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Food Quality Assurance: A Novel Freshness Assay

Automation and Challenge Testing of a Novel Freshness Assay Measuring Inosine Monophosphate (IMP), Inosine (Ino), and Hypoxanthine (Hx) as a % Ratio in Seafood Muscle Tissue

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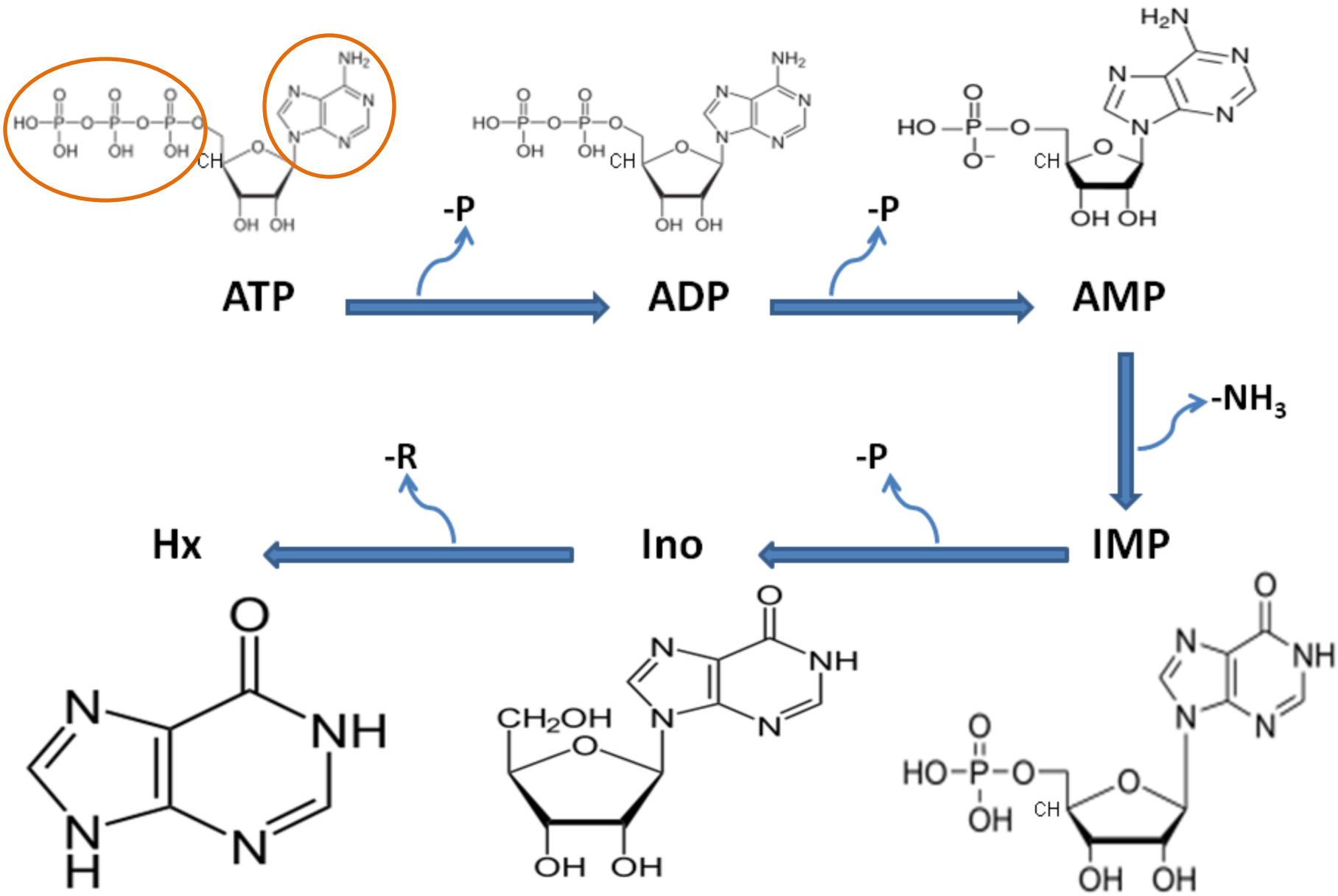
Introduction

