

Article

The Effect of Transglutaminase and Protease A2SD on Sensory Characters and Physical–Chemical Properties of Soy Sauce

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Abstract

N-acetyl-amino acids were additives for improving kokumi of soy sauce, which was obtained by Transglutaminase (TG) and protease A2SD using amino acid and acetic acid in aqueous solution. In this study, we studied sensory characters and physical–chemical properties of soy sauce treated with TG, TG and acetic acid, protease A2SD, protease A2SD and acetic acid (T, TA, P and PA). T and P had higher umami, sweetness, saltiness, kokumi, amino acid nitrogen and colour, and lower bitterness than control. Addition of acetic acid could increase this effect which increased by 103%, 17%, 18%, 18%, 5%, 21%, respectively, and decreased by 30% in TA. Chemical compound analysis indicated soy sauce treated with TG and protease A2SD had lower molecular weight distribution and higher free amino acids, *N*-acetyl-amino acids and *N*-acetyl-dipeptides content, which might resulted in taste difference of different soy sauces. This study offered an alternative method for improving kokumi of soy sauce by *N*-acetyl-amino acids.

Keywords: soy sauce; taste improvement; transglutaminase; protease A2SD; *N*-acetyl-amino acid

1. Introduction

Soy sauce, also called koji and *Jiangyou* in Chinese, is a popular condiment in asian countries. In China, soy sauce had 2000 years history and it has become an important part of the diet [1]. Traditional soy sauce is produced by two steps, including solid-state fermentation and brine fermentation. Solid-state fermentation was also called in koji, which used soybeans and wheat to produce microbes, usually *Aspergillus oryzae* or *Aspergillus sojae*. Moromi is conducted by immersing the resulting koji in a brine solution and left to ferment for several months up to 4 years by using the two-step [2,3] process. In this fermentation process, a variety of chemical compounds were produced to give special sensory quality to soy sauce, including vitamin, mineral, amino acids, peptides, etc. [4]. With rising quality of life, researchers focus on optimal production technology of soy sauce to improve quality of soy sauce.

Kokumi was defined as a feeling of interaction between food and mouth including mouth fullness, richness, and fullness, which was one of the most important sensory qualities of soy sauce deciding people's acceptability of soy sauce. Kokumi improvement became an important content of the soy sauce industry. For example, optimal ratio of soybean and brine, microorganisms types in the brewing process had a kokumi improvement on soy



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sauce, which was attributed to new kokumi compound production and different chemical composition [1–3,5–7]. Kokumi compound (producing kokumi) was the key to produce kokumi in soy sauce which could react with taste compound in soy sauce to kokumi, including γ -glutamyl peptide and MSG and NaCl, amadori, and non-proteinic amino acid derivatives (*N*-acetyl-amino acid, *N*-lactyl-amino acids and *N*-succinyl-amino acids) and MSG and NaCl [2,8]. These compounds lack in soy sauce and kokumi compound supplements and are the most common ways for improving kokumi of soy sauce. For example, Tominak et al. found that glutaminase could increase umami and kokumi of soy sauce which attributed to increase in free glutamate and glutamate polypeptides [9]. Lin et al. 2023 [10] found that umami, richness and fullness of soy sauce was increased by addition of 0.5 g/L of *N*-acetyl-amino acid. However, these compounds lack in nature and obtained through chemical preparation which increased production price of the related soy sauce.

TG and protease A2SD were important food additives which acted as crosslinker and hydrolase for protein, respectively [11,12]. They were main enzymes for preparing *N*-acetyl-amino acids applied in food, including *N*-acetyl-Val/Ile/Leu/Met/Phe [4]. Acetic acid and amino acids were substrates of this reaction, for example, *N*-acetyl-Val/Leu/Ile/Met/Trp/Phe could be synthesized by TG using 400 mM acetic acid and 100 mM Val/Leu/Ile/Met/Trp/Phe at pH = 6.0 and 40 °C, respectively, with a yield in a range of 3.62–22.75%. Soy sauce was rich in acetic acid and these amino acids (Val/Ile/Leu/Met/Phe), but whether TG and protease A2SD could synthesize *N*-acetyl-amino acid in soy sauce and its effect on sensory characters and physical–chemical properties of soy sauce remained unknown. Furthermore, acetic acid and amino acids (Val/Ile/Leu/Met/Phe) were sour and bitter compounds and its decrease caused by TG and protease A2SD might have reduced the bitterness of soy sauce and improved the quality of the soy sauce [10]. TG and protease A2SD were protease which had hydrolysis activity for soy protein which was beneficial for improving umami of soy sauce [10,13].

