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CHANGES IN FISH SAUCE QUALITY THROUGHOUT PRODUCTION

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Abstract

Fish sauce is a seasoning made by combining fish extracts that have been aged and cured in salt. Fish sauce contains various amino acids that contribute to umami flavoring. Since amino acids are produced *via* enzymatic protein decomposition, the composition of umami ingredients changes during fish sauce production. In this study, we have produced fish sauce using horse mackerel and measured levels of histidine, arginine, taurine, and alanine, which are abundant in horse mackerel, to investigate changes in umami components over time.

Horse mackerel was gutted, soaked in saturated brine, and stored at 25 ^{0}C for 13 weeks. The amino acids in the fish sauce were extracted with perchloric acid and extracts were neutralized with KOH. Extract samples were phenyl isothiocyanate-derivatized and analyzed by high-performance liquid chromatography - HPLC on the production start date and after 1, 3, 5, 9, and 13 weeks.

Except for arginine, the amount of each amino acid increased with time, indicating that these amino acids are being produced by proteases. An enzyme that rapidly degrades arginine has been reported to exist in Japanese anchovy. The presence of a homologous enzyme in horse mackerel could explain why arginine was not seen to increase in this study.

To investigate changes in fish sauce quality during production, histidine, alanine, taurine, and arginine levels were measured over time. Histidine, alanine, and taurine content increased over time, but arginine did not.

***Key words**: Fish sauce, Amino acids, HPLC.*

1. Introduction

Fish sauce is a seasoning obtained from the extracts of salt-cured and aged fish [1] and contains inosinic acid (IMP) and various amino acids as umami ingredients. Proteases produce amino acids when proteins are broken down [2], and accumulate in muscles. In contrast, IMPs accumulate in the muscle early postmortem through the breakdown of ATP but are broken down into the taste-neutral components inosine (HxR) and hypoxanthine (Hx) by IMP-degrading enzymes [3]. Therefore, it can be concluded that the composition of the umami components in fish sauce changes because these substances are degraded by enzymes during fish sauce production.

In this study, we prepared fish sauce from horse mackerel and measured the levels of histidine, arginine, taurine, alanine, IMP, and its degradation products, HxR and Hx, which are abundant in horse mackerel, to investigate the changes in quality over time.

2. Materials and Methods

Two horse mackerels (from Nagasaki) were gutted, soaked in 2 L saturated brine, and stored at 25 ^{0}C for 13 weeks.

2.1 Change in ATP-related compounds during fish sauce production

Each fish sauce sample (2.5 mL) was collected, and 4 mL of 10% perchloric acid (Nacalai Tesque Inc., Kyoto, Japan) was added. The solids were removed by centrifugation (11,000 rpm, 10 min, 5 ^{0}C, CR16RN, Eppendorf Himac Technologies Co., Ltd., Ibaraki, Japan), the supernatant was transferred to a 15-mL centrifuge tube, and the volume was fixed to 10 mL with 10% perchloric acid. One milliliter of the supernatant was neutralized with KOH (Kanto Chemical Co. Inc., Tokyo, Japan), the precipitate was removed by centrifugation (12,000 rpm, 5 min, 5 ^{0}C), and the supernatant was transferred to a new 15 mL centrifuge tube. One milliliter of pure water was added to the remaining precipitate, stirred, and centrifuged under the same

conditions, and the supernatant was transferred to a centrifuge tube. This process was repeated and the supernatant was transferred to a new 15 mL centrifuge tube and volume was fixed at 5 mL using pure water. The resultant solution was filtered through a 0.2 µm filter and measured using high-performance liquid chromatography (HPLC, Shimadzu Corporation, Kyoto, Japan). The HPLC conditions were as follows: Column: Cosmosil Packed Column 5C18-PAQ, 4.6 mm I. D. × 150 mm, mobile phase: 20 mM KH_2PO_4 solution (pH 6.0), flow rate: 0.5 mL/min, temperature: 40 °C, detector: UV, wavelength: 260 nm, injection volume: 20 µL, and the measurements were taken at the beginning of production and 1, 3, 5, 9, and 13 weeks later.

