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NON-PROTEIN NITROGENOUS (NPN) COMPOUNDS IN SEAFOODS

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Non-protein nitrogenous (NPN) compounds are a diverse group of nitrogen-containing compounds that play a crucial role in the chemistry and nutritional composition of seafood. Seafood's are known for their high protein content, but NPN compounds also contribute significantly to their overall nutritional value and sensory characteristics. This article provides an overview of the types, sources, and roles of NPN compounds in seafood, highlighting their impact on human health and the seafood industry. Various NPN compounds can be found in seafood, including free amino acids, nucleotides, urea, creatine, and trimethylamine oxide (TMAO). These compounds originate from endogenous sources within the fish, as well as exogenous sources such as their diet and the surrounding aquatic environment. NPN compounds contribute to the flavor, aroma, and overall sensory quality of seafood, enhancing their palatability and consumer acceptance. From a nutritional perspective, NPN compounds in seafood offer several health benefits. Free amino acids derived from protein breakdown are readily absorbed and utilized by the human body as building blocks for various physiological processes. Nucleotides play essential roles in energy metabolism, cellular signaling, and immune function. TMAO acts as an osmolyte and an antioxidant, protecting fish tissues against osmotic stress and oxidative damage. However, NPN compounds in seafood can also pose challenges for food safety and quality. High levels of certain NPN compounds, such as histamine, can lead to allergic reactions and foodborne illnesses in susceptible individuals. Moreover, the breakdown of proteinaceous compounds during storage and processing can result in the formation of undesirable compounds, affecting the sensory attributes and shelf life of seafood products.

Keywords

Peptides, Nucleotides, Seafood, Urea, TMAO

Introduction

Non-protein nitrogenous compounds or nitrogen containing extractives are water soluble low molecular weight nitrogen containing compounds of non-protein nature. The NPN fraction constitutes from 9-18**%** of the total nitrogen content in teleosts and 33-38**%** in elasmobranches. Their content depends on the species, habitat, life cycle and state of freshness after catch. The dark meat contains more NPN than the white meat of fish. In mollusks and crustaceans, the NPN fraction constitutes 20-25% of the total nitrogen content and in Atlantic krill about 20%.

Most marine fishes contain trimethylamine oxide (TMAO); this colorless, odorless, and flavourless compound is degraded to trimethylamine, which gives a 'fishy' odor and causes consumer rejection. This compound is not present in land animals and freshwater species (except for Nile perch and tilapia from Lake Victoria). TMAO reductase catalyzes the reaction and is found in several fish species (in the red muscle of scombroid fishes and in the white and red muscle of gadoids) and in certain microorganisms (Enterobacteriaceae, She-wanella putrefaciens). Creatine is quantitatively the main component of the NPN fraction. This molecule plays an important role in fish muscle metabolism in its phosphorylated form; it isabsent in crustaceans and molluscs.

Major components

The major components of the NPN fraction are volatile bases (ammonia and TMAO), guanidine compounds (phosphagens, creatine and creatinine), free amino acids, nucleotides and purine bases, urea (in cartilaginous fish), imidazole dipeptides (carnosine, anserine and balenine) and betaines. These compounds impart unique and delicate taste to seafoods as well as contribute to the spoilage of fish products.

Distribution of NPN fractions (%) in various seafoods

Class of compound	Teleosts (mackerel)	Elasmobranches (shark)	Crustacean (shrimp)	Cephalopods (squid)
Free amino acids	25	5	65	50
Peptides	5	5	15	15
Nucleotides	15	5	5	5
Creatine compound	50	10	-	-
TMAO	15	20	5	15
Urea	-	55	-	-
Betaines	-	-	10	5
Ammonia and amides	5	-	-	-
Octopine	-	-	-	10

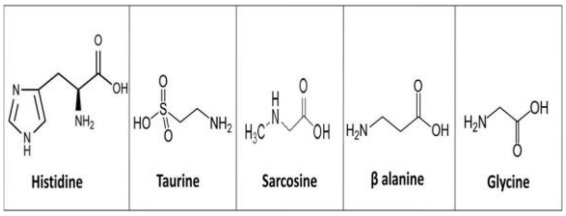
1. Free amino acids

Free amino acid content in seafood's range from 0.5-2.0% of muscle weight. Free amino acids contribute to osmoregulation and get depleted in fish muscle during starvation. Crustaceans have higher content of free amino acids than finfish and mollusks. Cultured fish have less free amino acids than wild fish. Free amino acids found in fish are taurine, sarcosine, □-alanine, methyl-histidine, glycine and □- amino butyric acid. Crustaceans and mollusks are rich in taurine, proline, glycine, alanine and arginine. Cephalopods have more proline and less glutamic acid than mollusks.

