HEADSPACE GAS CHROMATOGRAPHY FOR QUALITY ASSESSMENT OF CANNED PACIFIC SALMON

by

BENOIT GIRARD

B.Sc., L'Université Laval, 1984
M.Sc., The University of British Columbia, 1987

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

Department of Food Science

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 1991

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Food Science

The University of British Columbia
Vancouver, Canada

Date Sept. 23, 1991

ABSTRACT

The method currently established to assess the quality of canned Pacific salmon relies on sensory evaluation. Among the sensory attributes of importance, odour plays a determining role in grade assignment. It would therefore be useful to obtain information about the volatile components which can be indicative of various quality criteria. This study was primarily undertaken: (1) to analyze the headspace volatiles of canned salmon with a rapid method, (2) to apply multivariate statistics on the headspace volatile data for classifying canned salmon in terms of species, sexual maturity, and degree of decomposition, and (3) to investigate the perceived odour of canned salmon volatiles separated by dynamic headspace gas chromatographic methods.

Sample weight, incubation temperature and time were studied to develop a static headspace sampling method for volatiles in canned salmon. A random-centroid optimization program (RCO) simultaneously searched for the optimal levels of other factors, namely, initial oven temperature, column headpressure, and total flowrate. RCO was found to be an effective optimization program which allowed the performance of several treatment runs at a time. Optimal conditions of operation permitted the detection of 80 volatile compounds, 34 of which were identified including aldehydes, alkanes, aromatic compounds, sulfur-containing compounds, alkenes, ketones, several other compounds plus an alcohol and an acid.

Forty-four selected headspace volatiles from cans of 4 species of Pacific salmon (chum, coho, pink, sockeye), chum salmon at 3 stages of sexual maturity, and pink salmon of 3 quality grades were quantitatively determined using the static headspace gas chromatographic (SHGC) method, and analyzed by multivariate statistical methods. Principal component analysis (PCA) and common factor analysis (CFA) facilitated the interpretation and further handling of the collected gas chromatographic data by transforming them into ten or fewer important dimensional factors. Discriminant analyses (DA) were applied to the PCA scores for group classification. In light of non-compliance of statistical assumptions by the newly generated variables, error rates of linear, quadratic, and non-parametric functions computed by the resubstitution and cross-validation methods were compared. The non-parametric (NPAR) Epanechnikov kernel method maintained a 90% rate or higher of effectiveness at segregating canned salmon of different species, stages of sexual maturity, and quality levels. NPAR-DA also provided a high degree of discrimination at the beginning of refrigerated decomposition where the detection of spoilage by sensory grading is uncertain. Ethanol and 3-methyl-1-butanol contributed significantly to classification of quality grade of canned pink salmon.

Dynamic headspace concentration by Tenax trap sampling/gas chromatography/mass spectrometry (TTS/GC/MS) and cryofocussing concentration sampling/gas chromatography/odour evaluation (CCS/GC/OE) were other means used to analyze volatile components of canned pink salmon, grade A and reject, and canned late-run chum salmon. A total of

130 compounds were identified; hydrocarbons and ketones were found in large numbers followed by sulfur-containing compounds, nitrogen-containing compounds, alcohols, aldehydes, and acids. The headspace profile of all analyzed samples possessed several odour attributes which were associated with the chemical structures identified. No single compound was responsible for the characteristic aromas of canned pink salmon, grade A or reject. 2-Methyl-butanal and two lower boiling point unknowns had a hay or straw-like, cooked-malt odour typical of canned chum salmon of spawning maturity.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I wish to express my appreciation and gratitude to Dr. Shuryo Nakai for his support, council, and invaluable assistance throughout the course of this research project. I would also like to acknowledge and thank the other members of my supervisory committee, Dr. Brent J. Skura, Dr. Timothy D. Durance, Dr. William D. Powrie, and Dr. George K. Iwama for their time, efforts, and critical contribution.

