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CHEMICAL STRUCTURE OF AROMA AND VOLATILE FLAVOR COMPOUNDS OF SOME FISH, PROCESSED FISH, FISHERY PRODUCTS AND PRAWN OF JAPAN

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ABSTRACT

This research is a part of a research project of identification of volatile flavor compounds of fish, processed fish, fishery products, prawn species of Japan by GC-MS method in a series of laboratory experiments. First part of the research was identification and confirmation of aroma and volatile compounds by GC-MS method and comparison of mass spectrum of each compound with that of Mass spectral data base. The result was confirmed by using authentic compounds following the same method and analytical conditions. Second part (current research) is the identification of chemical structure of the volatile flavor compounds of fish, processed fish, fishery products and prawn species of Japan through computer matching of result (obtained from first part of the research) with the stored data in mass spectral database (NIST, National Institute of Science and Technology). Chemical structure of the aroma compounds and volatile flavor compounds indicate that majority of the components were of low molecular weight (less than 100), some of molecular weight between 100-150 and a few above this figure. Among the components majority were aliphatic hydrocarbons (alkane, alkene, cyclic hydrocarbon), some were carbonyl compounds (aldehyde, ketone), some were alcohols, an organic acid and two were aromatic compounds according to their chemical structure. Each component of these groups were identified and reported previously. Here we are reporting the chemical structure of the components. Chemical structures were obtained by computer matching of GC-MS peak and mass spectrum with the stored data in mass spectral database.

KEYWORDS: Aroma compounds, fish volatiles, molecular structure, fish, processed fish, fishery products.

INTRODUCTION

The term 'flavour' has evolved to a usage that implies an overall integrated perception of all of the contributing senses (smell, taste, sight, feeling and sound)at the time of food consumption. The ability of specialized cells of the olfactory epithelium of the nasal cavity to detect trace amount of volatile odorants accounts for the nearly unlimited variations in intensity and quality of odour and flavour. Taste buds located on the tongue and back of the oral cavity enable consumer to detect/sense sweetness, sourness, saltiness, bitterness and these sensations contribute to the taste compounds of flavor. Nonspecific neural responses also provide important contribution to flavor perception through detection of pungency, cooling, umami, or delicious attributes, as well as other chemically induced sensation. The nonchemical or indirect senses (sight, sound, feeling) influence the perception of taste and flavor, hence food acceptance (Lindsay, 1996).

Flavour is an important criterion for consumers' preference of food commodities specially fish and fishery products. The state of freshness or quality of fish and prawn can be apparently determined by the consumers or buyers on the basis of flavor. The desirable aroma and flavor of fish and fishery product influence

consumers' choice. Fishery products and processed fish are characterized by their specific taste, flavor and sometimes by texture which are in general referred to as "Sensory attributes". Fish Processi8ng Technology and Fishery Product Technology need to keep the original fresh flavor (i.e. no spoilage, not unpleasant) of fish, at the same time to make a product of pleasant flavor and taste. So flavor of fish and fishery product is very important. Taste and flavor of fishery products vary with the processing technique although the same species of fish is processed to produce the product.

Sensory attributes of the processed fish and fishery products are criteria for consumer preference. It is more important if such processed fish fishery products are eaten without any further heat treatment or cooking. In such case flavor is the most important attribute of the product. Perfect flavor or desired flavor of the fishery products is important for the consumers to find the expected quality product. Thus flavor of processed fish and fishery products are important for the consumers for their dietary satisfaction, nutritional benefit, health benefit. It is important for the fish industries to raise the market share of their products, as well as, consumers' acceptance.

