

## EVALUATION OF NON-VOLATILE KEY TASTE COMPOUNDS OF AIR-DRIED HERRING (*Clupea pallasii*) FILLET

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### ABSTRACT

An experiment was conducted to quantify the non-volatile key taste compounds, including free amino acids (FAAs), nucleotides, inorganic ions, organic acids, and bases of dried herring (*Clupea pallasii*) fillet (DHF). A taste recombinate was also prepared using those key taste compounds to compare the taste profiles with water soluble extracts (WSE) by sensory perception. The total FAA of DHF muscle was 12.25 mg/g on dry matter basis. The most dominant FAAs were alanine, glycine, lysine, and histidine. The nucleotides were identified as 5'-adenosine monophosphate (AMP) (1.74 mg/100 g) and 5'-inosine monophosphate (IMP) (5.28 mg/100 g). Potassium, phosphate, and chloride were the major inorganic ions while trimethylamine oxide, creatine, and lactic acid were the major organic bases. Sensory evaluation showed that WSE markedly increased the intensities of *umami*, mouthfulness, thickness, and taste continuity of Japanese noodle soup (JNS). However, addition of taste recombinate to the JNS did not bring any changes in the taste intensities of saltiness, *umami*, mouthfulness, taste continuity, and thickness of the soup. Results of this study indicated that the taste recombinate was lacking of some important key taste compounds that might be formed during drying of herring fillet.

**Keywords:** *Clupea pallasii*, taste compounds, free amino acids, nucleotides, sensory perception

### INTRODUCTION

Dried herring fillet (DHF) is a Japanese popular and well accepted food for its unique taste-augmenting characteristics. Specially, addition of DHF to the savory dishes enhance characteristic taste and flavor such as mouthfulness, thickness, and taste continuity. These flavor characteristic are usually known as *kokumi* in Japanese. Water soluble compounds like free amino acids (FAAs), peptides, nucleotides, inorganic ions, organic acids, and bases are mainly responsible for the taste of fish and fishery products (Konosu and Yamaguchi, 1982). Moreover, some FAAs and peptides play an important role in masking or intensifying the characteristic taste of a particular food item (Maga, 1994). Generally, extractive components viz. FAAs and peptides (low molecular weight) have some flavor enhancing properties of a variety of food. In addition, lactate, creatine and creatinine were recognized as active compounds for the development of taste and flavor in dried skipjack (*Katsuwonus pelamis*) (Fuks and Konosu, 1991). Creatine and creatinine were also identified as *kokumi* flavor enhancers in dried herring fillet (Shah et al., 2010a). Moreover, creatine, creatinine, succinate, lactate and hypoxanthine were found to be active compounds for generating characteristic taste in stewed beef juice (Schlichtherle-Cerny and Grosch, 1998). Twelve components such as alanine, glutamic acid, arginine, glycine, betaine, 5'-cytidine monophosphate, AMP, 5'-guanosine monophosphate (GMP), Na<sup>+</sup>, Cl<sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and K<sup>+</sup> were found to be active compound for the generation of characteristic taste in snow crab (*Chionoecetes opilio*) (Hayashi et al., 1981). Fuks and Ueda (1996) established that monosodium glutamate (MSG) with disodium salts of IMP, GMP, and AMP produces characteristic taste named *umami*. These compounds are naturally present in seafood, cheese, meat, and vegetables. Moreover, MSG and nucleotides showed synergistic effects when added to a food matrix (Yamaguchi et al., 1971). It is also reported that nucleotides enhance the flavor intensities of MSG, and the flavor attributes of IMP also improves by MSG (Furukawa, 1991).

Taste activity value (TAV) is mainly used to evaluate the effect of a taste animated substance in a food matrix. TAV is calculated based on the ratio between the concentration of a taste animated compounds and its taste recognition threshold. The TAV more than 1 is considered as active substances that enhancing the flavor of a certain food (Scharbert and Hofmann, 2005; Rotzoll et al., 2006). However, no studies have so far been conducted on non-

volatile key taste compounds of DHF. Thus, this study was aimed to determine the non-volatile key taste substances and evaluate their effect based on TAV. A taste recombinate was also prepared using those compounds, and compare the taste profiles with water soluble extracts of DHF by sensory perception.