- Essence of Delicious Taste – Umami (うま味)
- ‘Umami’ = taste of amino acid L-glutamate + 5’-ribonucleotides including inosine monophosphate (IMP) (1985)
- Highest levels of IMP are present in the early *post-mortem* ATP degradation pathway in fish and meat tissue (freshness pathway)

(*Y. Kawamura and M.R. Kare, ed. (1987). Umami: A basic taste. New York,NJ: Marcel Dekker)*
(*Yamaguchi S, Kumiko N (April 2000). "Umami and Food Palatability". Journal of Nutrition **130** (4): 921S–26S. PMID 10736353)*



Biochemical structure of freshness pathway characterized by ATP depletion following the arrest of cellular respiration *post-mortem* in muscle tissue

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Freshness Comes First...

•Saito *et al (*late 1950's)

K-value (%) = (**Ino**+**Hx**)/(**ATP**+**ADP**+**AMP**+**IMP**+**Ino**+**Hx**)

The lower the K-value the fresher the fish

Sashimi = K < 20%

▪ Karube *et al.* (1984)

K_i (%) = (**Ino** + **Hx**)/(**IMP** + **Ino** + **Hx**)

▪ % **IMP** and % **Hx** can be expressed as an equivalent freshness index by their relationship in K_i

% **IMP** = **IMP**/(**IMP**+**Ino**+**Hx**)

*The higher % **IMP** the fresher the fish*

% **Hx** = **Hx**/(**IMP**+**Ino**+**Hx**)

A higher % Hx indicates muscle aging and has been correlated with bacterial spoilage in fish muscle

(Surette et al, 1988; van Spreekens,1977; Dalgaard, 1993)
(Battle et al., 2001; Tsai, Cassesn, Briskey, and Greaser, 1972; Yano et al., 1995; A.S. Hernandez-Casares et al. 2010)
Huss, Hans H. Quality and Quality Changes in Fresh Fish. FAO Corporate Document Repository, 1995. http://www.fao.org/docrep/v7180e/v7180e00.htm

NovoCIB Precice® Freshness Assay

Nucleotides – Freshness, Nutrition, and Taste

Purine and Pyrimidine Nucleotides

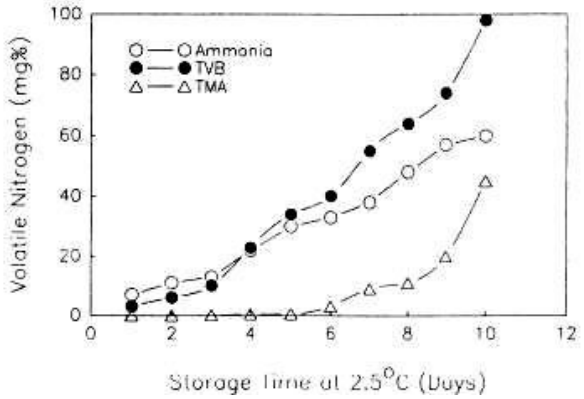
- Energy carrier abundant in animal muscle (ATP)
- Building blocks of nucleic acids
- Nutrients essential for immune and digestive systems
- Flavour enhancers (IMP)
- Feeding stimulants (aquaculture and farms)

Existing methods for seafood freshness evaluation

- Sensory assessment ⟶ **Rapid evaluation of <u>whole</u> wet fish**

- TVB
(Total volatile basic amines)

www.fao.org



- **Most commonly used criteria for assessing fish quality**
- **Detects only late phases of alteration**

