

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES

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*Ex parte* DANIEL M. GORAL,  
Appellant

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Appeal 2008-3504  
Application 10/348,790<sup>1</sup>  
Technology Center 1700

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Decided: June 27, 2008

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Before TEDDY S. GRON, CHUNG K. PAK, and CAROL A. SPIEGEL,  
*Administrative Patent Judges.*

SPIEGEL, *Administrative Patent Judge.*

DECISION ON APPEAL

I. Introduction

Appellant appeals under 35 U.S.C. § 134 from a final rejection of all pending claims, claims 1-7, 9-11, and 13-18. We have jurisdiction under 35 U.S.C. § 6(b). We REVERSE and add a NEW GROUND OF REJECTION.

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<sup>1</sup> Application 10/348,790 ("the 790 application") was filed 22 January 2003. The real party in interest is said to be DANIEL M. GORAL (Appeal Brief under 37 C.F.R., filed 31 October 2005 ("App. Br."), 1).

The subject matter on appeal relates to an umami-enhancing food additive made from a combination of a fermented anchovy product made from fish of Southeast Asian origin and a fermented anchovy product made from fish originating from Spain, Morocco, Argentina, Chile, and/or Peru.

Claim 1 is illustrative and reads (App. Br. 9):

1. A flavor-enhancing food additive, consisting essentially of a first fermented anchovy product blended with a second fermented anchovy product, wherein the first fermented anchovy product is made from fish of Southeast Asian origin and comprises at least one free amino acid, and the second fermented anchovy product is made from fish originating from at least one of the group consisting of Spain, Morocco, Argentina, Chile, and Peru and comprises at least one nucleotide, and the flavor-enhancing food additive exhibits a greater umami expression than either the first fermented anchovy product or the second fermented anchovy product.

The Examiner relies upon the following references of record:

Masahiro                                      JP 2002-027943                                      Jan. 29, 2002.

"Vietnamese Dipping Sauce,"  
<http://www.recipelands.com/recipe/25273>, posted 22 January 1991, by  
Stephen Ceideberg ("VDS").

Irma S. Rombauer and Marion Rombauer Becker, JOY OF  
COOKING, pages 343 and 350 (The Bobbs-Merrill Company, Inc.,  
Indianapolis/New York) (1975) ("Rombauer").

The Examiner rejected claims 1-7, 9-11, and 13-18 under 35 U.S.C.  
§ 103(a) as unpatentable over Masahiro, VDS, and Rombauer (Ans.<sup>2</sup> 4).

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<sup>2</sup> Examiner's Answer mailed 6 September 2007 ("Ans.").

II. Findings of Fact ("FF")

The following findings of fact are supported by a preponderance of the evidence of record.

A. Background

- [1] "Umami can be defined as the taste properties resulting from the natural occurrence or intentional addition of certain compounds such as monosodium glutamate (MSG) and certain 5'-nucleotides such as 5'-inosine monophosphate (IMP) and 5'-guanosine monophosphate (GMP)" (Maga<sup>3</sup> 198, ¶1).
- [2] "[I]t has been postulated (Hashimoto, 1965) that all types of marine products possess umami compounds due to the high amounts of glutamic acid and nucleotides they contain" (Maga 199, ¶1).
- [3] Since glutamic acid is usually the major amino acid in proteins, processing or intentional hydrolysis of protein frees glutamic acid, which in turn can result in the formation of MSG (Maga 204, ¶3).
- [4] "One of the most fascinating aspects of umami compounds . . . is their ability to act synergistically when used in combination with each other" (Maga 201, ¶2).

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<sup>3</sup> J.A. Maga, "Umami flavour of meat," Chapter 9, pages 197-216, in FLAVOR OF MEAT, MEAT PRODUCTS AND SEAFOODS, second edition, F. Shahidi, ed., Blackie Academic and Professional, St. Edmundsbury Press, Suffolk, Great Britain (1998) ("Maga") (copy enclosed). Appellant submitted a portion of Maga, namely pages 202-205, in an Information Disclosure Statement filed concurrently with the 790 application on 22 January 2003.

- [5] "[I]f MSG is assigned a umami intensity of 1.0, the addition of an equal amount of GMP increases relative flavour intensity 30 times" (Maga 201, ¶2).
- [6] "The role of synergism . . . is also evident in Table 9.5 when all three compounds [MSG, IMP, and GMP] are utilized in equal proportions" (Maga 202, ¶1)
- [7] Maga Table 9.5 summarizes the individual taste thresholds for MSG, IMP, and GMP, alone and in combination, and is reproduced below (Maga 202):

**Table 9.5** Umami compound taste thresholds

Compound	Taste threshold (%)
MSG	0.012
IMP	0.014
GMP	0.0035
IMP + GMP	0.0063
IMP + GMP + MSG	0.000031

From Maga (1983).

{Maga Table 9.5 lists umami compound taste thresholds.}

- [8] Maga Table 9.5 shows the findings of J.A. Maga, published in *Critical Reviews in Food Science and Nutrition*, Vol. 18, pages 231-312 (1983) (Maga 202 and 215).
- [9] It is well known that fish contain nucleotides and adenosine triphosphate (ATP), the main nucleotide in live fish, is degraded to IMP after death (see e.g., Mendes<sup>4</sup> 143, ¶¶5-6).

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<sup>4</sup>Mendes et al., "Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portugese coast," *Eur. Food Res. Technol.*, Vol. 212, page 141-146 (2001) ("Mendes") (copy enclosed).

B. Appellant's Specification ("Spec.")

- [10] The 790 specification states "IMP has been shown to greatly amplify the umami response . . . in the presence of various free amino acids and glutamate" (Spec. 1:25-26).
- [11] "Trained sensory panelists have been able to correlate the intensity of the umami taste with the actual content of individual umami compounds in a statistically significant manner" (Spec. 1:32 through 2:2).
- [12] Flavor enhancer manufacturers are said to promote the concept that "you can use less MSG when combined with a small amount of IMP or GMP, and even less MSG when combined with an IMP/GMP blend" (Spec. 2:3-6).
- [13] "There are no known references in the literature to blending natural products that contain these substances to achieve umami synergy" (Spec. 2:7-8).
- [14] The 790 specification describes Appellant's invention as an all-natural blend of a fish sauce having a high free amino acid and high glutamate content and an anchovy paste having a relatively high nucleotide content (Spec. 2:11-16 and 3:24-27).
- [15] The 790 specification, page 7, reproduces the identical "Umami compound taste thresholds" table found in J.A. Maga, published in *Critical Reviews in Food Science and Nutrition*, Vol. 18, pages 231-312 (1983) shown in Maga (see FF 7).
- [16] According to the 790 specification,

The flavor-enhancing product of the invention can be made by first procuring the fish sauce made from Southeast Asian anchovies and

the anchovy paste made from anchovies from Spain, Morocco, Argentina, Chile, or Peru. Alternatively, naturally fermented anchovy products of another form or originating from other areas around the world may be analyzed to determine whether appropriate umami compounds are present, namely the same umami compounds present in Southeast Asian fish sauce and Spanish, Moroccan, or South American anchovy paste, as described herein.

Quantitative analytical tests have been developed to prove the existence of MSG, IMP, GMP, and other free amino acids in anchovy products. Using chromatographic analysis, it has been found that cured anchovy paste fish contain a significant amount of the nucleotides IMP and GMP. More specifically, the anchovy paste used to form the flavor enhancer . . . should have at least 3.0 milligrams/gram (0.30%) IMP and at least 0.05 mg/g (0.005%) GMP. Also, the fish sauce used to form the flavor enhancer . . . should have at least 1.0% glutamate. [Spec. 8:9-22.]

Other findings of fact follow below.

## II. Discussion

### A. Legal Principles

A claimed invention is not patentable if it would have been obvious to a person of ordinary skill in the art. 35 U.S.C. § 103(a); *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727 (2007); *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966). Facts relevant to a determination of obviousness include: (1) the scope and content of the prior art, (2) any differences between the claimed invention and the prior art, (3) the level of ordinary skill in the art and (4) relevant objective evidence of nonobviousness. *KSR*,

127 S.Ct. at 1734; *Graham*, 383 U.S. at 17-18. All claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 984 (CCPA 1974).

It is well settled that the transition term "consisting essentially of," when used with compositions, means that other ingredients in any amounts may be included in the scope of the composition as long as these ingredients do not materially affect the basic and novel characteristics of the composition. *See PPG Indus. v. Guardian Indus. Corp.*, 156 F.3d 1351, 1354 (Fed. Cir. 1998). Appellant has the burden of establishing what is excluded by "consisting essentially of," especially when the specification clearly indicates that other components may be present as well. *See In re Herz*, 537 F.2d 549, 551-52 (CCPA 1976).

B. The Examiner's position

- [17] The Examiner found Masahiro "(JP 2002-027943) disclose[s] a traditional fish sauce (see abstract)," Rombauer "disclose[s] the conventional use of anchovy paste (see pages 343 and 350)," and VDS "discloses the combination of anchovy sauce and fish sauce in a 1:1 ratio, where anchovy cream may be substituted for the anchovy sauce (see entire document)" (Ans. 4).
- [18] According to the Examiner, Appellant's specification establishes "the presence of free amino acids in fish sauce and nucleotides in anchovy paste are inherent..." (Ans. 4).
- [19] The Examiner concluded the claimed invention is a combination of conventional condiments known in the art and "the source of fish is seen to be no more than a matter of choice and well within the skill of the art" (Ans. 4).

C. Appellant's position

[20] Appellant argues

(i) none of Masahiro, Rombauer, and/or VDS teach or suggest combining a fermented anchovy product made from fish of Southeast Asian origin and another fermented anchovy product made from fish originating from Spain, Morocco, Argentina, Chile, and/or Peru (App. Br. 4; Reply Br.<sup>5</sup> 3),

(ii) "Many other kinds of salted fish exist, but few if any have the characteristic umami functionality and nucleotide content necessary to provide the synergy of the combination of the fermented anchovy products disclosed..." (App. Br. 4-5) (see also Reply Br. 3 ("The differences in the amino acid composition based on the differences in origin results in two different classes of umami compounds.")), and

(iii) "consisting essentially of" claim language excludes food products containing ingredients that would materially affect the novel heightened/synergistic umami sensation of the claimed combination of anchovy products (App. Br. 7-8; Reply Br. 2-3). Specifically, the pineapple, garlic, red chili pepper, sugar, lemon juice, and vinegar in VDS and the butter, onion juice, lemon juice, and cayenne pepper in Rombauer possess strong, distinctive flavors "that would most certainly affect the flavor profile, as well as the umami expression, of the fermented anchovy products" (Reply Br. 3).