The flavor of processed fish and fishery products differ according to processing technology. Such difference is noticeable with the difference of fish size, area of capture, season, storage condition. For example the flavor of smoked salmon is different from the salted salmon. Even the flavor of dried tiny sardine is different from the flavor of dried horse mackerel. Therefore, flavor research and studies on fish, processed fish, fishery products is important. Components contributing to the flavor of fish and fishery products are studied by many scientists and researchers. Jones (1961) described in detail the flavor substances present in fish. The main categories of these substances were mentioned as sugar, sugar phosphate, amino acids and peptides, nucleotides and derivatives, lower fatty acids, other carboxylic acids, fats and its degradation products, nitrogenous bases and other nitrogenous compounds, sulphur compounds, carbonyl compounds, inorganic constituents. Lindsay (1990) discussed the volatiles in fish and seafood flavor. Characterizing flavors in seafood cover a broader range of flavor qualities than those occurring in other muscle foods. The broad range of animals involved fin fish, shell fish and crustaceans, and the variable flavor and aroma qualities related to freshness each account for the different flavours encountered. Fish aroma characterized by the terms as 'oxidized fish oil' and 'cod liver oil' like are largely caused by carbonyl compounds produced from the autoxidation of long chain ω-3-polyunsaturated fatty acids. These characteristic aroma result from 2-4-7-decatrienal isomers, and c-4-heptanal potentiates the fishy character of the decatrienals. Josephson *et. al.* (1983) identified the volatile aroma compounds contributing to the overall characterizing aroma of fresh white fish (*Coregonus clupeaformis*). Josephson et. al. (1987) reported the influence of processing on the volatile compounds characterizing the flavor of pickled fish Lake Michigan Smelt (*Osmerus mordax*) processed traditionally in brine (48 hrs), vinegar (48 hrs), and sugar vinegar (final solution). Pokorny (1987) studied the effect of browning reactions on the formation of flavor substances in food during processing and storage. He stated that the browning reactions are the mostb important reactions taking place in food during processing and storage and involved in the formation of flavor substances. Two types of browning reactions have been reported to occur in food material e.g. enzymatic browning and non- enzymic browning. He reported that the non enzymatic browning reactions affect the flavor as they are accompanied by production of volatile substances. Mansur (1995) identified the aroma and volatile flavor compounds of pickled herring (*Clupea harengus*) of Britain.

Early research on fish flavor was conducted by taste panel members. Trained taste panel members used to detect the compounds contributing to flavor of fish, then this compound was confirmed by tasting the standard chemical compound. Later classical chemical tests were followed in flavor research. Advancement was done in flavor research by the invention of apparatus like Gas Chromatography. Latest advancement of flavor research is carried out by the modern apparatus named GC-MS, LC-MS etc. The state of freshness, quality of a fish and fishery product can be perceived (assessed) by the consumers on the basis of its flavor. Development of laboratory techniques facilitates such flavor research of fish, processed fish, fishery products and other food.

Aroma and volatiles of some sea fish and prawn of Japan was studied which we have reported in our previous publications Mansur (2001), Mansur et. al. (2003). In the previous part of our research the aroma and fish volatiles were detected by the mass spectrum of the GC-MS peaks for each components, later confirmed by comparing the mass spectrum with those of the standard authentic compounds. In the current part of our research the components were been confirmed by computer matching of the mass spectrum of the components and obtaining the chemical structure of each aroma and flavor components of fish and prawn. Chemical structure were obtained by computer matching of GC-MS peak and mass spectrum with the stored data in mass spectral database.

MATERIALS AND METHOD

Source of experimental material

Materials used this research were mainly two types: (a) Raw fish and prawn, (b) Processed fish and prawn, and fishery products.

Raw fish and prawn

Red sea bream, Chum salmon, Horse mackerel, Pacific mackerel, Sardine, Tuna, Tiger prawn were bought from Nara City of Japan. Tuna was purchased as a piece of

tuna meat. Tiger prawn was purchased as chilled in ice. Tiger prawn was bought as chilled prawn which was frozen at frozen stored in the laboratory freezer at -20℃.
Processed fish, prawn, and fishery products.

Smoked salmon, dried horse mackerel, salted pacific mackerel, canned sardine, canned tuna meat, kamaboko, chikuwa were also bought from Nara City of Japan.
All experimental materials were bought from an approved and licensed fish shop of Nara City of Japan.