The financial assistance to this project by the Science Council of British Columbia through the Graduate Research Engineering and Technology (GREAT) Award is gratefully acknowledged. Furthermore, my appreciation is extended to the Fisheries Council of British Columbia for their cooperation and collaboration, and B.C. Packers Ltd. who allowed me to use their facilities.

Finally, I would like to thank my families, Girard and Weintraub, for their continued support and understanding during the past several years. I want to acknowledge the fellow students and staff in the Department of Food Science whose friendship and enthusiasm provided rewarding and enjoyable times. Most of all, a special thought is directed to Sara Weintraub for her encouragement, inspiration, motivation, patience, and love.

I. GENERAL INTRODUCTION

Canada's fishing grounds yield around 70 marketable species of fish and seafood. In economics, fish are second only to grain, the country's most valuable food export. In 1987, the Canadian commercial fishing industry harvested 1.5 million tonnes with an estimated value of $1.64 billion. This landed value was transformed into 839,800 tonnes of seafood products, worth $3.3 billions (Department of Fisheries and Oceans, 1988). Approximately 80% of the value of the Canadian fishery production is currently exported. Salmon and herring are the main export products from the Pacific coast.

Commercial fishing constitutes a major factor in the economies of the coastal provinces and northern communities, although it contributes only a small portion of our Gross National Product (0.5%). In British Columbia, commercial fishing ranks fourth among the primary industries, and fish processing accounts for over 25% of all food manufacturing activities. More than half of the $1 billion wholesale value of BC fish production came from ocean-caught salmon in 1988; 40% of which was sold in the form of canned salmon. Pink salmon accounted for 62%, chum 18%, sockeye 17%, and coho 2% of the number of 48-lb cases of cans (Aquaculture and Commercial Fisheries Branch, 1989).

Along with its economical significance, fish occupies an important place as a part of the human diet. In order to maintain a high level of quality, it is essential to ensure that fish products going to market are

wholesome, meet certain commercial requirements, and are aesthetically acceptable to the consumer. Good manufacturing practices, quality assurance and inspection are parts of the strategy.

The method currently established to evaluate the quality of fresh and canned salmon is largely based on sensory evaluation. Among the sensory attributes of texture, colour, taste, and odour, the latter two play important roles in the quality assurance of fish products. Rejections of canned salmon, that may be classed as poor quality, are mostly due to the presence of off-odours indicative of spoilage.

Regulatory agencies presently rely on trained assessors to ensure a minimum level of quality for canned salmon. The procedure is considered quick; one essential criterion of a method needed to judge quality of numerous lots of canned products during busy fishing seasons. Although a very useful tool, sensory flavour tests possess some limitations. Sensory tasters are used as measuring instruments, somewhat variable over time and among themselves, and prone to bias due to physiological and psychological factors. Extensive training aimed at removing possible built-in biases and preferences, developing the necessary skills, and familiarizing the future inspector with the procedure, are required to reach uniform and consistent grading. Attention should be paid to the grading of marginal lots where the potential for misclassification could be higher. Errors in the assignment of grade and release of questionable products may initiate strong consumer complaints, result in substantial monetary loss by a private company, and lead to plant closure with

consequent loss of employment. The 1985 incident of Starkist canned tuna, where "tainted" products (oxidized but safe for consumption) were released for retail and required a costly recall, is a good reminder.

The search for chemical compounds useful to indicate spoilage has been the subject of many investigations. In the past, research has mainly focussed on (a) amines such as total volatile bases (Botta et al. 1984), trimethylamine/dimethylamine (Hebard et al., 1982), ammonia (Leblanc and Gill, 1984), and biogenic amines (Mietz and Karmas, 1978), (b) nucleotides such as hypoxanthine (Jahns et al., 1976), and the K-value (Ehira and Uchiyama, 1987), and (c) ethanol (Hollingworth et al., 1986). Although some methods perform better than others, none were found to accurately reflect the quality of all fish products. This can be attributed to the diversity with regard to composition, mode of spoilage, and processing of different products. Despite constant improvements of the chemical methods, only sensory evaluation presently meets the practical requirements, and therefore constitutes the major routine procedures.