[21] In other words, "Appellant took the initiative to obtain the analytical test data of various fermented anchovy products and combined this

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<sup>5</sup> Appellant's Reply Brief under 37 CFR 41.41 filed 26 October 2007 ("Reply Br.").

data with the findings of J.A. Maga to arrive at the claimed invention. There is no suggestion or motivation in any of the cited references . . . to apply the findings of J.A. Maga to any actual foods, particularly to Southeast Asian fish sauce and Mediteranean/South American anchovy paste." (Reply Br. 6).

D. Analysis

The claimed food additive is a combination "consisting essentially of" MSG and IMP and/or GMP. The MSG is supplied as a "natural product," i.e., a first fermented anchovy product made from fish of Southeast Asian origin. The IMP and/or GMP is also supplied as a "natural product," i.e., a second fermented anchovy product made from fish of Spanish, Moroccan, Argentinian, Chilean or Peruvian origin.

The Examiner expressly relied on Masahiro's (JP 2002-027943) disclosure of a fish sauce to reject claims 1-7, 9-11, and 13-18 (Ans. 4). However, neither the Masahiro reference nor a credible, verified English translation thereof is of record.<sup>6</sup> A conclusion of obviousness is based on underlying findings of fact. Here, the factual content of Masahiro being relied upon by the Examiner is unclear. A rejection under 35 U.S.C. §103

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<sup>6</sup> The Examiner provided a machine translation of JP 2002-027943 with the Examiner's Answer (Ans. 3). The machine translation contains a disclaimer stating that it may contain errors and that texts in the figures are not translated. Thus, the machine translation is, at a minimum, incomplete. Further, the Examiner did not cite to any specific paragraphs of the machine translation. We decline to determine the accuracy of the machine translation and the particular support therein for the Examiner's conclusion of obviousness in the first instance. Indeed, the machine translation does not even contain an "abstract," which the Examiner purportedly relies on (Ans. 3).

should not be based on speculations and assumptions. *In re Steele*, 305 F.2d 859, 862 (CCPA 1962).

Furthermore, the Examiner apparently predicated her conclusion of obviousness exclusively on Appellant's teaching that free amino acids and nucleotides are inherently present in fish sauce and anchovy paste, respectively (see FF 18). The Examiner implies, without citing prior art support, e.g., in the disclosure of VDS, Rombauer, and/or Masahiro, or the general knowledge of one of ordinary skill in the art, that the source of the fish is seen to be no more than a matter of choice and well within the skill in the art to select because all fermented fish products contain umami compounds MSG, IMP, and/or GMP (see Ans. 4). The Examiner did not respond to Appellant's argument that differences in the origin of the fish results in different umami compounds in the fermented fish products.

Therefore, we will reverse the rejection of claims 1-7, 9-11, and 13-18 under § 103(a) as obvious over VDS, Rombauer, and Masahiro.

We add the following comments for completeness. It is Appellant's burden to establish what is excluded by "consisting essentially of." Attorney argument and conclusory statements, absent evidence, are entitled to little, if any, weight. *In re Pearson*, 494 F.2d 1399, 1405 (CCPA 1974). Therefore, Appellant's unsupported argument that additional ingredients in VDS and Rombauer "would most certainly affect the flavor profile, as well as the umami expression, of the fermented anchovy products" carries little, if any, weight. (Reply Br. 3). Finally, the prior art, e.g., the 1983 Maga article incorporated by reference into Appellant's specification (Spec. 7:21-22), teaches MSG's synergistic reaction with IMP and/or GMP to provide an

enhanced umami effect. Here, we agree with the Examiner (Ans. 6) that the claimed synergistic umami expression is an expected result.

III. New Ground of Rejection

We make the following new ground of rejection pursuant to 37 C.F.R. § 41.50(b). Claims 1 and 17 are rejected under 35 U.S.C. § 103(a) as obvious over the combined teachings of Maga and Mendes.

Claim 1 has been recited above. Claim 17 reads (App. Br. 11):

17. A method of making a flavor-enhancing food additive, comprising the steps of:

providing fish sauce made from fish of Southeast Asian origin and comprising at least one free amino acid;

providing anchovy paste made from fish originating from at least one of the group consisting of Spain, Morocco, Argentina, Chile, and Peru and comprising at least one nucleotide; and

blending the fish sauce and the anchovy paste together to form the flavor-enhancing food additive consisting essentially of the fish sauce and the anchovy paste.

*KSR Int'l Co. v. Teleflex, Inc.*, 127 S.Ct. 1727, 1732 (2007), instructs that when, there is motivation

or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to

try might show that it was obvious under § 103.  
*Id.*

This reasoning is applicable here. The "problem" facing those in the art was production of a "natural-based" versus "chemical-based" flavor additive that enhanced umami expression. Maga teaches that umami is a taste resulting from "the natural occurrence or intentional addition of" MSG and certain nucleotides, e.g., IMP and GMP (FF 1). Maga suggests that all types of marine products contain umami compounds such as glutamate and nucleotides (FF 2). Indeed, a skilled artisan would have reasonably expected salt-processed dead fish to contain MSG due to degradation of fish protein, as suggested by Maga (FF 3), and to contain IMP due to degradation of ATP, as suggested by Mendes (FF 9). Furthermore, Maga teaches expression of umami is synergistically enhanced when MSG and IMP and/or GMP are used in combination (FFs 4-8). Therefore, the skilled artisan would have had reason to intentionally add naturally occurring sources of MSG and IMP and/or GMP to obtain a natural flavor additive that enhanced umami expression as claimed. Thus, analyzing the amino acid (MSG) content and nucleotide (IMP) content of various fermented fish products and combining the fish products to produce a "natural-based" umami-enhancing flavor additive would have been "the product not of innovation but of ordinary skill and common sense." Synergy in the combined fish products would have been expected due to the known synergy existing in combined MSG and IMP and/or GMP (FFs 4-8). The art reasonably suggests that the MSG and IMP and/or GMP content of the fish products determines enhanced umami expression, not the fish source *per se* of the fish products. Indeed, Appellant's specification states, "Alternatively, naturally fermented

anchovy products of another form or originating from other areas around the world may be analyzed to determine whether appropriate umami compounds are present, namely the same umami compounds present in Southeast Asian fish sauce and Spanish, Moroccan, or South American anchovy paste" (FF 16).

We leave it to the Examiner to determine the applicability of Maga and Mendes and other prior art to claims 2-7, 9-11, 13-16, and 18 in the first instance.

#### IV. Order

Upon consideration of the record, and for the reasons given, it is

ORDERED that the Examiner's decision to reject claims 1-7, 9-11, and 13-18 under 35 U.S.C. § 103(a) as unpatentable over Masahiro, VDS, and Rombauer is REVERSED;

FURTHER ORDERED that a new ground of rejection is entered against claims 1 and 17 under 35 U.S.C. § 103(a) as unpatentable over Maga in view of Mendes.

Section 41.50(b) also provides that *WITHIN TWO MONTHS FROM THE DATE OF THE DECISION*, Appellant must exercise one of the following two options with respect to the new ground of rejection to avoid termination of the appeal as to the rejected claims:

(1) *Reopen prosecution*. Submit an appropriate amendment of the claims so rejected or new evidence relating to the claims so rejected, or both, and have the matter reconsidered by the examiner, in which event the proceeding will be remanded to the examiner. . . .

(2) *Request rehearing*. Request that the proceeding be reheard under § 41.52 by the Board upon the same record. . . .

Should Appellant elect to prosecute further before the examiner pursuant to 37 CFR § 41.50(b)(1), in order to preserve the right to seek review under 35 U.S.C. §§ 141 or 145 with respect to the affirmed rejection, the effective date of the affirmance is deferred until conclusion of the prosecution before the Examiner unless, as a mere incident to the limited prosecution, the affirmed rejection is overcome.

If Appellant elects prosecution before the Examiner and this does not result in allowance of the application, abandonment or a second appeal, this case should be returned to the Board of Patent Appeals and Interferences for final action on the affirmed rejection, including any timely request for rehearing thereof.

REVERSED; 37 CFR § 41.50(b)

Enc.: J.A. Maga, "Umami flavour of meat," Chapter 9, pages 197-216, in *FLAVOR OF MEAT, MEAT PRODUCTS AND SEAFOODS*, second edition, F. Shahidi, ed., Blackie Academic and Professional, St. Edmundsbury Press, Suffolk, Great Britain (1998).

Mendes et al., "Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portuguese coast," *Eur. Food Res. Technol.*, Vol. 212, page 141-146 (2001).

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Appeal 2008-3504  
Application 10/348,790

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## 9 Umami flavour of meat

J.A. MAGA

### 9.1 Introduction

Many compounds have been shown to be present in the flavour fraction of food. However, the flavour chemist has to decide which compound or series of compounds are the major contributors to specific food flavours. This has been a long and difficult task, and as a result, the flavours of foods such as meats are still not completely understood.

Even more complex is the situation where certain compounds have been shown to intensify, modify or mask the flavours of certain foods. The fact that a specific compound or combination of compounds, when intentionally added or formed in foods by biological or thermal pathways, has the ability to change the perceived flavour properties of certain foods is a research area that is fascinating.

Over the years, various nomenclatures have been proposed for compounds that have the ability to modify flavour perception. These include terms such as flavour potentiators, flavour enhancers and umami. Currently, the scientific community appears to be adopting the name

umami, defined as the taste of monosodium glutamate (MSG) and 5-nucleotides such as 5'-inosinate (IMP) and 5'-guanylate (GMP).

The major objectives of this chapter are to define and discuss the properties of umami in model system studies, and to review the formation, identification, quantitation and stability together with the sensory significance in beef, pork, chicken, turkey and lamb. The influence of meat aging/processing and compound synergism is also discussed, in this attempt to update the role of umami in meat flavour chemistry.

In addition, the identification of certain naturally occurring peptides having umami-like properties has opened another interesting research area. Also, the incorporation of yeast extracts or hydrolysed proteins from both animal and plant sources has resulted in the flavour intensification/modification of various foods, including processed meats. This latter approach has specific application with the introduction of low-fat meat products, which can be typically described as lacking characteristic meat flavour. The intentional addition of these materials, which normally contain significant amounts of flavour enhancers, has resulted in increased acceptance of low-fat meat products.

