

## 10 畜肉熟成與水產品的美味形成

1. Taste components and conditioning of beef, pork and chicken
2. Taste-active Components of Seafoods with special reference to umami substances

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# 15

## Taste Components and Conditioning of Beef, Pork, and Chicken

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From: "Umami: A Basic Taste" (Y. Kawamura and M.R. Kare eds.), 1987, pp. 289-306, Marcel Dekker Inc. New York.

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熟成之前：	牛肉屠宰後 4°C貯藏	4 日
	豬肉	1 日
	雞肉	0 日

### C. Preparation of the Heated Soup

An equal weight of water was added to the minced meat, and the preparation was homogenized. The homogenate was heated in boiling water for 20 min and then subjected to centrifugation to obtain the meat soup.

### D. Preparation of the Synthetic Soup

The synthetic soup was prepared on the basis of the analytical data. The NaCl concentration and the pH of each soup were adjusted to 0.508% and 5.8, respectively.

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**Table 1.** Effect of Additional Storage on the Intensity of the Brothy Taste of Beef, Pork, and Chicken

The no. of samples judged to have a more intense brothy taste:				
Meat	Before additional storage	After additional storage	n	Difference <sup>a</sup>
Beef	12	4	16	NS
Pork	2	14	16	*
Chicken	8	23	31	*

<sup>a</sup>NS, not significant; \*, significant ( $p < 0.05$ ).

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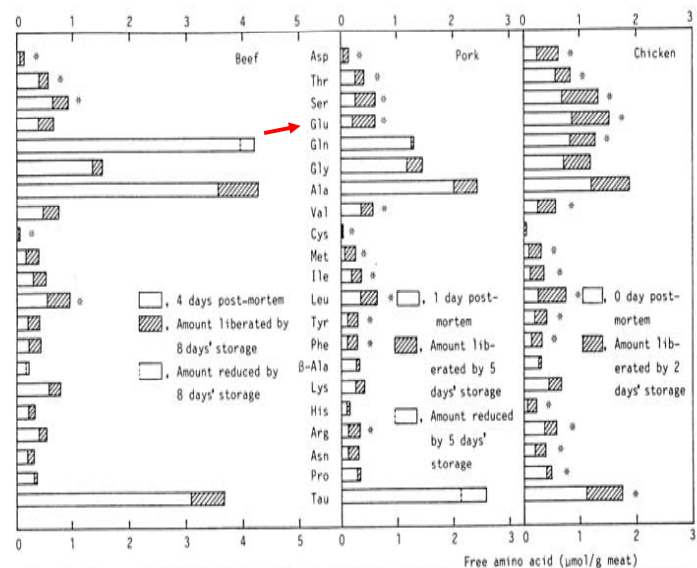


Figure 1. Changes in the levels of free amino acids during storage of beef, pork, and chicken. \*, Significantly different ( $p < 0.05$ ).

游離胺基  
酸含量的  
變化；  
\* 顯著變  
化

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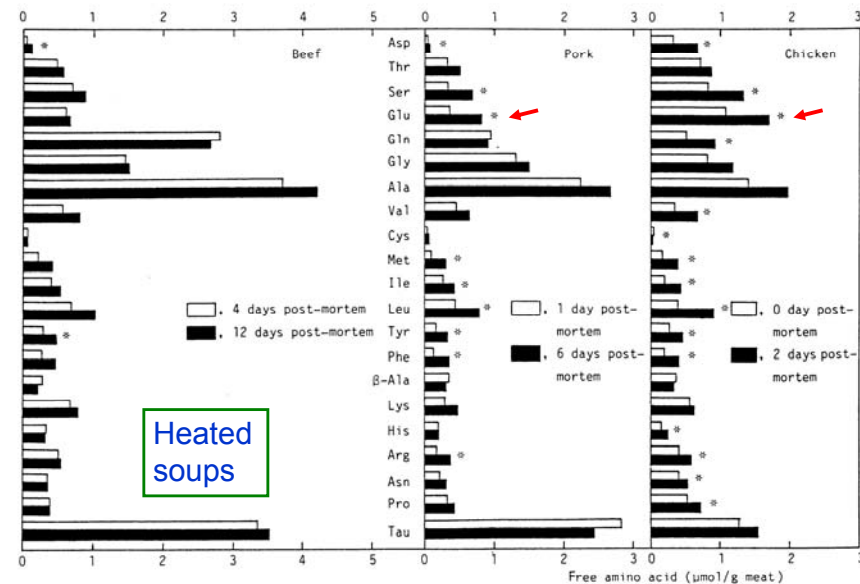


Figure 2. Free amino acids contained in heated soup of beef, pork, and chicken before and after additional storage. \*, Significantly different ( $p < 0.05$ ).

Heated  
soups

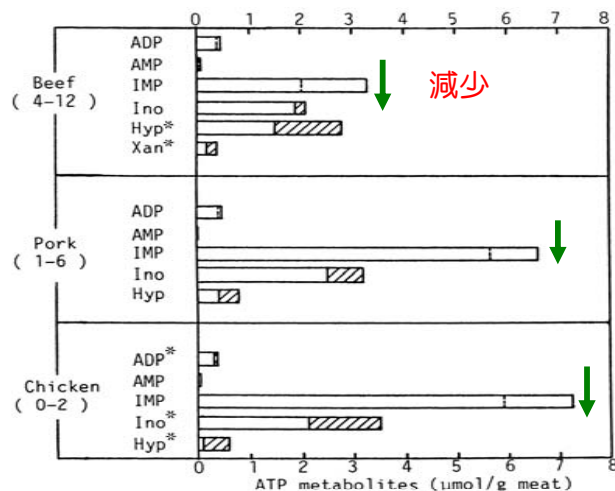


Figure 3. Changes in the levels of ATP metabolites during storage of beef, pork, and chicken. ATP metabolites were analyzed by HPLC. (□) Amount before the additional storage; (▨) amount increased by the additional storage; (▩) amount reduced by the additional storage; \*, Significantly different ( $p < 0.05$ ); numbers in parentheses, time post-mortem (days).

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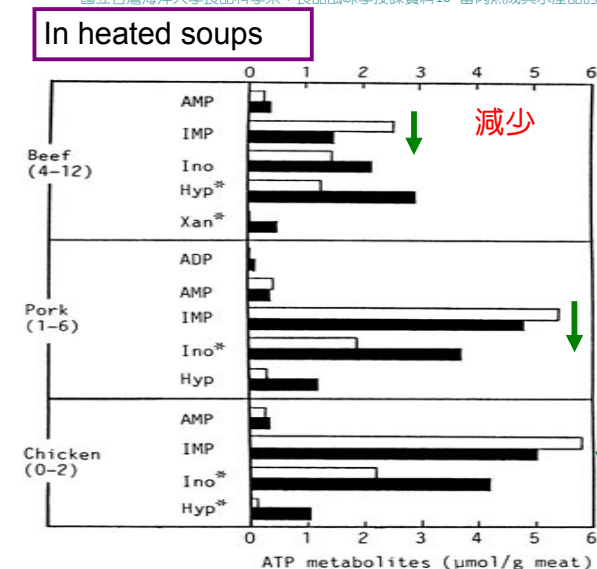
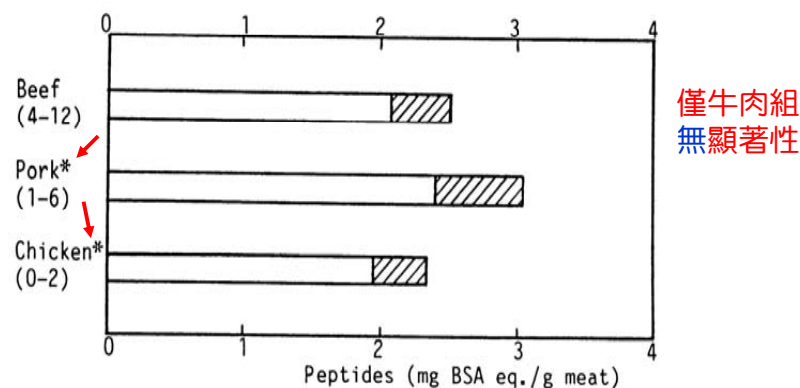


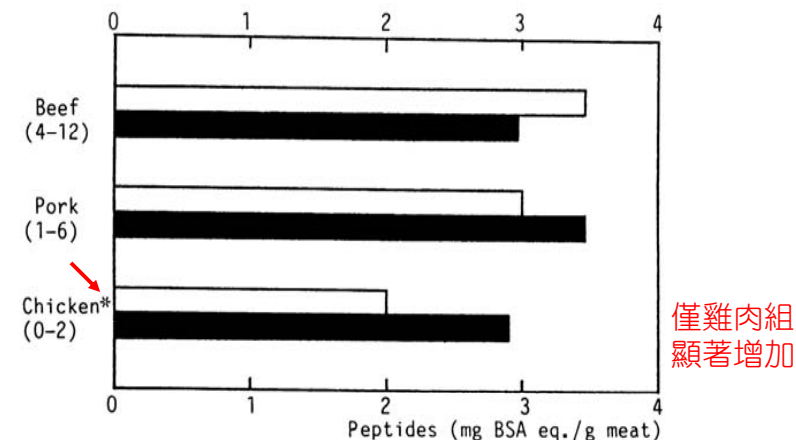
Figure 4. Levels of ATP metabolites in heated soup of meat before (□) and after (▨) additional storage. ATP metabolites were analyzed by HPLC. \*, Significantly different ( $p < 0.05$ ); numbers in parentheses, time post-mortem (days).