Acetic acid was the most easiest factor for mediating *N*-acetyl-amino acids content and type synthesized by TG and protease A2SD [4]. In this study, therefore, soy sauces were prepared by TG, TG and acetic acid, protease A2SD, and protease A2SD and acetic acid, respectively, at their optimal condition for synthesizing *N*-acetyl-amino acid, and their sensory characters and physical–chemical characters were evaluated. Astree II electronic tongue was a new diagnostic technique used to analyze food taste which was widely used for dairy products, dry ham, soy sauce, etc. [14–16]. For example, distinguishing sourness, sweetness, bitterness, saltiness, umami, and kokumi of different soy sauces by Astree II electronic tongue which was in agreement with human sensory results as well, fitted the logarithm model [15]. Therefore, taste character of soy sauce was evaluated by Astree II electronic tongue and physical–chemical properties including total nitrogen, amino acid nitrogen, colour, NaCl content, total acid, total sugar analyzed according to the related method. Key chemical compounds of soy sauce including molecular weight distribution, free amino acids composition, *N*-acetyl-amino acids and *N*-acetyl-dipeptides, and peptide contents were identified and quantified by HPLC and LC-MS-MS, respectively.

2. Materials and Methods

2.1. Materials

Soy sauce was purchased from Haoji Food Brewing Co., Ltd. (Jining, China). TG and protease A2SD were purchased from Qingrui Biological Technology Co., Ltd. (Shanghai, China) and Amano Enzyme Preparation Co., Ltd. (Shanghai, China), respectively. Other reagents were purchased from Plano Biotechnology Co. (Guangzhou, China) which were of analytical grade unless HPLC-grade acetonitrile and methanol.

2.2. Enzymic Treatment of Soy Sauce by TG and Protease A2SD

Firstly, five groups of soy sauce (300 g/group) were divided into two groups where one group was mixed with 0.5% (*w/w*) acetic acid and all soy sauces were adjusted to pH = 6.0 with 10 M NaOH and 6 M HCl. Then, 0.4% (*m/v*) TG and 0.1% (*m/v*) protease A2SD were added into the soy sauces with or without acetic acid, respectively. After reaction for 24 h, soy sauces were incubated in boiling water for 10 min for killing the enzymes. Soy sauces untreated with protease A2SD enzyme was named control and those treated with TG, TG and acetic acid, protease A2SD, and acetic acid were named T group, TA group, P group and PA group, respectively.

2.3. Taste Evaluation of Soy Sauce

Five gram of soy sauce was dissolved into 500 mL water and its taste was evaluated by Astree II electronic tongue system analysis equipped with seven sensors (ZZ, JE, BB, CA, GA, HA, and JB) (Alpha MOS, Toulouse, France) according to the previous method with a slight modification [15]. Before the experiment, the electronic tongue system was calibrated and diagnosed using distilled water, 0.01 mol/L sucrose, 0.01 mol/L NaCl, and 0.01 mol/L monosodium glutamate (MSG), respectively. Every sample was measured with seven replications, taste collection time, aftertaste collection time, and cleaning times were 30 s, 30 s, and 300 s, respectively. Finally, the basic tastes including sweet, bitter, salty, umami and thick taste were scored, and the last four data were selected for analysis using the PCA method.

2.4. Determination of Basic Indexes of Soy Sauce

Basic chemical indexes of soy sauce including total nitrogen, NaCl content, total sugar and colour were determined referring to GB5009.5-2016, GB18186-2000, GB/T12456-2008, and GB/T15672-2009 modified by previous studies, respectively [5,17]. Amino acid nitrogen and total acid were determined by the titration method [18].