## 9.2 Definitions

Umami can be defined as the taste properties resulting from the natural occurrence or intentional addition of compounds such as monosodium glutamate (MSG) and certain 5'-nucleotides such as 5'-inosine monophosphate (IMP) and 5'-guanosine monophosphate (GMP). Other researchers have used terms such as 'savory', 'beefy' and 'brothy' to describe the same taste sensations. These nucleotides have also been referred to respectively as inosinic and guanylic acids, 5'-inosine and 5'-guanylic acids, inosine 5'- and guanosine 5'-phosphates and disodium 5'-inosinate or disodium 5'-guanylate. IMP and GMP have also been marketed under the trade name Ribotide®.

Compounds of these types are especially interesting in that they have the ability to modify taste, even though they do not possess characteristic flavours of their own, especially at the low concentrations at which they affect food flavour. In using the above definition of flavour alteration without taste contribution, one could also consider that sodium chloride, if used as subthreshold levels, also possesses umami properties. This is an area that deserves research attention.

## 9.3 Historical background

Many cultures throughout the world have long used ingredients or food preparation techniques that result in the presence of umami compounds that intensify certain food flavours. Experience has taught cooks what it took scientists many years to discover.

It was not until the early 1900s that a specific compound that was proven to be responsible for an umami sensation was isolated. Ikeda (1909) was able to identify the compound monosodium glutamate (MSG) in the naturally occurring form in an extract from dried kombu or sea tangle, a type of seaweed. The importance of his discovery was soon evident because the commercial production of MSG for the intentional addition to foods began shortly thereafter. Apparently, Ikeda was the first to propose the name 'umami', which means 'deliciousness' in Japanese, for the taste sensation associated with MSG. Today MSG is produced in many countries and is consumed internationally.

A few years later, another food common to Oriental cuisine, dried bonito tuna, was the source for the identification of another umami compound, namely inosine monophosphate (IMP). It was reported in the initial study (Kodama, 1913) that the compound in question was the histidine salt of 5'-inosinic acid. However, it was later concluded that histidine was not a significant contributor to umami. In contrast to MSG, the commercialization of IMP was not begun until the early 1960s.

In the early 1960s another compound, guanosine monophosphate (GMP), was identified from another natural source, the Shiitake black mushroom (Nakajima *et al.*, 1961; Shimazono, 1964). It is quite interesting to note that the three commercially available umami compounds were first identified from natural sources. In fact, it has been postulated (Hashimoto, 1965) that all types of marine products possess umami compounds due to the high amounts of glutamic acid and nucleotides that they contain.

More recently, other umami compounds, including ibotenic and tricholomic acids, have been identified as naturally occurring compounds in other types of Japanese mushrooms (Takemoto and Nakajima, 1964; Takemoto *et al.*, 1964). Currently these umami compounds are not commercially available due to the effectiveness and ready availability of MSG, IMP and GMP. In the meantime, over 80 years of research on umami compounds has accumulated and their commercialization now represents an international industry worth several hundred million dollars.

#### 9.4 Structural considerations

To date, umami compounds have been found to structurally contain either L-amino acids containing five carbon atoms or a purine ribonucleotide 5'-monophosphate having an oxy group in the 6-position. These structures are representative of MSG, IMP and GMP as shown in Figure 9.1.

In commercial practice the amino acid based compound is in the monosodium salt form whereas the nucleotides are in the disodium form. In addition, MSG can also be obtained in the potassium, ammonium or calcium forms for utilization in products where low sodium levels are desired.

The isomeric structure of umami compounds can also dramatically influence their taste properties. In the case of MSG, the D-form, which is not

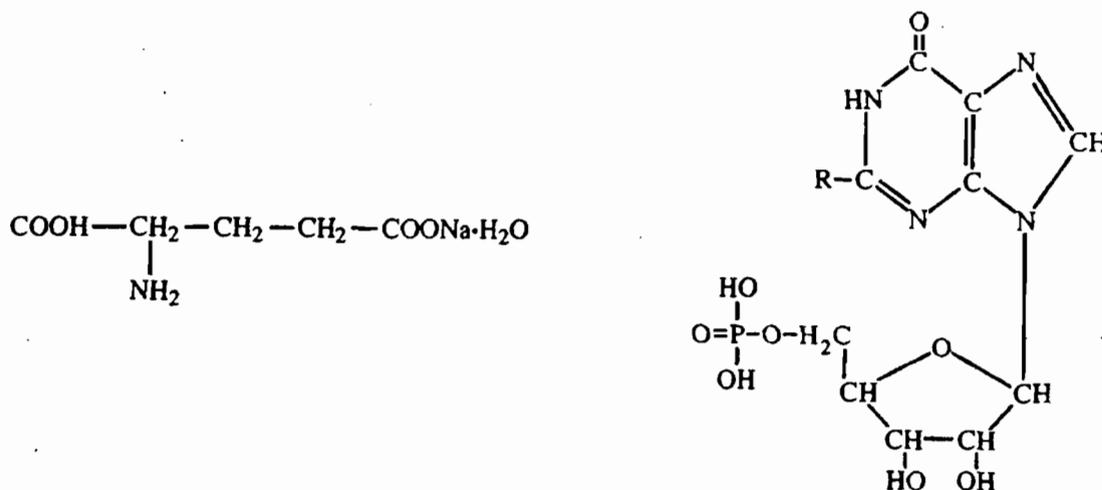


Figure 9.1 Umami structures.

naturally occurring, has no umami properties, whereas the L-form, which is naturally occurring, does. The nucleotides IMP and GMP can occur as the 2'-, 3'- or 5'- forms but only the 5'-form is taste active.

In the case of MSG, it is interesting to note that L-glutamine, which has the same basic structure as MSG, has no umami taste. The umami properties of other structurally related amino acids have been investigated (Akabori, 1939) and it was concluded that the L-forms of  $\alpha$ -amino dicarboxylic acids with four to seven carbons also have umami properties similar to those of L-glutamic acid. The flavour properties of various substituted nucleotides have also been reported (Yamazaki *et al.*, 1968a,b) and as expected, some structures do result in compounds possessing umami properties.

### 9.5 Stability

When in solution, MSG acts as an ampholyte and as such can exist in a number of ionic forms, in equilibrium, which are pH dependent. At various pHs forms such as glutamic acid hydrochloride, free glutamic acid, neutral MSG and basic disodium glutamate can exist. The influence of pH on these forms is summarized in Table 9.1, and as can be seen, the neutral MSG form is predominant over most of the pH scale, except in very acid conditions. The neutral MSG form in turn possesses the most potent umami sensation.

The thermal stability of IMP and GMP has been shown to be also pH dependent. As seen in Table 9.2, acidic conditions decrease compound stability and IMP appears to be slightly more stable than GMP. Their thermal degradation has been shown to follow first order kinetics with the major degradation products being nucleosides and phosphoric acid, which would suggest degradation via hydrolysis of the phosphoric ester bond (Matoba *et al.*, 1988).

**Table 9.1** Ionic form distribution of MSG as influenced by pH

pH	Distribution (%)			
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
8.0	0	0	96.9	3.1
7.0	0	0	99.8	0
6.0	0	1.8	98.2	0
5.5	0	5.3	94.7	0
5.0	0	15.1	84.9	0
4.5	0	36.0	64.0	0
4.0	0.9	63.1	36.0	0
3.5	4.0	80.9	15.1	0
3.0	13.4	81.3	5.3	0

**Table 9.2** Stability of GMP and IMP to temperature and pH

Compound	pH	Temperature (°C)	Half-life (h)
GMP	4.0	100	6.4
	7.0	100	8.2
	9.0	100	38.5
IMP	4.0	100	8.7
	7.0	100	13.1
	9.0	100	46.2

A 10°C increase in temperature lowered all half-lives by one-third. From Matoba *et al.* (1988).

**Table 9.3** Aqueous hydrolysis rates for nucleotides at 121°C and various pH values

pH	Compound	Half-life (min)
3.0	IMP	32.4
	GMP	31.5
4.0	IMP	45.3
	GMP	62.4
5.0	IMP	45.9
	GMP	57.8
6.0	IMP	63.0
	GMP	41.0

From Shaoul and Sporns (1987).

Nucleotide stability at temperatures normally used in retorting also presents problems, especially for larger-size cans where extended retorting times are required. The data summarized in Table 9.3 show that under highly acidic conditions half of either IMP or GMP can be lost in approximately 30 min. In contrast, it has been estimated that at pH 5 and at 23°C the half-life for IMP and GMP is 36 and 19 years, respectively (Shaoul and Sporns, 1987).

## 9.6 Synergism

One of the most fascinating aspects of umami compounds that is far from understood is their ability to act synergistically when used in combination with each other. This concept has been extensively reviewed (Maga, 1983) and thus will only be briefly touched upon. As shown in Table 9.4, if MSG is assigned a umami intensity of 1.0, the addition of an equal amount of GMP increases relative flavour intensity 30 times. Even addition of as little as 1% GMP to MSG significantly increases flavour intensity. Similar effects are noted for combinations of IMP and MSG.

**Table 9.4** Synergistic flavour intensity of MSG-GMP

Ratio of MSG:GMP	Relative flavour intensity
1:0	1.0
1:1	30.0
10:1	18.8
20:1	12.5
50:1	6.4
100:1	5.5

From Ribotide® Product Data Sheet No. 3.

## 9.7 Taste properties

The individual taste thresholds for various umami compounds, alone and in combination with each other, are summarized in Table 9.5. It can be seen that when used alone, GMP has a threshold an order of magnitude lower than that for MSG and IMP. The role of synergism as described above is also evident in Table 9.5 when all three compounds are utilized in equal proportions. Therefore, if naturally present in combination or intentionally added in combination, a relatively small total amount is required to elicit a umami response.

Relative to the basic tastes of sweet, salt, sour and bitter, the taste threshold of MSG is well within their range (Table 9.6). This is quite interesting in light of the fact that there are many studies attempting to relate the taste of MSG to the other basic tastes. As seen in Figure 9.2, various portions of the MSG molecule have been proposed to possess the potential to elicit other tastes. This approach has led Birch (1987) to propose that MSG not only possesss a umami taste but also a salty one (Table 9.7). In addition, he proposes that aspartic and glutamic acids also have a umami taste in combination with an acidic sensation.

Yamaguchi (1987) has attempted to locate the relative position of umami taste to the other basic tastes as well as to that of various foods, utilizing multi-dimensional plotting. As seen in Figure 9.3, umami falls outside the regions occupied by sweet, salt, sour and bitter tastes but is closely associated with tastes derived from various marine and meat prod-

**Table 9.5** Umami compound taste thresholds

Compound	Taste threshold (%)
MSG	0.012
IMP	0.014
GMP	0.0035
IMP + GMP	0.0063
IMP + GMP + MSG	0.000031

From Maga (1983).

