Reduction of salt content of fish sauce by ethanol treatment

Yu Liu¹ · Ying Xu² · Xiaoxia He³ · Dongfeng Wang² · Shiwei Hu¹ ·
Shijie Li¹ · Wei Jiang^{1,2}

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Abstract Fish sauce is a traditional condiment in Southeast Asia, normally containing high concentration of salt. The solubility of salt is lower in ethanol than in water. In the present study, fish sauce was desalted by ethanol treatment (including the processes of ethanol addition, mixing, standing and rotary evaporation). The salt concentration of fish sauce decreased significantly from 29.72 to 19.72 g/100 mL when the treated ethanol concentration was 21% (v/v). The addition of more than 12% (v/v) of ethanol significantly reduced dry weight, total soluble nitrogen content and amino acids nitrogen content. Besides, the quality of fish sauce remained first grade if no more than 21% (v/v) of ethanol was used. Furthermore, sensory analyses showed that ethanol treatment significantly reduced the taste of salty and the odor of ammonia. This study demonstrates that ethanol treatment is a potential way to decrease salt content in fish sauce, which meanwhile limits the losses of nutritional and sensorial values within an acceptable range.

Keywords Fish sauce · Reduction · Salt · Ethanol · Evaporation · Sensory

Introduction

Fish sauce is a traditional condiment widely used in Southeast Asia to impart an appetizing flavor to several cooking foods. This product is always produced by unsterilized fermentation (Zarei et al. 2012). To avoid harmful microbial contamination, more than 30% (w/w) of salt is normally added in fish fermentation (Lopetcharat et al. 2001; Gowda et al. 2016). Thus, high salt content is a well-known characteristic of fish sauce product. Jiang (2014) reported that salt concentrations of thirty-five commercial fish sauce samples from three production areas in China are between 20.44 and 35.44 g/100 mL. Since medical studies have demonstrated a significant relationship between high-salt food consumption and several health risk (i.e., high blood pressure, heart disease and kidney problem), a technique that can be used for production of low-salt fish sauce is desired in the food industry (Galvis-Sánchez et al. 2011; Fidaleo et al. 2012; Israr et al. 2016).

Several technologies (i.e., ion exchange, reverse osmosis, nanofiltration, ultrafiltration, freezing electrodialysis and extration) may be used to separate salt from solution, but only a few of them can be applied to reduce salt content of fish sauce on an industrial scale because of economic reasons. Electrodialysis is one of the separation techniques in which ion-exchange membranes and electric potential are utilized to separate ion species from an aqueous solution (Choi et al. 2003). Recently, some researchers have tried to reduce salt concentration in fish sauce by electrodialysis (Chindapan et al. 2009, 2010, 2011; Jundee et al.

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✉ Wei Jiang
jiangwei_zjou@163.com

¹ Laboratory of Seafood Processing, Innovative and Application Institute, Zhejiang Ocean University, Zhoushan 316022, China

² Laboratory of Food Chemistry and Nutrition, College of Food Science and Engineering, Ocean University of China, Qingdao 266003, China

³ Qingdao Entry-Exit Inspection and Quarantine Bureau, Qingdao 266002, China

2012; Bawornruttanaboonya et al. 2015). The research results indicate that electrodialysis can remove salt from fish sauce effectively, but the loss of color and the consumption of energy, ion-exchange membranes or time are inevitable. Besides, a special electrodialyser is needed whose size is related to the processing capacity of fish sauce. Therefore, a more convenient and economical technique for reducing salt content of fish sauce is expected.

It is known that salt was less soluble in ethanol than in water. Moreover, ethanol is miscible with water. Thus, salt in water may precipitate by the addition of enough ethanol, which results from the decrease of its solubility. In our experiment, we found that ethanol could precipitate salt in fish sauce. The schematic diagram of salt reduction of fish sauce by ethanol treatment was showed in Fig. 1. As a never-before-reported method to salt reduction of fish sauce, the effects of ethanol treatment on salt-removal level and selected physicochemical properties were investigated in this study.