Fish is one of our most perishable protein resources. When it spoils, unpleasant odours and flavours are released. Seafood decomposition is a complex process involving autolytic degradation, chemical oxidation, and bacterial activity. Since the decomposition process could follow any combination of pathways, measurement of several indices may be essential. Most breakdown products are smaller than their precursors, have higher vapour pressures, and are more likely to contribute to the total flavour of the product.

An instrumental method that measures a number of volatile compounds simultaneously and correlates well with sensory analysis would be highly desirable. It should also be particularly effective at the early stages of decomposition, where controversial situations are more likely to arise. Gas chromatographs are contemporary instruments extensively used for volatile analysis. A method applied to canned salmon could be developed with such an instrument. Multivariate statistics are foreseen as necessary to interpret the generated complex patterns of peaks from canned salmon of various quality and relate them to concurrent sensory assessments. Furthermore, it would be of interest to identify the various volatile compounds encountered in canned salmon and this could be accomplished by gas chromatography-mass spectrometry (GC-MS).

A. Quality of seafood products

In relation to fishery products, the term 'quality' sometimes leads to confusion as its meaning can be defined in many ways. Very often, it is synonymous with freshness, or degree of spoilage. However, species of salmon and stage of sexual maturity affect the appearance and other sensory characteristics perceived by the consumer, and affect acceptance as well as market value. There are therefore a number of factors encompassing quality in fishery products and the extent to which they affect that quality varies. The remainder of this introduction takes into consideration some background information covering the effects of various important quality factors, on odour in particular.

1. Species

Differences in the genetic material between species of salmon may be the cause of major influences on quality. For example, pink salmon (Oncorhynchus gorbusha) possesses a more delicate flesh than other species. Sockeye salmon (O. nerka) is noted for its distinctive red colour not matched by pink or chum (O. keta). Intrinsic variations in composition of protein, fat, pigment, low molecular weight compounds, etc., result in the differences seen in colour, microstructure and texture, and flavour (Wheaton and Lawson, 1985).

2. Diets

What a fish eats has a significant effect on the colour and flavour of its flesh. Fish, phytoplankton, zooplankton, invertebrates and other organisms constitute the main diet of wild salmon. The carotenoid pigments responsible for the orange coloration of salmon flesh are derived from its food and consist primarily of astaxanthin (Simpson, 1982). For aquaculture salmon, diets that are deficient in these carotenoids result in flesh lacking orange coloration (Chen et. al., 1984). High degree of pigmentation is associated with consumer acceptance and often with richer flavour (Skrede and Storebakken, 1986).

A common flavour defect described as blackberry, weedy, diesel, or sulfide flavour has been known to develop in cod and other gadoids, mackerel, and salmon (e.g., chum). This has been attributed to the presence of dimethylsulfide, or DMS (Connell, 1980). It generally occurs in fish feeding on a planktonic molluscs known as pteropods. The pteropods contain dimethyl-ß-propiothetin, which may be converted to DMS in the fish. A low concentration of this compound may not be detected in fresh fillets on ice. The odour becomes more pronounced in flesh such as salmon that is canned.

The location of the fishing ground plays an indirect role in the quality of the fishery product (Jones, 1969). Flavour can vary from one fishing ground to the next, and can also vary from one season to the next. Winds, tides, water conditions, and migratory patterns influence the

quality of a fish before harvest. These factors have repercussions on the type and abundance of food organisms available, which could affect the physiological condition of the fish.

3. Spawning

Salmon are anadromous fish that begin life in fresh water, migrate to the sea for a period of feeding and development, and return to freshwater to spawn. They do not take food during the spawning migration; feeding may cease while still in the ocean, or at the time they enter the brackish water at the mouth of the rivers (Childerhose and Trim, 1981). Their muscles contain minute amounts of carbohydrate and consist mainly of protein and fat. During the maturation process, the energy and nutrients needed for physical activity and for gonadal growth are taken from the viscera and the flesh. As the depletion of fat and protein in muscle occurs, the water content increases, probably mostly in the intercellular space (Greene, 1926; Love, 1980; Aksnes et al., 1986).