2.2 Change in amino acid compounds during fish sauce production

Forty microliters of the solution were prepared as described in Section 2.1., 70 µL ethanol (Imazu Chemical Co. Ltd., Tokyo, Japan), 20 µL triethylamine (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan), and 20 µL phenyl isocyanate (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) were added, and the resulting solutions were allowed to stand for 30 min at 25 °C (PITC derivatization), followed by the addition of 500 µL of a 97 : 3 mixture of buffer acetate (Nacalai Tesque, Inc., Kyoto, Japan) and acetonitrile (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) and filtration through a 0.22 µm filter. The HPLC conditions were as follows: column: 5C18-MS-II, 4.6 I.D. × 150 mm, mobile phase A: 250 mmol/L acetic acid buffer (pH 6.6) : acetonitrile (97 : 3), mobile phase B: acetonitrile : pure water (6 : 4), flow rate: 1 mL/min temperature: 40 °C, detector: UV, wavelength: 254 nm, injection volume 20 µL, gradient: (0 min (B5%)→16 min (B 100%)→20 min (B 5%)→25 min (B 5%). Measurements were taken at the beginning of production and 1, 3, 5, 9, and 13 weeks later.

2.3 Data collection and statistical analysis

Data were collected in triplicate for each sample. The differences between the means of the two groups were tested using the *t*-test, and the three groups were compared using analysis of variance, each at a significance level of 5%. The *t*-test and analysis of variance were performed using Microsoft Excel.

3. Results and Discussion

3.1 Change in ATP-related compounds during fish sauce production

Figure 1 shows the change over time of each ATP-related compound during fish sauce production: from 0.0 µmol/mL in week 0 to 0.78 µmol/mL in week 1, HxR concentration increased ($p < 0.05$), and then decreased to just 0.82 µmol/mL in week 3 ($p > 0.05$) and in week 13, the value dropped to 0 µmol/mL ($p < 0.05$). For Hx, the concentration increased from 0.0 µmol/mL at week 0 to 0.94 µmol/mL at week 9 ($p < 0.05$) and decreased to 0.84 µmol/mL at week 13 ($p < 0.05$). For IMPs without internal organs, the dose decreased from 0.25 µmol/mL at week 0 to 0.0 µmol/mL at week 1 ($p < 0.05$) and then remained unchanged until week 13 ($p < 0.05$).

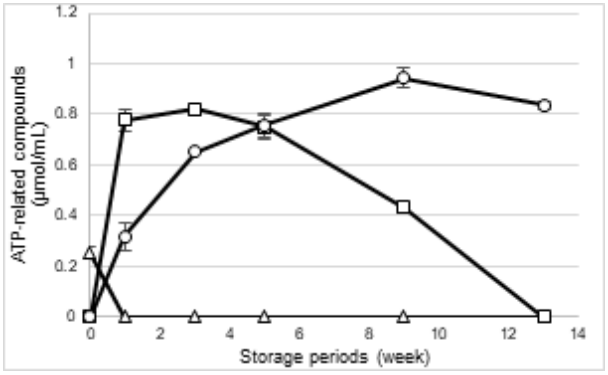

Figure 1. Change over time of each ATP-related compound during fish sauce production
Measurements n = 5.
Error bars indicate the standard deviation.
△:IMP, □:HxR, ○:Hx

Figure 1 shows that IMP disappeared in fish sauce one week after the start of production; HxR concentration increased until week 5 after the start of production, but disappeared by week 13, and Hx concentration increased over time from the start date of production to week 13. The IMP concentration was reported to be 0 mg/100 g when fermented for one month in Chinese anchovy fish sauce [4]. In addition, Hx concentration was reported to be 19.7 mg/100 mL in commercial nuoc mam, Vietnamese fish sauce and 27.1 mg/100 mL in commercial fish sauce, indicating that IMP and HxR were lost in both fish sauce and nuoc mam [5]. Nampula, Thai fish sauce, and nuoc mam were stored for 24 - 60 weeks. In this study, only Hx was detected at 13 weeks of age. IMP is not considered an umami component of fish sauce.