Materials and methods

Materials

About 50 kg of fish sauce was obtained from a local distributor in Guangdong province of China. The samples were transported in glass bottles to the laboratory and stored at 4 °C in the refrigerator (Haier, China). This fish sauce was produced by fermenting anchovy (*Engraulis japonicus*) and sea salt (3:1, w/w) in a cement cistern for six months. The characteristics of the untreated fish sauce are given in Supplementary Table 1. Ethanol of analytical grade ($\geq 99.7\%$, v/v) was provided by Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

Reduction of salt content of fish sauce

The fish sauce stored in the refrigerator was equilibrated to room temperature before use. Then 100 mL of fish sauce and different concentrations of ethanol were poured into a 300 mL beaker. The mixture was stirred for 15 s by a magnetic stirrer at room temperature, and subsequently was

Fig. 1 Schematic diagram of salt reduction of fish sauce

kept standing for 5 h. The supernatant was poured out and treated by a rotary evaporator (IKA, German) for ethanol removal. The rotary evaporator was equipped with IKA HB 10 Heating Bath, IKA RV 10 Control and IKA RC2 basic Refrigerating Constant Temperature Circulator. The automatic distillation program for ethanol was used. The bath temperature, rotation and vacuum were 60 °C, 100 r/min and 175 mbar, respectively. After evaporation, fish sauce was used for physical and chemical analyses.

Determination of salt concentration

Salt concentration of fish sauce was measured using the method of (AOAC 1995). Briefly, 5.0 mL of fish sauce was transferred to a 100 mL volumetric flask and diluted with distilled water to volume. Two milliliters of the diluted sample was mixed with 20.0 mL of 0.1 mol/L AgNO_3 and 20.0 mL of concentrated HNO_3 in a conical flask. The mixture was boiled gently on a hot plate for 30 min, and subsequently cooled using running water. Then 50.0 mL of distilled water and 5.0 mL of ferric alum indicator were added. The mixture was titrated with 0.1 mol/L potassium thiocyanate until the solution became permanent light brown. Salt concentration was calculated and expressed as g NaCl/100 mL.

Determination of dry weight

Dry weight of fish sauce was measured according to the method (Gildberg 2001) with some modifications. The fish sauce samples were dried to constant weight at 105 °C in a far-infrared drying oven (766-5AS, Jingpin, Shanghai, China). Dry weight was expressed as g/100 mL.

Determination of total soluble nitrogen content

Total soluble nitrogen content of fish sauce was measured by an Autoanalyzer (Kjeltec 2300, Foss TecatorAB, Hogans, Sweden) according to the Kjeldahl method. Total soluble nitrogen content was expressed as g nitrogen/100 mL.

Determination of amino acids nitrogen content

Amino acids nitrogen content of fish sauce was measured by the method described before (Klomklao et al. 2006). Amino acids nitrogen content was expressed as g nitrogen/100 mL.

Determination of free amino acids compositions

Free amino acids compositions of fish sauce samples were analyzed by amino acid analyzer (L-8900, HITACHI,

Japan) according to the method described before (Yongsawatdigul et al. 2007).

Sensory analyses

Flavor characteristics of fish sauce samples were determined by quantitative descriptive analysis (QDA). Analysis was carried out according to the method described by Jiang et al. (2007) with some modifications. Four kinds of tastes (meaty, sour, umami, salty) and seven kinds of odors (ammonia, cheesy, fecal, fishy, rancid, alcohol and roast) were compared with sensory panel. Taste panels were composed of ten members (five men and five women, aged 24–27) who had been trained to the standard level of proficiency of panelists for sensory evaluation. The samples were scored along seven scales (0–6) for seven kinds of tastes or four kinds of odors. Among these scores, ‘0’ accounts for a not perceivable intensity while ‘6’ accounts for an extreme intensity of an attribute.

Statistical analyses

Statistical analyses were performed using the software IBM SPSS Statistics 22.0 (IBM, USA). Data were analyzed by one-way analysis of variance (ANOVA) with the Tukey test. The experiments were performed in triplicate and the data were expressed as average $\pm$ standard deviation.