Existing methods for seafood freshness evaluation

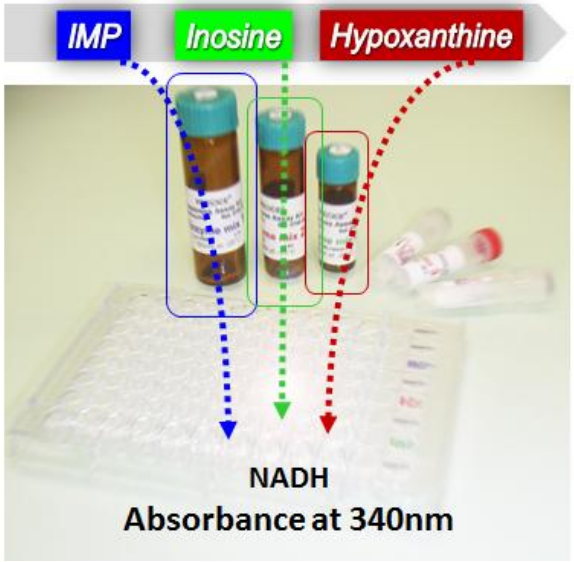
- Sensory assessment ⟶ **Subjective**
- TVB ⟶ **Late alteration**
- Nucleotides quantification by HLPC

PRECICE® Freshness Assay

First method based on enzymatic conversion of IMP, inosine, and hypoxanthine into NADH. Once converted to NADH, each nucleotide (IMP, inosine, and hypoxanthine) is quantified by measuring absorbance at 340nm using microplate reader.

The kit includes
3 enzymatic mixtures,
Cofactors, and
Microplate prefilled with nucleotide standard.

All enzymes and cofactors are provided in stable lyophilized form and are shipped without dry ice or cold blocks.



PRECICE® Freshness Assay

➢ **Wide application range:** Fish, crustaceans, mollusks, meat (pork, chicken, beef), by-products of animal origin, frozen and fresh, salted, smoked, sterilized and canned products

The rate of enzymatic degradation of ATP depends on the temperature

1) Freezing stops ATP degradation process. Hence, the measurement of ATP catabolites in frozen filets can reveal their freshness, which is difficult to evaluate by other methods.

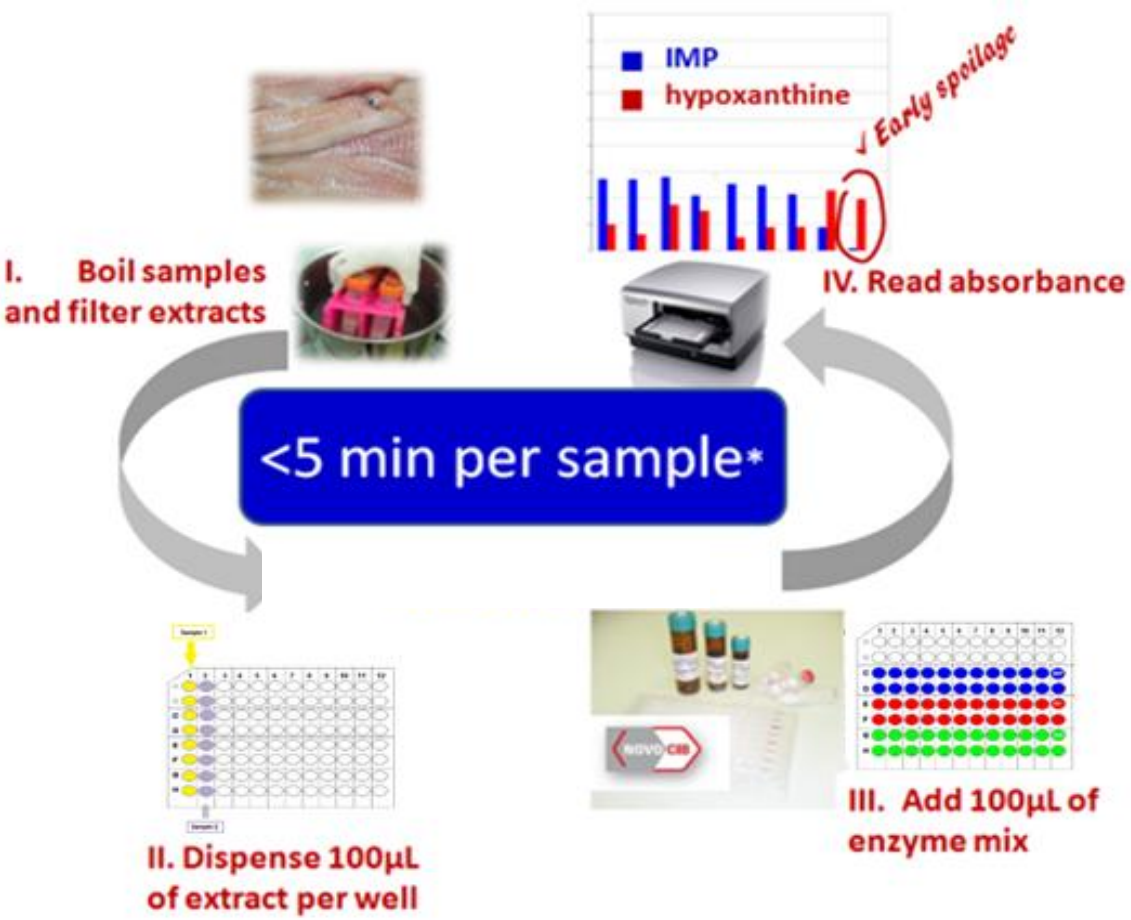
2) Thermal treatment stops ATP degradation process while leaving ATP catabolites intact. The content of nucleotides in cooked, canned or dried products can be used as freshness indicator for raw material of animal origin used before thermal treatment.