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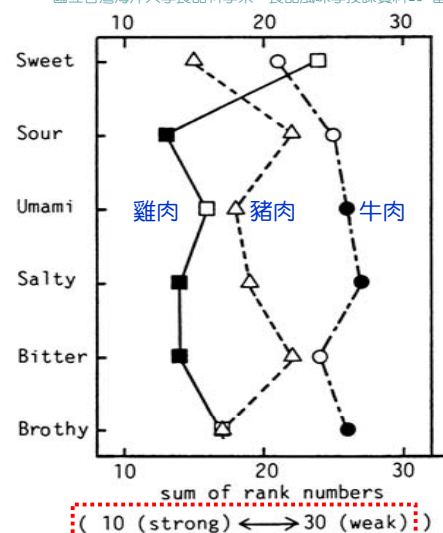
**Figure 5.** Changes in the levels of peptides during storage of beef, pork, and chicken. The levels of peptides were obtained from the difference between the values of phenol reagent-positive materials before and after the addition of  $\text{Cu}^{2+}$  into the phenol reagent. (□) Amount before additional storage; (▨) amount increased by additional storage; numbers in parentheses, time post-mortem (days).

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**Figure 6.** Peptides contained in heated soup of meat before and after additional storage. Analysis of peptides was the same as in Figure 5. (□) Before additional storage; (■) after additional storage; \*, Significantly different ( $p < 0.05$ ); numbers in parentheses, time post-mortem (days).

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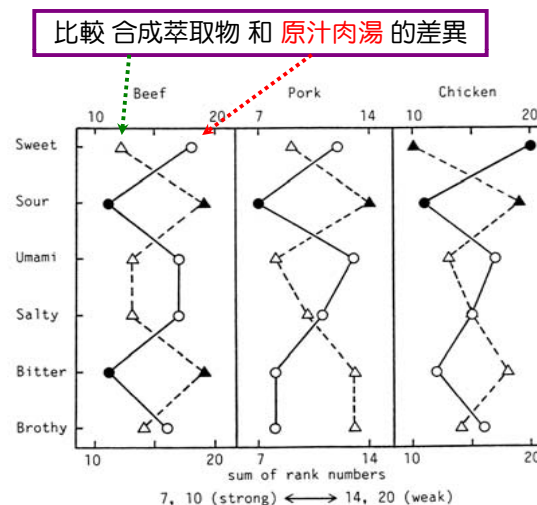
Sweet除外的每一種滋味強度，牛肉湯組都比豬肉、雞肉湯組較弱

每種肉湯都具有一特徵的滋味，由基本味彼此間平衡的不同。

很訝異：牛肉組umami及brothy強度最弱

尋找是哪些成分造成上述之差異

**Figure 7.** Relative strength of each taste among beef, pork, and chicken soups. The NaCl concentration of each soup was adjusted to 0.508%. (○---○) Beef; (△---△) pork; (□---□) chicken. Closed symbols, significantly different from the others ( $p < 0.05$ ).



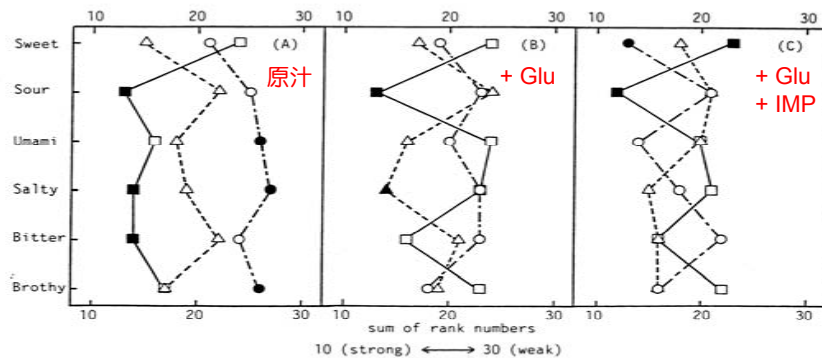
**Figure 8.** Comparison between authentic and synthetic soups of beef, pork, and chicken. Each soup consisted of IMP and free amino acids. The NaCl concentration of each soup was adjusted to 0.508%. (○---○) Authentic soup; (△---△) synthetic soup. Closed symbols, significantly different from others ( $p < 0.05$ ).

原汁組的鮮、鹹、肉湯味和合成組幾乎相同，酸味則較弱；雞肉合成組的甜味也顯著強於原汁組。

游離胺基酸和IMP已知貢獻於鮮味及肉汁味；合成組甜味較強之原因不明，有可能是酸味及苦味的強度更大時，使得可感覺的甜味就較弱。

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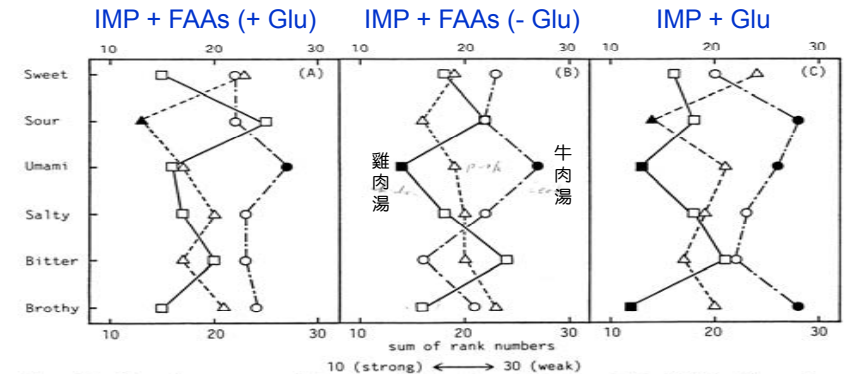
**Figure 9.** Effect of adding glutamic acid (Glu) or Glu + IMP to beef and pork soups at the same level as found in chicken soup. The NaCl concentration of each soup was adjusted to 0.508%. (A) Authentic soup; (B) with addition of Glu to beef and pork soups; (C) with addition of Glu + IMP to beef and pork soups. (○-○) beef; (△-△) pork; (□-□) chicken. Closed symbols, significantly different from others ( $p < 0.05$ ).

牛肉及豬肉湯中再加入Glu 或 Glu + IMP，使濃度等同於雞肉湯中。

牛肉湯組的 umami及brothy 提升至等同或甚強於豬雞肉湯組。表示對於umami及brothy，Glu扮演很重要的角色。

另一方面，酸味強度未受影響；牛及豬肉湯鹹味強度被增大；牛肉湯的甜味強度也增大。

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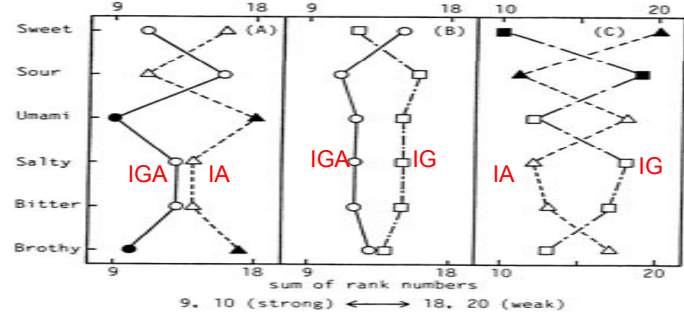
**Figure 10.** Comparison among synthetic soups of beef, pork, and chicken consisting of IMP and free amino acids. The NaCl concentration of each soup was adjusted to 0.508%. (A) IMP + free amino acids (+ Glu); (B) IMP + free amino acids (- Glu); (C) IMP + Glu (○-○) Beef (△-△) pork (□-□) chicken. Closed symbols, significantly different from others ( $p < 0.05$ ).

(A): 合成萃取物由IMP及全部的游離胺基酸組成。三組之中，牛肉湯組umami及brothy最弱。

(B): 欠缺 Glu，umami強度以雞肉組最大，牛肉組最低。欠缺 Glu，則Glu + IMP的相乘作用喪失。

(C): 僅Glu+IMP，雞湯組umami及brothy強度最大，牛肉湯組最低，和在(A)合成萃取物之情形有些類似。

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**Figure 11.** Contribution of Glu to each taste quality of chicken synthetic soup consisting of IMP and amino acids. The NaCl concentration of each soup was adjusted to 0.508%. (A) IGA vs. IA; (B) IGA vs. IG; (C) IA vs. IG. (○-○) IGA: IMP + amino acids (+ Glu). (△-△) IA: IMP + amino acids (- Glu). (□-□) IG: IMP + Glu. Closed symbols, significantly different from others ( $p < 0.05$ ).