Although the fat content decreases, the fatty acid composition is not strongly affected. However, the increase in the hydroxyproline in protein indicates that the metabolism of protein from the muscle during maturation takes place at the expense of non-collagenous and cellular proteins. These occurrences have been documented by Lovern (1934), Kaneko et al. (1966), Love (1970), and Aksnes et al. (1986). After spawning, some muscle fibres have been found to be in the process of disintegration.

Striation disappeared and the myoplasm eventually presented the appearance of a structureless but swollen colloid (Greene, 1926).

The sensory attributes of the cooked product are affected by the maturation process. The first evident signs of maturation are usually the change in the skin pigmentation from silvery to various shades of brown, green, or red depending on the species. The colour of the flesh is also affected as red colours fade due to pigment mobilization.

The depletion of fat and protein correlates with the gradual disappearance of odour and flavour of the cooked product. During maturation of Atlantic salmon, the odour of cooked samples becomes less pronounced and is often classified as neutral. The original flavour gradually changes and the maturing salmon is eventually perceived as tasteless (Aksnes et al., 1986). In the case of chum salmon, the so-called "late-run odours" appear. The texture of immature fish is evaluated as filamentous and firm while mature fish have a more watery, distinctively soft and tough consistency. It is possibly explained by the higher water content and the higher relative amount of collagenous tissue (Aksnes et al., 1986). In extreme cases, the depletion of protein results in a gelatinous state which renders the flesh useless.

B. Postmortem deteriorative factors

Deterioration of fresh fish begins as soon as the animal dies. It is a complex situation for which no single factor is responsible, but, rather, is a combination of several interrelated processes. Eventually undesirable odours and off-flavours develop as well as softening of the flesh and loss of cellular fluid containing various nutrients. There are three basic modes of deterioration in fish: microbial, enzymatic, and chemical.

1. Enzymatic degradation

When captured or harvested before starting the spawning migration, salmon usually contain food in their gut as well as digestive enzymes and bacteria. Enzymes present in the flesh and stomach remain active after death of the fish, and are particularly involved in flavour changes that take place during the first few days of storage before bacterial spoilage becomes significant.

In the postmortem muscle, one of the first metabolites to appear is lactic acid. Lactic acid accumulates because of the glycolytic conversion of storage glycogen in the fish muscle after the cessation of respiration. Lactic acid build-up can cause a drop in pH, resulting in the liberation and activation of inherent acid cell-proteases, cathepsins (Connell, 1980). These proteases contribute to the weakening and softening of the flesh. Hemoglobin, particularly concentrated in the kidney, can be

released and may migrate toward the body cavity to cause red discoloration called belly-burn (Department of Fisheries and Oceans, 1989). Spoilage products released from the intestine may diffuse throughout the flesh of the fish, producing offensive odours and discoloration (Wheaton and Lawson, 1985).

In the muscle of live animals, ATP predominates among the nucleotides under normal conditions. More than 90% of the nucleotides in the muscle of fish and shellfish are accounted for by purine derivatives (Seki, 1971). From the food chemistry point of view, some nucleotide degradation products serve as important umami-producing factors. Five prime nucleotides such as inosine 5'-monophosphate (IMP) and guanosine 5'-monophosphate (GMP) show a distinct taste-enhancing effect in combination with glutamic acid. However, during the process of nucleotide degradation, ammonia is produced and may contribute to off-flavours (Gunnar, 1982).

2. Microbial spoilage

Aided by enzymatic activity, microbial spoilage is by far the main mode of spoilage of chilled fish and shellfish. Large numbers of bacteria are normally present in the surface slime, on the gills, and in the intestines of the live fish. However, bacterial spoilage does not begin until the passage of rigor mortis when cellular fluids are released from the muscle fibres.