PRECICE® Freshness Assay Protocol

- Quantitative extraction of nucleotides is achieved by simple boiling of samples in provided extraction buffer
- Filtered extracts are dispensed into the wells of microplate followed by addition of enzymatic mixtures
- During 30minutes of incubation, added enzymes specifically and irreversibly convert nucleotides into NADH
- At the end of incubation the absorbance data are read at 340nm using microplate reader

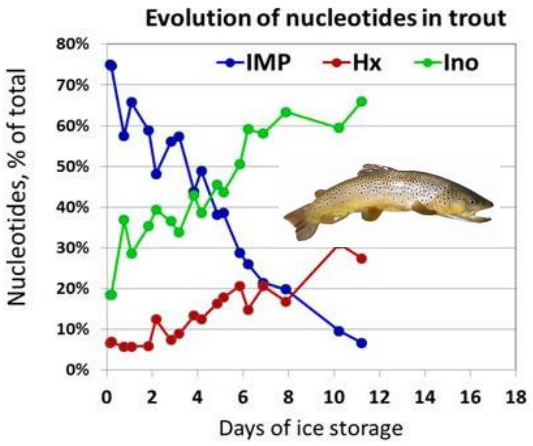


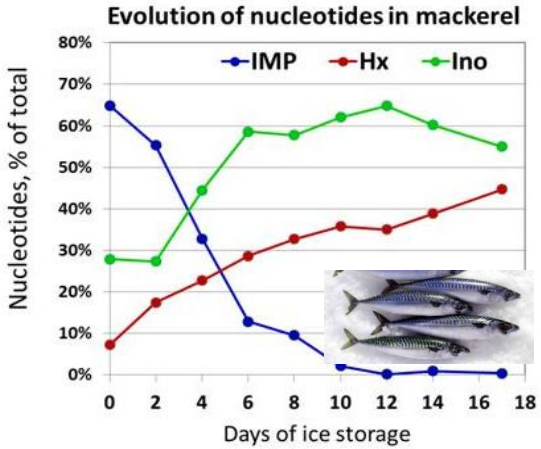
Convenient microplate format

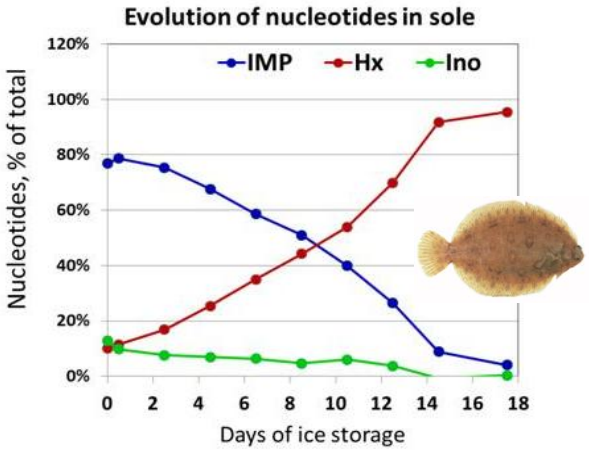
ATP → IMP → Ino → Hx

The possibility of using ATP catabolites for freshness evaluation has been demonstrated for the first time by Saito and colleagues in late 50's and confirmed by numerous scientific publications. However, wide application of nucleotides for Quality Control in Food industry was hampered by:

1) the absence of rapid method for accurate nucleotide quantification,
2) the variations observed in the rate of ATP-catabolism between species**.**

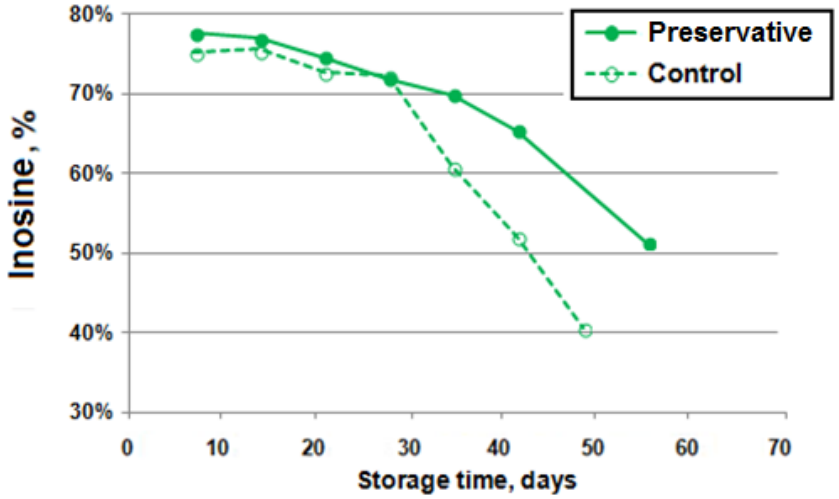


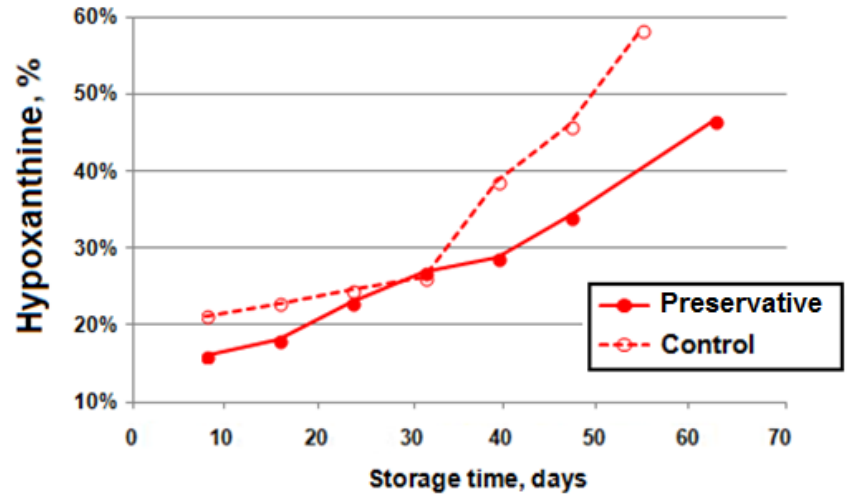




PRECICE® Freshness Assay is first microplate assay allowing the measurement of ATP catabolites in 22 samples in parallel. This convenient microplate format offers the opportunity for systematic caracterisation of ATP degradation rate in wide range of fish species and at various conditions (temperature, packaging).