(A): IMP + all FAAs (IGA) vs.欠缺 Glu (IA)。欠缺Glu減弱甜、鮮、肉味等，但鹹及苦味未受影響。

(B): IGA vs.IG+IMP (IG)。全部項目都無顯著差異。IG組的酸、鮮、鹹、苦味等強度較低，似受到Glu以外FAAs的影響。

(C): IA vs. IG。缺少Glu，減弱甜、鮮、肉味，但增強酸、鹹、苦味等。綜合以上的結果，Glu是貢獻umami及brothy taste之最重要的成分，其餘的胺基也貢獻一部分的鮮味。

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明確的回答，實非易事。

本節中，為避免重複第三章3-6 節內容（魚介類肌肉的萃取物成分），主要僅針對會刺激味覺產生之物質（呈味成分）加以闡述（7-10）。

#### 4-2-1 主要的呈味成分

1) 胺基酸 魚介類，毫無例外地，其所含的游離胺基酸是呈味成分中最為重要的成分。胺基酸各有其獨特的風味，而到底在食品中發揮怎樣的呈味作用，基本上是受到各胺基酸的濃度及含量，或者和其它成分之間的相互作用等因素所決定。下面就將截至目前為止，已經報告過而和魚介類的味道有關的主要胺基酸作一敘述。

麩胺酸 它是一種鈉鹽（麩胺酸鈉，monosodium glutamate, MSG）帶有鮮味（ $\text{Umami}$ ）的成分。所有的魚介類，沒有例外，都含有麩胺酸，只是含量大多低於MSG的濃度（0.03%）。可是由於和死後肉中所含肌苷酸（IMP）之間具有鮮味的相乘作用，因此於肌苷酸並存的情況下，即使含量低於閾值，仍有助於呈味（參見核苷酸項）。

甘胺酸 為帶有爽快甜味之胺基酸。無脊椎動物，特別是甲殼類及海扇貝柱中的含量高，其大部份的甜味即來自甘胺酸。蝦類的美味與甘胺酸的含量之間也關係密切。

\* 以麩胺酸(MSG)及肌苷酸(IMP)、鳥嘌呤核苷酸(GMP)等核苷酸鹽類所表現出來的味稱為鮮味。雖然曾有以基本味如甜味、酸味、苦味、鹹味可合成出鮮味之說法，但最近味覺心理學的研究顯示，鮮味乃是上述基本味所無法合成之另一種獨立的味道。另外，現在已經知道對麩胺酸具有特異感受之受容體(receptor)，也存於人類及老鼠的化學感受器官中，因此，將鮮味視為基本味之一，乃更為妥當之看法，此在國際上已逐漸得到認同。鮮味並非本身即是鮮美的味道，而是鮮味的成分具有提高基味的作用；所以，將鮮味與美味予以區別，有其必要性。「鮮味」(日語為「うま味」一詞，為日本人所創，外語中找不到適當的譯詞，所以外國的專家們也就直接採用該詞的日語發音「umami」)。

丙胺酸 為帶有弱苦味之甜味胺基酸。其含量大抵低於甘胺酸，但無脊椎動物中則含相當多的量，在慈愛蟹(snow crab)及海扇貝已確知係提供甜味之成分。

組胺酸 紅肉魚肉中蓄積有多量的這種胺基酸，但有的報告指出它和呈味有關，有的則認為無關，因此尚未獲得明確的結論。不過有報告指出鯷魚肉中含有很高的組胺酸，和並存的多量的乳酸與磷酸二氫鉀( $\text{KH}_2\text{PO}_4$ )共同地提高緩衝能，而有增強呈味之作用。由於和組胺酸一樣具有異噁唑基(imidazole group)之鰻肌肽(anserine)，鯷魚肉中亦含有多量，今後，這些成分的呈味作用有必要再詳細地探討。

精胺酸 為帶有苦味之胺基酸。因而富含精胺酸的魚介類往往被認為味道不佳，但相反地，含多量精胺酸的無脊椎動物則大多具有美味。探討慈愛蟹及海扇貝柱合成萃取物的結果，發現精胺酸並沒發出苦味，反而可增強風味的複雜性及濃厚性，具有提高整體美味之作用。

甲硫胺酸 它雖係苦味的胺基酸，卻是現出海膽的特有風味所不可欠缺的成分。而且有報告指出微量的甲硫胺酸能使麩胺酸鈉的呈味感覺更加濃厚。

脯胺酸 報告指出它與海膽特有的苦味有關。

脯胺酸 是帶有苦味之甜味胺基酸。因此有記載稱它對脯胺酸含量豐富的魚貝類的風味有所助益；但是在慈愛蟹合成萃取物的官能試驗結果，即使在每100mg中含163mg脯胺酸這種相當多量情況下，對美味仍未能有所幫助。附帶地說，L-脯胺酸的刺激閾值高達0.3%。

2) 肌肽 肌肽(carnosine)被認為與牛肉萃取物的美味有關；而鰻魚肌肉中亦含有多量之此雙胺酸，但它與鰻魚的風味之間的關係尚無人探討。在鼠鯊肉合成萃取物中研究鰻肌肽(anserine)的結果，加入該胺酸可稍增強酸味及引出濃厚感。Balanine在鰻鯊肉合成萃取物中的官能試驗結果，被認為具有增強鮮味及濃厚感之作用。由於這三種胺酸在中性酸鹼度附近均具有

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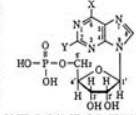
甘強緩衝能，其所以引出濃厚感，可能與此性質有關。

3) **核苷酸** 為探討鰵魚煮汁的鮮味成分，1913年小玉氏分離得到肌苷酸組酸鹽，之後並了解其鈉鹽可以呈現相同強度的鮮味。鳥嘌呤核苷酸為天然食品的鮮味成分一事，係由國中氏於1960年研究香菇的呈味成分時最早發現的。鳥嘌呤核苷酸在乾香菇及松茸中的含量高，魚介類中亦有少量存在。

國中氏的研究並闡明核苷酸的化學構造與鮮味產生之間的關係，即嘌呤基的第6位置上結合羧基和核糖的5'位置上接上磷酸酯，乃產生鮮味之必要條件。肌苷酸、鳥嘌呤核苷酸及5'-黃苷酸(xanthylic acid; XMP)都能符合這個必要條件，而腺苷酸(adenylic acid; AMP)則因第6位置上鍵結羧基而幾乎無味。

核苷酸的閾值，IMP · Na<sub>2</sub> · 7.5H<sub>2</sub>O為0.025%，GMP · Na<sub>2</sub> · 7H<sub>2</sub>O為0.0125%，都相當低，和羧酸鈉所不同的是其鮮味強度不因用量增加而有明顯的提高。是故以核苷酸作為呈味成分之意義，應是考慮其與羧酸鈉之間所產生的鮮味相乘效果。該兩成分的相乘作用可以 $y = u + 120uv$ 關係式來表示， $u$ 及 $v$ 分別代表混合液中羧酸鈉及肌苷酸的濃度， $y$ 則是具有和混合液相同的風味強度之羧酸鈉單獨的濃度(g/dl)。

羧酸鈉和核苷酸間的相乘作用所產生的鮮味，構成魚介類的主要風味；但有趣的是本身幾乎無味的腺苷酸也能夠增強羧酸鈉的鮮味。雖異於蓄積肌苷酸之魚肉，這個事實足以說明蓄積腺苷酸之無脊椎動物肌肉為何也具有強鮮味之原因。像這樣的羧酸鈉和核苷酸間的相乘作用，不僅是人類的感覺，從老鼠的味神經感應也獲得證明，亦即核苷酸結合於味受容體之後，可提高羧酸鈉對受容體的親和力之故。



4) **次黃嘌呤** 為具有苦味之嘌呤鹽基，由肌苷酸分解而生成。一般認為它是造成冰凍鰵魚肉產生苦味之原因。

5) **甘胺甜菜鹼(glycinebetaine)** 因具有甜味，一般認為它有助於含量豐富之無脊椎動物肌肉甜味的產生。但是以慈愛蟹肉合成萃取物(100mℓ中含357mg甘胺甜菜鹼)所作的官能試驗，雖承認它對甜味有所助益，但在其含量降為179mg時，其效果卻沒有太大的影響。另外，在海扇貝柱合成萃取物的官能試驗結果，於100mℓ中含237mg甘胺甜菜鹼時，其呈味效果並不顯著，惟有趣的是合成萃取物中若再加入甘胺甜菜鹼，則會引出水產物般的香味(seafood-like flavor)。

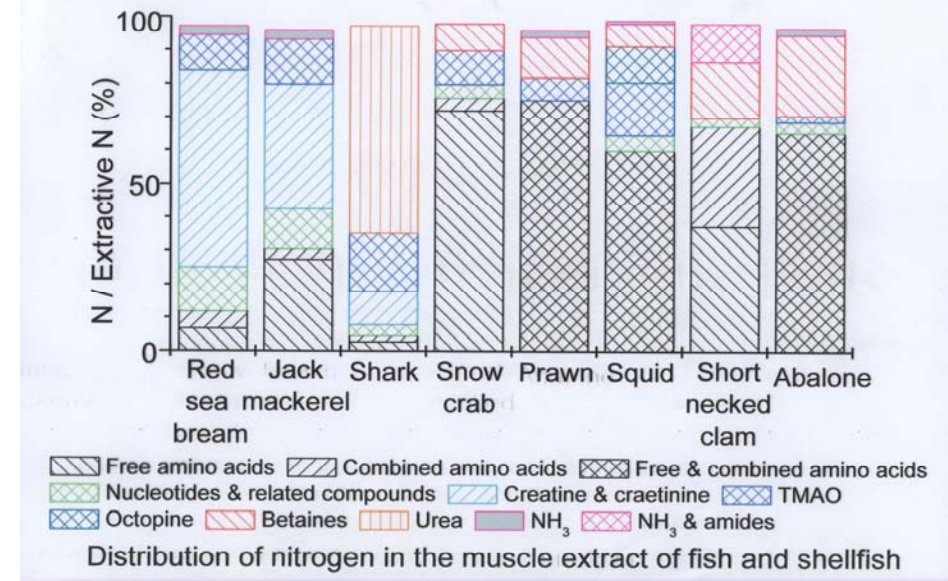
6) **氧化三甲胺** 因具有甜味，一般認為它能夠對含量高的魚貝類提供甜味，但在慈愛蟹肉合成萃取物(100mℓ中含169mg氧化三甲胺，TMAO)的官能試驗中，被斷定不具呈味效果；而在鳳凰肉合成萃取物(100mℓ中含1.12g氧化三甲胺)的官能試驗中，報告指出氧化三甲胺之加入可稍增強甜味。從上述的實驗結果，可知氧化三甲胺唯有存在相當多量時，始能顯現出呈味效果。