### MATERIAL AND METHODS

#### Materials

The DHF was collected from a fish processing industry, located at Hakodate, Japan. Herring was harvested at the Kamchatka Peninsula coast, Russia. The fish was kept frozen until processing. All chemicals used were of analytical or HPLC grade.

#### Preparation of DHF

The collected fish was thawed, eviscerated, cleaned, and filleted for air drying after arrival at the fish processing industry. The fish fillet was air-dried using electric fans under controlled condition (room temperature: 14 °C; relative humidity: 45%). After 10 days of drying, DHF was arbitrarily sampled for chemical and sensory analyses.

#### Water-soluble extracts preparation

The water-soluble extracts (WSE) were prepared following the method described by Shah et al. (2010a). Briefly, freeze dried DHF (20 g) was defatted using *n*-hexane and homogenized with a 10-fold volume of distilled water. After centrifugation of the homogenate, obtained supernatant was extracted with ethanol. The filtrate was then evaporated and freeze-dried to obtain the WSE of DHF.

#### Quantitative analyses

The DHF muscle was extracted using perchloric acid (PCA) to quantify FAAs, nucleotides, inorganic ions, organic acids, and bases following the method of Kani et al. (2007). Briefly, DHF (5 g) was homogenized with cold 10% PCA (25

mL) using an ace homogenizer (Nissei A-72, Nihonseiki Kaisha Ltd., Tokyo, Japan). The homogenate was centrifuged and the residue was re-extracted twice with cold 5% PCA (10 mL). The supernatants obtained were neutralized with KOH and centrifuged again. Finally, the sample solution was made up to 100 mL with de-ionized water and designated as PCA extract.

#### Free amino acid (FAA) analysis

The PCA extract was analyzed to determine FAAs by an amino acid analyzer (JEOL JLC-500/V, Nihon Denshi Datem Co. Ltd., Tokyo, Japan). All the analyses were done in triplicate.

#### Nucleotides determination

The PCA extract was analyzed to determine nucleotides and their related compounds by reversed-phase HPLC (Hitachi 665A, Hitachi Ltd., Tokyo, Japan) following the method of Yokoyama *et al.* (1992).

#### Inorganic ions, trimethylamine (TMA) and trimethylamine oxide (TMAO) determination

Inorganic ions, TMA and TMAO were determined by a personal ion analyzer (PIA-1000, Shimadzu Corp., Kyoto, Japan) following the method described by Kani *et al.* (2007).

#### Lactic acid measurement

The lactic acid content of DHF was measured using a Lactate test kit (Roche Diagnostics, Penzberg, Germany).

#### Creatine and creatinine measurement

Creatine and creatinine content were determined by hydrophilic interaction liquid chromatography (HILIC) following the chromatographic conditions described by Mora *et al.* (2007).

#### TAV measurement

It was measured according to the method of Rotzoll *et al.* (2006).

#### Sensory evaluation

To perform the sensory evaluation, WSE and a taste recombinant (the “natural” amount of the 32 compounds were determined in the DHF) were added to the Japanese noodle soup (JNS), according to the method of Ueda *et al.* (1997) with slight modification. The JNS was made following the method described by Shah *et al.* (2009a). The soup powder was dissolved into six-fold volumes of deionized water. Then the WSE and taste recombinant were added at a concentration of 0.1% and heated at 60 °C in a water bath. The sip-and-spit method was used to perform the sensory evaluation (Running and Hayes, 2017). Scoring was done based on sweetness, saltiness, sourness, bitterness, *umami*, thickness, mouthfulness, and taste continuity using a 1-7 point scale, where 3 points were assigned to the control. The panel was poised of seven trained assessors (ages between 24 and 38 years) from the Laboratory of Food Research & Development, MC Food Specialties Inc., Ibaraki, Japan.

