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Amino Acid Analysis of three Imported Fish Species Consumed in Abakaliki, Nigeria

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Abstract: Fish has been widely used as an excellent source of animal protein and other nutrients, with a wide variety of species eaten in various parts of the world. This study seeks to profile the amino acid content of three imported fish species, Croaker, Mackerel and Scumbria in Abakaliki, Nigeria. The methodology used was that described by Benitez. Results of the comparative study showed all three fish species contained eighteen (18) of the twenty (20) amino acids needed by most biological systems. Croaker hadsignificantly higher($p<0.05$) concentrations ofmost of the various amino acids with concentrations ranging from 13.02to0.47g/ 100g of protein,followed byMackerel with amino acid concentrations range of 10.29to0.58 (g/ 100g of protein) while the least was Scumbria, with concentrations ranging from 9.99to0.31 (g/ 100g of protein). Glutamic acid, aspartic acid and serine were the most abundant amino acidsin concentration while cysteine and tryptophan had the least concentration across the samples.For the nutritionally essential amino acids, leucine (6.01- 2.39 g/ 100g of protein), lysine (5.94-1.01 g/ 100g of protein) and arginine (4.99-3.96 g/ 100g of protein) were the most abundant. Although all three fishes had appreciable amounts of the various amino acids, croaker could be said to be the most preferable.

Key words: Imported fish · Amino Acids · Frozen · Mackerel · Scumbria and Croaker

INTRODUCTION

Aquatic foods, including fish represent an essential component of the global food basket to improve the nutrition, health and wellbeing of all peoples [1]. As a vital source of food for all people, fish is man's most important source of high quality protein. It provides about 16% of the animal protein consumed by the world's population [2]. It is a particularly important protein source in regions where livestock is relatively scarce, supplying about <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (Food and Agriculture Organization, 2000). The FAO estimates that about one billion people world-wide rely on fish as their primary source of animal protein [5]. Beside its consumption as food, fish also has substantial social and economic importance. In fact, the FAO estimates the value of fish traded internationally to be US$ 51 billion per annum[5]. It is also reported that over 36 million people are employed directly through fishing and aquaculture and that as many as 200 million people derive direct and indirect income from fish [3].

Amino acids had been classified traditionally as nutritionally essential or nonessential based on growth or nitrogen (N) balance of animals [4]. Nutritionally, essential amino acids (EAAs) are those amino acids whose carbon skeletons are not synthesized de novo. Put in another way, they are those amino acids that usually are not synthesized in adequate amounts to meet the animal's needs and, therefore, must be provided in diets to sustain life [5]. They include Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine. On the other hand, amino acids that are synthesized de novo in animal cells are considered to be dispensable in diets and therefore, nutritionally nonessential amino acids (NEAAs) [6]. These include Norleucine, Proline, Arginine, Tyrosine, Cystine, Alanine, Glutamic acid, Serine and Aspartic acid.

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Amino acids synthesis depends on a number of factors. These include substrate availability, species, developmental stage, physiological status, micro biota in the lumen of the gastrointestinal tract, environmental factors and pathological states [7]. However, their availability and balance (proportion) in diets is of paramount importance in protein nutrition. This is because an imbalance among chemically or structurally similar amino acids causes a phenomenon known as amino acid antagonism. This is manifested as reduced food intake, abnormal behavior and impaired growth of animals [8]. This scenario mimics that in man and underscores the importance of availability and balance of amino acids in fish foods for consumption. This (amino acid balance) is especially so, considering the vital role they play, which includes constituting the major structural component of muscle and other tissues in the body, production of hormones, enzymes and hemoglobin. Against this backdrop therefore, this study seeks to examine the amino acid profile of three fish species (Scumbria, Croaker and Mackerel).

