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Free amino acids and their relationship to taste in (salt)ripened pelagic fish species

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Introduction.

The salting of pelagic fish species is a common practice in many European countries. Salting as such is a traditional preservation process and herring (*Clupea harengus*) is salted for instance in Russia, Norway, Finland, Denmark, Iceland, Holland, England and Germany, whereas (salt)ripened anchovies (*Engraulis encrasicholus*) are commonly produced in the southern part of Europe, Spain, Portugal and France (Voskresensky, 1965; Cheftel, 1965). In recent years a lack of catches has been observed for anchovies which has led to the use of other pelagic fish species (e.g. *Sardina pilchardus, Engraulis anchoita*, and *Engraulis ringens*) for the production of anchovy-like products (Mendez *et al.*, 1994; Ayensa *et al.*, 1994). Anchovy type products are also known in the Scandinavian countries and are commonly produced from sprats (*Sprattus sprattus*; Alm, 1965).

During the salting various changes occur that change the chemical and physicochemical properties of the fish. The changes can in general be divided into two phases, salting and ripening. Salting as such is characterised by salt penetration into the fish tissues and it ends when the concentration of the salt in the tissues equals that of the surrounding brine. Changes occurring during the salting stage, salt uptake, water content changes and fish weight changes, have been fairly well documented (Del Valle and Nickerson, 1967a, b; Zugarramurdi and Lupin, 1976; 1980).

The ripening consists of chemical and biochemical processes that change the characteristics of the fish tissues and thus the sensory properties of the fish. As yet these changes are not well understood. During the ripening or maturing stage macromolecules of the fish musculature, such as proteins and fat, are broken down thus producing low molecular weight compounds, e.g. peptides, amino acids and fatty acids. The texture of the fish slowly becomes softer and more tender and a pleasant taste (ripened taste) and aroma is formed. The ripening is chiefly believed to be caused by enzymes but the origin of the enzymes has as yet not been clearly identified. Endogenous proteolytic enzymes from the internal organs of the herring are considered to be of prime importance, but enzymes from muscle tissue may also be important (Voskresensky, 1965; Alm, 1965; Kiesvaara, 1975). It is commonly believed in the herring industry, based on earlier studies (Luijpen, 1959; Voskresensky, 1965) that herring which has been thoroughly cleaned of intestines ripens slowly and does not acquire the characteristic taste and odour. This indicates that enzymes from the internal organs play a major role in the ripening of salted herring. Muscle enzymes can however not be ruled out as some studies indicate that eviscerated herring ripens albeit more slowly than an ungutted herring (Alm, 1965; Stefansson and Nielsen, 1994). Micro-

organisms and their enzymes have however been shown to play only a minor role, if any, during ripening of herring (Varga *et al.*, 1979; Knøchel and Huss, 1984) although their role can not be ruled out during the ripening of anchovies (Péres-Villarreal and Pozo, 1992).

During ripening of the pelagic fish species the characteristic raw taste of the fish slowly disappears and gradually a typical salt-ripened taste and flavour is formed in the fish. The compounds affecting the taste of ripened or matured fish products are believed to be principally soluble nitrogen containing compounds: amino acids, peptides and nucleotides and their decomposition products (Kiesvaara, 1975).

In this paper an attempt will be made to look at some of the chemical changes taking place during ripening, especially the breakdown of proteins and the formation of free amino acids and how these changes may affect the taste of the resulting salted products.

The amino acid composition of pelagic fish species.

The proteins of herring, as do most fish proteins, contain mostly glutamic acid, alanine, aspartic acid, leucine and lysine whereas the amino acids cystine, tryptophan, methionine, tyrosine and histidine are found in lower content (Connell and Howgate, 1959). During spawning considerable changes take place in the muscle of fish, that result for instance in decreased content of arginine, methionine, tryptophan and tyrosin (Love, 1970).