#### Statistical analysis

All the values are presented as means  $\pm$  standard deviation. Statistical analyses were performed using Microsoft Office Excel 2010. Student's *t* test was performed to identify level of significance ( $p < 0.05$ ) (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

#### Effects of free amino acids on the taste of DHF

The FAA contents, taste recognition thresholds and their TAV are depicted in Table 1. The total amount of FAA was 12.25 mg/g in DHF. The major FAA were alanine, glycine, histidine, and lysine, which contain about 72% of the total FAAs. These FAAs were mostly found in dried skipjack (Fuks *et al.*, 1989) and dried sardine (Takiguchi, 1999). However, a comparatively higher amount (20.9 mg/g) of total FAA was found in Chinese mitten crab than that of DHF (Chen and Zhang, 2007). Besides, alanine and glycine were the predominant amino acids in DHF (>2 mg/g of dried muscle), and both the amino acids are desirable for their sweet taste. In the DHF, glutamic acid (desirable for *umami*), histidine (desirable for bitter), and lysine (desirable for sweet) were found to be predominant, which suggests that these free amino acids produce delicious taste of DHF. Fuks and Konosu (1991) documented that the taste active FAAs in snow crab, short-necked clam, abalone, sea urchin, and scallop were glycine and glutamic acid. Furthermore, glutamic acid enhances the overall taste and flavor

attributes such as fullness, mildness, complexity, and taste continuity when added to the artificial extracts (Yamaguchi and Kimizuka, 1979).

#### Effects of nucleotides and related components on the taste of DHF

The contents, taste recognition thresholds and TAV of nucleotides and related compounds of DHF are shown in Table 2. The amount of AMP and IMP of DHF were 1.74 mg/100 g and 5.28 mg/100 g, respectively on a dry matter basis. The TAV of AMP and IMP were 0.03 and 0.21, respectively. The amount of AMP and IMP were much higher in the crab meat than DHF (Chen and Zhang, 2007). In contrast, a comparatively higher amount of hypoxanthine (181.45 mg/100 g) and inosine (145 mg/100 g) were found in the DHF. Yamaguchi *et al.* (1971) found that GMP and IMP give intense *umami* taste, and their flavor intensities are stronger than MSG. It has been reported that AMP gives sweet taste even at a low concentration but has no effect on the *umami* taste. On the other hand, a minimum amount of IMP enhances sweet taste, *umami* intensities, and thickness in a food matrix (Fuks and Ueda, 1996).

**Table 1** The concentrations, taste characteristics (pleasant (+), unpleasant (-)), taste recognition thresholds, and TAV of FAAs in the DHF

Free Amino Acid	Concentration (mg/g) <sup>a</sup>	Taste characteristic	Taste threshold <sup>b</sup> (mg/ml)	TAV
Glutamic acid	0.65 $\pm$ 0.01	Umami (+)	0.3	2.17
Aspartic acid	0.05 $\pm$ 0.1	Umami (+)	1	0.05
Glycine	2.13 $\pm$ 0.03	Sweet (+)	1.3	1.64
Serine	0.18 $\pm$ 0.02	Sweet (+)	1.5	0.12
Alanine	4.55 $\pm$ 0.07	Sweet (+)	0.6	7.58
Phenylalanine	0.27 $\pm$ 0.01	Bitter (-)	0.9	0.30
Histidine	0.74 $\pm$ 0.07	Bitter (-)	0.2	3.70
Threonine	0.19 $\pm$ 0.02	Sweet (+)	2.6	0.07
Arginine	0.13 $\pm$ 0.01	Bitter/sweet (+)	0.5	0.26
Methionine	0.16 $\pm$ 0.01	Bitter/sweet/sulfurous (-)	0.3	0.53
Tyrosine	0.19 $\pm$ 0.00	Bitter (-)	ND	ND
Lysine	1.47 $\pm$ 0.03	Sweet/bitter (-)	0.5	2.94
Valine	0.46 $\pm$ 0.02	Sweet/bitter (-)	0.4	1.15
Leucine	0.48 $\pm$ 0.01	Bitter (-)	1.9	0.25
Isoleucine	0.24 $\pm$ 0.01	Bitter (-)	0.9	0.27
Proline	0.37 $\pm$ 0.01	Sweet/bitter (+)	3	0.12
<b>Total</b>	<b>12.25 <math>\pm</math> 0.06</b>			