This is a pelagic, 'oceanodromous' species. It is abundant in cold and temperate shelf areas and forms large schools near the surface. They 'overwinter' (meaning - spend the winter) in deeper waters but move closer to the shore in spring when water temperatures range between 11° and 14°C. Mainly diurnal, it feeds on zooplankton and small fish. Eggs and larvae are pelagic. Maximum size for this species is 66 cm, although fish greater than 50 cm are uncommon. This species matures at approximately age two [9], with 100% maturity at age seven in some populations of the eastern stock [10]. For the Western stock longevity is estimated to be about 12 years [11] and for the Eastern stock longevity is estimated to be 18 years [12]. Generation length is therefore conservatively estimated to be about 3.5 years in the Western stock and 6.5 years in the Eastern stock [13]. Disparities in longevity between stocks may valid, or may be due to differences in methods of age determination, environmental factors and/or response to fishing pressure over time. Maximum Size is 66 cm fork length (FL). The all-tackle game fish record is of a 1.2 kg fish caught in the Kraakvaag Fjord, Norway in 1992 (IGFA, 2014).

The Atlantic mackerel is a popular fish found in Nigerian rural and urban markets. In it many processed forms (fresh, dried, fried, etc.) consumers have found it relatively more affordable compared with other exotic species. This is beside its cherished flavor and taste. In the western Atlantic this species is present from Labrador to Cape Lookout, U.S. and in the eastern Atlantic from Iceland to Mauritania, including the southwestern Baltic Sea, the Mediterranean and Black seas. The croaker is the only representative of the genus in the western North Atlantic. This species gets its name from the deep croaking sounds created by muscular action on the air bladder. It is one of 23 members of the family Sciaenidae found along the Atlantic and Gulf of Mexico coasts [14]. The species has a typical fusiform shape, although it is somewhat vertically compressed. The fish is silvery overall with a faint pinkish-bronze cast. The back and upper sides are grayish, with brassy or brown spots forming wavy lines on the side [15]. The gill cover has three to five prominent spines and there are three to five small chin barbels. It has a slightly convex caudal fin.

In Nigeria also, the species is a prominent commercial species sold in both rural and urban markets. Like other freshwater and marine water fishes, various preservation methods have been employed to extend its shelf life and enhance the quality. This, amongst other reasons justifies why it may be necessary to examine its profile post preservation, especially with regards to its amino acid contents.

Since the early 1970s, the vast majority of these catches (20-75%) were composed of the Chilean jack mackerel (*Trachurus murphy*), caught in the southeastern Pacific Ocean. In recent years, the most northern representative of the trachurid family,*T. trachurus* has ranked second in catches (2000: 275,000 t, >10% of total catch; FAO[5]. In the northeast Atlantic Ocean and adjacent areas, horse mackerel commonly occurs on the continental shelf: from the West African Cape Verde Islands [16], northwards to the Norwegian Sea and North Sea including Iceland, as well as in the Mediterranean Sea and Black Sea.

Horse mackerel are a fairly long-lived species, reaching a maximum age of well over 30 years [4]. Therefore, an occasional strong year class can lead to high abundance of horse mackerel. In the case of the western horse mackerel, the extraordinary strong 1982 year-class created a substantial fishery in the northern areas, which continued for more than a decade. As a result, *T. trachurus*became an important commercial species in the 1980s and 90s and is now one of the three most important pelagic species in the European fish industry [17]. Literature shows a plethora of research done on the species and its keeping quality after harvest. For example, treatment with Rosmol-P, a commercial plant

extract prominent for its role in retarding lipid oxidation in meat products enhanced the shelf life of horse mackerel fillets [18].