Results and discussion

Reduction of salt content of fish sauce

To visually view the salt reduction process, the images of untreated fish sauce and fish sauce sequentially treated by ethanol addition, mixing, standing and rotary evaporation were presented in Fig. 2. The untreated fish sauce was brown and clear (Fig. 2a). With the addition of ethanol and

Fig. 2 Images of **a** untreated fish sauce, and fish sauce sequentially treated by **b** ethanol addition, **c** mixing, **d** standing and **e** rotary evaporation. The added ethanol concentration was 21%

mixing, a large amount of white insoluble substances appeared in fish sauce (Fig. 2c). Then these substances sunk to the bottom in five hours' standing (Fig. 2d). Besides, no significant difference was observed between the untreated fish sauce (Fig. 2a) and the obtained desalted fish sauce (Fig. 2e) regarding color and clarity, indicating that ethanol treatment did not significantly change the appearance of fish sauce.

Figure 3a shows the decrease of salt concentration in fish sauce through ethanol treatment. As no precipitation was observed when the added ethanol concentration was lower than 9% (v/v), the salt concentration in fish sauce did not change significantly ($P > 0.05$). As the ethanol concentration rose, the solubility of salt decreased. When the ethanol concentration increased to 12% (v/v), some white precipitation formed and sunk to the bottom, and the salt concentration decreased significantly from 29.72 to 22.14 g/100 mL ($P < 0.05$). Moreover, the salt concentration continued to decline if higher proportion of ethanol added to the fish sauce. The salt contents in fish sauce decreased by 33.65 and 51.88% when the ethanol concentrations were 21% (v/v) and 51% (v/v), respectively ($P < 0.05$). The results show that ethanol treatment significantly reduces salt content in fish sauce.

Effect of ethanol treatment on dry weight

The dry substances in fish sauce include soluble protein, amino acids, peptides, inorganic salts and other non-volatile compounds. Figure 3b shows dry weight changes of fish sauce by ethanol treatment. The results exhibited similar trend to that of salt concentration (Fig. 3b). The dry weight did not change significantly when treated by ethanol concentration ranged from 0% (v/v) to 9% (v/v) ($P > 0.05$), but decreased from 39.71 to 28.84 g/100 mL

when the ethanol concentration increased to 12% (v/v) ($P < 0.05$). The dry weights in fish sauce decreased to 25.70 and 19.99 g/100 mL when the ethanol concentrations increased to 21% (v/v) and 51% (v/v), respectively ($P < 0.05$). The results show that ethanol treatment significantly reduces dry substances in fish sauce when the ethanol concentration is more than 12% (v/v), indicating possible loss of protein, salt, peptides, amino acids and so on.

Effect of ethanol treatment on total soluble nitrogen content, amino acids nitrogen content and the ratio of total soluble nitrogen content/amino acids nitrogen content

Both total soluble nitrogen and amino acids nitrogen contents are regarded as important quality indexes for fish sauce. Amino acids nitrogen is used as a convenient index of the degree of protein hydrolysis during fermentation, and it contributes to the specific aroma and flavor (Jiang et al. 2007). According to the Standard for Fish Sauce (CODEX STAN 302-2011) published by Codex Alimentarius Commission, fish sauce should contain no less than 1.0 g/100 mL total soluble nitrogen content, and amino acids nitrogen content must account for no less than 40% of the total soluble nitrogen content (Codex Alimentarius Commission 2013). According to the Recommended Commercial Standard for Fish Sauce (SB/T 10324-1999) published by State Bureau of Internal Trade (State Bureau of Internal Trade 1999), fish sauce was classified into three grades by both total soluble nitrogen and amino acids nitrogen contents as follows: the first grade (total soluble nitrogen content no less than 1.2 g/100 mL, amino acids nitrogen content no less than 0.9 g/100 mL), the second grade (total soluble nitrogen content no less than 0.87 g/

Fig. 3 Changes in salt concentrations (a) and dry weights (b) of fish sauce by ethanol treatment. Values with different letters mean that the values are significantly different ($P < 0.05$)

100 mL, amino acids nitrogen content no less than 0.65 g/100 mL) and the third grade (total soluble nitrogen content no less than 0.54 g/100 mL, amino acids nitrogen content no less than 0.4 g/100 mL). As shown in Supplementary Table 1, the fish sauce used in this study was evaluated as the first grade.