Distribution of NPN fraction (quantity wise) in various seafoods

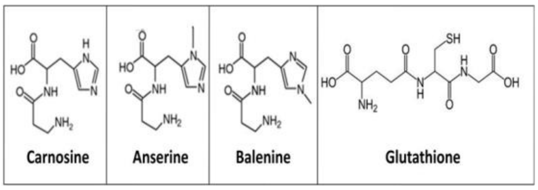
Compounds (mg/100g)	Cod	Herring	Shark	Lobster
Total extractives	1200	1200	3000	5500
Free amino acids	75	300	100	3000
Creatine	400	400	300	-
Betaines	-	-	150	100
TMAO	350	250	500-1000	100
Anserine	150	-	-	-
Carnosine	-	-	-	-
Urea	-	-	2000	-

Histidine is the predominant free amino acid with dark flesh fish like tuna, mackerel and mahi-mahi. Their content in the flesh is **0.6-2%** fresh weight. Taurine is a sulfonic acid, which is common and abundant in marine invertebrates. Their content is as high as 500 mg/kg. In fish, white muscle contains more taurine than dark muscle. Taurine serves as food reserve and has a function in osmoregulation. It is very active in the maillard browning reaction and contributes to the discoloration. Crab tends to accumulate more taurine. Abalone contains 1000 mg/kg of taurine**.** □-alanine appears to be most common in cold-water fish. □-alanine and sarcosine are abundant in elasmobranches', crustaceans (in crabs) and fishes of cold water. Glycine is found in high concentration (1%) in prawns than crabs. Glycine gives the sweetness to prawn tissue. Glycine content varies from 1455 mg/kg in scallops to 10 mg/kg in squids.



2. Peptides

Peptides include carnosine (□-alanyl histidine), anserine (□-alanyl 1-methyl histidine) and balenine (□-alanyl 3-methyl histidine) and glutathione. Dark muscle contains more peptides than white muscle. The ratio of carnosine to anserine is higher for freshwater fish than for marine fish. Carnosine is abundant in eel and skipjack tuna. Eel muscle contains 500-600 mg/kg. It is found in the muscle of prawns, squids and crabs. Carnosine has antioxidant properties. Anserine is abundant in skipjack tuna and some species of shark. It is also found in oysters. Balenine is a major part in baleen whale (>1500 mg/kg) but found in small quantities in tuna, shark, crustacean and mollusks. Glutathione is present in carp (4mg/kg) and salmon (13-20mg/kg).



3. Nucleotides

Majority (90%) of nucleotides in fish muscle are purine derivatives and small amounts of uracil and cytosine derivatives. The purine content in fish muscle is about 300 mg/kg. Nucleotide derivatives are generated as a result of ATP catabolism. After the death of fish, ATP is enzymatically degraded to hypoxanthine by the consequence of endogenous enzymes. In addition, bacterial inosine nucleotide also influences this reaction.

ATP □ ADP □ AMP □ IMP □ Inosine □ Hypoxanthine

The reaction IMP□ Inosine is rather slow, hence IMP accumulates in fresh fish muscle. In crustaceans, AMP tends to accumulate more because of low AMP deaminase activity. In the leg meat of boiled crab, AMP is detected as a main component in addition to small amounts of ADP, IMP, GMP and UMP. Mollusks rarely have AMP deaminase and the major pathway of ATP degradation is

ATP → ADP → AMP → Adenosine → Inosine → Hypoxanthine

ATP catabolism differs with species of fish. For instance, ATP is depleted within 1 h after death of Pacific rock fish, but it gets depleted in almost 1 week in farmed sturgeon. Other nucleotides include nicotinamide adenine nucleotides (NAD/NADH – 4-38 mg/kg; NADP/NADPH – 0.3-11 mg/kg). Dark muscle contains twice these compounds than white muscle.