Example 1. Shelf-life time study of cold-smoked salmon



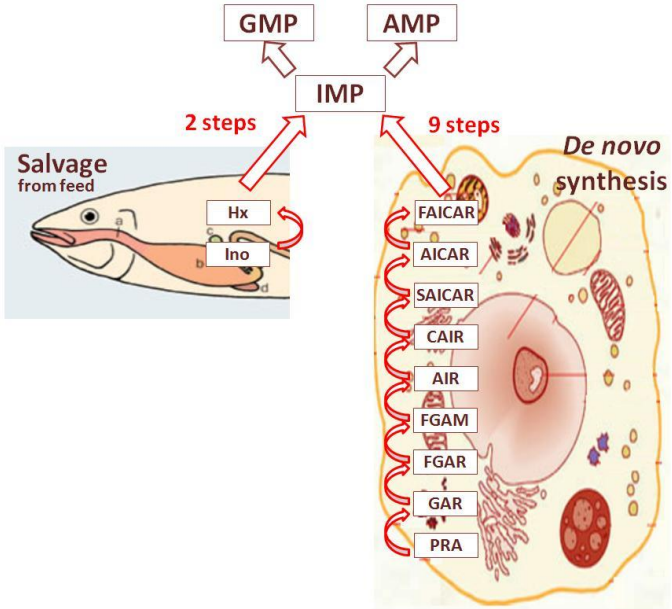


High blood pressure is a major health problem affecting one third of adults.
Reducing salt level in foods is on-going process but remains a major challenge because the salt was historically added to the food to enhance its shelf-life time.
While reducing the salt content, particular attention should be paid to maintain the freshness of new products.

PRECICE® Freshness Assay Kit provides an objective scientific way for characterization of quality changes in new recipes.

Example 2. Dietary Nucleotides - Fish Meal

With the continuing growth of the aquaculture industry, more attention is given to fish welfare since it has significant impacts on fish health and its resistance to diseases. Overpopulation and stress may provoke an immunosuppression and the decrease in resistance to bacterial infection.