Changes in total soluble nitrogen content, amino acids nitrogen content and the ratio of amino acids nitrogen content/total soluble nitrogen content of fish sauce by ethanol treatment are depicted in Fig. 4. Total soluble nitrogen in fish sauce is mainly composed of protein nitrogen, free amino acids, nucleotide, peptides, ammonia, urea, trimethylamine oxide and so on (Klomklao et al. 2006). Figure 4a showed that the total soluble nitrogen content did not change significantly if the ethanol concentration was lower than 9% (v/v) ($P > 0.05$), which was in accordance with the salt concentration (Fig. 3a) and dry weight (Fig. 3b). The total soluble nitrogen content decreased from 1.54 to 1.05 g/100 mL when the fish sauce was treated by 51% (v/v) of ethanol, which still met the

criteria of the Standard for Fish Sauce (Codex Alimentarius Commission 2013). However, the fish sauce fell to the second grade if treated by more than 36% (v/v) of ethanol based on the total soluble nitrogen criteria of the Recommended Commercial Standard for Fish Sauce (State Bureau of Internal Trade 1999). The downward trend of amino acids nitrogen content in fish sauce is similar to that of total soluble nitrogen (Fig. 4b). Specifically, the amino acids nitrogen content slightly changed between 1.04 and 1.05 g/100 mL when treated by less than 9% ethanol (v/v) ($P > 0.05$), but declined rapidly to 0.93 g/100 mL if 12% (v/v) of ethanol was added ($P < 0.05$). The fish sauce fell to the second grade if treated by more than 21% (v/v) of ethanol based on the amino acids nitrogen content criteria of the Recommended Commercial Standard for Fish Sauce (State Bureau of Internal Trade 1999). Figure 4c showed that the ratio of amino acids nitrogen content/total soluble nitrogen content of fish sauce did not change significantly ($P > 0.05$) and maintained around 60% when treated by different concentrations of ethanol. This index well met the

Fig. 4 Changes in **a** total soluble nitrogen contents, **b** amino acids nitrogen contents and **c** the ratios between amino acids nitrogen content and total soluble nitrogen content of fish sauce by ethanol

treatment. Values with different letters mean that the values are significantly different ($P < 0.05$)

Table 1 Free amino acids compositions of fish sauce by ethanol treatment

Amino acid (mg/100 mL)	Ethanol concentration (% v/v)					
	0	3	6	9	12	15
Aspartic acid	475.39 ± 28.84 c	465.22 ± 46.50 bc	468.74 ± 60.86 c	465.61 ± 39.95 bc	429.92 ± 31.03 abc	414.07 ± 41.20 abc
Threonine	361.03 ± 21.54 bc	361.65 ± 35.78 c	364.47 ± 46.96 c	367.78 ± 31.21 c	321.90 ± 22.83 abc	323.70 ± 31.85 abc
Serine	259.21 ± 15.03 c	248.17 ± 24.03 bc	254.86 ± 32.34 bc	258.33 ± 21.42 c	228.96 ± 15.77 abc	229.23 ± 22.08 abc
Glutamic acid	836.31 ± 51.89 c	810.68 ± 82.28 bc	847.10 ± 111.32 c	829.29 ± 72.43 c	728.71 ± 53.72 abc	726.67 ± 73.57 abc
Glycine	3072.78 ± 194.76 cd	3091.88 ± 318.49 d	3105.23 ± 412.44 d	3049.78 ± 270.81 cd	2734.19 ± 206.03 abcd	2769.94 ± 285.15 bcd
Alanine	188.37 ± 10.53 d	184.35 ± 17.43 d	195.04 ± 24.36 d	189.03 ± 15.25 d	166.39 ± 11.02 bcd	168.68 ± 15.79 cd
Valine	521.91 ± 31.81 cde	559.68 ± 56.28 e	534.96 ± 69.70 de	533.84 ± 46.03 de	471.00 ± 34.15 bcde	463.49 ± 46.33 abcde
Methionine	193.26 ± 10.83 fg	185.03 ± 17.50 efg	182.58 ± 22.69 efg	199.46 ± 16.16 g	178.71 ± 11.96 defg	167.93 ± 15.73 bcdefg
Isoleucine	366.89 ± 21.91 d	357.26 ± 35.32 cd	370.92 ± 47.81 d	373.10 ± 31.67 d	324.08 ± 23.00 abcd	328.79 ± 32.37 bcd
Leucine	668.60 ± 41.18 cd	670.12 ± 67.74 cd	681.26 ± 89.19 d	647.02 ± 56.15 bcd	598.57 ± 43.84 abcd	570.95 ± 57.46 abcd
Tyrosine	22.14 ± 0.79 e	22.10 ± 0.66 e	21.57 ± 1.24 de	21.99 ± 0.47 e	20.56 ± 0.54 cde	19.24 ± 0.39 abc
Phenylalanine	219.02 ± 12.47 d	200.69 ± 19.12 bcd	203.52 ± 25.49 cd	198.85 ± 16.11 abcd	191.46 ± 12.93 abcd	181.31 ± 17.11 abcd
Lysine	445.50 ± 26.93 d	439.52 ± 43.84 cd	433.96 ± 56.22 cd	424.47 ± 36.26 bcd	398.51 ± 28.64 abcd	384.09 ± 38.10 abcd
Histidine	208.54 ± 11.81 de	202.65 ± 19.33 de	229.77 ± 29.00 e	199.33 ± 16.15 cde	189.20 ± 12.76 bcde	184.20 ± 17.41 abcde
Arginine	9.12 ± 1.23 a	8.23 ± 0.83 a	8.48 ± 0.52 a	8.78 ± 0.94 a	8.06 ± 1.14 a	8.93 ± 0.77 a
Proline	126.26 ± 6.58 cd	122.88 ± 11.06 cd	124.32 ± 14.93 cd	124.63 ± 9.49 d	110.53 ± 6.80 abcd	111.71 ± 9.90 bcd
Total	7974.34 ± 484.96 c	7930.11 ± 794.50 c	8026.76 ± 1044.04 c	7891.30 ± 678.52 bc	7100.74 ± 513.37 abc	7052.91 ± 703.63 abc

