 2002).

3.5. Quantitative descriptive analysis (QDA)

The sensory profiles of samples based on the QDA test-ten attributes by nine panelists are shown in Fig. 5. The scores for umami, fishy, salty, meaty and roasty were increased with the fermentation ($p < 0.05$). Umami score for 187 days (after incubation) was the highest among all samples. The scores for fishy, salty and cheesy also increased obviously after incubation at 50 °C for a week. Fecal and rancid smell of samples before increase incubation temperature were much stronger than that after incubation at 50 °C. It indicated that the off-flavor of Yu-lu could become weak after rising incubation temperature.

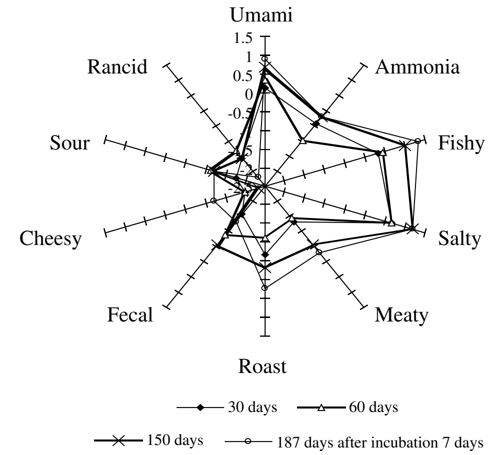

Fig. 5. The sensory profiles of 5 samples based on the QDA test-ten attributes by nine panelists.

4. Conclusions

The study conducted here showed that the Yu-lu process is mixed culture fermentation. This is characteristic of many traditional processes. The spontaneous fermentation occurs in Yu-lu processing and the process favors the growth of halotolerant and halophiles generally. The fermentation process seems to increase the total soluble nitrogen, TCA soluble peptides, formaldehyde nitrogen, total titratable acid and free amino acid concentration of the fish sauce, thus increase the nutritive value of the products. Rising incubation temperature in the last stage of fermentation could develop the flavor and accelerate the process. Yu-lu is not consumed as much normally, but they may be rather used as condiment to flavor soup and more prepared foods. The fermentation of Yu-lu should be modified to yield more hygienic and health condiment. Further investigations on how Its flavor forming should be conducted. The research results of Yu-lu should also be applied to develop wide variety of seafood-like flavor condiments in the future.

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