Rate of microbial growth depends on the numbers and types of microorganisms present and the temperature at which the fish is held. The bacterial flora of fish is influenced by a number of factors such as season and environment. The flora is a reflection of the flora in the water in which the fish is caught. The surface flora may also reflect post-mortem handling and contamination on board a vessel, during unloading, or in the fish plant. While the fish are chilled, the psychrotrophic Pseudomonas species predominates, followed by Achromobacter and Flavobacterium species. At higher temperatures, the genera Micrococcus and Bacillus appear to increase in numbers (Frazier and Westhoff, 1978).

The primary substrate for bacterial growth and the main source of spoilage products is the soluble material in the muscle. Since free carbohydrate is very low in fish, Pseudomonas rapidly utilize the nonprotein nitrogen (NPN) fraction of muscle, and this is a major reason for their rapid domination of the microflora during spoilage. The major components of NPN in fish muscle are peptides, amino acids, nucleotides, trimethylamine oxide, urea, taurine, and related purine-based compounds. The free amino acids of Atlantic salmon (Salmo salar) include moderate amounts of taurine, glutamic acid, glycine, and alanine (Cowey et al., 1962). According to Krzynowek and Murphy (1987), all species of Pacific salmon have a similar amino acid composition and contain relatively high amounts of leucine (1.6g/100g), lysine (1.8g/100g), aspartic acid (2.0g/100g), and glutamic acid (3.0g/100g). With their decarboxylation capacity, bacteria can convert some of these amino acids to volatile bases

such as the diamines, cadaverine and putrescine, as well as histamine. Histamine, a very potent capillary dilator in man, is the major cause of scombroid food poisoning (Eitenmiller et al., 1982).

In some species of the gadoid family, trimethylamine oxide (TMAO) is found in relatively high amounts. The odourless compounds TMAO and lactic acid can be metabolized by bacterial action to yield trimethylamine (TMA) and acetic acid (Hebard et al., 1982). TMA is characterized by an ammoniacal odour, but in combination with other compounds, such as fat, may give a "fishy" odour. Also, ammonia is ultimately produced from the breakdown of these nitrogen-containing compounds. Some proteolysis seems to occur in the early stages of spoilage, but there is evidence that protease production by bacteria is initially repressed. This appears to be due to the presence of free amino acids at a high level. Proteolysis becomes more vigorous in the later stages of spoilage as the amino acids are utilized (Chung, 1968). Various products can be formed directly by enzymatic deamination of amino acids. The end-products are categorized as alcohols, ketones, fatty acids, aldehydes, sulfides, thiols, mercaptans, etc. These products, indicative of putrefaction, increase in concentration as the fish become unacceptable. A correlation has been made between bacterial counts and the occurrence of volatile sulfur compound producing bacteria. When the sulfide producers reach 40% of the population (often corresponding to a bacterial count exceeding $10^6/cm^2$), overt spoilage occurs (Liston, 1982).

Microorganisms may also initiate the hydrolysis of triacylglycerols. The resulting free fatty acids are particularly susceptible to oxidation which proceeds via hydroperoxides. Most microorganisms can degrade peroxides and the formation of secondary fat oxidation products by microorganisms is very complex (Alford et al., 1971). This leads to numerous stable end products, such as saturated and unsaturated aldehydes, ketones, dicarbonyl compounds, alcohols, alkanes, alkenes, and methyl furans (Grosch, 1984). The carbonyl compounds are odorous even in trace concentrations and cause the rancid aroma of oxidized fat.