Amino acid (mg/100 mL)	Ethanol concentration (% v/v)						
	18	21	24	27	30	33	36
Aspartic acid	408.51 ± 43.63 abc	397.67 ± 26.13 abc	385.17 ± 19.81 abc	375.58 ± 30.87 abc	359.69 ± 22.65 ab	349.13 ± 31.50 a	343.18 ± 34.37 a
Threonine	314.20 ± 33.17 abc	294.73 ± 18.96 abc	289.47 ± 14.55 abc	279.63 ± 22.56 ab	264.36 ± 16.24 a	261.62 ± 23.17 a	262.14 ± 25.86 a
Serine	222.56 ± 23.01 abc	225.19 ± 14.12 abc	217.83 ± 10.64 abc	199.33 ± 15.60 ab	200.39 ± 11.93 ab	189.27 ± 16.30 a	189.21 ± 18.21 a
Glutamic acid	715.62 ± 77.67 abc	693.30 ± 46.74 abc	677.55 ± 35.84 abc	663.12 ± 55.75 abc	623.97 ± 40.44 a	600.67 ± 55.37 a	607.24 ± 62.09 a
Glycine	2614.63 ± 288.20 abcd	2541.03 ± 175.54 abcd	2363.76 ± 128.38 abc	2262.97 ± 194.29 ab	2292.60 ± 152.77 ab	2263.75 ± 213.27 ab	2251.15 ± 234.70 ab
Alanine	160.29 ± 16.11 bcd	156.39 ± 9.33 abcd	158.04 ± 7.38 abcd	154.86 ± 11.76 abcd	140.55 ± 7.93 abc	139.55 ± 11.59 abc	132.78 ± 12.28 abc
Valine	434.09 ± 46.46 abcd	433.29 ± 28.61 abcd	428.47 ± 22.17 abcd	401.35 ± 33.10 abc	381.40 ± 24.12 ab	381.10 ± 34.51 ab	384.96 ± 38.76 ab
Methionine	174.45 ± 17.68 cdefg	160.45 ± 9.61 abcdefg	158.96 ± 7.44 abcdefg	152.62 ± 11.57 abcdef	154.19 ± 8.83 abcdef	149.12 ± 12.50 abcde	147.33 ± 13.80 abcde
Isoleucine	322.19 ± 34.05 abcd	314.59 ± 20.35 abcd	294.17 ± 14.82 abcd	282.14 ± 22.78 abc	279.74 ± 17.28 abc	267.90 ± 23.78 ab	266.40 ± 26.31 ab
Leucine	550.91 ± 59.41 abcd	545.07 ± 36.40 abcd	516.08 ± 26.98 abc	514.15 ± 42.86 ab	498.84 ± 32.01 ab	498.21 ± 45.64 ab	485.47 ± 49.31 a
Tyrosine	19.84 ± 0.56 bcd	18.38 ± 0.72ab	18.75 ± 1.14 abc	18.03 ± 0.40 ab	17.82 ± 0.79 ab	18.17 ± 0.29 ab	19.08 ± 0.40 abc
Phenylalanine	186.37 ± 19.00 abcd	183.83 ± 11.24 abcd	170.61 ± 8.07 abc	168.43 ± 12.94 abc	167.41 ± 9.72 abc	162.93 ± 13.81 abc	159.03 ± 15.04 abc
Lysine	372.07 ± 39.58 abcd	364.85 ± 23.85 abcd	351.20 ± 17.93 abcd	349.59 ± 28.62 abcd	342.38 ± 21.49 abc	325.30 ± 29.22 ab	329.47 ± 32.92 ab
Histidine	178.00 ± 18.07 abcd	173.13 ± 10.50 abcd	167.20 ± 7.89 abcd	169.36 ± 13.01 abcd	155.59 ± 8.93 abc	150.27 ± 12.61 ab	154.42 ± 14.55 abc
Arginine	7.96 ± 0.78 a	8.07 ± 1.22 a	7.31 ± 1.45 a	7.71 ± 1.05 a	6.81 ± 1.32 a	7.66 ± 0.98 a	7.62 ± 0.89 a
Proline	107.44 ± 10.25 abcd	99.82 ± 5.40 abc	101.24 ± 4.32 abcd	97.09 ± 6.76 ab	92.20 ± 4.69 ab	94.20 ± 7.29 ab	91.68 ± 7.96 ab
Total	6789.13 ± 726.05 abc	6609.80 ± 435.37 abc	6305.83 ± 324.66 abc	6095.97 ± 501.37 ab	5977.96 ± 377.43 a	5858.87 ± 529.67 a	5831.17 ± 585.63 a