The soluble nitrogen fraction of fresh herring is about 16-18% of the total nitrogen fraction (Kiesvaara, 1975). This fraction contains evaporating bases, trimethylammonium bases, guanine and imidazole derivatives, peptides and free amino acids. The most abundant free amino acids in the muscle of fresh herring have been shown to be taurine, histidine, glycine and alanine (Kiesvaara, 1975; Hughes, 1959). Dark fleshed fish contain in general high amounts of histidine (Konosu and Yamaguchi, 1982). Although the content of muscle amino acids and ovaries change considerably in herring with maturity the levels of free amino acids show little change with maturity or age (Hughes, 1959; Love, 1970). The main exception appears to be histidine as the content of free histidine appears to decrease during spawning in herring (Konosu and Yamaguchi, 1982).

Histidine, taurine and lysine have been reported to be the most abundant free amino acids in herring used for maatjes production (Luten *et al.*, 1994).

Although histidine is a major component of many pelagic fish species there is controversy about it's role in the flavour of the species; Konosu (1979) points out that

although histidine makes up to about 80% of the free amino acids in dried skipjack (katsowobushi) it does not contribute appreciably to the taste.

It would be of interest to study the effect of spawning on the ripening of pelagic fish species; unpublished results (Stefansson, 1992) suggest that spent herring still has a raw taste after 20 weeks salt storage. This finding may point to changes in muscle amino acids resulting in different formation of soluble nitrogen compounds and/or lack of enzymatic activity.

Proteolytic changes occurring during ripening.

During salt-ripening of herring the soluble nitrogen fraction of the total nitrogen in fish muscle increases. Kiesvaara (1975) found that during the first part of ripening of salted herring there was a gradual increase both in the content of free amino acids and peptides; however the content of peptides appeared to decrease somewhat at the time the herring was judged ripe (by sensory analysis [taste, texture] and instrumental means [texture]). Kiesvaara (1975) found that in unripe fish the portion of soluble nitrogen was less than 20% of the total nitrogen, in ripe fish 20 to 30% and over 30% in overripe fish. The total amount of free amino acids increases during salt-ripening of herring; Kiesvaara (1975) found approximately 6-7 fold increase in the total content of free amino acids during 4-6 months cold storage of spice-salted herring. The content of individual amino acids changes considerably during the ripening and it has been shown by Kiesvaara (1975) that the proportion of basic free amino acids (lysine, histidine and arginine) decreases from about 40% in fresh fish to about 15% in ripe herring. The portion of acidic amino acids (aspartic acid, threonine, serine, proline and glutamic acid) increased during the ripening from 10 to 30% (Kiesvaara, 1975). Furthermore Kiesvaara (1975) suggested using the ratio of basic free amino acids (lys, his, arg) versus acidic free amino acids (asp, thr, ser, glu) and proline to indicate the degree of ripening in salted herring. This ratio changed from 4 to 0,5 during the ripening.

The amino acids alanine, glutamic acid, leucine and lysine have been reported to appear most abundantly in the fractions of free amino acids of ripened herring (Kiesvaara, 1975). Skåra and Olsen (1994) have obtained similar results during the ripening of headed herring; they observed approximately 30 fold increase in glutamine/glutamic acid, a 10 fold increase in the content of alanine and a general slight decrease in the basic amino acids (histidine, arginine and lysine).

The content of free amino acids increases during the production of gibbed maatjes herring (one day storage) although the content of only a few amino acids (arginine,

tyrosine and phenylalanine) was statistically significantly higher than that of the raw material (Luten *et al.*, 1994).

It has been reported that during processing of rice-bran salt-fermented sardine (*Etrumeus teres*) a general increase is observed in the content of free amino acids; the acidic amino acid, aspartic acid increased about 50 fold whereas both histidine and taurine decreased (Chang *et al.*, 1992). Mendes *et al.* (1994) found similarly a general increase in the total content of free amino acids during the ripening of salted sardines. They found that the ratio of basic amino acids versus acidic amino acids declined to a value of about 1 in approximately 150 days from salting in partially gutted sardine (Mendes *et al.*, 1994).

The taste of ripened and fermented fish products.

It is well known that amino acids are important taste contributors in foods (Kirimura *et al.*, 1969; Solms, 1969). Amino acids can influence the taste of foods, e.g. as taste enhancers, act synergistically with other components to affect taste and contribute with their own typical taste if their concentration exceeds a certain threshold value. Each amino acid has its own threshold value.