3. Chemical spoilage

As with many other seafoods, monounsaturated and polyunsaturated fatty acids are more abundant in Pacific salmon than saturated fatty acids (Hearn et al., 1987). Among the wide array of fatty acids, palmitic (16:0), oleic (18:1n9), and docosahexaenoic (22:6n3) acids dominate in fresh wild Pacific salmon and remain dominant after cooking or canning (Ackman and McLeod, 1988; Barber et al., 1987; Barber et al., 1988; Braddock and Dugan, 1972). Fish normally have a much higher degree of lipid unsaturation than most other foods and, therefore, are particularly prone to oxidative deterioration and rancidity. This is the case for salmon, mackerel, tuna, and herring.

Microbial enzymes may be involved in the oxidation of fats and oils, but autoxidation is more common during frozen storage. Freezing temperature, oxygen concentration, relative humidity, and catalyst

concentration are important factors in lipid oxidation (Powrie, 1984). These factors determine the rate at which oxygen reaches the muscle surface and ultimately reacts with the lipids. However, endogenous enzyme systems, metallo- and hemoproteins, and metal ions in the tissue play a role in oxidation by acting as pro-oxidants. Hardy et al. (1979) found that lipolysis was responsible for the major changes in the lipid components during the frozen storage of cod. Oxidation was slow and occured primarily in the phospholipid fraction. In the isolated microsomal membrane fraction from fish skeletal muscle, enzymic oxidation of lipid components occured at temperatures as low as -20°C (Apgar and Hultin, 1982). Lipases and phospholipases release free fatty acids from the lipids and these free fatty acids may then undergo oxidation producing lower molecular weight compounds. Pretreatment with microwave heating is another factor found to reduce the storage life of fish by catalyzing the development of oxidative rancidity (Ke et al., 1978). Lipid oxidation results principally in undesirable flavours often described as "painty". In addition, there may be some effects on the nutritive components of the fish tissue due to degradation of oxidizable nutrients such as essential fatty acids, some amino acids, and some vitamins. Oxidation of heme pigments can yield discoloration, and texture changes may occur due to protein cross-linking (Nawar, 1985).

C. Formation of flavour volatiles from muscle food

Fresh raw meat and fish have little odour although it is usually possible to distinguish between animal species by sniffing. Recent investigations using various chromatographic and biochemical techniques have identified volatile components of several fish species. Raw freshwater and marine fish possess mild flavours that result from the conversions of polyunsaturated fatty acids by lipoxygenases (Josephson et al., 1984a). Josephson (1987) found that, during each of the stages of life cycle, Pacific salmon (Oncorhynchus spp.) possessed 8-carbon alcohols and carbonyls which contributed distinct plant-like aromas to the fish. Spawning condition salmon in freshwater environments additionally possessed a group of 9-carbon alcohols and carbonyls that added cucumber- or melon-like aroma notes. During storage, the initial changes in the aroma of freshly caught fish usually involve a shift from these fresh, planty aroma notes to a neutral, or flat-sweet odour and eventually detectable amounts of malodorous spoilage compounds are produced.

In general, meat and fish must be cooked in some fashion in order to develop desirable odour and flavour. During cooking or canning of salmon, flavour compounds are formed which replace the fresh fish compounds, to yield the characteristic flavour of cooked salmon. Although little information has been published about the flavours of cooked seafoods, volatile carbonyls have been suggested as significant contributors to cooked fish flavours (Pokorny, 1980). In general, meat and fish aroma is not the result of one constituent but the sum of the

sensory effects of a multitude of volatiles arising from the thermal process. However, certain groups of compounds appear to be important contributors to meat flavours. Chang and Peterson (1977) suggested that lactones, furanoid compounds, acyclic sulfur-containing compounds and heterocyclic compounds containing S, N, and O may have a large flavour impact. It is recognized that the low molecular weight fraction arising from the degradation of sugars, proteins, and lipids are responsible for the development of meat and fish flavours upon heating. The various reactions occurring in the process depend on the type and concentration of the non-volatile precursors, the heating temperature, the pH of the medium, and the water activity of the meat.