2.5. Molecular Weight (MW) Distribution

Molecular weight of soy sauces were performed by size exclusion chromatography (SEC) equipped by SEC column (TSK gel 2000-SWXL, 7.8 mm × 30 cm, particle size 5 µm; Tosoh Corporation, Tokyo, Japan) according to the previous method [19]. Sample was detected at a condition of 70% solvent A (0.04% TFA in purified water), 30% solvent B (0.034% TFA in acetonitrile), sample injection volume of 10 µL, flow rate of 0.3 mL/min and detected wavelength of 220 nm. The molecular weight standards used were cytochrome c (12,327 Da), insulin (5733.49 Da), thymosin-α (3108.32 Da), vitamin B₁₂ (1355.37 Da), and uridine (244.2 Da).

2.6. Free Amino Acid Analysis of Soy Sauce

Free amino acids were analyzed by automatic amino acid analyzer (A300 advanced, Membrapure, Berlin, Germany) according to the previous method [20]. Soy sauce was diluted to 5 mg/mL using distilled water and was placed at room temperature for 30 min. Then, 10% sulfosalicylic acid was added into soy sauce with at a volume ratio of 4:1 (*v/v*) and incubated at 4 °C for 60 min for deproteinization. The final sample was centrifugated at a speed of 10,000 r/min with 4 °C for 15 min and the supernatant was collected and filtered with a 0.22 µm Millex® PVDF needle filter which was used for amino acids analysis.

2.7. N-Acetyl-Amino Acid and N-Acetyl-Dipeptides Analysis of Soy Sauce

Soy sauce was mixed with ethyl acetate according to the ratio of 1:10, and the mixture was stirred for 30 min in room temperature. Then, ethyl acetate was obtained and dried in rotary evaporator at a condition of 60 °C and 50 bar. Finally, the obtained solid was

prepared at a concentration of 5 mg/mL with aqueous solution for LC-MS-MS analysis according to previous methods with a slight modification [21]. LC-MS-MS consisted of Ultra-high phase liquid chromatograph (Agilent 1290, Bruker, German) (UPLC) coupled with a RRHD SB-C18 column (Agilent ZORBAX 2.1 mm × 50 mm, 1.8 µm, 30 °C) and mass spectrometer (Bruker maXis, Bruker, German). Firstly, HPLC included two mobile phases (A: 0.1% formic acid–acetonitrile solution and B: 0.1% formic acid–water solution) and its separation condition was 0–5 min A:B = 100:0, 10 min A:B = 90:10, 12 min A:B = 85:15, and 13 min A:B = 100:0, injection volume of 5 µL. Mass spectrometer conditions referred to previous method, and the obtained wiff2 date was converted into Mgf files using ProteoWizard 3.0. Short peptides were automatically despectrured by PepOS peptideomics comprehensive analysis software (Wuyi University, 2022) according to the following steps: peptide identification length set 2–5, primary ion deviation 0.005 Da, secondary ion deviation 0.02 Da, solution core number 6, product ion cluster matching types a, b and y, set the complete matching species of product ion cluster to at least one, Set the N-terminal modification to acetyl modification.

2.8. Statistical Analysis

Statistical analysis was done by SPSS 8.0 using a binomial distribution and one-way ANOVA with Fisher LSD posthoc test for taste re-engineering experiments and taste profile of the fractions, respectively, and $p < 0.05$ indicated the significantly difference in different samples.

3. Results and Discussion

3.1. Effect of TG and Protease A2SD on Taste Characters of Soy Sauce

Difference in taste of soy sauces treated with TG and protease A2SD are shown in Figure 1. Soy sauce presented five tastes including umami, sweetness, saltiness, thickness and bitterness with a score value of 2.9, 4.1, 5.1, 5.1 and 6.1, respectively. Soy sauce treated with TG and protease A2SD had higher umami, sweetness, saltiness, and kokumi significantly increasing to 4.2, 4.3, 5.2, and 5.2, and 4.1, 4.3, 6.0, and 5.2, respectively, and lower bitterness decreasing to 4.2. Umami, sweetness, saltiness and thickness further significantly increased to 5.9, 4.8, 6.0, and 6.1, and 4.3, 4.4, 6.0 and 6.0 when soy sauce was treated with TG or protease A2SD combined with acetic acid, respectively, but bitterness had slightly increased to 4.9 for soy sauce treated with protease A2SD and acetic acid. This indicated that TG and protease A2SD treatment were effective for improving the taste of soy sauce, especially when combined with acetic acid. This improvement was better than previous method of adding *N-acetyl-amino acid* and umami-enhancing effect than glutaminase [9,10]. It might be due to that TG and protease A2SD reacted with soy sauce which changed the taste compound composition of soy sauce. For example, soy sauce was rich in free amino acid and acetic acid which could be used to synthesize *N-acetyl-amino acids* by TG and protease A2SD in aqueous solution. *N-acetyl-amino acids* were kokumi compounds which could give kokumi and increase umami, saltiness and sweetness of the soy sauce [10]. When acetic acid was added, more *N-acetyl-amino acids* was synthesized by TG and protease A2SD, which gave the soy sauce a stronger kokumi [10]. Meanwhile, decrease in bitter amino acids Val/Ile/Leu/Phe/Met/Trp might caused decrease in bitterness of the soy sauce. However, *N-acetyl-amino acids* caused bitterness which was more than 10 fold than corresponding amino acid when its content was over the kokumi threshold [7]. This might explain why bitterness increased in soy sauce treated with TG and acetic acid. Additionally, peptide and protein in soy sauce could be hydrolyse by TG and protease A2SD due to their hydrolysis activity of protease which further increased umami and decreased bitterness