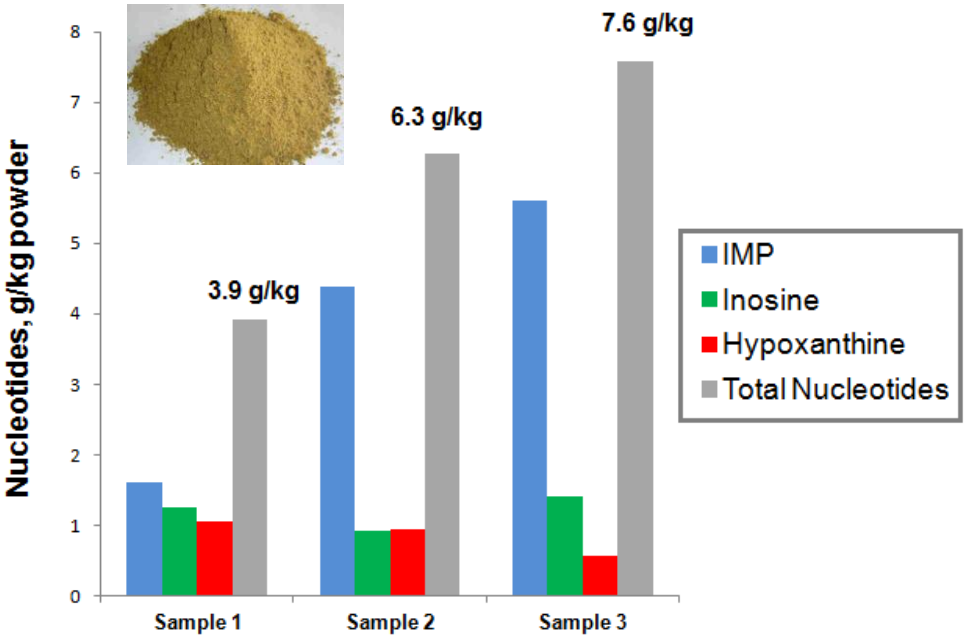


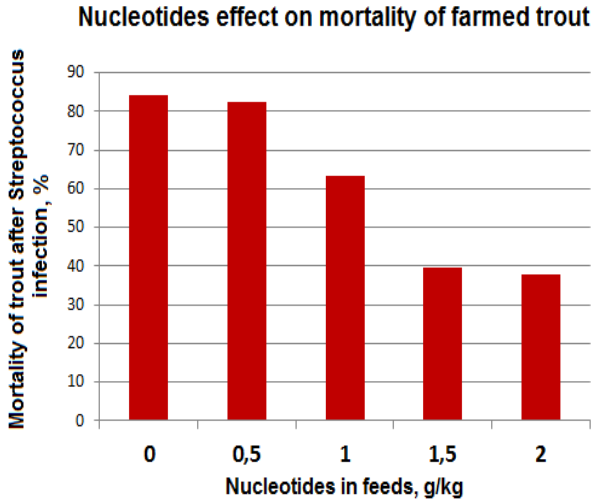
Since nucleotides can be synthesized *de novo* and recycled through salvage pathways, they are considered as semi-essential nutrients. However, *de novo* synthesis of nucleotides is metabolically a costly process compared to salvage pathway.

An exogenous source of nucleotides from the diet may optimize the function of