The taste of amino acids in their free state (as L, D, and DL forms) has been determined by Haefeli and Glaser (1990). A few of the L-forms of the amino acids taste sweet, that is glycine, alanine, threonine, valine (with a bitter aftertaste), serine (with a bitter/sour aftertaste), lysine and proline (bitter aftertaste). L-aspartic and glutamic acids taste sour whereas isoleucine, leucine, arginine, cysteine, methionine, phenylalanine, tryptophan and histidine taste bitter (Shallenberger, 1993). Glutamine has a salty-bitter taste. Kirimura *et al* (1969) have described the taste of the amino acids aspartic and glutamic acids and their salts as meaty. Many of the L-amino acids have a pleasant taste but all the bitter tasting amino acids, valine, lysine and glutamine were considered by the taste panel to have an unpleasant taste (Haefeli and Glaser, 1990).

In contrast to the L-form, the D-form of the amino acids generally taste sweet (Haefeli and Glaser, 1990). They point out that some fermented milk products such as yoghurt, kefir, quark and cheese may contain up to 17% D-amino acids (Haefeli and Glaser, 1990).

Umami taste (glutamates, inosinates and guanylates) is considered to contribute largely to the taste of many seafoods, e.g. sea urchins, abalone, scallop, shrimp and lobster (Komata, 1990). Glutamic acid provides the umami taste through a synergistic effect with 5´-nucleotides. Komata (1990) suggested that umami taste may be the signal

which announces the intake of protein to prepare the body to digest and absorb protein, whereas sweet taste may be the signal for carbohydrate intake.

The flavour of many fermented fish products is believed to be mainly due to free amino acids and peptides; e.g. in fermented anchovies (Lee *et al.*, 1982a) in fermented shrimp (Chung and Lee, 1976) and in fermented squid (Lee *et al.*, 1982b). The taste of fish sauce is contributed mainly to free amino acids (Chayovan *et al.*, 1983) and recently a direct correlation has been observed between the content of free amino acids and preference scores for fish sauce (Raksakulthai and Haard, 1992). Multiple regression analysis showed that the preference scores correlated best with the concentration of aspartic acid, glutamic acid, glycine and peptide nitrogen (Raksakulthai and Haard, 1992). It is interesting to note that in the study of Raksakulthai and Haard (1992) the typical flavour (especially the brothy taste) of the fish sauce was apparently also correlated with large peptides. Kiesvaara (1975) points out in his studies that the amino acids aspartic, glutamic, methionine, lysine, histidine, alanine, valine and arginine were all found in excess of their threshold value in spice-salted herring and titbits.

Peptides can be important taste contributors of foods. Kirimura *et al.* (1969) classified the taste of dipeptides into three groups: 1. Sour taste (two acidic amino acids, acidic and neutral amino acids, or acidic and aromatic amino acids). 2. Bitter taste (neutral amino acids with either large alkyl groups or a combination of large and small alkyl groups, neutral and aromatic amino acids, or neutral and basic amino acids). 3. Little or no taste (two amino acids with small alkyl groups, acidic and basic amino acids, or two aromatic amino acids). Dipeptides consisting of two bitter or tasteless amino acids can taste intensely sweet, a good example is aspartame. Only the L,L-isomer is sweet as both D,L-, L,D- and D,D- isomers are bitter (Shallenberger, 1993).

Peptides can have an undesirable effect on the taste of foods. During proteolytic hydrolysis peptides of varying sizes are formed but it is well known that some peptides, especially those rich in hydrophobic amino acids taste bitter (Adler-Nissen, 1986). Little information appears to be available on the possible effect of bitter tasting peptides on ripened fish products. It has been pointed out by Raa and Gildberg (1982) that bitter tasting peptides are apparently not observed during the production of traditional fish sauce possibly because of the high salt concentration.

Conclusion.

It appears looking at the research in the past few years that free amino acids and peptides may be the main contributors of taste in ripened and fermented fish species.