Table 1 continued

Amino acid (mg/100 mL)	Ethanol concentration (% v/v)				
	39	42	45	48	51
Aspartic acid	348.43 ± 27.58 a	338.34 ± 24.50 a	331.30 ± 43.04 a	327.45 ± 24.08 a	324.55 ± 31.00 a
Threonine	263.53 ± 20.46 a	250.19 ± 17.70 a	249.88 ± 32.06 a	251.72 ± 18.14 a	247.75 ± 23.28 a
Serine	184.62 ± 13.84 a	181.93 ± 12.44 a	179.27 ± 22.54 a	180.72 ± 12.57 a	177.59 ± 16.21 a
Glutamic acid	610.88 ± 49.58 a	602.45 ± 44.90 a	588.09 ± 77.67 a	581.09 ± 43.99 a	566.22 ± 55.35 a
Glycine	2210.42 ± 183.72 ab	2180.45 ± 166.79 ab	2160.78 ± 289.78 ab	2192.41 ± 170.56 ab	2017.15 ± 201.37 a
Alanine	134.89 ± 9.67 abc	133.67 ± 8.70 abc	132.46 ± 16.22 abc	127.34 ± 8.38 ab	119.79 ± 10.40 a
Valine	379.74 ± 30.20 ab	370.35 ± 26.97 ab	377.01 ± 49.20 ab	343.89 ± 25.38 a	349.60 ± 33.52 a
Methionine	138.40 ± 9.97 abcd	140.14 ± 9.21 abcd	135.90 ± 16.69 abc	129.75 ± 8.57 ab	123.16 ± 10.73 a
Isoleucine	262.73 ± 20.39 ab	270.35 ± 19.26 ab	258.00 ± 33.15 ab	245.80 ± 17.67 a	242.79 ± 22.77 a
Leucine	482.78 ± 38.83 a	474.44 ± 35.01 a	479.16 ± 62.98 a	476.16 ± 35.77 a	460.66 ± 44.71 a
Tyrosine	18.86 ± 0.42 abc	17.71 ± 0.59 a	18.57 ± 0.86 abc	18.44 ± 0.53 ab	18.26 ± 0.29 ab
Phenylalanine	159.84 ± 11.76 abc	158.42 ± 10.63 abc	159.02 ± 19.80 abc	156.71 ± 10.68 ab	153.91 ± 13.84 a
Lysine	328.02 ± 25.86 ab	316.12 ± 22.79 a	313.27 ± 40.61 a	319.61 ± 23.48 a	301.95 ± 28.73 a
Histidine	155.33 ± 11.38 abc	149.98 ± 9.97 ab	144.64 ± 17.87 ab	145.40 ± 9.79 ab	139.68 ± 12.40 a
Arginine	7.53 ± 1.10 a	7.87 ± 1.15 a	7.41 ± 0.65 a	7.36 ± 1.17 a	7.00 ± 1.00 a
Proline	94.42 ± 6.28 ab	89.59 ± 5.31 ab	87.79 ± 10.19 ab	89.03 ± 5.38 ab	85.99 ± 7.00 a
Total	5780.43 ± 458.40 a	5681.99 ± 412.83 a	5622.56 ± 732.00 a	5592.87 ± 413.15 a	5336.03 ± 510.50 a