Table 1: List of the experimental materials.

Experimental materials	
Raw fish and prawn	Processed fish and prawn, fishery products
Red sea bream	Smoked salmon
Chum salmon	Dried horse mackle
Horse mackerel	Lightly Salted pacific mackerel
Pacific mackerel	Canned sardine
Sardine	Canned tuna meat
Tuna	Kamaboko
Tiger prawn and pink shrimp	Chikuwa

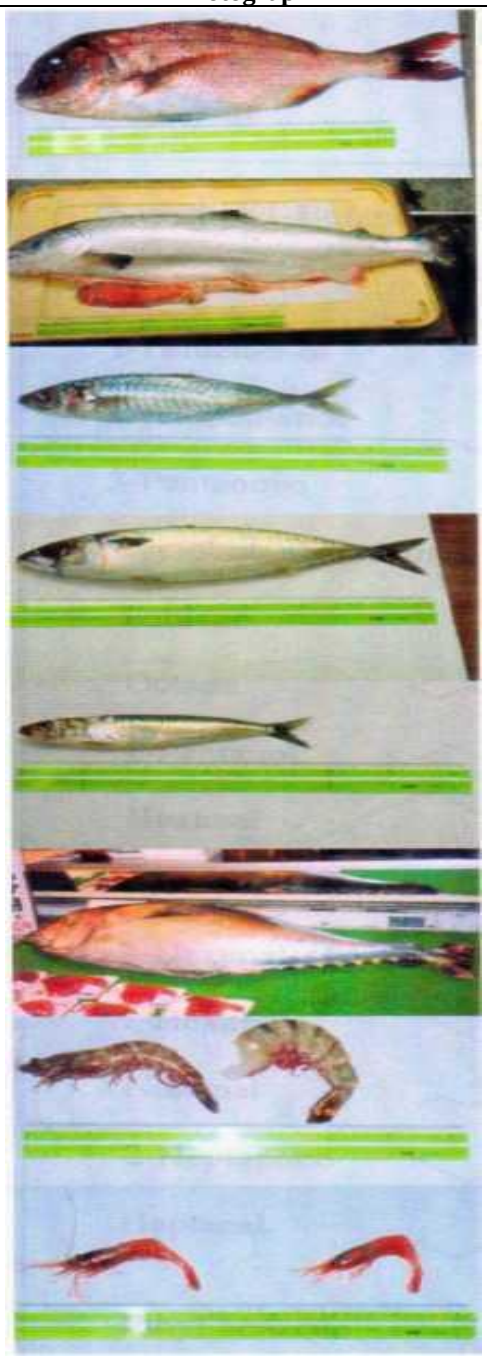
Sample Preparation
The experimental materials i.e. fresh fish and prawn, processed fish, fishery products were taken in a small plate and the edible portion was separated by knife, scissor, forceps and were cut into small pieces from at least three specimens. Frozen tiger prawn was taken in a polyethylene packet and thawed at room temperature in the laboratory. After thawing, shell was removed and the muscle was cut into small pieces. All of the samples were cut into as small pieces as the grains are. For all of the experimental materials at least three specimens were used for sample preparation. Small pieces of each sample were mixed thoroughly to make a representative sample. For the experiment a few grain size small pieces were taken carefully by the forceps.

Extraction of headspace volatiles
Accurately weighed 5g of representative sample was taken in a 20 ml vial (Perkin Elmer) which was sealed with Teflon lined rubber septum to make the vial airtight. The vial containing the sample was heated at 70℃ for 30 minutes in an automated headspace sampler to evaporate the volatile flavor compounds from the sample but remain in the vial. The needle of the SPME (Solid Phase Micro Extraction) fibre holder (Spelco) was pierced through the septum and the volatile flavor compounds were extracted to SPME fused silica fibre (Carboxen-PDMS) for 5 minutes. The fused silica fibre of the needle of SPME was then retracted and the needle was taken out of the vial. Before the extraction of each sample's volatile flavor compounds the SPME fused silica fibre was conditioned by thermal desorption in GC column through the injection port of the GC-MS. Such blank analysis was done to make it sure that the fibre does not contain any other volatile compound before the extraction of sample's volatile flavor compounds. In some experiments it was necessary to conduct blank analysis twice or thrice to make the SPME fused silica fibre free of any compounds.