of soy sauce [22]. This was similar to molecular weight distribution, free amino acid and *N*-acetyl-amino acid content.

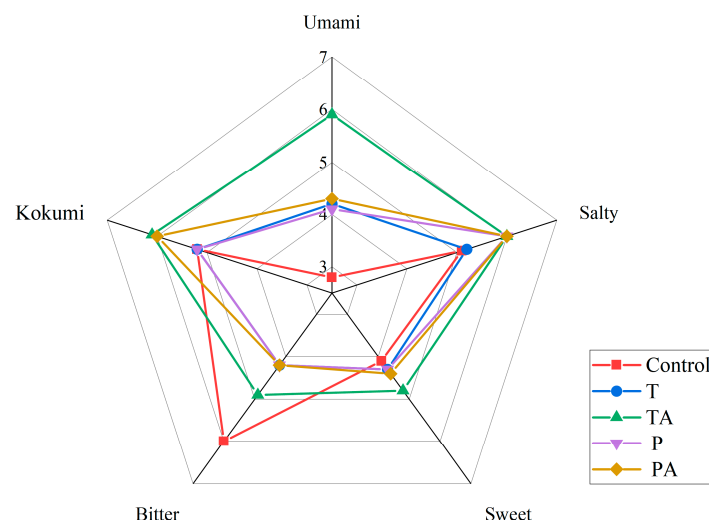


Figure 1. The effect of TG and Protease A2SD treatments on taste of soy sauce.

3.2. Effect of TG and Protease A2SD on Basic Characters of Soy Sauce

Except taste, total nitrogen, amino acid nitrogen, sugar content, total acid, NaCl content, and colour were important indexes deciding on the quality of soy sauces. As shown in Table 1, amino acid nitrogen and colour were significantly affected by TG and Protease A2SD, and total nitrogen, sugar content, total acid, and NaCl content were not affected by these two enzymes. As shown in Table 1, amino acid nitrogen content was significantly higher in soy sauce treated with TG and protease A2SD than control which were 1.28 g/100 mL, 1.34, and 1.36 g/100 mL, respectively. Compared to these soy sauces, amino acid nitrogen content slightly decreased in soy sauce treated with TG (or Protease A2SD) and acetic acid. This might be due to that TG and protease A2SD degraded protein and peptide in soy sauces into short peptides and free amino acids which were two proteases [23,24]. In presence of acetic acid, TG and protease A2SD catalyst a large amount of amino acids to react with acetic acid to synthesize *N*-acetyl-amino acid. Therefore, amino acid nitrogen decreased at a condition of combining addition of TG and protease A2SD with acetic acid. This indicated that TG and protease A2SD was more suitable for improving amino acid nitrogen of soy sauce.

Table 1. The effect of TG and protease A2SD treatment on basic indexes of soy sauce.