Data in the same row with different letters are significantly different ($P < 0.05$)

Fig. 5 The sensory profiles of four ethanol-treated fish sauce samples based on the QDA test-eleven attributes by ten panelists. Values with different letters mean that the values are significantly different ($P < 0.05$)

Standard for Fish Sauce (Codex Alimentarius Commission 2013).

The results indicate that ethanol treatment decreases total soluble nitrogen and amino acids nitrogen contents in fish sauce. However, the fish sauce keeps in the first grade if treated by no more than 21% (v/v) of ethanol.

Effect of ethanol treatment on free amino acids composition

The free amino acids of fish sauce are nutritionally important especially in some coastal regions where fish sauce serves as a significant source of dietary protein (Hjalmarsson et al. 2007). Besides, it has also been reported that free amino acids contribute to the odor and taste of fish sauce (Yimdee and Wang 2016). Table 1 shows the free amino acids compositions of the untreated fish sauce and ethanol-treated fish sauce. Glycine, glutamic acid, leucine and valine were prominently high in the untreated fish sauce. Specially, glycine (3072.78 mg/100 mL) accounted for 38.53% of the total free amino acids. The amounts of all free amino acids contents decreased with the increased ethanol concentration. As compared with that of the untreated fish sauce, the total free amino acids content reduced by 17.12 and 33.08% when treated by 21% (v/v) and 51% (v/v) of ethanol, respectively. The results indicate that ethanol treatment decreases all sixteen kind of free amino acids detected in this study, but the decrease rate was acceptable when the ethanol concentration was not more than 21% (v/v). The reduction of free amino acids content might be attributed to

the decrease of solubility by the addition of ethanol (Nozaki and Tanford 1971).

Sensory analyses

The sensory profiles of fish sauce samples based on quantitative descriptive analysis (QDA) test-eleven attributes by ten panelists are shown in Fig. 5. It was observed that the taste of salty became significantly weaker by ethanol treatment than that of the untreated fish sauce ($P < 0.05$). Besides, ethanol treatment decreased the scores for meaty, sour, fishy and umami while increased the scores for alcohol, rancid and fecal, but there was no significant difference ($P > 0.05$). Cheesy and roast smell became much stronger in ethanol-treated fish sauce than that of the untreated fish sauce ($P < 0.05$). It was worth noting that the salt reduction process in this study significantly weakened the odor of ammonia ($P < 0.05$), which was the off-flavor of fish sauce. The results indicate that ethanol treatment significantly weakens the taste of salty and the off-flavor odor of ammonia, but enhances cheesy and roast smell.

Conclusion

The salt concentration of fish sauce significantly decreased if treated by more than 12% (v/v) of ethanol. Besides, ethanol treatment reduced dry weight, total soluble nitrogen content and amino acids nitrogen content of fish sauce, but the decline was acceptable when the ethanol

concentration was not more than 21% (v/v). Sensory analyses showed that the taste of salty and the odor of ammonia were significantly weakened by ethanol treatment. This study demonstrated that ethanol treatment might be used as a potential way to reduce salt concentration in high-salt fish sauce.