7) **有機酸** 琥珀酸在鰵、鮭、海扇貝等貝類中的含量多，一直被認為是貝類的重要呈味成分。但琥珀酸係貝類死後或在嫌氧狀態下存活時所蓄積的，剛捕獲的貝類含量低。由於這樣的試料一定就不鮮美，故琥珀酸在呈味上的作用有待檢討。乳酸在鰵魚煮汁中係提高緩衝能之要因，一般認為有增強味道的作用。

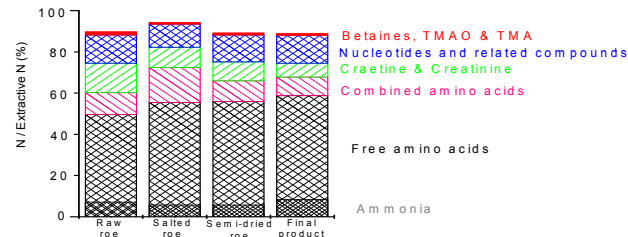
8) **無機成分** 第三章中曾述及無機成分並不屬於一般的萃取物成分，然而鈉、鉀、氯、磷酸根等的離子，特別是鈉及氯離子對於風味的產生非常重要，將於下節舉例說明。

#### 4-2-2 數種魚介類的風味組成

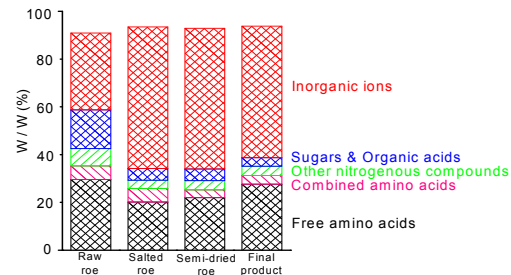
自食品中製造其萃取物，詳細分析其中的組成成份及其含量之後，依照分析結果之組成，混合高純度的試劑，這樣就能夠得到充分再現該食品



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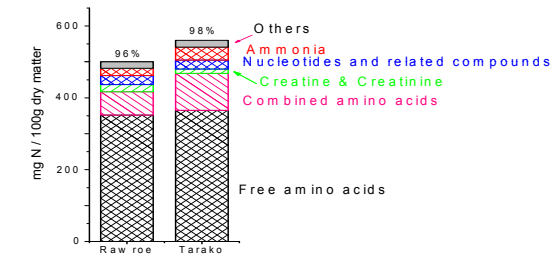


Distribution of nitrogen in the extracts at different stages of dried mullet roe processing.

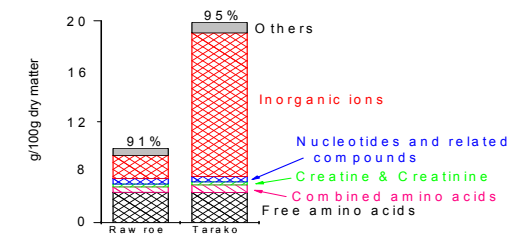


Distribution of various components in the extracts at different stages of the dried mullet roe processing.

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Nitrogen distribution in the extract of raw and salted Alaska pollack roes (Tarako). The figures at the top of the columns indicate the recovery on nitrogen basis.



Distribution of various components in the extracts of raw and salted Alaska pollack roes (Tarako). The figures at the top of the columns indicate the recovery on weight basis.

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## 8 Taste-active components of seafoods with special reference to umami substances

### S. FUKE

#### 8.1 What are umami and umami substances?

##### 8.1.1 Introduction

From the age of Aristotle or earlier, many scientists have tried to analyse the taste of foods especially in western Europe, and advocated sweetness, sourness, saltiness, bitterness, astringent, metallic and other tastes as fundamental elements contributing to food taste. Henning systematically summarized these tastes and proposed the famous taste tetrahedron theory, in which four basic tastes (sweetness, sourness, bitterness and saltiness) were located on each apex of the tetrahedron and other tastes were located somewhere on the surface (Komata, 1986). This tetrahedron theory has been very influential and has been supported by many food scientists.

At the beginning of this century, Ikeda (1909) isolated monosodium glutamate (MSG) as a substance responsible for the palatable taste of sea tangle which had been used popularly in Japan to prepare savoury soup. He noticed that the taste of MSG was a new type of taste, different from the conventional basic tastes and designated it as umami. Since then the concept of umami has been widely accepted in Japan and many works on umami substances have been conducted. Kodama (1913) identified inosine 5'-monophosphate (IMP) in dried skipjack and Kuninaka (1964) recognized guanosine 5'-monophosphate (GMP) in black mushroom as umami substances. In Europe and America, umami substances have been believed to be only flavour potentiators or enhancers, and umami has not been accepted as one of the basic tastes. In recent years however, new psychometric and neurophysiological approaches to recognize umami as one of the basic tastes have been developed. The multidimensional scaling method, applied to measure the distances between the conventional four basic tastes represented by sucrose, tartaric acid, sodium chloride and quinine sulphate, respectively, found that umami was independent of the other four tastes (Yamaguchi, 1987). The occurrence in mice of glossopharyngeal nerve responding strongly to umami substance but not to the other substances (Ninomiya and Funakoshi, 1987) also supported the

From: "Seafoods- Chemistry, Processing and Quality" (F. Shahidi and J.R. Botta eds.), 1994, pp. 115-139, Chapman & Hall, London.

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## What are umami and umami substances?

- The taste of foods  
Taste tetrahedron theory: 4 basic tastes (sweetness, sourness, bitterness and saltiness)  
"Umami" substances:  
Monosodium glutamate (MSG) - sea tangle  
Inosine 5'-monophosphate - dried skipjack  
Guanosine 5'-monophosphate - black mushroom  
Flavor potentiators or enhancers
- A substances to be labeled as having umami should fulfill the following conditions:
  - it should stimulate neurofibres specific to umami substances;
  - in the multidimensional scaling analyses, it should be located in a different dimension from the other tastes;
  - it should show a synergistic taste effect in coexistence with monosodium glutamate.

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### Free L-glutamate in natural foods (mg/100g)

Kelp	2240
Parmesan cheese	1200
Green tea	668
Seaweed	640
Sardine	280
Fresh tomato juice	260
Champignon	180
Cuttlefish	146
Tomato	140
Oyster	137
Potato	102
Chinese cabbage	100
Fresh shiitake mushroom	67
Soybean	66
Sweet potato	60
Dried sardine	50
Prawn	43
Clam	41
Chicken bones	40
Cabbage	37
Carrot	33
Bonito flakes	26
Pork fillet	23

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### 5'-inosinate in natural foods (mg/100g)

Dried sardine	886
Bonito flake	687
Bonito	285
Horse mackerel	265
Mackerel pike	242
Sea bream	215
Mackerel	215
Sardine	193
Tuna	188
Pork	122
Beef	107
Prawn	92
Chicken	76
Cod	44

### 5'-guanylate in natural foods (mg/100g)

Dried shiitake mushroom	156.5
Matsutake	64.6
Enokitake mushroom	21.8
Fresh shiitake mushroom	16-45
Truffle mushroom	5.8
Pork	2.5
Beef	2.2
Chicken	1.5

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## Synergistic Taste Effect

### • MSG with 5'-ribonucleotides (IMP or GMP)

Model system

Soup of various foods

**Table 8.2** Relative intensity of synergism between MSG and various 5'-ribonucleotides<sup>a</sup>

Substances (disodium salt)	Relative intensity
5'-IMP·7.5H <sub>2</sub> O	1
5'-GMP·7H <sub>2</sub> O	2.3
5'-XMP·3H <sub>2</sub> O	0.61
5'-AMP	0.18

<sup>a</sup>Abbreviations used: IMP, inosine monophosphate; GMP, guanine monophosphate; XMP, xanthine monophosphate; AMP, adenine monophosphate.