***Gas Chromatography-Mass Spectrometry* (GC-MS)**
The volatile flavor compounds extracted into the fused silica fibre of SPME needle were injected and thermally desorbed for 5 minutes to the capillary column DB 624 (60 m x 0.322 mm ID, 1.80 µm film thickness) through the injection port of GC-MS (Shimadzu QP 5050A). The desorbed components were subjected to GC-MS (Shimadzu GC-17A gas chromatograph with Shimadzu QP-5050A mass spectrometer, Shimadzu, Kyoto, Japan) analysis under standard conditions. The mass spectrum of each peak of GC was analysed by the Mass Spectrometer and the volatile flavor compounds were identified by computer matching of mass spectra of the volatile flavor compounds with those of the data stored in the mass spectral data base (NIST). In each experiment the compounds of highest possibility is reported. Result of each experiment was checked in a subsequent set of experiment.

Analytical conditions
Capillary column DB 624 (60 m x 0.322 mm ID, 1.80 µm film thickness was used. Helium was used as carrier gas. The analytical conditions were as follows.
Oven temperature 40℃, Oven equilibration time 3 minutes, Injection temperature 280℃, Interface temperature 230℃, Column pressure 35.0 (KPa), Column flow 1.5 (ml/min) and linear velocity 30.7, split ratio 25, total flow 40.0 (ml/min), carrier flow 40.0 (ml/min). Mass range (40-350 m/z). Scan interval (0.50 sec) threshold (5000), scan speed 1000 amu/sec.

Plate 1: Species identification and Photograph of fish and prawn.

Species identification	Photograph
1. English name: Red sea bream Japanese name: Madai Scientific name: *Pagrus major* 2. English name: Chum salmon Japanese name: Sake Scientific name: *Oncorhynchus kata* 3. English name: Horse mackerel Japanese name: Maaji Scientific name: *Trachurus japonicus* 4. English name: Pacific mackerel Japanese name: Masaba Scientific name: *Scomber japonicus* 5. English name: Sardine Japanese name: Maiwashi Scientific name: *Sardinops melanostica* 6. English name: Tuna Japanese name: Kuromaguro Scientific name: *Thynnus thynnus* 7. English name: Tiger prawn Japanese name: Ebi Scientific name: *Penaeus orientalis* 8. English name: Pink shrimp Japanese name: Ama ebi Scientific name: *Pandalus hypsinotus*	
*Tuna was 1.4 m long. Length of other species are indicated by a measuring scale (36 cm).	

CONFIRMATION OF RESULT

The result of these experiments was confirmed by the result of another set of experiments conducted by using standard authentic compounds (Nacalai Tesque). The experimental method and analytical conditions were same as for the fish and prawn, processed fish and fishery products except heating at 70℃ for 30 minutes. Result obtained from GC-MS analysis by using authentic compounds were compared with those of the previous experiments to confirm the findings of compound identification as well as to sort out the unusual compounds and peak, artifact appeared from unknown source.

Determination of chemical structure

Total number of identified aroma and volatile flavor compounds was 32. Determination of chemical structure of these 32 compounds was done by the computer matching/search of the compounds and chemical structure. After a thorough search the chemical structure of the compounds were been done. The identification of chemical structure of the volatile flavor compounds of fish, processed fish, fishery products and prawn species

of Japan was confirmed through computer matching of the mass spectrum of the identified compounds with those of the stored data in mass spectral database (NIST, National Institute of Science and Technology). Thus the determination of the chemical structure of the aroma and volatile flavor compounds were finalized.