rapidly dividing tissues, such as lymphoid and intestinal tissues, particularly when growth is rapid and the diet is low in nucleotides.

Example 3. Fish Meal Nucleotides

ATP → IMP → Ino → Hx





The level of ATP-catabolites in fishmeal measured with PRECICE® Freshness Assay Kit provides information on:

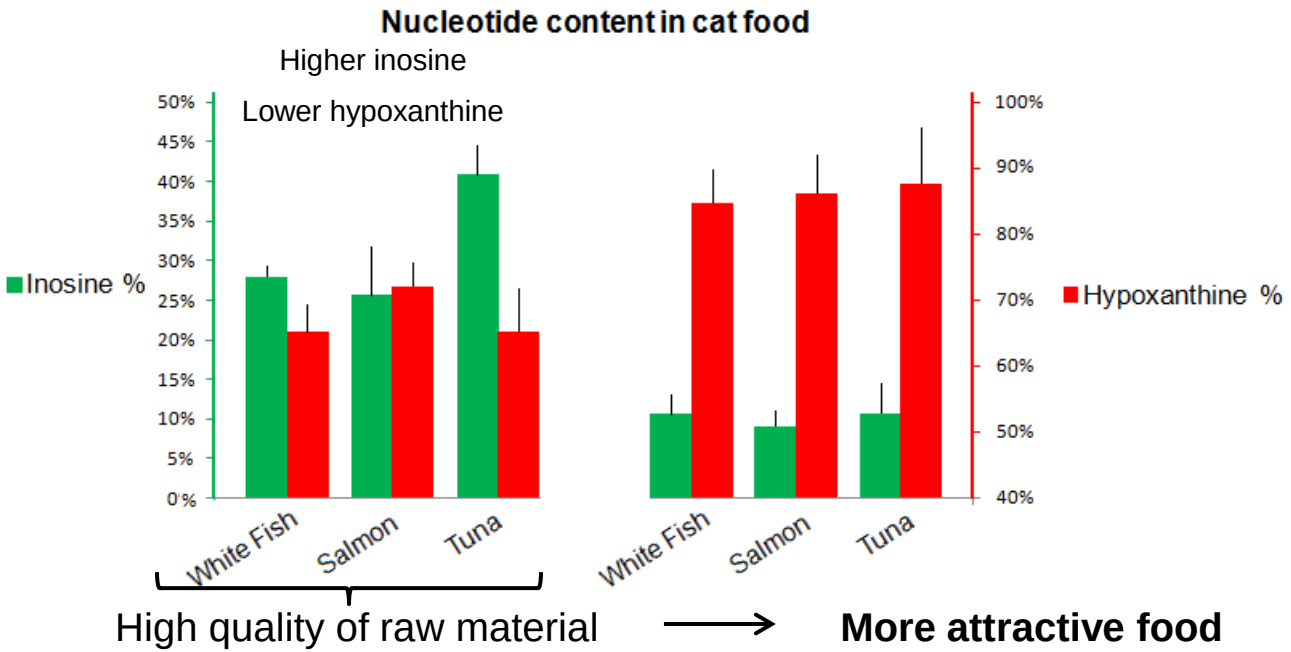
- Nutritional value
- Quality and freshness
- Attractiveness