**Table 9.6** Detection thresholds in water of various taste substances

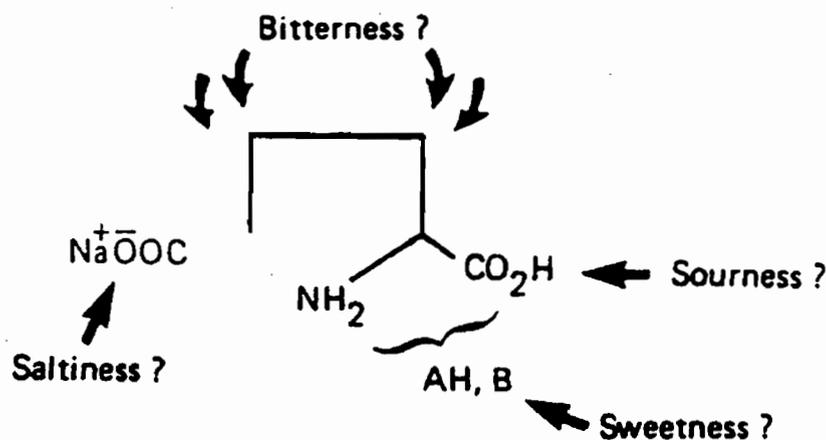
Compound	Threshold (%)
Sucrose	0.086
Sodium chloride	0.0037
Tartaric acid	0.00094
Quinine sulphate	0.000049
MSG	0.012

From Yamaguchi (1987).

**Table 9.7** Compounds related to MSG and having umami taste

Compound	Tastes
MSG	Umami and salty
Aspartic acid	Acidic and umami
Glutamic acid	Acidic and umami

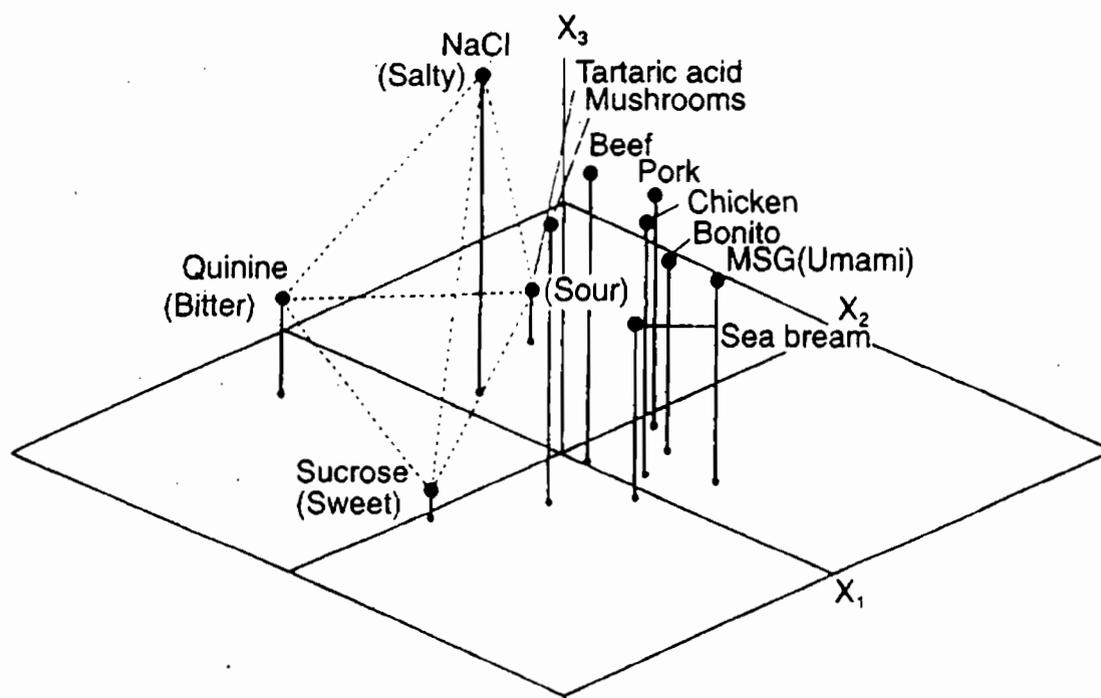
From Birch (1987).

**Figure 9.2** Proposed MSG taste properties (Birch, 1987).

ucts. These data can be interpreted in at least two different ways. First, the data clearly demonstrate that umami is neither associated with, nor is the result of, the four acknowledged basic tastes, from which one could conclude that it indeed is a separate and distinctive taste. On the other hand, one also conclude that umami is a specialized form of taste associated with meat or marine-based foods.

## 9.8 Food occurrence

Umami compounds and their precursors are present in a wide variety of foods. This can perhaps be best appreciated by viewing the information



**Figure 9.3** Relationship of umami taste to other tastes and foods (Yamaguchi, 1987).

summarized in Table 9.8. These foods include raw and processed, fermented and unfermented, as well as plant and animal sources. Therefore, those individuals who may be concerned with the intentional addition of umami compounds to various foods would be hard pressed to identify a varied diet that did not contain naturally occurring sources of the compounds in question.

If one looks at the free glutamate levels that are present in various foods (Table 9.9), it can quickly be appreciated that significant amounts of glutamate may be present, especially in cheeses that are aged for long periods.

Glutamic acid is usually the major amino acid in protein, and as seen in Table 9.10, it represents approximately 20% of all amino acids in various animal protein sources. When these proteins are processed or intentionally hydrolysed in the manufacture of hydrolysed protein, glutamic acid is freed, which in turn can result in the formation of MSG.

**Table 9.8** Foods containing umami compounds

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Beverages (beer, tea, wine)
Fruits (various)
Marine products (seaweed, fish, clams, crab, oysters, shrimp)
Meats (beef, chicken, lamb, pork)
Milk products (milk, cheese)
Plant proteins (barley, coconut, corn, cottonseed, peanut, soybean, wheat)
Vegetables (various)

---

From Maga (1983).

**Table 9.9** Free glutamate levels in various foods

Food	Glutamate level (mg/100 g)
Tomato	140
Tomato juice	260
Grape juice	258
Broccoli	176
Parmesan cheese	1 200
Gruyère cheese	1 050

From Giacometti (1979).

**Table 9.10** Glutamate levels in various animal proteins

Protein	Glutamate level (g/100 g)
$\alpha$ -Casein (milk)	22.5
$\beta$ -Lactoglobulin (milk)	20.0
Actin (muscle)	14.8
Myosin (muscle)	21.0
Albumin (egg white)	16.5

From Giacometti (1979).

If one was to rank the importance of umami compounds to the flavour properties of various foods, as shown in Table 9.11, it becomes quite apparent that they make major contributions to meat flavours. Therefore, the remainder of this review will be devoted to the role of umami compounds in meat flavour.

## 9.9 Umami compounds and meat flavour

Numerous early studies have demonstrated that the intentional addition of MSG and nucleotides significantly modifies meat flavour perception. For example, Girardot and Peryam (1954) showed that the addition of MSG to a variety of processed meats resulted in products that were preferred over the same products to which MSG was not added (Table 9.12). This was especially true in the case of chicken. Of the products they evaluated, meat loaf had the least improvement in flavour perception.

When using added IMP, other researchers (Kurtzman and Sjostrom, 1964) concluded that canned chicken-containing noodle soup was not flavour-enhanced (Table 9.13). Based on IMP thermal stability data presented earlier, perhaps most or all of the added IMP was degraded. However, other products evaluated, including canned beef noodle soup, did show improvement with IMP addition. For some unexplained reason, no improvement was found with canned beef hash.

**Table 9.11** Major taste-active compounds in foods

Compound	Meat	Vegetables	Fruit	Roots	Seeds
Inorganic ions	x x	x	x	x	x
Amino acids	x x x	x x	x	x x	x x
Peptides and proteins	x x x	x x		x	x x
Histidine dipeptides	x x x				
Nucleotides	x x x	x		x x	x x
Amines	x x	x			
Sugars			x x x	x x	x x
Phenols (simple)		x x	x x		x
Hydroxy compounds		x	x x		
Polyphenolic compounds	x x	x x			x
Carbonyl compounds		x x	x x x		x
Esters			x x		
Sulphur compounds	x x	x x			x
Acids		x x	x x x		
Furans		x x	x x	x	x
Lactones		x x	x x x		
N,S-heterocycles	x	x x x	x		x

From Grill and Flynn (1987).

x of minor importance; x x somewhat important;

x x x very important.

**Table 9.12** Preference of processed meats containing MSG

Product	% Preferring MSG sample
Beef stew	61
Beef and gravy	61
Boned chicken	75
Hamburger	65
Meat loaf	53
Pork and gravy	66

From Giradot and Peryam (1954).

**Table 9.13** Influence of IMP addition to the flavour of various meat products

Product	Effect
Beef bouillon	Enhanced
Beef noodle soup, canned	Enhanced
Chicken noodle soup, canned	Undecided
Canned luncheon meats	Enhanced
Corned beef hash	Not enhanced
Ham	Enhanced

From Kurtzman and Sjostrom (1964).

Bauer (1983) clearly demonstrated that the aging of meat clearly influences resulting glutamic acid content. He aged beef and pork for 4 and 7 days, and as seen in Table 9.14, in the case of both products, the longer-aged meat had significantly higher levels of glutamic acid. Apparently, microbial activity during aging resulted in partial protein hydrolysis, thereby liberating free glutamic acid. However, as seen in Table 9.15, the levels of glutamate present in a range of processed meats are quite variable. The relatively low levels found in some of these products could perhaps be attributed to the degree of heat processing or low pH.

The free glutamate content in a variety of cooked meats has been summarized in Table 9.16, and as can be seen, most of the values are relatively low except for the two poultry products and the free-run juice from beef rib roast. It is also interesting to note that cooking beef rib roast to well done compared to medium rare resulted in approximately an 80% reduction in the amount of free glutamate present. Other products that had low values included lamb and mutton as well as boiled frankfurters. In the case of the frankfurters, the low value could be attributed to one of two reasons; either their high fat content had a dilution effect on the actual amount of meat present, or the free glutamate was lost to the cooking water.

The nucleotide levels naturally found in beef and chicken are summarized in Table 9.17. These data indicate that beef actually has higher levels of IMP than does chicken whereas the levels of GMP are the same.

**Table 9.14** Meat age versus glutamic acid content

Product	Age (days)	Glutamic acid content (mg/100 g)
Beef	4	9.3
	7	21.1
Pork	4	11.4
	7	16.0

From Bauer (1983).