4. Guanidine compounds

Guanidine compounds constitute 600-700 mg/kg in the fresh fish muscle tissue. They include phosphogens, creatine, creatine phosphate, arginine and creatinine. They serve as high energy reservoirs. White muscle contains more of these compounds than dark muscle. Conversion of creatine phosphate to creatine by Lohman reaction occurs rapidly during stress and slaughter. In resting fish, most of the creatine is phosphorylated and supplies energy for muscular contraction. Creatinine is formed from creatine by cyclization and present in smaller amounts (6-35 mg/kg). It appears to be a metabolic end product for excretion. Cephalopods such as squid or scallop tend to accumulate arginine and octapine, which are intermediates of the arginine phosphate cycle. Octopine is a compound peculiar to mollusks and makes up 10-20**%** of the total extractive nitrogen in cephalopods. Octopine increases strikingly with a decrease of arginine phosphate in scallop, squid and octopus. In crustaceans, the role of creatine is taken over by arginine.

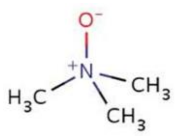
5. Urea

Urea is common in all fish tissues, It is <50 mg/kg in teleost fish and very high in marine elasmobranchs, 1-2.5g/kg. The function of urea is in osmoregulation and to detoxify the ammonia. Urea gets decomposed to ammonia and CO_2 by bacterial urease. It is water soluble and can readily permeate cell membranes. It can also be washed from fillets by the processor. In rays and sharks, the endogenous muscle urease itself, hydrolyse urea and cause a detectable ammonia odour and bitter taste. Special treatments are required to remove urea to make it suitable for human consumption.

i. Early and complete bleeding by severing the tail vein, gutting, filleting and washing shall remove much of urea
ii. Leaching of shark meat in a solution containing 2-2.5% NaCl and 0.02-0.4% acetic acid (1:5) for 2 h at room temperature

6. TMAO

TMAO constitutes a characteristic and important part of the NPN fraction in marine species. TMAO is common in marine teleosts (15-250 mg/kg) and present in very high concentration in marine elasmobranchs (1000-1800 mg/kg) and cephalopods. It is virtually absent in freshwater fish and terrestrial organisms. Dark muscles contain more TMAO than white muscles**.** The amount of TMAO in fish muscle depends on the species, season and fishing ground. Pelagic fish like sardines, tuna and mackerel have high TMAO in dark muscle, while demersal fish have high TMAO in white muscle.



TMAO

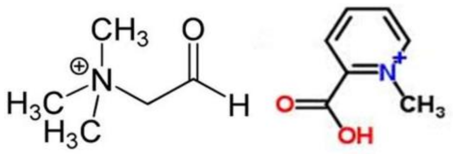
TMAO is formed by biosynthesis in certain zooplankton. Zooplankton possesses an enzyme, TMA monooxygenase, which oxidizes TMA to TMAO. TMA is commonly found in marine plants as other methylated amines (MMA and DMA). Plankton eating fish, therefore obtain TMAO from feeding on these zooplankton. Certain fish species also synthesis of TMAO from TMA. TMA-oxidase system is found in the microsomes of the cell and is dependent on the presence of NADPH.

(CH_3) + NADPH + H^+ + O_2 □ $(CH_3)_3NO$ + $NADP^+$ + H_2O

TMAO is essentially a waste product, a detoxified form of TMA. TMAO is an osmoregulator. TMAO functions as an ‘anti-freeze’ compound. Enzymatic decomposition of TMAO results in the formation of DMA and formaldehyde (FA). Freeze denaturation of proteins in fish is generally caused by the action of FA, which decrease the extractability of myofibrillar proteins.

7. Betaines

Betaines are minor compounds in fish but abundant in mollusks and crustaceans (400-1000 mg/kg). They contribute to the taste of the meat. The most common betaine is glycine betaine. The other betaines include □-alanine betaine, □-butyro betaine and homarine. Homarine is compound widely distributed among marine invertebrates. Betaine is an intermediate compound in choline metabolism.



Betaine Homarine

Importance of NPN compounds

Seafood contains higher content of NPN compounds compared to other muscle foods. Some of these compounds are responsible for rapid deterioration of seafood, because they serve as substrates for typical spoilage organisms that convert them to volatile bases with unfavorable odours.

NPN compounds provide unique and delicate flavor to the seafood. Free amino acids and nucleotides are important compounds contributing for desirable flavors. The 5’nucleotides also contribute to the taste of fish. ‘Umani’ taste of seaurchins and abalone is mainly due to nucleotides. Content of free glutamic acid, along with other free amino acids such as taurine, sarcosine, □-alanine, 1,3 methyl histidine and □-aminobutyric acids are responsible for the taste of seafoods. Inaddition, glycine betaine and certain dipeptides such as carnosine and anserine also contribute to the taste of seafoods.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Conflict of interest

The authors declare that the manuscript was formulated in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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