- Enhance growth
- Strenghten immune system
- Increase survival rate
- Avoid use of antibiotics

Ref.: A. Tahmasebi-Kohyani *et al.*: **Dietary administration of nucleotides to enhance growth, humoral immune responses, and disease resistance of the rainbow trout (Oncorhynchus mykiss) fingerlings** *Fish & Shellfish Immunology 30* (2011) 189-193

Example 4. Pet Food

ATP → IMP → Ino → Hx

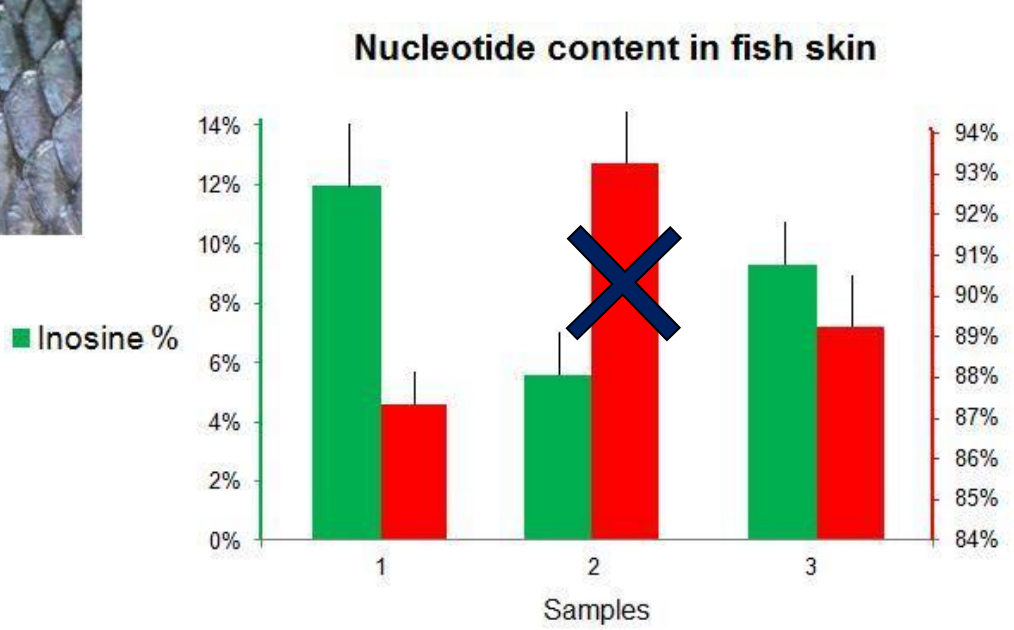


High quality of raw material → **More attractive food**

Petfood ingredients (meat and chicken by-products) are subjects for rapid decay by microbial growth or autoxidation. The freshness of petfood ingredients can affect the palatability of the resulting product. Flavors caused by chemical or microbiological deterioration of the ingredients can result in food refusal, since animals rely on their sense of smell and taste to differentiate safe, nutritious foods from those that taste bad or may contain toxic substances. Measuring Inosine/Hypoxanthine ratio with **PRECICE® Freshness Assay kit** provides a rapid method for qualification of the quality of petfood ingredients.

Example 5. Marine Collagen

ATP → IMP → Ino → Hx



Processing of seafood inevitably generates a large amount of by-products (skin, bones, liver, head, blood etc.) that may contain valuable bioactive materials such as marine collagen for pharmaceutical or cosmetic industry. Because of the presence of bacteria on the surface of fish skin and intestine, the spoilage of by-products is a very fast process resulting in the degradation of biomolecules with high-added values. The content of Inosine and Hypoxanthine in fish skin was measured using **PRECICE® Freshness Assay Kit** and compared in 3 different samples. This analysis has allowed the identification of **highly spoiled** sample in 1 hour.

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