MATERIALS AND METHODS

Fresh samples of Scumbria, Croaker and Mackerel fish species were purchased from artisans at the Abacha market in Abakaliki. The Amino Acid profile was determined using methods described by [19]. The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Defatting of Samples: The samples were defatted by method of (Bligh and Dyer [20]. The method has been applied to fish muscle and may easily be adapted to use with other tissues. The entire procedure can be carried out in approximately 10 minutes; it is efficient, reproducible and free from deleterious manipulations. The wet tissue is miscible system is formed with the water in the tissue. Dilution with chloroform and methanol (ratio of each solvent??) separates the homogenate into two layers, the chloroform layer containing all the lipids and the methanolic layer containing all the non-lipids. A purified lipid extract is obtained merely by isolating the chloroform layer. A known weight (2.0g) of ground tissue was weighed into separating funnel.This was followed by addition of 15ml methanol, 30ml distilled water and 15ml chloroform. The separating funnel was shaken vigorously for 2 minutes and the liquid layer was decanted into 250ml conical flask after 10 minutes. The fat free tissue was put into a clean petri dish and dried overnight at room temperature.

Nitrogen Determination: About, 115mg of ground sample was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeidhal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10m1) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point [21].

$$\text{Percentage Nitrogen} = \frac{(a-b)\ x\ 0.01\ x\ 14\ x\ V\ x\ 100}{W\ x\ C}$$

where:

a. = Titre value of the digested sample,
b. = Titre value of blank sample,
v. = Volume after dilution (100ml),
W. = Weight of dried sample (mg),
C. = Aliquot of the sample used (10ml),
14. = Nitrogen constant in mg.

Hydrolysis of the Sample: A known weight of the defatted sample was weighed into glass ampoule. 7ml of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C± 5°C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins.

It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer [22].

Since tryptophan is destroyed by acid hydrolysis, it is hydrolyzed using alkaline hydrolysis. Tryptophan in the known sample was hydrolyzed with 4.2 M Sodium hydroxide. The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer [23].

Loading of the Hydrolysate into Analyzer: The amount loaded was 60 microlitre. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Method of Calculating Amino Acid Values: An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

RESULTS

In the mackerel sample, amino acid values ranged from 0.58 - 10.29g/ 100g of protein, with the most abundant value from glutaminc acid (10.29g/ 100g of protein) and the least being tryptophan (0.58g/ 100g of protein). The six most abundant amino acids in the mackerel sample was glutamic acid (10.29g/ 100g of protein), alanine 6.07g/ 100g of protein, arginine 4.99g/ 100g of protein, lysine 4.67g/ 100g of protein, proline 4.47g/ 100g of protein and leucine 4.32g/ 100g of protein. In mackerel aside from tryptophan (0.58g/ 100g of protein) cysteine (0.73g/ 100g of protein) all other amino acid values were of concentrations above 3.0g/ 100g of protein as can be observed in Figure 1.

The croaker samples had similar amino acid contents although in different concentrations. Glutamic acid still remained the most abundant amino acid with concentration of 13.02g/ 100g of protein while cysteine and tryptophan appeared to be the least in concentration with cysteine surpassing the concentration of tryptophan with 0.01g. The six most abundant amino acids are Glutamic acid (13.02g), Aspartic acid (7.88g), Serine (6.1g), Leucine (6.01g), lysine (5.94g) and Arginie (3.96g). Isoleucine, Phenylalanine, Valine, Alanine and glycine all had amino acid concentration range from 3.57g (valine) to 3.01g (Phenylalanine). Methionine, Proline, Tyrosine, Histidine, Threonine had amino acid concentration of 2.89g while histidine had concentration of 1.66g and tryptophan with cysteine had concentrations below 1g as could be observed in Figure 2.

Results from the amino acid analysis on the scumbria sample showed glutamic acid had the highest amino acid (9.99g) and tryptophan the least abundant (0.31g). The six (6) most abundant amino acids includes glutamic acid (9.99g), glycine (5.22g), aspartic acid (5.08g), alanine (4.25g), proline (3.96g) and Arginine (3.27g). Apart from the six most abundant specie the rest had concentrations below 3.0g with Cysteine and tryptophan been the least abundant 0.36g and 0.31g respectively as shown in Figure 3.