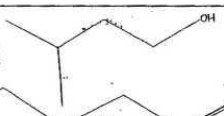
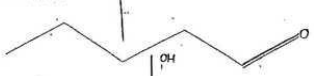
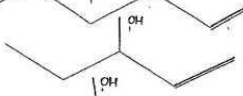
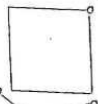
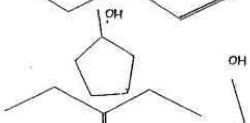
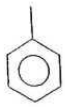
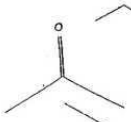
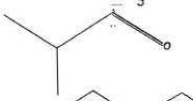
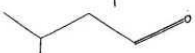
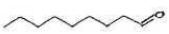
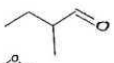
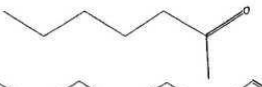
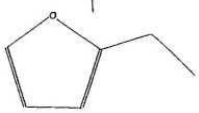
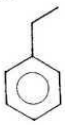
RESULT AND DISCUSSION

A total of 33 volatile flavor compounds were identified. Chemical/Molecular structure of the flavor compounds are shown in the Table 2. Frozen fish and prawn contained less number of flavor compound than the fresh fish and prawn, oily fish contains more flavor components than the lean fis which we have reported in our previous publication (Mansur *et. al*., 2002 and 2003)., Among the identified and confirmed volatile flavor compounds majority were aliphatic hydrocarbons (alkane, alkene, cyclic hydrocarbons) some were carbonyl compounds (aldehydes, ketone); some were alcohols, an organic acid and two were aromatic compounds according to their chemical/molecular structure. Molecular weight of the most of the volatile flavor compounds were less than 100, some were of molecular weight between 100 and 150; and a few were of molecular weight above 150. Some of the flavor compounds were originally present in the raw fish muscle. Rest of the flavor compounds were formed during processing because during processing techniques a series of complicated chemical and biochemical reaction takes place. In general the number and concentration of each flavor compound in the processed fish was much higher than the raw unprocessed fish. But the frozen prawn possessed less number and concentration of flavor components than the raw/chilled fish. The chemical/molecular structure of each volatile flavor compounds has been shown in Table 2. In this table the chemical/molecular structure of the volatile flavor compounds showing that the volatile flavor compounds were of the following three categories.

1. Aliphatic hydrocarbon
2. Carbonyl compounds
3. Alcohol
4. Organic acid
5. Aromatic compounds.

Raw fish muscle and skin contained similar types of volatile flavor compounds than the prawn. In the processed fish and fishery products volatile flavor compounds were higher than the raw fish. During smoking and baking of smoking the main cause of higher number of volatile flavor compounds in the final product is the deposition or settling of smoke components to the fish. Biochemical changes due to slightly higher temperature may also partially contributed to the formation of such flavor compounds (Josephson and Lindsay, 1987). During canning some flavor compounds were formed as a result degradation of original biomolecules of fish as well as the contribution ingredients used in fish during canning. Dried horse mackerel also possessed a number of volatile flavor compounds than the original raw fish. These flavour compounds may be formed as a result of oxidation of fat as well as enzymatic hydrolysis of the original composition of fish e.g. protein, fat etc. In the surimi based products e.g. kamaboko and chikuwa, processing steps influenced the formation of flavor compounds. During processing of fish after bone separation and during texture formation step the enzymic hydrolysis of the original component of fish e.g. protein, fat resulted the formation or bio-generation of flavor components in surimi based products e.g. kamaboko, chikuwa. A certain degree of oxidation may also contributed for this phenomenon. Thermal condition may accelerated retro-aldol degradation of aldehydes which lead to altered flavor in these products (Josephson and Lindsay, 1987). In the lightly salted pacific mackerel (Shio saba) the flavor compounds were comparatively less than the heavily salted fish. In lightly salted pacific mackerel the ratio was salt : fish = 1 : 20, the process continues for only 2 to 3 days at chilling temperature (0-4℃). It may be the reason that the lightly salted pacific mackerel contained less number of volatile flavor compounds compared to the heavily salted fish. The mechanism of formation of flavor compounds in the lightly salted pacific mackerel may be the oxidation of fat. Pokorny (1987) has reviewed the flavor compounds formed by the browning reactions of oxidized fat. In almost all of the processing and storage methods the process increased the flavor compounds. But freezing and frozen storage of prawn cause loss of volatile flavor compounds (Dimethyl sulfide and hexane) due to condensation, but acetone was retained in the frozen stored prawn (both tiger prawn and pink shrimp).