<sup>a</sup> Data are expressed as mean  $\pm$  SD. ND = Not detected.

<sup>b</sup> Taste recognition threshold (mg/ml) of FAAs in water (Shallenberger, 1993; Kato *et al.*, 1989).

**Table 2** The contents, taste recognition thresholds and TAV of nucleotides and related compounds in the DHF

Compounds	Content <sup>a</sup> (mg/100 g)	Taste threshold <sup>b</sup> (mg/100 ml)	TAV
Adenosine-5'-monophosphate (AMP)	1.74 $\pm$ 0.02	50	0.03
Inosine-5'-monophosphate (IMP)	5.28 $\pm$ 0.15	25	0.21
Hypoxanthine	181.45 $\pm$ 8.19	ND	ND
Inosine	145.74 $\pm$ 4.31	ND	ND

<sup>a</sup> Data are expressed as mean  $\pm$  SD. ND = Not detected.

<sup>b</sup> Taste recognition threshold (mg/100 ml) of nucleotides in water (Yamaguchi *et al.*, 1971; Fuks and Ueda, 1996).

#### Effects of inorganic ions, organic acids, and bases on the taste of DHF

The amount of inorganic ions, organic acids, and bases in the DHF are shown in Table 3. Quantitative analyses showed that PO<sub>4</sub><sup>3-</sup> and K<sup>+</sup> were found to be predominant inorganic ions in the DHF at a concentration of 1465.31 mg/100 g and 706.81 mg/100 g, respectively on dry weight. The amount of Cl<sup>-</sup>, Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> were 356.13, 237.78, 142.67, 29.42, and 16.66 mg/100 g, respectively on the dry weight. Fuks and Konosu (1991) found that sodium and chloride ions significantly enhance the taste of snow crab, scallop, and short necked clam. They also reported that a synthetic extract lacking sodium ion significantly decreases *umami*, saltiness, sweetness, and characteristic flavor, but increases the bitterness of the extract. Moreover, a synthetic extract turns into a tasteless solution when chloride ion was omitted. The taste attributes of saltiness, sweetness, and *umami* were slightly decreased from the synthetic snow crab extract when phosphate ion was omitted. A substantial amount of creatinine (161.48 mg/100 g) was determined in the DHF, which mainly converted from creatine by non-enzymatic intramolecular dehydration. It has been reported that creatine content was decreased while creatinine content increased during the drying of herring fillet (Shah *et al.*, 2013). Snider and Baldwin (1981) found that the flavor intensity of cooked beef positively influenced by the creatinine content. Moreover, flavor characteristics of meat extracts were intensely increased when creatine was added to the broths (Schlichterle-Cerny and

Grosch, 1998; Cambero *et al.*, 2000). Conversely, a synthetic extract without creatinine was more acceptable than that with creatinine (Fuke and Konosu, 1991). In the DHF, the amount of TMAO and TMA were 441.75 mg/100 g and 37.16 mg/100 g, respectively. The amount of lactic acid was 532 mg/100 g, which is an important compound for producing sourness as well as enhancing overall preference (Fuke and Konosu, 1991).