The ratio of basic free amino acids (lys, his, arg) versus acidic free amino acids (asp, thr, ser, glu) and proline has been observed to decrease during ripening of some products and may be a useful indicator of ripening of fish products and possibly also point to some chemical constituents of the ripened taste in the products (Kiesvaara, 1975; Mendes *et al,* 1994; Skåra and Olsen, 1994). Some studies indicate (Kiesvaara, 1975; Mendes *et al.*, 1994; Skåra and Olsen, 1994; Raksakulthai and Haard, 1992) that the content of glutamic acid increases considerably in the products and may in combination with other constituents (nucleotides and other free amino acid) contribute to a meaty (umami?) taste in the products. Glycine and alanine also appear to increase in (some of?) the ripened products but both can contribute to a sweet taste. A sweet taste is often observed in salted herring at the peak of ripening, just before it starts to spoil.

Peptides appear to play a role in forming part of the typical flavour (especially the brothy taste) of fish sauce (Raksakulthai and Haard, 1992) and their role should not be overlooked in the taste of ripened pelagic fish products.

Although some information is already available on the proteolytic changes occurring during ripening of pelagic fish species which affect the sensorial properties of the final products, very little appears to be known about the part the free amino acids and peptides play in the taste of the ripened pelagic fish species. Even though the nitrogen containing compounds are likely to be the main contributors in the taste of ripened pelagic fish products the role of other components, e.g. fatty acids, nucleotides and their decomposition products, should not be overlooked.

Suggestions for further research.

Further research is required on the proteolytic breakdown that takes place during ripening and the role played by the resulting compounds in the flavour of the final products. Research is also required on the possible role of other compounds in forming the taste of the products, e.g. the possible role of free fatty acids, rancidity products, nucleotides, etc.

It is suggested that possibly more information may be obtained on the taste of salt-ripened pelagic fish products by preparing extracts from fully ripened products and analyse the component composition. By this it is assumed that the characteristic taste of the products can be extracted. Secondly, to prepare a synthetic extract based on the

chemical composition of the natural extract(s) that should have the identical taste as the natural extract(s). The importance of the chemical components on the taste of the synthetic extract can then be evaluated by omission testing.

Considerable work has been carried out in Japan on fresh seafood using these techniques and in many cases the taste active components in the seafoods have been identified (Konusu, 1979). Commonly hot water is used as an extraction medium and deproteinization is carried out using 80% ethanol. The extract is then analysed for free and combined amino acids, nucleotides and related compounds, quarternary ammonium bases, sugars, organic acids and inorganic salts.

In a recent article by Konosu *et al.* (1987) an example is given on the role of extractive components in the taste of boiled crab. They found out after testing several different extraction procedures that the use of hot water followed by deproteinization with 80% ethanol was the best method for their purposes. The extraction procedures were evaluated by comparing total nitrogen, α-amino acid and by determining the flavour of the extracts. The extracts were then analysed for free amino acids, total amino acids (after hydrolysis), nucleotides and related compounds, quaternary ammonium bases, sugars and organic acids and minerals. A synthetic extract was then prepared using 40 pure chemicals that simulated the taste of boiled crab. Organoleptic tests of the reconstructed extract, using omission and addition test methods, revealed that 12 of the compounds were taste active. Those were glycine, arginine, alanine, glutamic acid, AMP, CMP, GMP, Na^+, K^+, Cl^-, PO_4^{3-} and glycine betaine. The taste of a synthetic extract containing those compounds had the taste characteristics of crab meat extract although lacking in intensity.

The researchers, Konosu *et al.* (1987), found that glycine imparts a sweet taste to the seafood and alanine as well. Arginine did not in their view impart a bitter taste but rather contributed to fullness and seafood like flavour. Glutamic acid with the nucleotides contributed umami taste but also a sweet taste. NaCl enhanced the flavour of the product. They point out that although proline, taurine and TMAO were found in large amounts they had little effect on the taste of the extracts.

The use of similar methodology, as briefly described above, on enzymatically ripened pelagic fish species may be a valuable tool in linking together (bio)chemical changes taking place during ripening and sensory attributes, especially taste and flavour.

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