Groups	Amino Acid Nitrogen	Total Nitrogen	Total Acid	NaCl Content	Sugar Content	Colour
Control	1.28 ± 0.02 ^a	2.17 ± 0.02 ^a	1.30 ± 0.03 ^a	15.26 ± 0.41 ^a	1.61 ± 0.03 ^a	5.12 ± 0.32 ^a
T	1.37 ± 0.03 ^b	2.21 ± 0.04 ^a	1.30 ± 0.01 ^a	15.21 ± 0.43 ^a	1.89 ± 0.06 ^a	6.16 ± 0.09 ^b
TA	1.35 ± 0.02 ^{ab}	2.18 ± 0.06 ^a	1.30 ± 0.03 ^a	14.77 ± 0.16 ^a	1.85 ± 0.09 ^a	6.17 ± 0.08 ^b
P	1.36 ± 0.04 ^b	2.24 ± 0.08 ^a	1.30 ± 0.02 ^a	15.06 ± 0.38 ^a	1.65 ± 0.01 ^a	6.91 ± 0.16 ^c
PA	1.31 ± 0.02 ^a	2.17 ± 0.13 ^a	1.30 ± 0.01 ^a	14.77 ± 0.62 ^a	1.57 ± 0.13 ^a	6.46 ± 0.14 ^b

Note: 'a', 'b', 'c' represented significantly difference in column ($p < 0.05$).

The dye content in the soy sauce was constant. As shown in Table 1, colour of soy sauce was higher treated with TG and protease A2SD than control which were 5.12 g/100 mL, 6.16 g/100 mL, and 6.91 g/100 mL, respectively. Colour was lower when soy sauce added TG (or Protease A2SD) and acetic acid than enzymes, decreasing to 6.46 g/100 mL compared to soy sauce treated with protease A2SD. This might be due to that soy sauce

was hydrolysed by TG and protease A2SD to produce amino acid and peptides, resulting in increase in colour, which were reported to have hydrolysis of protein [23,24]. In presence of acetic acid, free amino acids in soy sauce reacted with synthesized *N*-acetyl-amino acids and water with TG and Protease A2SD, which had a diluted effect on solid content in soy sauce [10]. This indicated that TG and protease A2SD combined acetic acid were more suitable for improving the colour.

3.3. Molecular Weight Distribution of Soy Sauce

The effect of TG and protease A2SD treatment on molecular weight distribution of soy sauce are shown in Figure 2. Molecular weight of soy sauce consisted of 0–500 Da and 500–1k Da, where 0–500 Da account for 65.2%. Molecular content (<500 Da) increased to 70% and 68% with addition of TG and protease A2SD, respectively. When acetic acid was added, molecular content (<500 Da) was further increased to 69% and 67%, respectively. This indicated that TG and protease A2SD treatment resulting in molecular weight decrease in soy sauces, which might resulted in increase in umami [23,24]. This might be due to that TG and protease A2SD were protease which could degrade peptide and soy protein in soy sauce. Acetic acid is an organic acid which has hydrolysis activity for protein in high temperature condition, and thus lowest molecular weight was found in soy sauces in the presence of acetic acid.

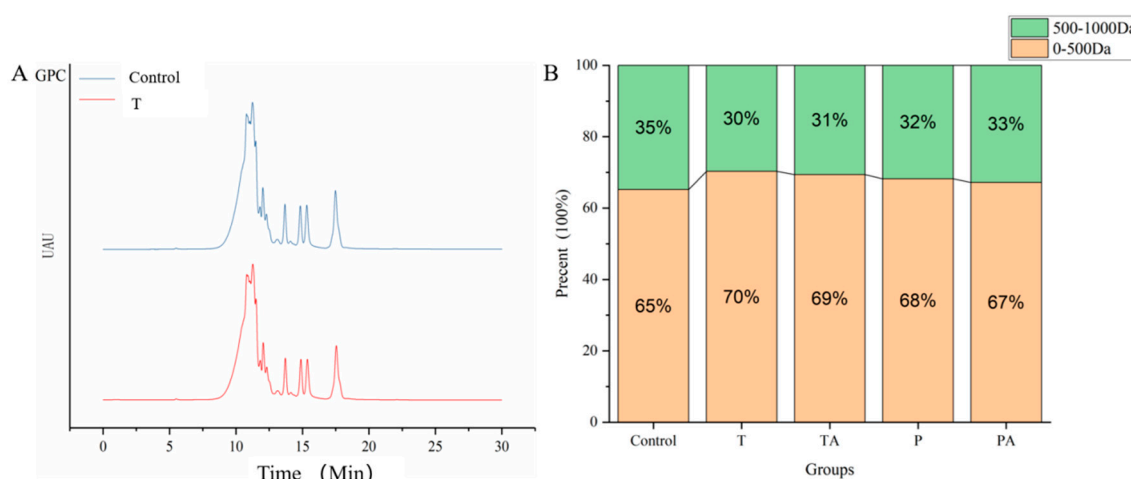


Figure 2. The effect of TG and protease A2SD treatment on molecular weight distribution of soy sauce (A) figure of different soy sauces; (B) chemical compound composition and relative content of soy sauce).