From the results, comparative analysis on all three samples reveal glutamic acid was the most abundant across all the samples with croaker having the highest concentrations while tryptophan was the least abundant with scumbria having the least concentration than its other counterparts as shown in Figure 4.

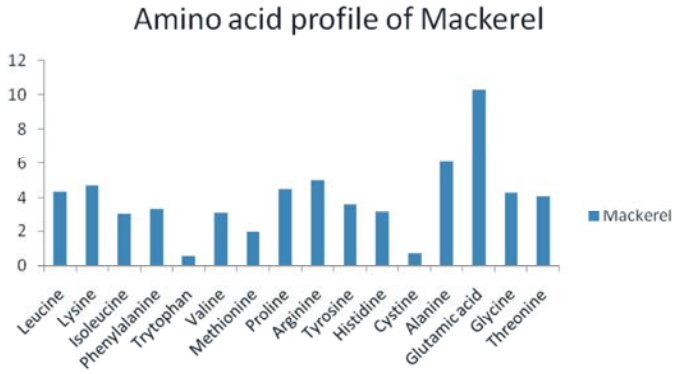

Fig. 1: Amino acid profile of Mackerel.

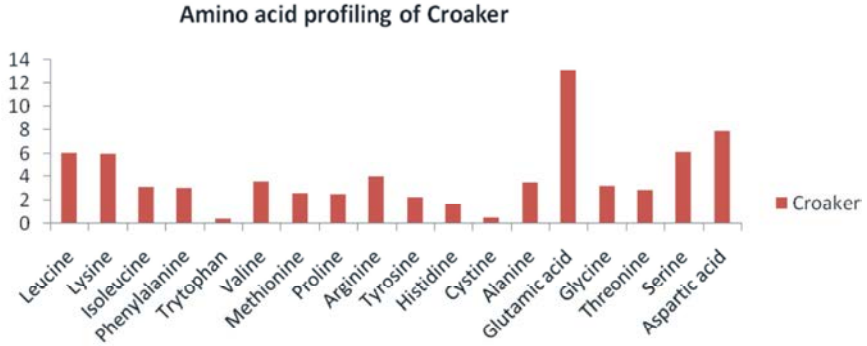

Fig. 2: Amino acid profile of Croaker.

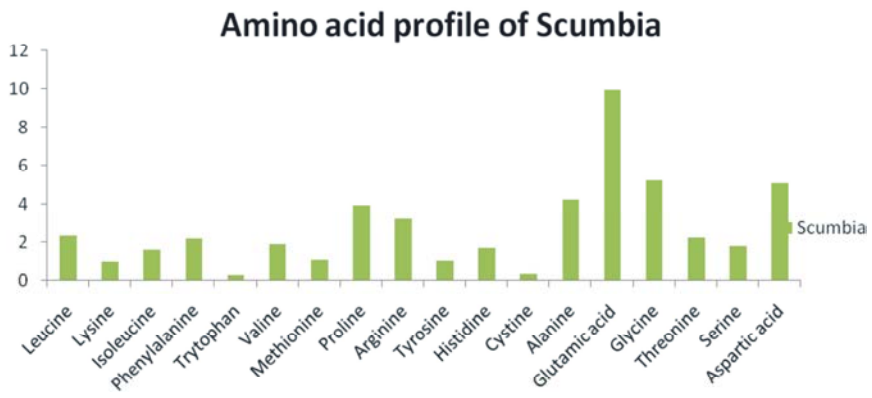

Fig. 3: Amino acid profile of Scumbria.