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**Table 8.3** Effect of subthreshold amount of various amino acids and succinic acid on lowering the threshold value of IMP

Substances <sup>a</sup>	IMP added <sup>b</sup>	Significance <sup>c</sup>	Ratio <sup>d</sup>
Aspartate	0.0025	***	
	0.00125	*	1/20
Glutamate	0.0004	***	
	0.0003	**	1/83
Aminoadipate	0.0050	***	
	0.0025	***	1/10
Oxyglutamate	0.0002	**	
	0.0001	*	1/250
Homocysteinate	0.0005	***	
	0.00025	*	1/100
Succinic acid	0.030	***	
	0.020	*	1/1

<sup>a</sup>Substances used were all sodium salts at the concentration of 0.1 g/dl in 1% sodium chloride solution except succinic acid whose concentration was 0.05 g/dl in the same solution.

<sup>b</sup>Disodium salt of 5'-IMP was used.

<sup>c</sup>\*\*\*, \*\* and \*: Significant at 0.1, 1 and 5% levels, respectively.

<sup>d</sup>The concentration ratio of each substance against the threshold value of IMP, 0.025%.

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### • α-Amino dicarboxylates and L-homocysteinate with IMP

IMP·2Na和 α-Amino dicarboxylates (aspartate·Na, D,L-threo-β-hydroxy- glutamate·Na, D,L-α-aminoadipate·Na, MSG 及 L-cysteinate·Na)之間的滋味相乘效應

閾值(水中)：aspartate 0.16%, hydroxyglutamate 0.03%, MSG 0.03%, IMP 0.025%

**Table 8.3** Effect of subthreshold amount of various amino acids and succinic acid on lowering the threshold value of IMP

Substances <sup>a</sup>	IMP added <sup>b</sup>	Significance <sup>c</sup>	Ratio <sup>d</sup>
Aspartate	0.0025	***	
	0.00125	*	1/20
Glutamate	0.0004	***	
	0.0003	**	1/83
Aminoadipate	0.0050	***	
	0.0025	***	1/10
Oxyglutamate	0.0002	**	
	0.0001	*	1/250
Homocysteinate	0.0005	***	
	0.00025	*	1/100
Succinic acid	0.030	***	
	0.020	*	1/1

<sup>a</sup>Substances used were all sodium salts at the concentration of 0.1 g/dl in 1% sodium chloride solution except succinic acid whose concentration was 0.05 g/dl in the same solution.

<sup>b</sup>Disodium salt of 5'-IMP was used.

<sup>c</sup>\*\*\*, \*\* and \*: Significant at 0.1, 1 and 5% levels, respectively.

<sup>d</sup>The concentration ratio of each substance against the threshold value of IMP, 0.025%.

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## The taste of free amino acids

**Table 8.4** Comparison of the predominant taste of L-amino acids reported by several authors

Amino acids	A <sup>a</sup> (1989)	B (1977)	C (1982)	D (1985)
Gly	sw <sup>b</sup>	sw	sw	sw
Ala	sw	sw	sw	sw
Ser	sw	sw	fl/sw	fl/sw
Pro	sw	sw/bi	—	fl
Lys	sw/ot	sw/bi	—	fl
His	bi/tl	bi	fl/bi	fl
Arg	bi	bi	—	fl
Ile	bi	bi	fl/bi	fl
Val	bi/sw	bi	tl	fl
Leu	bi	bi	fl/bi	bi
Met	bi	bi	fl/bi	su/mt/bi
Trp	bi	bi	fl/bi	bi
Asp	so	—	fl/so	fl
Glu	tl	nt	fl/sw/mt	—

<sup>a</sup>A, B, C and D: Cited from Birch and Kemp (1989), Wieser *et al.* (1977), Shiffman *et al.* (1982) and Solms *et al.* (1965).

<sup>b</sup>Abbreviations used: sw, sweet; bi, bitter; fl, flat; tl, tasteless; ot, other taste; mt, meaty; su, sulphurous, so, sour; nt, neutral.

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**Table 8.5** Contribution of basic tastes to the tastes of free amino acids (%)

	Sweet	Salt	Sour	Bitter	Umami	Others
Gly <sup>a</sup>	83.0	0.4	8.4	4.6	5.3	0.2
Ala	75.1	1.3	2.7	8.3	13.1	1.0
Thr	87.9	0.4	4.4	2.7	3.9	0.8
Ser	64.7	2.0	5.6	7.2	19.1	1.4
Pro	51.6	0.4	3.4	42.8	2.2	0
Lys·HCl <sup>b</sup>	38.6	8.7	7.9	28.8	10.5	5.5
Gln	41.8	0.3	1.5	10.2	26.0	10.9
Phe	0	0.7	0	98.7	0	0.7
His	7.2	1.0	8.5	64.1	11.5	7.8
Arg	2.1	0.4	1.2	85.9	1.5	8.9
Val	16.1	0.4	3.8	73.5	1.8	4.4
Leu	3.0	1.4	2.0	87.0	3.0	1.6
Met	7.4	0.2	4.0	61.6	16.6	9.4
Trp	1.6	0	2.2	95.0	0.2	1.0
Asp	1.7	3.1	81.1	4.3	8.5	1.6
Glu	5.0	5.4	60.0	3.8	24.8	1.0
Asp·Na <sup>b</sup>	5.3	43.5	8.6	4.8	35.5	2.3
Glu·Na <sup>b</sup>	9.8	13.3	3.4	1.7	71.4	0.4

<sup>a</sup>All amino acids (L-form except Gly) were tasted in powder.<sup>b</sup>Lys·HCl; lysine hydrochloride, Asp·Na; monosodium aspartate, Glu·Na; monosodium glutamate.

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### • Synergistic taste effect among three compounds

The strength of umami giving by the mixtures in 1% NaCl solution comprising 2 or 3 out of 3 elements (amino acids, 低於刺激閾值; nucleotides, 0.01% IMP/GMP; 0.1% MSG), was evaluated and compared with that of the 1% NaCl solution containing either the same amount of amino acid only or the same amounts of nucleotides and MSG.

各種胺基酸(濃度低於閾值)溶液中

Exp 1: 加入 Glu·Na 或 Asp·Na

Exp 2: 加入 IMP/GMP

Exp 3: 加入 Glu·Na or Asp·Na/IMP/GMP

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Exp 1: + Glu·Na or Asp·Na;      Exp 3: + Glu·Na or Asp·Na/IMP/GMP  
Exp 2: + IMP/GMP;

**Table 8.6** Taste enhancing effect of α-amino acids

	Threshold (g/dl)	Tested (g/dl)	Exp. 1	Exp. 2	Exp. 3
Gly	0.11	0.10	—	—	**
Ala	0.06	0.05	—	—	**
Thr	0.26	0.20	—	—	*
Ser	0.15	0.10	—	—	**
Pro	0.30	0.20	—	—	**
Lys·HCl	0.05	0.04	—	—	—
His	0.02	0.01	—	—	—
His·HCl	0.0005	0.004	—	—	**
Arg	0.01	0.008	—	—	—
Arg·HCl	0.03	0.02	—	—	—
Ile	0.09	0.09	—	—	—
Val	0.15	0.15	—	—	**
Cys	NG	0.05	—	—	**
D,L-Tyr	0.02	0.15	—	—	**
Tyr	NG	0.10	—	—	—
Met	0.03	0.03	—	—	**
Phe	0.15	0.05	—	—	—
Asp	0.003	0.003	—	—	*
Asp·Na	0.10	0.10	NA	**	NA
Glu·Na	0.03	0.02	NA	**	NA

Exp. 1: Enhancing effect of amino acids on sodium salt of Glu or Asp.

Exp. 2: Enhancing effect of amino acids on 5'-ribonucleotides.

Exp. 3: Enhancing effect of amino acids on mixtures of 5'-ribonucleotides and sodium salt of Glu or Asp.

\*\* and \*: Significant at 1% and 5% levels, respectively. —: Insignificant. NA, not analysed. NG, value was not given.

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### • Sulphur-containing compounds as flavor enhancers

The addition of 0.1 and 0.4% freeze-dried garlic extract to Chinese or curry soup, respectively, improved their flavor, especially "kokumi" flavor.

**Table 8.7** Enhancing effect of freeze-dried garlic extract on 'Kokumi' flavour

Test solutions	Effect
Water	—
0.05% MSG	+
0.05% IMP	+
3.1% MSG	++
0.05% MSG + 0.05% IMP	++

++ and +: Significant at 0.1% and 1% levels, respectively. —: Insignificant.

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**Table 8.8** Effect of sulphur compounds in garlic on 'kokumi' flavour of umami solution

Components	Effect
Alliin	+++
Cycloalliin	+
MeCSO	++
GAC	++
GACSO	+
Glutathione	+++
Cys	+
Met	+

Abbreviations used: MeCSO, (+)-*S*-methyl-L-cysteine sulfoxide; GAC,  $\gamma$ -L-glutamyl-*S*-allyl-L-cysteine; GACSO,  $\gamma$ -glutamyl-*S*-allyl-L-cysteine sulfoxide.

+++ , ++ and +: Enhancing effect is very strong, strong, and a little, respectively.