3.4. Free Amino Acids Composition

The effect of TG and protease A2SD on free amino acid composition of soy sauce are shown in Table 2. Soy sauce mainly included Glu, Asp, Val, Leu, Ile, Met, Phe, Trp, and Tyr and total free amino acid content was 42.54 mg/g. Free amino acid contents are 56.82 mg/g and 54.56 mg/g with addition of TG and protease A2SD, respectively, where Thr, Glu, Ile, and Leu content increased over 35%. When acetic acid was added into soy sauce, free amino acid content decreased to 49.87 mg/g and 50.52 mg/g, respectively, especially for Val, Met, Leu, Phe and Trp. This might be due to that protein and peptides were degraded into free amino acid by TG and protease A2SD which were the two proteases [23,24]. In presence of acetic acid, a large amount of amino acid reacted with acetic acid to synthesize *N*-acetyl-amino acid under catalyst of TG and protease A2SD, and thus resulting in free amino acid content decrease when acetic acid was added [10].

Table 2. Free amino acid composition of soy sauces.

Taste Attributes	Amino Acid Type	Amino Acid Content (mg/g)					Threshold Values (g/L ⁻¹) in Water
		Control	T	TA	P	PA	
Umami	Asp	3.66	4.97	4.25	3.73	3.45	1.00 ^a
Umami	Glu	8.81	11.88	10.53	11.76	10.60	0.30 ^a
Sweet	Ser	2.52	3.47	2.92	3.00	2.71	2.60 ^a
Sweet	Thr	1.97	2.63	2.31	2.48	2.21	1.50 ^a
Sweet	Gly	1.67	2.29	1.94	3.73	3.45	1.30 ^a
Sweet	Ala	2.52	3.43	3.04	3.55	3.18	0.60 ^a
Sweet/bitter	Lys	2.12	2.80	2.49	2.91	2.57	0.50 ^a
Sweet/bitter	Pro	2.53	2.50	2.60	2.73	2.13	3.00 ^a
Bitter	Val	3.06	4.09	3.60	4.05	3.71	0.40 ^a
Bitter	Met	0.72	1.01	0.86	0.93	0.89	0.30 ^a
Bitter	Ile	2.73	3.73	3.24	3.65	3.33	0.90 ^a
Bitter	Leu	4.03	5.56	4.83	5.53	5.50	1.90 ^a
Bitter	Phe	2.76	3.78	3.32	3.80	3.44	0.91 ^a
Bitter	His	1.38	1.81	1.61	1.78	1.65	0.90 ^a
Bitter	Arg	1.12	1.49	1.27	1.43	1.31	0.20 ^a
Bitter/sweet	Tyr	0.89	1.25	0.98	1.03	0.92	0.82 ^a
Tasteless	(Cys) ₂	0.05	0.13	0.08	0.09	0.06	0.50 ^a
	Total content	42.54	56.82	49.87	54.56	50.52	

Note: ^a [5].

Free amino acids were important taste compounds in soy sauce. For example, Asp and Glu were umami with a taste threshold of 1.00 g/L and 0.30 g/L, respectively; Gly, Ala, Thr, and Ser were sweet with a taste threshold of 2.60 g/L, 1.50 g/L, 1.30 g/L, 0.60 g/L, 3.00 g/L, and 0.50 g/L, respectively; Val, Met, Ile, Leu, Phe, His, and Arg were bitter and their taste threshold were 0.40 g/L, 0.30 g/L, 0.90 g/L, 1.90 g/L, 0.91 g/L, 0.90 g/L, 0.20 g/L, and 0.82 g/L, respectively [5]. The increased or decreased amino acid content in soy sauce treated with TG and protease A2SD was higher in their taste threshold, which might contributed to the increase corresponding to the taste of soy sauce.