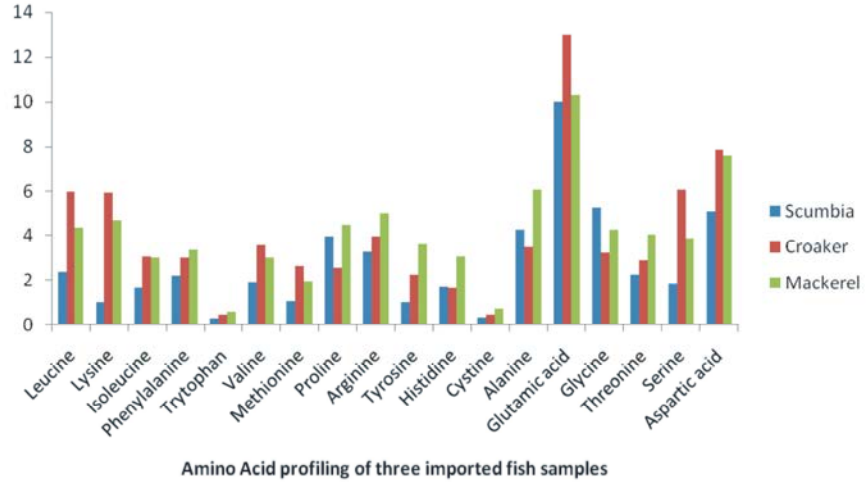

Fig. 4: Comparative amino acid profile of the three samples.

Nutritionally essential amino acid found in the three samples (Scumbria, Croaker and Mackerel) included leucine (2.39, 6.01, 4.32 g/100g of protein), lysine (1.01, 5.94, 4.67g/ 100g of protein), valine (1.93, 3.57, 3.04 g/100g of protein), arginine (3.27, 3.96, 4.99 g/100g of protein), threonine (2.27, 2.89, 4.05 g/100g of protein), tyrosine (1.03, 2.24, 3.67 g/100g of protein), tryptophan (0.31, 0.47,0.58 g/100g of protein), cysteine (0.36, 0.48, 0.73 g/100g of protein), methionine (1.07,2.62, 1.97 g/100g of protein) and histidine (1.72, 1.66, 3.07 g/100g of protein) as shown in figure 4.

Nutritionally non-essential amino acids were of higher concentrations, those detected were glutamic acid (9.99, 13.02, 10.29 g/100g of protein), serine (1.84, 6.1, 3.89 g/100g of protein), glycine (5.22, 3.23, 4.27 g/100g of protein), isoleucine (1.64, 3.08, 3.01 g/100g of protein), phenyalanine (2.22, 3.01, 3.37 g/100g of protein), proline (1.07, 2.62, 1.97 g/100g of protein), alanine (4.25, 3.49, 6.07 g/100g of protein) and Aspartic acid (5.08, 7.88, 7.63 g/100g of protein).

DISCUSSION

Results from the three samples used showed the presence of eighteen (18) amino acids. They all contained the eight essential amino acids in different concentrations [24] also reported similar findings of 18 amino acids in four commercial Nile fishes in Sudan.

Glutamic acid was the most abundant in all samples used. Glutamic acid, aspartic acid, Alanine, serine, leucine and lysine were found to be the most abundant amino acids amongst the samples. Skagen [25] reported similar findings for *Penaeuskerathurus* when he reported the amino acid composition of glutamate, aspartate, proline, lysine, arginine and glycine made up 50% of the entire protein available in his sample. Glutamic acid plays an important role in amino acid metabolism because of its role in transamination reactions and is necessary for the synthesis of key molecules, such as glutathione which are required for removal of highly toxic peroxides and the polyglutamatefolate cofactors. This amino acid was found

to be one of the most abundant amino acids in all the three samples with Croaker having the most abundant concentration (13.02g). Similar values of glutamic acid have been reported in other fish species like red salmon [26] and also in beef [27]. The high concentration of glutamic acid may be as a result of it being a nonessential amino acid and as such produced in higher quantities by the fish.