3.2 Change in amino acid compounds during fish sauce production

Figure 2 shows the changes in the amino acid content over time during fish sauce production. Histidine concentration increased from 0.12 µmol/mL at week 0 to 4.91 µmol/mL at week 3 ($p < 0.05$), significantly decreased to 4.31 µmol/mL at week 5 ($p < 0.05$), increased to 5.85 µmol/mL at week 9 ($p < 0.05$), and then decreased to 5.85 µmol/mL at week 13 ($p < 0.05$). Regarding arginine concentration, there was no change from 0 µmol/mL at week 0 to 0 µmol/mL at week 1 ($p < 0.05$); however, its concentration increased to 0.77 µmol/mL at week 5 ($p < 0.05$). It then decreased to 0 µmol/mL at week 9 ($p < 0.05$) and did not change

until week 13 ($p > 0.05$). Taurine concentration increased from 0 µmol/mL at week 0 to 4.16 µmol/mL at week 13 ($p < 0.05$). Alanine concentration increased from 0 µmol/mL at week 0 to 3.56 µmol/L at week 13 ($p < 0.05$).

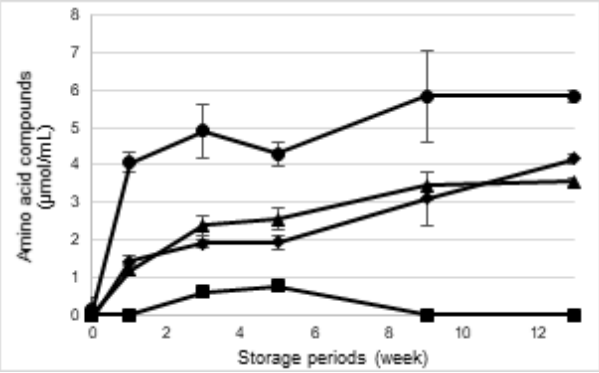

Figure 2. Change in amino acids over time during fish sauce production
Measurements n=5.
Error bars indicate standard deviation.
●:Histidine, ■:Arginine, ◆:Taurine, ▲:Alanine

Figure 2 shows that, during fish sauce production, amino acids showed an overall increasing trend with increasing production time, except for arginine. Chinese anchovy fish sauce made from only salt and fish was reported to contain 199 mg/100 g histidine, 322 mg/100 g alanine, and 2.2 mg/100 g arginine in one month [4]. In the present study, histidine, taurine, alanine, and arginine levels were 67 mg/100 g, 24 mg/100 g, 23 mg/100 g, and 13 mg/100 g, respectively. They showed lower overall amino acid levels than previously reported. Japanese anchovy and horse mackerel have been reported to contain 1100 mg/100 g and 790 mg/100 g histidine, respectively. Histidine is a hydrophilic amino acid; therefore, the high histidine content in Japanese anchovy could be due to the dissolution of histidine in fish sauce. However, arginine levels were lower than histidine levels in this study. It has been reported that the arginine concentration in Japanese anchovies tends to decrease during the production of fish sauce and that there may be an enzyme that rapidly breaks down arginine. Because the composition of free amino acids in fish soy sauce is reported to change depending on the type of fish used, salt concentration of the pickling liquid, and temperature during production [6], it is believed that the amount of amino acids varies greatly depending on fish species.

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4. Conclusions

- In this study, IMP, HxR, Hx, histidine, alanine, taurine, and arginine levels were measured over time to investigate quality changes in fish sauce production with brine.
- ATP-related compounds were found to be IMP at the start of production; however, after one week, only HxR and Hx were detected. The contents of most of the examined amino acids increased over time.
- These results suggest that fish sauces with a high amino acid content can be prepared by extending the ripening time.

5. References

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