**Table 9.15** Glutamate content of processed meats

Product	Glutamate content (%)
Dry sausages	0.03–0.25
Frankfurters	0.02–0.15
Liver sausages	0.01–0.29
Hams	0.01–0.11
Pickled tongue	0.07–0.17

From Gerhardt and Schulz (1985).

**Table 9.16** Free glutamate content of various cooked meats

Product	Glutamate content (%)
Beef, tenderloin	0.042
Beef, shank	0.014
Beef, standing rib, medium rare	0.057
Juice from above	0.088
Beef, standing rib, well done	0.013
Bologna	0.004
Chicken	0.055
Duck	0.064
Frankfurters, boiled	0.001
Lamb	0.003
Mutton	0.008
Pork, loin	0.029

From Maga (1983).

**Table 9.17** Nucleotide composition (%) of various meats

Nucleotide	Beef	Chicken
IMP	0.106-0.443	0.075-0.122
GMP	0.002	0.002
AMP	0.007	0.007-0.013
CMP	0.001	0.002
UMP	0.001	0.003

From Maga (1983).

Interestingly, chicken has somewhat higher levels of other nucleotides (AMP, CMP, UMP), which have limited umami properties, than beef.

Kato and Nishimura (1987) measured the IMP levels in beef, pork and chicken that had been aged for varying lengths of time, and as seen in Table 9.18, chicken had the highest IMP level even though it had only been aged 2 days. Pork had the next highest level while beef, although aged the longest, had the lowest amount of IMP. When a panel was asked to judge whether samples had a more intense umami taste before or after again, no difference was found for beef, which had the lowest IMP level, while storage significantly influenced umami taste intensity for pork and chicken (Table 9.19). Interestingly, cooking stored beef, pork and chicken

**Table 9.18** Effect of meat storage on IMP levels

Meat and storage days	IMP level ( $\mu\text{mol/g}$ meat)
Beef (12)	3.2
Pork (6)	6.7
Chicken (2)	7.2

From Kato and Nishimura (1987).

**Table 9.19** Effect of meat storage on umami taste intensity

Meat and storage days	Number of samples judged to have a more intense umami taste		Significance
	Before storage	After storage	
Beef (12)	12	4	NS
Pork (6)	2	14	S
Chicken (2)	8	23	S

NS, not significant; S, significant.  
From Kato and Nishimura (1987).

(Table 9.20) resulted in levels of IMP that were 50% higher in all three products, as compared to their noncooked but aged counterparts.

Most researchers have exclusively investigated the influence of umami compounds on taste perception but one should also question if these compounds can influence odour perception. A limited number of articles has appeared addressing this issue. Maga and Lorenz (1972) prepared beef stock samples to which they added either no umami compounds, or a total of 0.05% of only MSG, IMP or GMP, or a combination of IMP, GMP and MSG. They sampled the headspace above the various stocks and measured total peak areas using gas chromatography. The stock sample which had no added compounds served as the control. As seen in Table 9.21, the addition of MSG alone as well as combinations of umami compounds significantly increased peak area ratios thereby indicating that these compounds can also influence aroma properties.

**Table 9.20** Effect of storage on IMP levels in cooked meats

Meat and storage days	IMP level ( $\mu\text{mol/g}$ meat)
Beef (12)	4.1
Pork (6)	10.5
Chicken (2)	10.9

From Kato and Nishimura (1987).

**Table 9.21** Beef broth GLC headspace peak area ratios versus umami additions

Peak area ratio <sup>a</sup>	Additive
1.66	0.05% MSG
2.30	IMP + GMP (0.05% total)
2.35	IMP + GMP + MSG (0.05% total)

<sup>a</sup> Control versus compound addition.  
From Maga and Lorenz (1972).

Yamaguchi and Kimizuka (1979) performed a flavour profile analysis on cooked hamburgers to which 1% MSG had been added as compared to a no additive control. As seen in Table 9.22, they observed a slight increase in the 'meaty' and 'acceptability' descriptors associated with the aroma portion of the profile of hamburgers containing added MSG. It is also interesting to note that added MSG also intensified many of the other sensory properties.

Yamaguchi (1987) has conducted extensive research on umami compounds in meat stocks. For example, she found that the major nucleotide in chicken stock was IMP (Table 9.23). Interestingly, no GMP was detected in this study. In addition to IMP, relatively high levels of glutamic acid were found. In comparing beef and chicken stocks, she reported (Table 9.24) significant differences between minimum detectable levels for MSG and IMP. Twice the amount of MSG was required before it was detected in chicken stock as compared to beef stock, whereas only one-third the amount of IMP required for beef stock needed to be added to chicken stock before it was detected.

**Table 9.22** Flavour profile of cooked hamburger with 1% MSG

Descriptor	Score
<b>Aroma</b>	
Whole aroma	0
Meaty	0.2
Beefy	0
Acceptability	0.4
<b>Basic taste</b>	
Whole taste	0.6
Salty	0.3
Sweet	0.1
Sour	0.2
Bitter	0
<b>Flavour characteristic</b>	
Continuity	0.5
Mouthfulness	0.6
Impact	0.5
Mildness	0.5
Thickness	0.4
<b>Other flavours</b>	
Spicy	0.2
Oily	0
Meaty	0.2
Beefy	0.3
<b>Overall preference</b>	
Palatability	0.6

0, Same as control (no MSG); 1, slight increase; 2, marked increase.

From Yamaguchi and Kimizuka (1979).

**Table 9.23** Umami content (mg/100 ml) in chicken stock

Compound	Amount
AMP	2.26
IMP	5.84
GMP	0
ATP	ND
Glutamic acid	15.00

From Yamaguchi (1987).

ND = not detected.

**Table 9.24** Detection levels of umami compounds added to stocks

Stock	Detection level (%)	
	MSG	IMP
Beef	0.00625	0.025
Chicken	0.0125	0.00625

From Yamaguchi (1987).

Maga (1987) attempted to evaluate the role of MSG, IMP and GMP on the taste intensities of various purified meat proteins including beef, pork, lamb, chicken and turkey as compared to the same meat proteins containing no added compounds. From Table 9.25 it can be seen that with no additions, beef and pork had the highest intensity ratings while lamb had the lowest. In all cases, the addition of any of the three compounds increased taste intensity although the increase for lamb was minimal. In looking at percentage increases over the control (Table 9.26) it is apparent that the most affected meat protein was chicken. Also, not all additives were equally effective. Therefore, these data would indicate that the functionality and perhaps mechanism of interaction between umami compounds and meat proteins vary with protein source.

**Table 9.25** Taste intensities (0-100) of various 1% meat proteins with added umami compounds

Meat	No added umami	Added umami compound		
		MSG (0.015%)	IMP (0.010%)	GMP (0.004%)
Beef	64	83	80	89
Pork	60	68	70	76
Lamb	41	46	45	48
Chicken	53	84	86	90
Turkey	49	61	60	65

From Maga (1987).

**Table 9.26** Percentage increase in taste intensity caused by umami addition to meats

Meat	Added umami compound		
	MSG	IMP	GMP
Beef	30	25	39
Pork	13	17	27
Lamb	12	10	17
Chicken	58	62	70
Turkey	24	22	33

From Maga (1987).

### 9.10 Yeast extracts

The fact that yeast is generally high in ribonucleic acid (RNA), and that it is a readily available by-product from various food processing operations, has led to the widespread use of yeast extracts or powders to modify or intensify meat flavour. As a result, 5'-nucleotides as well as MSG can be derived from the nucleotide associated with yeast RNA. Yeast extracts in turn can be used in conjunction with added MSG to produce an extremely effective umami effect.

Basically, yeast extract is a concentrated form of soluble material obtained from yeast cells that have undergone hydrolysis by various techniques, including autolysis, plasmolysis or acid hydrolysis. By controlling temperature and pH, autolysis is an autodegradation process that minimizes the loss of inherent hydrolytic enzymes such as proteases, nucleases and carbohydrases. Plasmolysis is usually brought about by the addition of salt, while acid addition is used to induce acid hydrolysis. Based on the type and source of yeast, along with the type of hydrolysis employed, a wide range of umami types and concentrations can result. In addition, most yeast extracts have a wide array of precursors such as amino acids, sugars and B-vitamins that can react during subsequent heat treatment when added to meat systems to produce additional flavour compounds.

### 9.11 Hydrolysed proteins

Historically the use of products such as soy sauce as a flavour stimulant is well documented. In actuality, soy sauce represents a hydrolysed protein obtained by either natural fermentation or via chemical acid hydrolysis. Both processes result in a product high in MSG.

Through the past 30 years, various plant protein materials, such as wheat gluten, corn gluten, defatted legume flours (soy, peanut) and defatted cottonseed flour have served as starting materials for the manufacture of

what is commonly known as hydrolysed vegetable protein (HVP). Hydrolysis can be either by enzymatic addition or by acid/base addition. Acid-treated products are normally neutralized with sodium hydroxide, thereby resulting in the formation of sodium chloride, up to levels of 45%, which in turn can add to umami flavour properties and interactions via synergism. Depending on the protein source, as well as processing conditions, typical HVP can contain up to 16% MSG. The utilization of hydrolysed meat by-products also can serve as a source of umami compounds, as well as numerous meat-like flavours.

### 9.12 Peptides

Throughout the flavour literature, numerous reports have appeared where peptides of varying structure and length possess unique taste properties including sweet, salty, sour, bitter and umami/beefy (Otagiri *et al.*, 1985; Asao *et al.*, 1987; Mojarro-Guerra *et al.*, 1991; Izzo and Ho, 1992).

Relative to beef, the octapeptide (H-Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala-OH) was first isolated by Yamasaki and Maekawa (1978) from papain-treated beef. They reported that a panel of three people described the isolated peptide as having a 'delicious' taste. In a later publication, Yamasaki and Maekawa (1980) reported on the synthesis of the octapeptide in question and described the taste of a 5% solution as meat-like. They also synthesized a series of structurally similar peptides and reported that the meat-like character was not evident. Similar structures primarily possessed sour, bitter and astringent taste properties.

A similar approach was utilized by Tamura *et al.* (1989) to synthesize the originally described 'delicious' peptide as well as structurally related compounds. They concluded that the octapeptide in question possessed both sour and umami properties and had a taste threshold of 1.4 mM. Based on the taste properties of similar peptides, they concluded that the taste observed with the octapeptide in question was due to the interaction between the basic and acidic fractions of the peptide.