Histidine which is a nutritionally essential amino acid needed for growth and repair of tissue, maintenance of the myelin sheaths and in removing heavy metals from the body [28]. The histidine composition in Croaker and Scumbria were below the minimum requirement for standard protein in human protein (<1.9g/100g of protein) but was more abundant in Mackerel (3.07g) and met the standard protein requirement for histidine. Values from this research were lower than values obtained from the marine fish *Rastrelligerkanagurta* by Wesselinova [24] who had values of 7.9g. The difference in the concentration of histidine may be related to the species of fish, size and feeding habits of the specie.

Lysine also an essential amino acid needed for optimal growth and its ability to help form collagen and repair tissue in the body. It plays a vital role in maintaining energy and building muscle protein and tissue repair, making it important to those with sports injuries. Lysine is also needed for antibody formation, bone formation and the production of hormones and enzymes. It is often used to reduce the incidence of herpes infections [25]. The croaker sample had the greatest availability of lysine (5.94g) which was lower than values obtained byZakhariev, Ibrishimovand Monov [26] on *Channamicropeltes* (10.9 ± 1.05%) and *Channa Lucius* (10.1 ± 1.42%). The difference could be as a result of preservation and length of storage asZuraini, Somchit and Solihahetal [27] proved in his study with Hake (*Merluccius sp*.) that lysine reduced when stored at -12°C for 4 months due to its reaction to formaldehyde and is converted to formallysine.

Leucine is an important amino acid in physiological conditions like burn, trauma, sepsis and stimulation of muscle growth. It was found in high concentration in the croaker sample (6.01g) and least in scumbria (2.39g). Similar values were obtained from studies on European seabass (7.21 ± 0.56%), *Gilthead seabream* (7.27 ± 0.80%) and turbot (5.91 ± 0.69%) [8]. While Mackerel and Croaker could make good sources of leucine, Scrumbria could not meet the dietary requirement of man. The difference in values could be as a result of difference in specie as seen in the different values obtained from various species.

Arginine plays an important role in cell division, wound healing, ammonia removal, immune function and hormone release. The mackerel sample had the most abundant of arginine content (4.99g). Similar levels of arginine have been reported in the small forage fish capelin (*Mallotusvillosus*) (5.70 ± 0.02%) [28]. Also Arginine contents of cold water fishes *O. mykiss* (6.5±0.3 $g100g^{-1}$ protein), *T. putitora* and *N. hexagonolepis* were found to be very high among the fishes studied and can be recommended in arginine deficiency [9]. The difference in the concentration could be attributed to a number of factors like storage conditions, size and species of the fish sample.

Glycine plays an important role in metabolic regulation, preventing tissue injury, enhancing anti-antioxidant activity, promoting protein synthesis and wound healing and improving immunity and treatment of metabolic disorders in obesity, diabetes, cardiovascular disease, ischemia-reperfusion injuries, cancer and various inflammatory diseases [11]. This is what makes it an important amino acid and it was found in higher concentrations in the Scumbria sample (5.22g), which was similar to the glycine content of fresh *Penaeusnotialis* (4.20±0.16) while the catfish *H. fossilis* was found to contain higher amount of glycine (15.4±3.6g/100g of protein) and in same study *Cyprinuscarpio* had concentration of 3.2±0.9g/100g of protein. This shows that Glycine content is specie specific.

Methionine is used for treating liver disorders, improving wound healing and treating depression, alcoholism, allergies, asthma, copper poisoning, radiation side effects, schizophrenia, drug withdrawal and Parkinson's disease [20]. Methionine content of the Croaker sample was the highest (2.62g 100^{-1} g protein) and similar studies conducted with *Clariasbatrachus* had methionine values with 2.85 ± 0.3 g 100^{-1} g protein, *Anabastestudineus* had 1.6 ± 0.1 g 100^{-1} g protein and the cold water fish *T. putitora* had concentrations of 3.6 ± 0.3 g 100^{-1} g protein [2]. Methionine is affected by long storage time as proposed byMohammed and Alim [13] stating that methionine transitions to methionine sulfoxide.

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