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### • Methyl xanthines and IMP

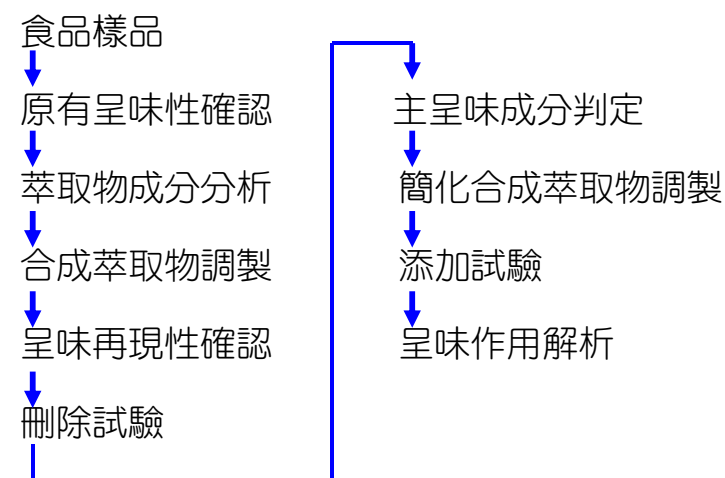
Methyl xanthines such as **caffeine**, **theobromine** and **theophylline** enhance the taste of compounds which is not enhanced by IMP, and conversely IMP enhances the taste of compounds which are not enhanced by methyl xanthines.

The panelist's tongue was adapted to one of the methyl xanthines at concentrations ranging from  $10^{-5}$  to  $10^{-2}$  M and then the stimulus to be tested was introduced to the tongue.

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- (1) The tastes of 6 sweeteners ( $10^{-5}$  M acesulfame-K,  $3.00 \times 10^{-5}$  to  $10^{-4}$  M neohesperidin dihydrochalcone,  $1.47 \times 10^{-2}$  M D-tryptophan,  $2.78 \times 10^{-5}$  M thaumatin,  $1.17 \times 10^{-3}$  M stevioside and  $1.87 \times 10^{-3}$  M sodium saccharin) were potentiated by either of the methyl xanthines.
- (2) The tastes of aspartame, sucrose, fructose and sodium cyclamate were not enhanced by methyl xanthines.
- (3) The same effect was observed for amino acids (enantiomers of Phe, His, Arg, Ala and Asn) except for enantiomers of Glu which were potentiated by IMP.
- (4) The tastes of quinine hydrochloride, KCl, NaCl and urea were enhanced by methyl xanthines as well.

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### 水產食品呈味機制之解析流程

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Table 8.10 Extractive components in snow crab (mg/100 g)

Tau	243	Phe	17	Ino	13
Asp	10	Orn	1	Gua	1
Thr	14	Lys	25	Cyt	1
Ser	14	His	8	Bet	357
Sar	77	$\tau$ -MeHis	3	TMAO	338
Glu	19	Trp	10	Hom	63
Pro	327	Arg	579	Glc	17
Gly	623	CMP	6	Rib	4
Ala	187	AMP	32	Lac	100
$\alpha$ -Abu	2	GMP	4	Suc	9
Val	30	IMP	5	Na <sup>+</sup>	191
Met	19	ADP	7	K <sup>+</sup>	197
Ile	29	Ade	1	Cl <sup>-</sup>	336
Leu	30	Ado	26	PO <sub>4</sub> <sup>3-</sup>	217
Tyr	19	Hyp	7		

Abbreviations used: Sar, sarcosine;  $\alpha$ -Abu,  $\alpha$ -aminobutyric acid; Orn, ornithine;  $\tau$ -MeHis,  $\tau$ -methylhistidine; Ade, adenine; Ado, adenosine; Hyp, hypoxanthine; Ino, inosine; Gua, guanine; Cyt, cytosine; Hom, homarine; Glc, glucose; Rib, ribose; Lac, lactic acid; Suc, succinic acid.

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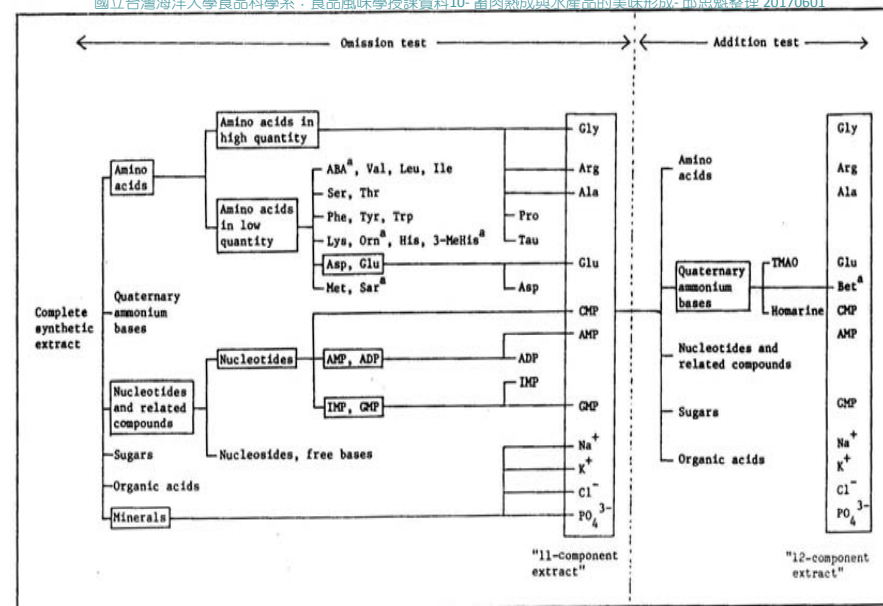
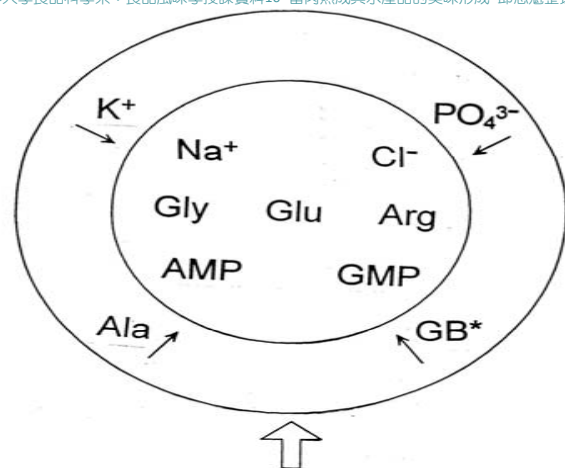


Fig. 1—Summary of omission and addition tests. The components enclosed in boxes were judged to contribute to the taste of snow crab. (ABA,  $\alpha$ -amino-n-butyric acid; Orn, ornithine; 3-MeHis, 3-methylhistidine; Sar, sarcosine; Bet, glycine betaine.)



其餘的萃取物成分

慈愛蟹(snow crab)肉呈味的構成模式  
(GB\*: glycine betaine)

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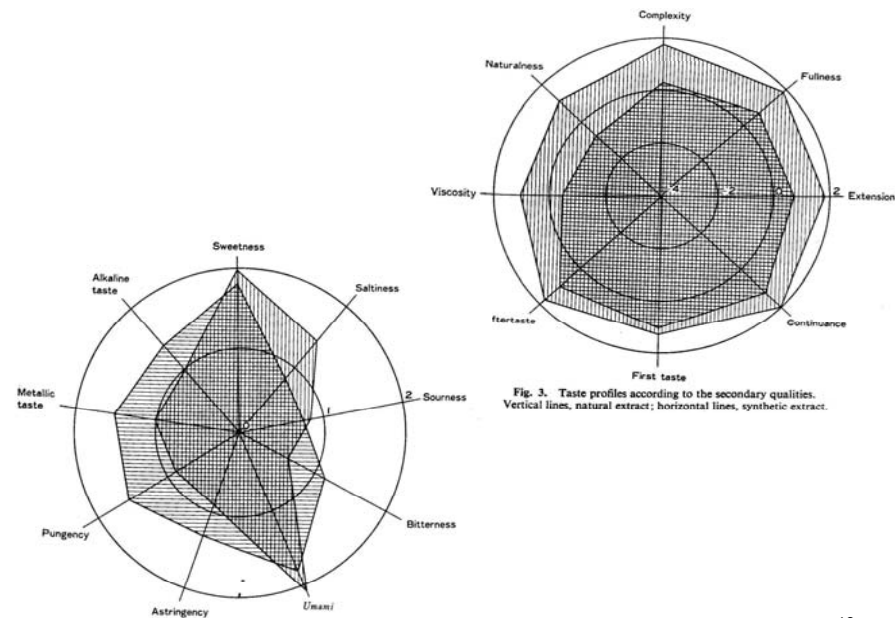


Fig. 2. Taste profiles according to the primary qualities. Vertical lines, natural extract; horizontal lines, synthetic extract.

Fig. 3. Taste profiles according to the secondary qualities. Vertical lines, natural extract; horizontal lines, synthetic extract.