Later, Spanier (1992) and Spanier *et al.* (1992) reported that the octapeptide was naturally present in beef that had not undergone enzymatic treatment and proposed the name beefy meaty peptide (BMP). Earlier, Spanier and Edwards (1987) were able to isolate two polypeptide fractions from cooked beef. One group was composed of hydrophilic peptides, which can lead to sweet taste, while the other group represented hydrophobic peptides, which are normally associated with sour and bitter tastes. Also, Cagan (1984) reported that a portion of the BMP structure is similar to that of protein-based monellin, which has a strong sweet taste.

More recently, Spanier *et al.* (1995) reported on the taste properties of BMP as compared to MSG. In a beef-flavoured gravy system, flavour

enhancement by BMP was found to be more pronounced than with MSG, especially at sub-threshold levels. Both BMP and MSG were reported to have their optimum effect at their threshold levels (1.41 mM/0.16% for BMP and 1.56 mM/0.26% for MSG). They also reported that the addition of MSG at its taste threshold increased the salty note in a beef gravy, whereas the addition of BMP at its taste threshold did not enhance perceived saltiness.

Recently, Wang *et al.* (1996) reported on the taste response of BMP as influenced by pH and in conjunction with salt and MSG additions. They determined BMP taste thresholds at pH 3.5, 6.5 and 9.5. From their data they concluded that BMP taste threshold was not influenced by pH but taste descriptors used by the panel did change with pH. For example, at pH 3.5, BMP taste was described as sour, whereas at pH 6.5, the predominant taste was umami, while at 9.5, sweet and sour notes were also noted, along with umami.

Wang *et al.* (1996) clearly demonstrated that BMP displays synergism with added salt and MSG. For example, the taste threshold for BMP decreased approximately 3.5-fold when either salt or MSG was present at their individual taste thresholds. When both salt and MSG were added to BMP, the threshold for BMP decreased 9-fold. The major taste descriptor reported for combinations of BMP, salt and MSG was umami.

Wang *et al.* (1966) added 2 mM of synthesized BMP to a beef extract prepared from a heated ground beef and water mixture as compared to the same beef extract without added BMP. Triangle test results demonstrated that the panel could statistically distinguish between the two variables. Sensory descriptors used by the panel to describe the taste of added BMP to beef extract included meaty, salty, savoury, sweet and umami.

Since BMP is protein-based, a practical question is compound stability, especially during heating. Wang *et al.* (1995) subjected a synthesized 0.35 mM solution of BMP to heating at 71°C for 15 s (pasteurization) and 121°C for 20 min (sterilization) to determine thermal compound stability. Unheated and heated solutions were analysed using both HPLC and mass spectrometry. From their data, they concluded that BMP was over 97% stable to both pasteurization and sterilization conditions. From these data, it can be concluded that either naturally occurring or intentionally added BMP to meat products would be stable to subsequent heat processing.

### 9.13 Conclusions

The literature clearly demonstrates that umami compounds have the ability to alter the taste and possibly aroma properties of a wide range of foods independent of the four basic tastes. Umami compounds react synergistically, therefore reducing the total amount required to elicit a response.

They and their precursors occur naturally in a wide range of foods and their effectiveness can be improved by their intentional addition to most foods. Acidic conditions and high processing temperatures minimize the effectiveness of 5'-nucleotide-based umami compounds. Umami compounds are very effective in contributing to meat flavour, especially chicken, and exhibit minimum effectiveness with lamb.

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## ORIGINAL PAPER

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## Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portuguese coast

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**Abstract** Adenine nucleotides and related compounds were measured in North Atlantic hake, monkfish, rockfish, Norway lobster, and red shrimp from the South of Portugal immediately after catch and after a 72-hours ice storage period. To preserve the samples until analysis, a freezing technique with liquid nitrogen was developed and data compared with those from immediate extraction. The use of plastic vials in liquid nitrogen freezing gave similar data as when immediate post mortem extraction was done. Inosine monophosphate (IMP) was the main nucleotide present in the fish species, whereas adenosine monophosphate (AMP) was the major component of crustaceans. During ice storage of fish, adenine nucleotides were almost completely converted to IMP. Rockfish showed a significant catabolism of IMP with conversion to hypoxanthine (Hx). Red shrimp and, especially, Norway lobster presented an important transformation of AMP into IMP. In general, Hx was either not detected (crustaceans) or was present in very low amounts, the highest levels being detected in rockfish after ice storage. This species presented also after 72 h the highest *K* value (58%), whereas in the other cases the values ranged between 7% and 11%. Trigonelline (Trigo), a UV-absorbing betaine, was also present in both crustacean species together with adenosine triphosphate (ATP) and related compounds.

### Introduction

Fish spoilage has been evaluated traditionally by measurements of the levels of trimethylamine nitrogen and total volatile basic nitrogen. Nevertheless, for most fish species these are not good criteria because the loss of

freshness, which often precedes microbial spoilage, is mainly associated with autolytic reactions controlled by endogenous enzymes [1]. The accumulation of inosine (HxR) and hypoxanthine (Hx) in fish species appears to be related to both autolytic and microbial action, although the former seems more important. On account of this, nucleotide degradation products have been widely used as indicators of storage age or freshness. Several authors have examined the degradation profiles of nucleotides and their relation with iced storage, and the *K* value or related values [2–4] have been regarded as reliable freshness indicators for some species.

While there have been various reports on the biochemical changes occurring post mortem in the muscle of many fish species, insufficient information has been available on the baseline levels of ATP and breakdown products, particularly for crustacean species immediately after catch. The effect of catching method on quality indices has been studied [5–7] and contradictory evidence has been reported regarding the changes in fish quality. To be able to understand the influence of the type of processing to which the fish was submitted to, it is therefore important to know the effect of catching methods on the levels of ATP and breakdown products, and their concentration in muscle immediately after catch and during normal commercial handling.

The objective of this study was to determine these baseline levels of adenine nucleotides and their related compounds in North Atlantic hake, monkfish, rockfish, Norway lobster, and red shrimp from the south coast of Portugal and compare these to values measured after a 72-hours iced storage period. *K* values were calculated from the levels of ATP-related compounds. Additionally, the effects of the iced storage conditions used on-board Portuguese crustacean trawlers on the quality of the products was determined. To allow the preservation of samples for later analysis in the laboratory, a freezing technique with liquid nitrogen was developed and the data obtained were compared with data from immediate extraction. Additionally a UV-absorbing be-

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taine, trigonelline (Trigo), which co-appeared in the HPLC of the acid-soluble fractions of both crustacean species, was determined.

## Materials and methods

### Samples

To study the influence of the type of freezing on the level of nucleotides, live gilthead seabream (*Sparus aurata*) of 150–200 g size were obtained from tanks at the aquaculture station of the Institute for Fisheries and Marine Research, Lisbon. After removal from the tanks the fish were immediately immobilized and killed by burying in ice during 1 h. Fish stress was limited to a minimum by swift action during the preceding procedures. Fish were gutted, and samples removed from skinned fillets. Samples were extracted, either (A) immediately after fish death (1 h in ice), (B) after freezing in a blast freezer at  $-30^{\circ}\text{C}$  for 1 day and thawing for 15 min at room temperature, (C) in the frozen state after freezing in a blast freezer at  $-30^{\circ}\text{C}$  for 1 day, and (D) after freezing in a blast freezer at  $-80^{\circ}\text{C}$ . In addition, samples (5–6 g) frozen in liquid nitrogen either (i) wrapped in aluminum foil, or (ii) inside Nalgene bags, and (iii) in cryogenic vials were extracted without thawing, and analyzed to determine the best sample container. Samples to study the influence of anatomic location on the level of nucleotides were removed from the anterior, median, and tail sections of the fish.

North Atlantic hake (*Merluccius merluccius*), monkfish (*Lophius piscatorius*), rockfish (*Helicolenus dactylopterus*), Norway lobster (*Nephrops norvegicus*), and red shrimp (*Aristeus antennatus*) used in this study were caught in June 1998 at a depth of 550 m, 12 miles southwest of Cape St. Vicente by bottom trawling during 5 h. After harvest, six individuals of each species were immediately (within 1 to 2 min) taken from the deck, gutted, and portions of the white muscle weighing 5–8 g were cut from the left-hand side near the dorsal midsection area. Samples were held in cryogenic vials in liquid nitrogen until extraction in the laboratory. The fish were iced using salted ice and an ice:fish ratio of 2:1. To reduce the effect of individual variation in fish, sampling after 72 h was done on the same fish on the corresponding opposite right-hand side. On account of the size of Norway lobster and red shrimp, six different individuals were analyzed.

### Nucleotide analysis

Nucleotides were extracted by homogenizing 5 g of muscle with 25 ml of 0.6 M perchloric acid at  $0^{\circ}\text{C}$  for 1 min with a Polytron homogenizer at 20,000 rpm. The homogenate was centrifuged (3,000 g, 10 min,  $0^{\circ}\text{C}$ ) and 10 ml of the supernatant neutralized to pH 6.5–6.8 with 1 M potassium hydroxide solution. The extract was maintained at  $0^{\circ}\text{C}$  for 30 min, then potassium perchlorate removed by filtration through sintered glass (#3) and the filtrate diluted to 20 ml. Aliquots were blast frozen (2 h) in 3 ml vials at  $-80^{\circ}\text{C}$  and stored at the same temperature until analysis.

Nucleotide analysis was done with a high performance liquid chromatographic method similar to that reported by Ryder [8]. A Hewlett Packard 1050 HPLC system was used, and a fixed wavelength detector (MWD HP-1050 Series) set to monitor at 254 nm. Separations were done with a Hewlett Packard LiChrosorb RP-18 column (10  $\mu\text{m}$ ,  $200 \times 4.6$  mm) operated isocratically at 1.7 ml/min with a mobile phase composed of 0.1 M phosphate buffer with a pH of 6.95. Standards were obtained from Sigma Chemicals Company. K values were calculated according to Karube et al. [2] substituting the contents ( $\mu\text{mol/g}$  wet muscle) of principal adenine nucleotides and their related compounds in the following equation:

$$K_{value}(\%) = \frac{(HxR + Hx) \times 100}{ATP + ADP + AMP + IMP + HxR + Hx}$$

### Statistical analysis

Since the data did not fit a normal distribution, non-parametric tests were used for statistical analysis [9]. The Kruskal-Wallis test for comparing several treatments was performed to see if there were significant differences between samples from different anatomical locations and between samples submitted to different types of freezing procedures. The Wilcoxon rank-sum test for comparison of two treatments was carried out to determine if there were significant differences between fresh and ice-stored fish.