Table 2: Chemical/Molecular structure of the flavor compounds.

Component name	Molecular structure	Component name	Molecular structure
Pentane		3-methyl-1-Butanol	
Ethanol		Pentanal	
Propanal		1-Penten-3-ol	
Trimethylene oxide		Cyclopentanol	
Acetic acid, anhydride		3-Pentanone	
2-methyl-Pentanal		Cyclobutanemethanol	
Acetone		Toluene	
Dimethyl sulfide		Octane	
2-methyl-Propanal		1-Pentanol	
Hexane		Hexanal	
Butanal		Cyclopentanone	
2-Butanone		2-Hexenal, (E)	
Ethyl acetate		1-Hexanol	
3-methyl-Butanal		Nonanal	
2-methyl-Butanal		2-Heptanone	
2-ethyl-Furan		Heptanal	
		Ethyl benzene	

Objective of this experiment was the determination of chemical/molecular structure of the volatile flavor compounds of some fish, prawn, processed fish and fishery products available in Japan. Determination of structures of the volatile flavor compounds of fish, prawn and shrimp, processed fish and fishery products were accomplished by laboratory experiments using GC-MS and computer matching of stored information or data. Knowledge about the chemistry and technology of flavours has expanded greatly in the last several decades and information has accumulated at this point where control and manipulation of many flavours in foods is possible. However, some of the areas of research likely to be fruitful in the forseeable future are binding of flavours to macromolecules, structure-activity relations in taste and olfaction as determined using computer techniques, control of reaction flavours chemistry, and flavor development in many research areas (Lindsay, 1996). The issue of authenticity of natural flavor continues to attract considerable attention, and research on the various aspects of analysis and structural relationships of closely related molecules can be expected to enhance knowledge about subtle molecular influences on the flavor quality of such optical isomers. Isotopic mass spectrometry techniques have made it possible in many cases to differentiate between natural and synthetic molecules, but carbon-13 enrichment alterations of synthetic can render such approaches, for detecting adulteration invalid. However, site specific natural isotope fractionation measured by nuclear magnetic resonance is developing to the point where it provides an isotropic fingerprint that makes it possible to detect adulteration natural origin flavours (Martin, 1993). The chemical accuracy of nature-identical synthetic substances continues to be challenged by new information that is accumulating on the unique odour and

flavor properties of enantiomers and other chiral components (Mossandl, 1988).

In this experiment we detected the chemical structure of the volatile flavor flavor compounds of prawn and shrimp, fresh fish, processed fish and fishery products available in Japan. Result of this experiment will be helpful for the detection of adulteration, spoilage and deterioration, quality reduction etc. This method is reliable, rapid and useful for international trade of prawn, shrimp, fish and fishery products, regional trade of prawn, shrimp, fish and fishery products. Such research results are useful for public health consideration where fish consumption is high enough by the people.

CONCLUSION

From the result of the present research it may be be concluded that the determination of chemical structure or molecular structure of the volatile flavor compounds of prawn, shrimp, fish, processed fish and fishery products is possible by GC-MS and computer technique. The chemical/molecular structure of the volatile flavor compounds of the prawn and shrimp, fish, processed fish and fishery products are broadly of 5 groups namely aliphatic hydrocarbon, Carbonyl compounds, Alcohol, Organic acid, Aromatic compounds. Majority of the volatile flavor compounds were of low molecular weight (less than 100), some were of molecular weight between 100-150 and a few above this figure.

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