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References

- AOAC (1995) Official method of analysis, 16th edn. Association of Official Analytical Chemists, Washington
- Bawornruttanaboonya K, Devahastin S, Yoovidhya T, Chindapan N (2015) Mathematical modeling of transport phenomena and quality changes of fish sauce undergoing electrodialysis desalination. *J Food Eng* 159:76–85
- Chindapan N, Devahastin S, Chiewchan N (2009) Electrodialysis desalination of fish sauce: electrodialysis performance and product quality. *J Food Sci* 74:E363–E371
- Chindapan N, Devahastin S, Chiewchan N (2010) Effect of electrodialysis pretreatment on physicochemical properties and morphology of spray-dried-fish sauce powder. *J Food Eng* 99:31–39
- Chindapan N, Devahastin S, Chiewchan N, Sablani SS (2011) Desalination of fish sauce by electrodialysis: effect on selected aroma compounds and amino acid compositions. *J Food Sci* 76:S451–S457
- Choi E-Y, Choi J-H, Moon S-H (2003) An electrodialysis model for determination of the optimal current density. *Desalination* 153:399–404
- Codex Alimentarius Commission (2013) Standard for fish sauce (Amendment 2012, 2013) (CODEX STAN 302-2011), Rome, Italy
- Fidaleo M, Moresi M, Cammaroto A et al (2012) Soy sauce desalting by electrodialysis. *J Food Eng* 110:175–181
- Galvis-Sánchez AC, Tóth IV, Portela A et al (2011) Monitoring sodium chloride during cod fish desalting process by flow injection spectrometry and infrared spectroscopy. *Food Control* 22:277–282
- Gildberg A (2001) Utilisation of male Arctic capelin and Atlantic cod intestines for fish sauce production—evaluation of fermentation conditions. *Bioresour Technol* 76:119–123
- Gowda SGS, Narayan B, Gopal S (2016) Bacteriological properties and health-related biochemical components of fermented fish sauce: an overview. *Food Rev Int* 32:203–229
- Hjalmarsson GH, Park JW, Kristbergsson K (2007) Seasonal effects on the physicochemical characteristics of fish sauce made from capelin (*Mallotus villosus*). *Food Chem* 103:495–504
- Israr T, Rakha A, Sohail M et al (2016) Salt reduction in baked products: strategies and constraints. *Trends Food Sci Technol* 51:98–105
- Jiang W (2014) Study on the screening, taxonomic analysis and application of one novel, salt-tolerant and high efficient biogenic amines degrading bacterium (doctoral thesis)
- Jiang J-J, Zeng Q-X, Zhu Z-W, Zhang L-Y (2007) Chemical and sensory changes associated Yu-lu fermentation process—a traditional Chinese fish sauce. *Food Chem* 104:1629–1634
- Jundee J, Devahastin S, Chiewchan N (2012) Development and testing of a pilot-scale electrodialyser for desalination of fish sauce. *Procedia Eng* 32:97–103
- Klomklao S, Benjakul S, Visessanguan W et al (2006) Effects of the addition of spleen of skipjack tuna (*Katsuwonus pelamis*) on the liquefaction and characteristics of fish sauce made from sardine (*Sardinella gibbosa*). *Food Chem* 98:440–452
- Lopetcharat K, Choi YJ, Park JW, Daeschel MA (2001) Fish sauce products and manufacturing: a review. *Food Rev Int* 17:65–88
- Nozaki Y, Tanford C (1971) The solubility of amino acids and two glycine peptides in aqueous ethanol and dioxane solutions establishment of a hydrophobicity scale. *J Biol Chem* 246(7):2211–2217
- State Bureau of Internal Trade (1999) Recommended commercial standard for fish sauce (SB/T 10324-1999), Beijing, China
- Yimdee T, Wang X-C (2016) Comparison of odor and taste of commercial brand fish sauces from east and south east Asian countries. *Int J Food Prop* 19:873–896
- Yongsawatdigul J, Rodtong S, Raksakulthai N (2007) Acceleration of Thai fish sauce fermentation using proteinases and bacterial starter cultures. *J Food Sci* 72:M382–M390
- Zarei M, Najafzadeh H, Eskandari MH et al (2012) Chemical and microbial properties of mahyaveh, a traditional Iranian fish sauce. *Food Control* 23:511–514