## Results and discussion

### Influence of anatomical location on ATP breakdown products

Extraction of nucleotides immediately after death revealed ATP contents between 5.0 and 6.1  $\mu\text{mol/g}$ . The contents of ADP and AMP were low, whereas IMP levels were high, reflecting a rapid conversion of ATP into IMP. The anatomic location of the samples taken did not affect ( $p < 0.05$ ) the measured level of nucleotide catabolites (Fig. 1). Nonetheless, the concentration of nucleotides varied considerably from fish to fish, showing the existence of different physiological conditions, probably as a result of different levels of struggle during the removal of the fish from the water tanks. On the other hand, when the total amount of ATP and IMP in individual fish is compared, in order to simulate the nucleotide contents in living fish, this variability is considerably reduced. In this case, total nucleotide amount ranged between  $10.6 \pm 1.7 \mu\text{mol/g}$  and  $12.5 \pm 4.1 \mu\text{mol/g}$ . Hattula et al. [10] reported similar results in small whitefish (*Coregonus wartmanni*) of comparable size, as did Kanoh et al. [11] for albacore, yellowfin tuna, and skipjack. On the contrary, Cappeln and Jessen [12], working with cod (*Gadus morhua*), found a large variation in the ATP content depending on the anatomic location of samples. The results suggest that in these

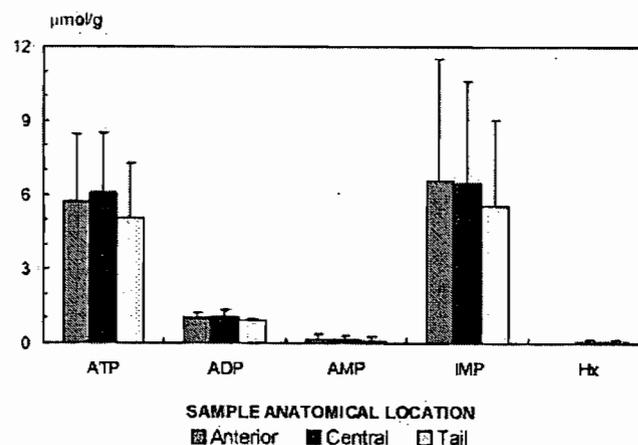


Fig. 1 ATP and breakdown products from samples of gilthead seabream from different anatomical locations. Data are given as  $\mu\text{mol/g}$  wet weight by mean  $\pm$  SD from six specimens.

small fish all the muscles are equally involved in the swimming motion.

Changes in ATP breakdown products due to freezing technique

As expected, higher levels ( $p < 0.05$ ) of ATP were determined in samples extracted immediately post mortem (Fig. 2), although these were slightly lower (mean  $3.27 \mu\text{mol/g}$ ) than those determined in the study of the influence of anatomic location. This may have been caused by the high number of samples processed and therefore a slight increase in the time of storage before extraction (~30 min). The higher levels of IMP measured here further confirm this degradation of ATP.

ATP levels were below the detection limit ( $0.05 \mu\text{mol/g}$ ) in samples frozen at  $-30^\circ\text{C}$  and thawed for 15 min (B) before extraction at room temperature. There was probably an accelerated ATP degradation caused by the high temperature at the beginning of the extraction, as well as the longer time taken to freeze the sample at  $-30^\circ\text{C}$ . Jessen and Capplen [13] demonstrated that ATP is decomposed at temperature as low as  $-39^\circ\text{C}$ .

Samples extracted directly from the frozen state, and in particular from those frozen at  $-80^\circ\text{C}$ , showed higher levels of ATP ( $p < 0.05$ ) than thawed ones. The higher freezing speed likely slowed the autolytic enzymes responsible for the ATP degradation. Samples wrapped in aluminum foil exhibited the lowest ATP levels (Fig. 3) and highest IMP levels ( $p < 0.05$ ), although this material enabled the fastest freezing. Samples frozen in cryogenic vials, despite exhibiting greater variability of the data, gave similar ATP results ( $p < 0.05$ ) to those frozen in Nalgene bags or extracted immediately post mortem (Fig. 2). Use of cryogenic vials was adopted for subsequent work on account of the lower IMP levels obtained by this method and the fragility of the bags

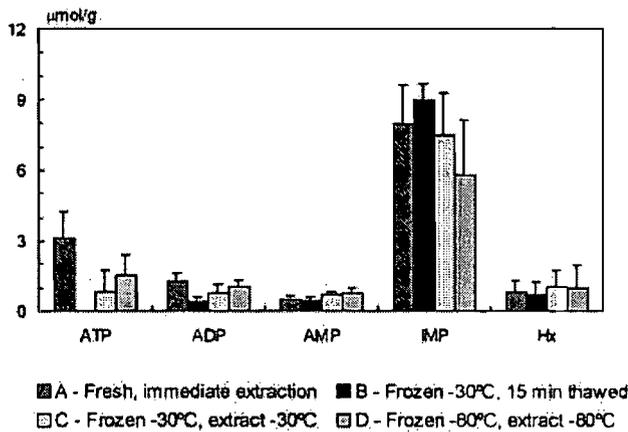


Fig. 2 Content of ATP and breakdown products in gilthead sea-bream in function of different sample preparation procedures. Data are given as  $\mu\text{mol/g}$  wet weight by mean  $\pm$  SD from six specimens.

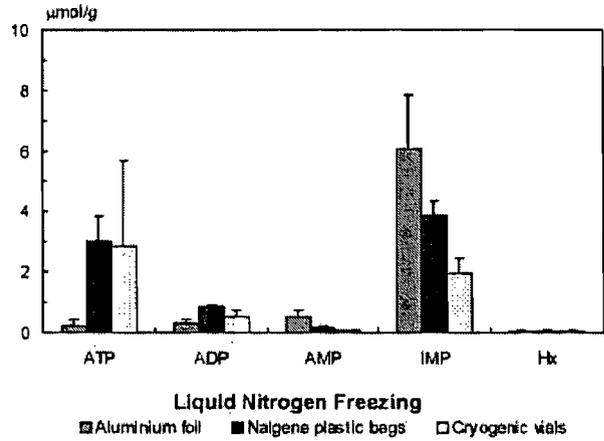


Fig. 3 Content of ATP and breakdown products in gilthead sea-bream samples frozen in liquid nitrogen in different containers. Data are given as  $\mu\text{mol/g}$  wet weight by mean  $\pm$  SD from six specimens.

when frozen at  $-196^\circ\text{C}$ . No clear explanation was found for the rapid breakdown of ATP to IMP in samples which were frozen faster. Nevertheless, it seems that the decrease in freezing speed caused by the use of the cryogenic vials had a positive effect in reducing the rate of ATP breakdown.

Changes in ATP and breakdown products in fish and crustaceans

The degradation sequence of adenosine triphosphate in the fish species,  $\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP} \rightarrow \text{HxR} \rightarrow \text{Hx}$ , is first and foremost oriented to the production of IMP (Fig. 4). The muscle baseline ATP concentrations for North Atlantic hake (*Merluccius merluccius*), monkfish (*Lophius piscatorius*), and rockfish (*Helicolenus dactylopterus*) were, respectively, 0.70, 0.44 and  $0.18 \mu\text{mol/g}$  with the dominant nucleotide present being IMP. These relatively low levels suggest that ATP breakdown began before landing of the fish on deck, and are probably a direct consequence of stress [14]. Due to the fishing technique used (bottom trawl), fish may have been struggling in the net for prolonged periods (approximately 5 h) during capture. Jones and Murray [15] report that trawled cod has little ATP and a substantial amount of IMP at death compared with well-fed fish. Hattula et al. [5] showed also that the contents of ATP, ADP, and AMP are very low (below  $0.2 \mu\text{mol/g}$ ) in Baltic herring from trawling, gillnetting, and poundnetting.

In the early post mortem stages, ATP in the muscle of all fish species degraded rapidly to IMP via ADP and AMP, while IMP tended to accumulate on account of slower subsequent breakdown to HxR. It is well documented that ATP, the main adenine nucleotide in live fish, undergoes a rapid degradation to IMP after death [16, 17] while IMP conversion to HxR goes at a slower rate [18, 19]. It is also well known that the white muscle

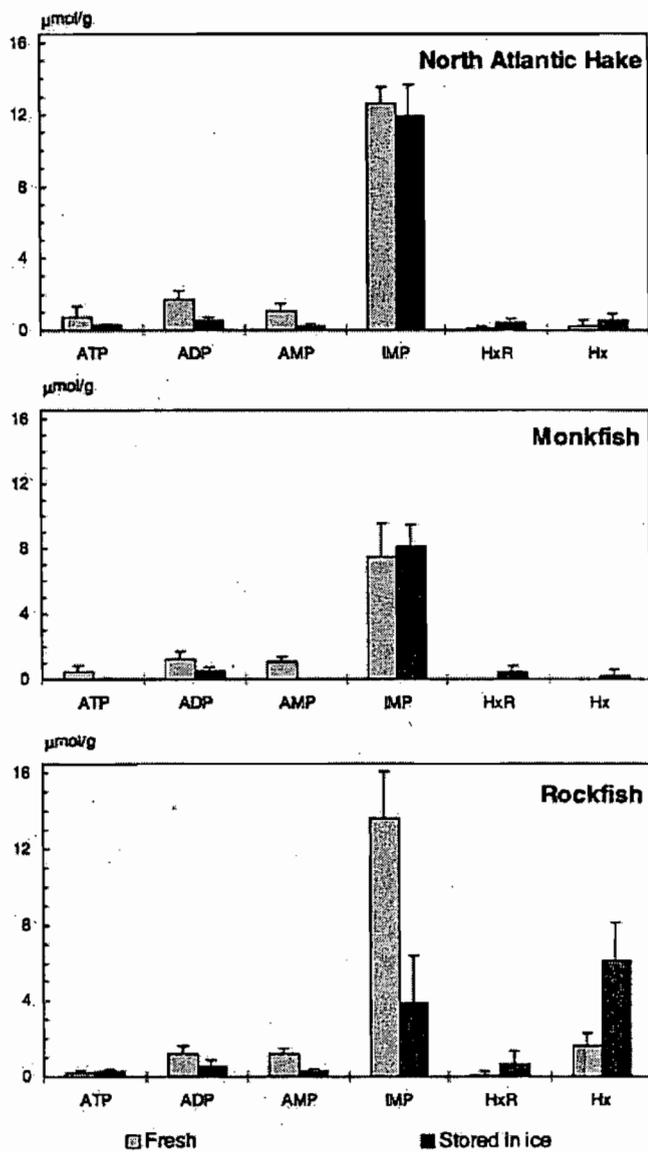


Fig. 4 ATP and breakdown products in fresh and stored in ice (72 hours) North Atlantic hake, monkfish and rockfish. Data are given as  $\mu\text{mol/g}$  wet weight by mean  $\pm$  SD from six specimens.

of many fish species has high AMP deaminase activity [20, 21], and also low levels of 5'-nucleotidases responsible for the IMP decline [22]. Nonetheless, the extent of IMP breakdown reportedly depends on the fish species and muscle type [23, 24]. The IMP levels determined for the fish species of this study ( $7.4 \pm 2.1 \mu\text{mol/g}$  to  $13.4 \pm 2.5 \mu\text{mol/g}$ ) are in accordance with reported data for fish of good quality [25, 26].