3.5. N-Acetyl-Amino Acid and N-Acetyl-Dipeptide Compositions of Soy Sauce

N-acetyl-amino acid and *N*-acetyl-dipeptides composition of soy sauces treated with TG and protease A2SD are shown in Tables 3 and 4. A total of 20 *N*-acetyl-amino acids and 51 *N*-acetyl-dipeptides were found in soy sauce. *N*-acetyl-amino acids and *N*-acetyl-dipeptides type and content increased with addition of TG and protease A2SD which had higher relative peak area, except for *N*-acetyl-G. In presence of acetic acid, these results become more significant. This might be due to that *N*-acetyl-amino acids and *N*-acetyl-dipeptides could be synthesized with TG and protease A2SD which were reported to synthesize *N*-acetyl-amino acids and *N*-acetyl-dipeptides using acetic acid and free amino acid and dipeptide in aqueous solution, respectively [10]. In presence of acetic acid, more *N*-acetyl-amino acids and *N*-acetyl-dipeptides were synthesized by TG and protease A2SD. When acetic acid was lacking, TG and protease A2SD could hydrolyse *N*-acetyl-amino acids to corresponding amino acid and acetic acid [10]. TG and protease A2SD might have the highest *K*_m for *N*-acetyl-G which acted as *N*-acetylation donor for synthesis of other *N*-acetyl-amino acids and *N*-acetyl-dipeptides. Additionally, soy sauce presented compound with *N*-acetylation, which could be used to produce *N*-acetyl-amino acids and *N*-acetyl-dipeptides, such as hydrolysis. Therefore, no *N*-acetyl-G was found in soy sauce treated with TG and protease A2SD.

Table 3. *N*-acetyl-amino acid composition of soy sauces.

<i>N</i> -Acetyl-Amino Acid Type	Relative Rebounded				
	Control	T	TA	P	PA
N-Ace-G	1.51×10^4	--	--	--	--
N-Ace-A	1.15×10^5	2.88×10^5	4.07×10^5	1.48×10^5	2.07×10^5
N-Ace-V	2.80×10^4	1.79×10^6	3.44×10^6	2.32×10^6	1.24×10^6
N-Ace-L	3.14×10^4	3.68×10^6	6.80×10^6	2.68×10^6	3.80×10^6
N-Ace-I	3.13×10^4	2.12×10^6	4.80×10^6	3.02×10^6	5.60×10^6
N-Ace-D	1.08×10^4	4.34×10^5	8.15×10^5	5.31×10^5	9.22×10^5
N-Ace-E	1.36×10^6	3.86×10^3	7.79×10^3	2.32×10^3	6.29×10^3
N-Ace-Q	5.42×10^3	3.42×10^5	5.42×10^5	4.16×10^5	8.49×10^5
N-Ace-P	9.40×10^5	3.44×10^6	6.40×10^6	3.14×10^6	6.26×10^6
N-Ace-F	1.05×10^5	8.02×10^6	1.48×10^7	4.32×10^6	2.02×10^7
N-Ace-M	5.46×10^4	8.17×10^5	1.58×10^6	7.76×10^5	2.21×10^6
N-Ace-W	6.10×10^3	7.52×10^5	1.12×10^6	8.63×10^5	2.24×10^6
N-Ace-S	1.17×10^5	2.53×10^5	4.69×10^6	3.11×10^5	5.78×10^6
N-Ace-T	1.42×10^3	3.64×10^5	5.69×10^5	3.44×10^5	6.97×10^5
N-Ace-C	--	6.59×10^4	9.42×10^4	5.03×10^4	8.42×10^4
N-Ace-N	1.08×10^4	4.34×10^5	8.15×10^5	5.34×10^5	9.73×10^5

Note: "--" indicated that no detected.

Table 4. *N*-acetyl-dipeptide composition of soy sauces.