40

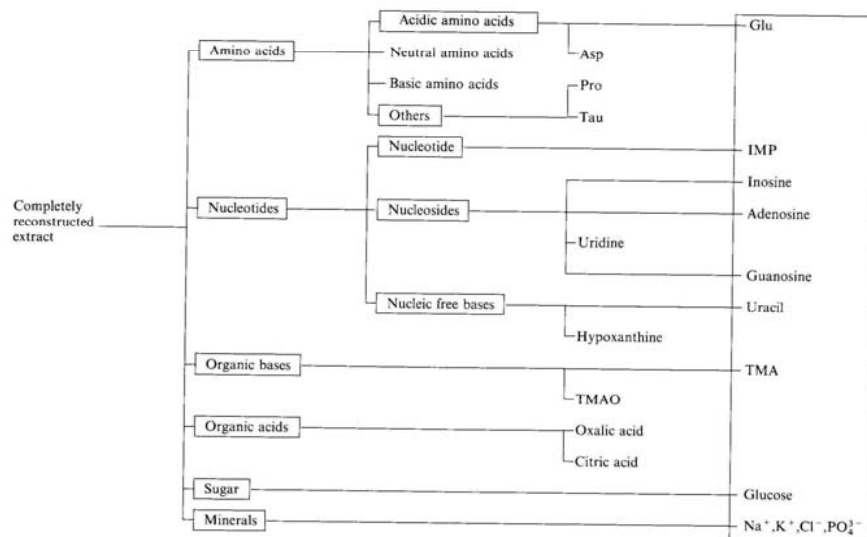


Fig 1. Summary of sensory omission test. The components enclosed in boxes were judged to contribute to the characteristic taste of ikura.

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Table 8.9 Extractive components in abalone muscle (mg/100 g)

Tau	946	Tyr	57
Asp	9	Phe	26
Thr	82	Trp	20
Ser	95	His	23
Glu	109	Lys	76
Pro	98	Arg	299
Gly	174	Bet	975
Ala	98	AMP	90
Val	37	ADP	12
Met	12	TMAO	3.2
Ile	18	TMA	1.1
Leu	24	NH <sub>3</sub>	8

Abbreviations used: Tau, taurine; Bet, glycine betaine; TMAO, trimethylamine oxide; TMA, trimethylamine.

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Extractive components in the adductor muscle of scallop (mg/100g)

Free amino acids	Tyrosine	-	Organic bases
Taurine	784	Phenylalanine	2
Aspartic acid	4	β-Alanine	2
Threonine	16	β-Aib	3
Serine	8	Ornithine	1
Glutamic acid	140*	Lysine	5
Proline	51	Histidine	2
Glycine	1925*	Arginine	323*
Alanine	256*	Ammonia	4
Valine	8	Nucleotides etc.	-
Cystine	8	AMP	172*
Methionine	3	Inosine	14
Cystathionine	4	Hypoxanthine	2
Isoleucine	2		
Leucine	3		
		Na <sup>+</sup>	73*
		K <sup>+</sup>	218*
		Ca <sup>2+</sup>	+
		Mg <sup>2+</sup>	+
		Cl <sup>-</sup>	95*
		PO <sub>4</sub> <sup>3-</sup>	213

β-Aib, β-aminoisobutyric acid; TMAO, trimethylamine oxide; TMA, trimethylamine. -, not detected; +, trace amount.

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Extractive components in short-necked clam (mg/100g)

Free amino acids		Phenylalanine	3	TMA	4
Taurine	<u>555*</u>	β-Alanine	2	TMAO	3
Aspartic acid	18	β-Aib	1	Organic acids	
Threonine	5	Ornithine	2	Oxalic acid	119
Serine	7	Trypyophan	3	Succinic acid	<u>65*</u>
Glutamic acid	<u>90*</u>	Lysine	6	Sugars	
Proline	3	Histidine	3	Mannose	3
Glycine	<u>180*</u>	Arginine	<u>53*</u>	Glucose	3
Alanine	74	Ammonia	1	Inorganic ions	
Valine	4	Nucleotides etc.		Na <sup>+</sup>	<u>378*</u>
Methionine	3	ADP	9	K <sup>+</sup>	<u>273*</u>
Cystathionine	2	AMP	<u>28*</u>	Ca <sup>2+</sup>	52
Isoleucine	3	Inosine	11	Mg <sup>2+</sup>	40
Leucine	5	Organic bases		Cl <sup>-</sup>	<u>452*</u>
Cystine	-	Glycine betaine	42	PO <sub>4</sub> <sup>3-</sup>	72

β-Aib, β-aminoisobutyric acid; TMAO, trimethylamine oxide; TMA, trimethylamine.

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**Table 8.11** Extractive components in dried skipjack 'Katsubushi' (mg/100 g)

Tau	32	Trp	15	Gyl	17
Gly	26	Orn	5	Ara	1
Ala	50	Arg	5	Rib	2
His	1992	(Cys) <sub>2</sub>	26	Man	5
Asp	2	$\beta$ -Ala	1	Glc	6
Glu	23	$\pi$ -MeHis	1	For	13
Leu	25	Ans	1250	Ace	52
Lys	29	Car	107	Prp	3
Met	17	AMP	52	Suc	96
Thr	11	IMP	474	Lac	3415
Ser	12	Ino	186	Na <sup>+</sup>	434
Pro	5	Hyp	12	K <sup>+</sup>	688
Val	16	TMA	19	Ca <sup>2+</sup>	39
Ile	8	TMAO	5	Mg <sup>2+</sup>	124
Tyr	20	Cre	540	Cl <sup>-</sup>	1600
Phe	15	Crn	1150	PO <sub>4</sub> <sup>3-</sup>	545

Abbreviations used: (Cys)<sub>2</sub>, cystine;  $\beta$ -Ala,  $\beta$ -alanine;  $\pi$ -MeHis,  $\pi$ -methylhistidine; Ans, anserine; Car, carnosine; Cre, creatine; Crn, creatinine; Gyl, glycerol; Ara, arabinose; Man, mannose; For, formic acid; Ace, acetic acid; Prp, propionic acid.

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Extractive components of salted salmon eggs\* (mg/100g)

Free amino acids		Tyrosine	5.6	Hypoxanthine	3.4
Taurine	13.6	Phenylalanine	7.6	Organic bases	
Aspartic acid	12.4	Lysine	9.0	TMA	1.7*
Threonine	6.6	Histidine	2.1	TMAO	62.3
Serine	14.0	Carnosine	2.8	Organic acids	
Glutamic acid	23.5*	Arginine	6.6	Oxalic acid	0.2
$\alpha$ -AAA	0.6	Nucleotides etc.		Citric acid	0.5
Proline	3.2	IMP	2.7*	Sugars	
Glycine	4.0	Uridine	5.1	Glucose	8.4*
Alanine	8.9	Inosine	7.8*	Inorganic ions	
Valine	9.6	Adenosine	4.2*	Na <sup>+</sup>	1165*
Methionine	3.1	Guanosine	4.3*	K <sup>+</sup>	191*
Isoleucine	8.6	Cytosine	25.1	Cl <sup>-</sup>	1055*
Leucine	11.7	Uracil	3.1*	PO <sub>4</sub> <sup>3-</sup>	33.3

\*Only the components used to prepare the synthetic extract are shown.

$\alpha$ -AAA,  $\alpha$ -aminoadipic acid; TMAO, trimethylamine oxide; TMA, trimethylamine.

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**Taste-active components in five kinds of seafoods (mg/100g)**

	Abalone	Sea-urchin	Snow crab	Scallop	Short-neck clam
Glu	109	103	19	140	90
Gly	174	842	623	1925	180
Ala	98	261	187	256	74
Val	37	154	30	8	4
Met	13	47	19	3	3
Arg	299	316	579	323	53
Tau	946	105	243	784	555
AMP	90	10	32	172	28
IMP	-	2	5	-	-
GMP	-	2	4	-	-
Bet	975	7	357	339	42
Suc	-	1.2	9	10	65
Na <sup>+</sup>	NA	NA	191	73	244
K <sup>+</sup>	NA	NA	197	218	273
Cl <sup>-</sup>	NA	NA	336	95	322
PO <sub>4</sub> <sup>3-</sup>	NA	NA	217	213	74

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**Taste-active components in dried skipjack and salted salmon eggs (mg/100g)**

	Dried skipjack	Salted salmon		Dried skipjack	Salted salmon
		eggs			eggs
Glu	23	18.6	TMA	19	2
Lys	29	9	Creatinine	1150	-
His	1992	2.1	Lactic acid	3415	-
Carnosine	107	2.8	Glucose	6	8.4
IMP	474	2.7	Na <sup>+</sup>	434	117
HxR	186	7.8	K <sup>+</sup>	688	19
Adenosine	-	4.2	Cl <sup>-</sup>	1600	106
Guanosine	-	4.3	PO <sub>4</sub> <sup>3-</sup>	545	33
Uracil	-	3.1			

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Table 8.14 Effect of omission of each component on the flavour of synthetic extract

	Sea-urchin	Snow crab	Scallop	Short-necked clam	Dried skipjack
Glu	um sw	um sw sf	um sw pl	um sw pl	um sw
Gly	sw sf bi	um sw	sw pl	sw	—
Ala	sw bi	sw	sw	—	—
Arg	um sw	sf pl	sf	sf	—
IMP	um at	—	—	—	um sw pl
AMP	—	um	um sw pl	um sw pl	—
Na <sup>+</sup>	NA	sw um sf	um sf pl	sw um sa	sa sf pl
Cl <sup>-</sup>	NA	tl	sw um pl	sw um sa pl	sa sf pl

Abbreviations used: um, umami; sw, sweetness; bi, bitterness; sf, specific flavour of the seafood; at, aftertaste; pl, palatability; sa, saltiness; —, no effect. Bold letters signify an increase, others a decrease in the specified items.