In general, the results are also in accordance with those of Konosu and Yamaguchi [27] who reported that a relatively high IMP concentration ( $>8 \mu\text{mol/g}$ ) is typical for bonefish, while in crustaceans and molluscs, AMP and ATP are the dominating nucleotides immediately post mortem. In the present study HxR and Hx were not detected in monkfish, whereas in North At-

lantic hake these nucleotides were measurable in very low amounts. From all the species analyzed, rockfish showed the highest level of IMP breakdown to Hx ( $p < 0.05$ ). These results are in accordance with those of Ehira and Uchiyama [28] who, in an extensive study of 98 species, classified rockfish among those that form Hx. Low levels of HxR were also found by Fraser et al. [29] in redbfish, indicating a rapid HxR to Hx conversion. The accumulation of HxR and Hx has been suggested to be related to both autolytic and microbial action [30]. Marseno et al. [22] studied the presence of 5'-nucleotidases in muscle homogenates of some vertebrates and invertebrates and reported the highest activity in black rockfish (*Sebastes inermis*), a closely related species to the studied rockfish (*Helicolenus dactylopterus*).

Immediately after harvest, within 1 to 2 min after crustaceans landing on deck, AMP was the predominant nucleotide ( $9.3\text{--}11.8 \mu\text{mol/g}$ ) in Norway lobster (*Nephrops norvegicus*) and red shrimp (*Aristeus antennatus*) (Fig. 5), far higher than in other species ( $p < 0.05$ ). Yokoama et al. [31] concluded that this AMP accumulation is due to a highly reduced or non-existent AMP-deaminase activity. After iced storage the nucleotide profile was considerably different

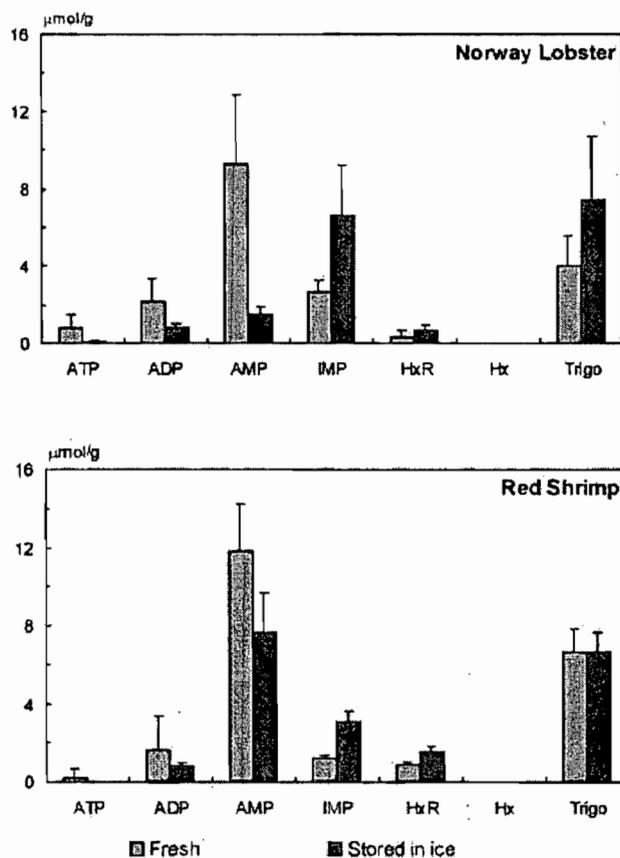
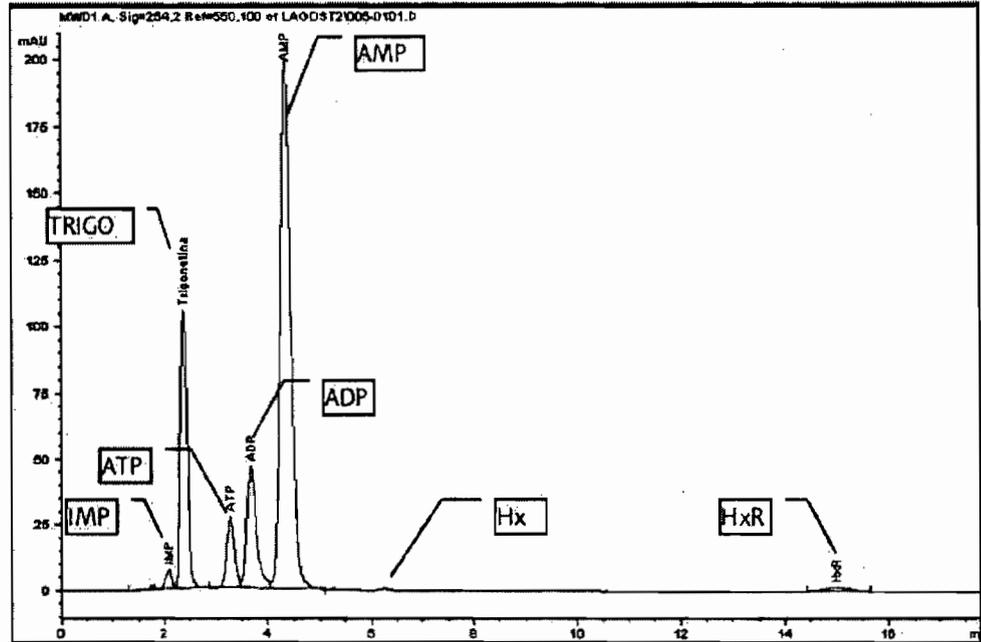


Fig. 5 ATP and breakdown products and trigonelline (Trigo) in fresh and stored in ice (72 hours) Norway lobster and red shrimp. Data are given as  $\mu\text{mol/g}$  wet weight by mean  $\pm$  SD from six specimens.

**Fig. 6** Chromatogram of the acid soluble fraction from the muscle of fresh Norway lobster with separation of the ATP-related compounds and trigonelline (TRIGO).

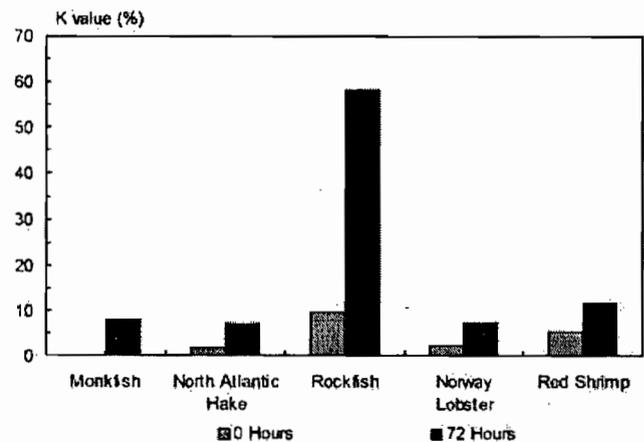


( $p < 0.05$ ) in Norway lobster, and as a direct result of AMP catabolism, IMP was the main nucleotide. According to Fatima et al. [32] the absence of IMP in shrimp muscle and values of Hx greater than  $2 \mu\text{mol/g}$  of shrimp muscle are indicators of doubtful quality. Because both the red shrimp and Norway lobster disembarked after a 72-h onboard iced storage period, exhibited high levels of AMP and IMP but levels of Hx below the detection limit ( $0.06 \mu\text{mol/g}$ ), the handling used onboard was considered to yield products of prime quality.

In the chromatographic separation of the crustacean extracts an unknown peak eluted between IMP and ATP, as shown in Fig. 6. This peak was identified by retention time comparisons with other compounds previously reported as occurring in crustaceans [33] like adenine, xanthine, adenosine, and the betaines homarine and trigonelline. The peak was tentatively identified as trigonelline, and for quantitation, a calibration curve was prepared within the range of  $0.085\text{--}0.205 \mu\text{mol/g}$ . The curve was found to be linear as were those previously obtained for the other nucleotides. Separation and quantitation of ATP and related compounds were not affected by the presence of this component. As shown in Fig. 5, the mean content of trigonelline in red shrimp ( $6.6 \pm 1.0 \mu\text{mol/g}$ ) was unaffected by iced storage ( $p < 0.05$ ), whereas in the Norway lobster trigonelline changed from  $4.0 \pm 1.5 \mu\text{mol/g}$  to  $7.4 \pm 3.2 \mu\text{mol/g}$ . Trigonelline has been only occasionally reported to occur naturally in muscle and is mainly associated with molluscs and crustaceans [31, 33]. The detected levels were rather high as compared with trigonelline levels reported for shrimp (*Penaeus japonicus*) after 10 days in ice ( $0.75\text{--}1.57 \mu\text{mol/g}$ ) [33], though no explanation was found for the behavior observed in the present study.

To evaluate the freshness of the fish and crustacean, changes in K values during iced storage were determined (Fig. 7). In general, these values were low immediately after catch, as well as after 72 hours in ice, and similar to those measured for other species [34]. Immediately after capture monkfish showed the lowest K value (0%) and rockfish showed the highest level (9.6%) ( $p < 0.05$ ). After 72 hours in ice rockfish displayed also the highest change in K value ( $p < 0.05$ ), attaining a value of 58.2%, while all the other species showed considerably lower values.

Based on the K values measured, these raw materials were classified as highly fresh, which was confirmed by sensory analysis (data not presented since no significant differences were determined after iced storage). Rockfish was an exception to this behavior and displayed K values in the range normally considered to be



**Fig. 7** Changes in K value (%) during ice storage of monkfish, North Atlantic hake, rockfish, Norway lobster, and red shrimp. Results are the mean value of six individuals from each species.

on the borderline of acceptability. Similar results were reported by Kennish and Kramer [17] for rockfish, which they report to be a high accumulator of Hx, and a species that loses its characteristic flavor very fast. From these data it can be concluded that the type of handling and processing which fish and crustaceans usually experience on board Portuguese trawlers is appropriate, providing at the time of landing (72 hours after catch), products of very good quality.

Further work is planned to determine the baseline levels of ATP and breakdown products in other fish and crustacean species captured with other fishing technologies and in different seasons. Use of K values as a freshness indicator will be studied further, particularly for crustaceans.

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