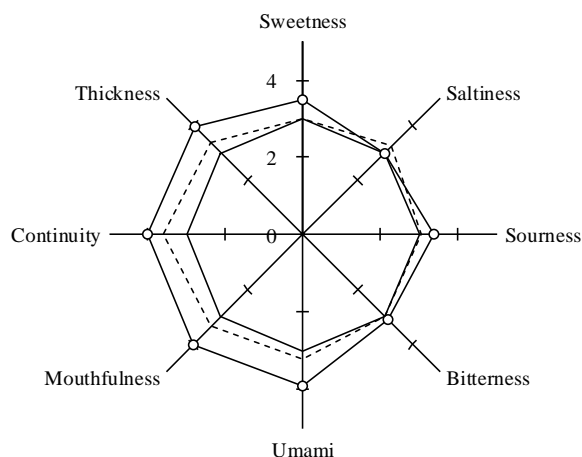
**Table 3** The concentrations (dry matter basis) of inorganic ions and organic bases in the DHF

Compounds	Concentration (mg/100 g) <sup>a</sup>	Compounds	Concentration (mg/100g) <sup>a</sup>
<b>Inorganic ions</b>		<b>Organic acid and bases</b>	
NH <sub>4</sub> <sup>+</sup>	142.67 ± 6.83	Trimethylamine oxide (TMAO)	441.75 ± 9.92
Na <sup>+</sup>	237.78 ± 7.93	Trimethylamine (TMA)	37.16 ± 2.18
K <sup>+</sup>	706.81 ± 12.68	Creatine	1062.35 ± 15.52
Mg <sup>2+</sup>	29.42 ± 2.51	Creatinine	161.48 ± 4.63
Ca <sup>2+</sup>	16.66 ± 1.79	Lactic acid	532.67 ± 12.59
PO <sub>4</sub> <sup>3-</sup>	1465.31 ± 26.37		
Cl <sup>-</sup>	356.13 ± 8.58		

<sup>a</sup> Data are expressed as mean ± SD.

### Sensory evaluation of taste recombinant and WSE

A taste recombinant and WSE were added to the JNS and presented to the trained sensory panelists. Sensory assessment results showed that addition of WSE to the JNS significantly ( $p < 0.05$ ) increased the flavor properties such as *umami*, mouthfulness, continuity, and thickness of the Japanese noodle soup, while WSE had no significant ( $p > 0.05$ ) effect on the basic taste attributes, for instance, saltiness, sweetness, sourness, and bitterness (Figure 1). On the other hand, there was no significant increment found on the taste attributes like *umami*, mouthfulness, continuity, and thickness when taste recombinant was added to Japanese noodle soup. It is suggested that the taste recombinant was lacking of some important taste components or Maillard reaction products, which are formed in the herring fillet during drying (Shah *et al.*, 2009b). Another report mentioned that the unique flavor of DHF might be generated during the drying period when free fatty acids or products of lipid oxidation react with the FAAs especially lysine (Shah *et al.*, 2010b). In this study, we did not perform the addition and omission test since the taste recombinants were not mimicked with the taste profiles of WSE. It has been reported that Maillard-reacted peptide contains key taste compounds, which generate a typical flavor of soybean paste seasoned for 20 months (Ogasawara *et al.*, 2006).



**Figure 1** Taste attribute of JNS treated with artificial taste recombinant and WSE of DHF. Where, a score of three for control (—), artificial taste recombinant (---), the WSE of DHF (···).

### CONCLUSION

The major FAA of DHF were alanine, glycine, histidine, and lysine. The amount of AMP and IMP of DHF were 1.74 mg/100 g and 5.24 mg/100 g, respectively that was much lower than those of taste threshold value. PO<sub>4</sub><sup>3-</sup> and K<sup>+</sup> were found to be predominant inorganic ions followed by Cl<sup>-</sup>, Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> ions. A substantial amount of creatinine was also determined in the DHF, which might be derived from creatine during the drying of herring fillet. Sensory evaluation showed that the taste recombinant was not mimicked with the taste profiles of WSE, which suggests that the taste recombinant was lacking of some important key taste components formed during drying of herring fillet.

**Conflicts of Interest:** The authors declare no conflict of interest.

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