<i>N</i> -Acetyl-Dipeptides	Relative Peak Area				
	Control	T	TA	P	PA
N-Ac-DF	4.53×10^4	7.89×10^4	1.19×10^6	5.98×10^4	0.22×10^6
N-Ac-DG	1.48×10^4	2.56×10^4	5.69×10^3	1.21×10^4	3.99×10^3
N-Ac-DM	1.00×10^5	1.54×10^5	1.40×10^5	0.64×10^5	0.50×10^5
N-Ac-DV	1.82×10^3	1.66×10^3	5.94×10^5	2.32×10^3	9.05×10^5
N-Ac-DY	5.79×10^4	9.08×10^4	2.25×10^4	4.08×10^4	2.33×10^4
N-Ac-EM	1.33×10^4	5.93×10^3	1.02×10^5	3.99×10^3	0.22×10^5
N-Ac-EF	2.21×10^4	4.79×10^4	9.51×10^5	2.35×10^4	6.23×10^5
N-Ac-FE	3.43×10^4	5.14×10^4	1.46×10^5	3.21×10^4	0.73×10^5
N-Ac-FF	1.02×10^5	2.38×10^5	2.83×10^5	4.43×10^5	6.22×10^5
N-Ac-FV	1.11×10^4	2.26×10^5	1.80×10^5	2.84×10^5	1.05×10^5
N-Ac-GP	6.37×10^4	3.76×10^4	3.40×10^5	--	--
N-Ac-HE	4.52×10^4	6.67×10^4	2.62×10^5	5.67×10^4	1.62×10^5
N-Ac-PV	1.22×10^6	2.28×10^6	1.40×10^9	4.28×10^6	3.40×10^9
N-Ac-QH	1.18×10^4	1.60×10^4	3.26×10^5	--	--
N-Ac-TY	--	--	1.86×10^6	--	--
N-Ac-VE	1.46×10^4	1.16×10^5	1.27×10^{11}	0.23×10^5	1.24×10^{11}
N-Ac-VF	1.38×10^5	2.55×10^5	2.38×10^{10}	4.65×10^5	5.31×10^{10}
N-Ac-VM	8.94×10^4	1.51×10^5	1.89×10^{10}	1.21×10^5	1.04×10^{10}
N-Ac-VP	2.57×10^5	4.28×10^5	1.82×10^6	3.32×10^5	0.65×10^6
N-Ac-VV	8.50×10^4	3.34×10^5	2.05×10^5	4.12×10^5	1.59×10^5
N-Ac-CP	8.78×10^5	1.27×10^6	1.05×10^4	--	--
N-Ac-MD	1.08×10^5	1.45×10^5	3.81×10^3	2.14×10^5	4.11×10^3
N-Ac-MV	6.64×10^5	1.04×10^6	5.82×10^4	--	1.19×10^4
N-Ac-QE	3.40×10^6	7.39×10^6	6.66×10^6	5.94×10^6	5.62×10^6
N-Ac-MQ	1.78×10^5	3.04×10^5	3.34×10^4	1.22×10^5	2.33×10^4
N-Ac-ZM	3.83×10^3	8.30×10^3	1.19×10^5	9.75×10^3	5.43×10^5
N-Ac-ZP	1.48×10^5	2.15×10^5	1.88×10^5	1.22×10^5	3.22×10^5
N-Ac-ZDH	9.84×10^4	1.57×10^5	1.53×10^5	--	--

Note: "--" represented no compound.

N-acetyl-amino acids were kokumi compounds which had umami-, salt- and sweet-enhancing effect on soy sauce and give fullness, richness and mouth fullness to soy sauce [10,25–27]. It showed bitter taste and the bitter strength was 10 fold than corresponding bitter amino acid at high concentration condition [28,29]. This could explain why soy sauce had stronger kokumi and slight bitter taste when treated with TG or protease A 2SD and acetic acid, but the specific results need to be further confirmed by determining its content and taste contributor in soy sauce.

4. Conclusions

In this study, the effect of TG and protease A2SD on sensory experiment and physical-chemical properties of soy sauce is studied. Soy sauce had best taste characters at a condition of TG combined with acetic acid, where umami, sweetness, saltiness, kokumi increased for 103%, 17%, 18%, 18%, respectively, and bitterness decreased by 30%. Amino acid nitrogen and colour of soy sauce was increased to 5.5% and 21% by TG combined with acetic acid, respectively. Chemical compound analysis indicated that TG-combined acetic acid lowered relative molecular weight of soy sauce, and increased free amino acids, *N-acetyl-amino acid* and *N-acetyl-dipeptide* content, which might resulted in taste differences in soy sauce. This study deduced preparation technology of *N-acetyl-amino acids* applied in soy sauce. However, taste improvement mechanism of TG-combined acetic acid on soy sauce remained unknown, which would be further studied in the future.

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