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Effect of omission of each component on the flavor of synthetic extract

	海膽	慈愛蟹	扇貝柱	蜆	鯉柴魚
Glu	鮮 / 甜	鮮 / 甜 / <b>SF</b>	鮮 / 甜 / 美味性	鮮 / 甜 / 美味性	鮮 / 甜
Gly	甜 / 苦 <b>SF</b>	鮮 / 甜	甜 / 美味性	甜	—
Ala	甜 / 苦	甜	甜	—	—
Arg	鮮 / 甜	<b>SF</b> 美味性	<b>SF</b>	<b>SF</b>	—
IMP	鮮 / 餘味	—	—	—	鮮 / 甜 / 美味性
AMP	—	鮮	鮮 / 甜 / 美味性	鮮 / 甜 / 美味性	—
Na <sup>+</sup>	/	鮮 / 甜 / <b>SF</b>	鮮 / <b>SF</b> / 美味性	甜 / 鮮 / 鹹	鹹 / <b>SF</b> 美味性
Cl <sup>-</sup>	/	鮮 / 甜 / 美味性	甜 / 鮮 / 美味性	甜 / 鮮 / 鹹 美味性	鹹 / <b>SF</b> 美味性

紅字表示增強；—表示no effect。SF: specific flavor the seafood；美味性 palatability.

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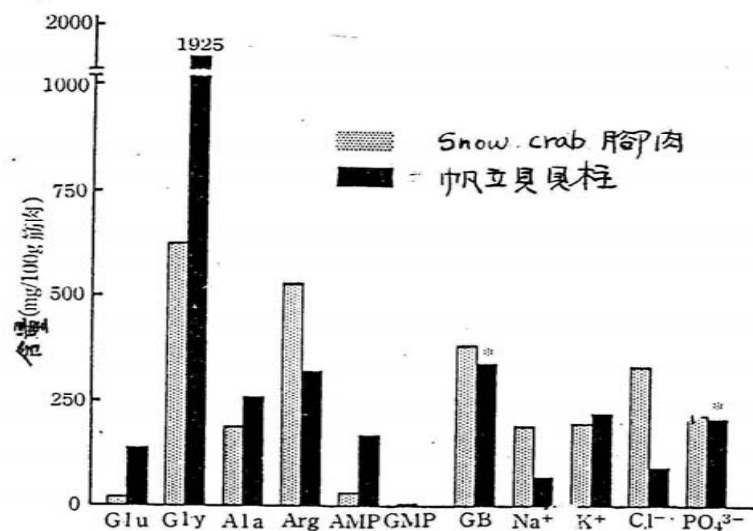


圖 8-2 スワイガニとホタテガイの呈味有効成分の含量の比較

\*：呈味無効と判定されたもの

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Nippon Suisan Gakkaishi 55(9), 1599-1603 (1989)

## The Effects of Phosphatase Treatment of Yellowtail Muscle Extracts and Subsequent Addition of IMP on Flavor Intensity

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(Received March 31, 1989)

Table 1. Effects of acid phosphatase treatment on flavor score of the boiled muscle extract

Flavor attributes	Flavor score
Umami	— 1.93 ± 0.19 <sup>+3</sup>
Sourness	1.43 ± 0.20 <sup>+3</sup>
Thickness	— 1.86 ± 0.14 <sup>+3</sup>
Fresh fish flavor	— 1.14 ± 0.40 <sup>+1</sup>
Overall taste quality	— 1.93 ± 0.19 <sup>+3</sup>

The original boiled muscle extract was presented as control. +1,  $p < 0.05$ ; +3,  $p < 0.001$ .

White muscle extract: 0.5 g/mL Dark muscle Extract: 0.33 g/mL

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Table 2. Effects of the addition of IMP to the enzyme-treated extract on flavor score

Flavor attributes	IMP added ( $\mu\text{mol/ml}$ )		
	0.125	0.25	0.5
Umami	$1.21 \pm 0.37^{+1}$	$0.93 \pm 0.07^{+3}$	$1.29 \pm 0.19^{+3}$
Sourness	$-0.21 \pm 0.29$	$-0.21 \pm 0.31$	$-1.07 \pm 0.23^{+2}$
Thickness	$0.21 \pm 0.33$	$1.21 \pm 0.37^{+1}$	$1.14 \pm 0.14^{+3}$
Fresh fish flavor	$0.14 \pm 0.40$	$0.36 \pm 0.36$	$0.86 \pm 0.14^{+3}$
Overall taste quality	$-0.29 \pm 0.34$	$0.36 \pm 0.28$	$1.00 \pm 0.01^{+3}$

The enzyme-treated extract was presented as control.  $+1, p < 0.05$ ;  $+2, p < 0.01$ ;  $+3, p < 0.001$ .

Table 3. Effect of the high level addition of IMP to the enzyme-treated extract on flavor score

Flavor attributes	IMP added ( $\mu\text{mol/ml}$ )			
	1.5	2	2.5	4.0
Umami	$-0.71 \pm 0.15^{+2}$	$-0.21 \pm 0.29$	$0.21 \pm 0.33$	$0.14 \pm 0.40$
Sourness	$0.71 \pm 0.19^{+2}$	$-0.36 \pm 0.32$	$-0.36 \pm 0.36$	$-0.29 \pm 0.29$
Thickness	$-1.00 \pm 0.01^{+3}$	$-0.29 \pm 0.36$	$-0.21 \pm 0.29$	$-0.29 \pm 0.36$
Fresh fish flavor	$-0.71 \pm 0.19^{+2}$	$-0.21 \pm 0.29$	$3.36 \pm 0.32$	$0 \pm 0.31$
Overall taste quality	$-1.00 \pm 0.01^{+3}$	$-0.29 \pm 0.34$	$-0.29 \pm 0.34$	$-0.29 \pm 0.36$

The original boiled muscle extract was presented as control.  $+2, p < 0.01$ ;  $+3, p < 0.001$ .

Table 4. Effects of the addition of IMP and Glu to the boiled muscle extracts on flavor score

Muscles	IMP added	Glu added
White (15-days storage)	$0.21 \pm 0.29$	$-0.29 \pm 0.29$
Dark (1-day storage)	$1.86 \pm 0.01^{+3}$	$0.14 \pm 0.31$

The original boiled muscle extracts were presented as control.  
 $+3, p < 0.001$ .

From:

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**Changes in Flavor Profile in Boiled  
Muscle Extracts of Yellowtail  
*Seriola quinqueradiata*  
Stored in Ice**

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(Received July 21, 1989)



**Table 1.** Changes in flavor score of the yellowtail white and dark muscle extracts during ice storage

	Storage period (days)						
	0	2	4	8	12	16	22
<b>WHITE MUSCLE</b>							
Umami	0	0.3 ±0.29	-0.1 ±0.46	0.1 ±0.26	0.1 ±0.26	0.1 ±0.36	-0.3 ±0.29
Sourness	0	-0.7 ±0.36	-0.7 ±0.42	-0.7 ±0.47	-0.6 ±0.37	-0.6 ±0.43	-0.1 ±0.40
Thickness	0	0.4 ±0.37	0.3 ±0.29	0.4 ±0.20	0.1 ±0.34	-0.3 ±0.36	-0.1 ±0.34
Astringency	0	0 ±0.22	0.1 ±0.26	0.3 ±0.19	0.3 ±0.29	0.1 ±0.34	0 ±0.00
Off-odor	0	0.1 ±0.26	-0.3 ±0.29	0 ±0.22	-0.1 ±0.40	-0.1 ±0.46	0 ±0.22
pH	5.9	6.1	6.0	6.1	6.1	6.0	6.1
<b>DARK MUSCLE</b>							
Umami	0	-0.9 ±0.26 <sup>*1</sup>	-1.0 ±0.31 <sup>*1</sup>	-1.0 ±0.38 <sup>*1</sup>	-1.0 ±0.34 <sup>*1</sup>	-1.0 ±0.28 <sup>*1</sup>	-1.3 ±0.28 <sup>*1</sup>
Sourness	0	-0.1 ±0.34	-0.4 ±0.37	0 ±0.43	-0.4 ±0.37	-0.3 ±0.19	-0.1 ±0.34
Thickness	0	-0.6 ±0.37	-1.1 ±0.34 <sup>*1</sup>	-1.3 ±0.36 <sup>*1</sup>	-1.5 ±0.30 <sup>*2</sup>	-1.0 ±0.43	-1.3 ±0.29 <sup>*2</sup>
Astringency	0	-0.1 ±0.26	-0.1 ±0.26	-0.1 ±0.26	0.4 ±0.30	0.9 ±0.26 <sup>*1</sup>	0.4 ±0.26
Off-odor	0	-0.6 ±0.43	-0.3 ±0.20	-0.3 ±0.36	-0.4 ±0.48	1.1 ±0.14 <sup>*2</sup>	1.3 ±0.19 <sup>*2</sup>
Bitterness	0	-0.4 ±0.30	-0.4 ±0.30	-0.1 ±0.26	0.1 ±0.26	1.3 ±0.29 <sup>*2</sup>	0.7 ±0.36
pH	6.2	6.3	6.3	6.2	6.3	6.2	6.3

Flavor score, mean ± SEM.

Symbols: <sup>\*1</sup>, significant at the 95% level; <sup>\*2</sup>, significant at the 99% level; <sup>\*3</sup>, significant at the 99.9% level.