II. STATIC HEADSPACE GAS CHROMATOGRAPHIC METHOD TO ANALYZE VOLATILES IN CANNED SALMON

A. Introduction

Food volatiles, usually found in small amounts, constitute an integral part of the flavours perceived by consumers. Gas chromatography (GC) is a widely employed analytical technique to analyze volatile compounds, and a number of methods have been developed for sample preparation. One way of analyzing the volatile constituents is by direct injection of the sample itself or of the sample headspace gases. More often, food volatiles must be isolated and concentrated in some manner before GC analysis. Samples can be subjected to extraction, adsorption, distillation, cryogenic procedures, or a combination of the above. A description of these methods as well as their advantages and disadvantages were recently reviewed by Heath and Reineccius (1986) and Parliment (1986).

Equilibrium or static headspace analysis (SHA) involves the chromatographic separation of a predetermined volume of vapour headspace above a sample held in a closed vial. Volatiles of many food commodities including macadamia nuts (Crain and Tang, 1975), cooked Brassicaceous vegetables (Maruyama, 1970), citrus juice (Davis and Chace, 1969), dehydrated potatoes (Sapers et al., 1970), beer (Hoff and Herwig, 1976), peanuts (Young and Hovis, 1990), wine (Noble et al., 1980), and spices (Pesek et al., 1985) have been analyzed by this technique. This method has also been applied to the determination of dimethyl sulphide in cod

(Sipos and Ackman, 1964), amines in hake, sole, cod, rockfish, perch and lingcod (Miller et al., 1972a), volatile halocarbons in eel, carp, striped bass, and spot fish (Entz and Hollifield, 1982), and ethanol in canned salmon (Hollingworth et al., 1986).

SHA has potential to be used for quality control purposes due to its simple and rapid operation. However, detection is limited to abundant compounds of low boiling point. One way to improve sensitivity is to raise the temperature of the sample. Furthermore, the injection procedure can be automated by a headspace sampler where the amount of headspace volume taken is constant, eliminating errors associated with manual handling. The main objective of this research was to develop a method based on static headspace gas chromatography (SHGC) that would simultaneously measure a large number of volatile compounds from canned salmon in a short period of time. More specifically, this investigation was aimed at: 1) studying some sample preparation and incubation related-factors which influenced chromatographic sensitivity; 2) optimizing factors involved in initial sample introduction and separation, and 3) identifying the headspace volatiles separated by the SHGC method.

B. Materials and methods

1. Collection and preparation of samples

Ocean caught pink salmon (Oncorhynchus gorbuscha) were collected from the fish pumped out of commercial boats at a local processing plant. After washing and butchering the fish, approx. 215g salmon steaks and 2g sodium chloride were put in 307 x 115 two-piece cans. The cans were then vacuum-sealed (10 in Hg), processed in a ten busse horizontal batch still steam retort, and cooled with chlorinated water. The installation and practice of the commercial plant followed established Canadian fisheries regulations ensuring a minimum lethality (F_o) of 5.6 min. Cans were treated as independent observations from a random sampling.

Before GC analysis, each can was opened and the liquor was drained for approx. 2 min. The "white" muscles of pink salmon were gently flaked (3-5 mm in size) with a spatula carefully avoiding dark meat, skin, and bones. The selected amount of fish flakes was transferred to 20 mL headspace vials (Hewlett Packard, Avondale, PA). Aluminum caps and teflon-faced silicone septums were then crimped on the vials. When all the factors described above were optimized, a 1.0 mL solution of 3-hexanol (Aldrich Chemical Co., Milwaukee, WI) at a concentration of 84.8 mg/L, in deionized distilled water, was added to the salmon flakes just before sealing and served as a reference compound.

2. Static headspace gas chromatography (SHGC)

The transfer line of an HP 19395A headspace sampler (Hewlett Packard, Avondale, PA) was attached to the injector port of a HP 5890 gas chromatograph equipped with a flame ionization detector (FID). Chromatograms were recorded with an HP 3396A integrator. Volatile separation was accomplished with an ULTRA 2 (cross-linked 5% phenyl methyl silicone) fused silica capillary column (0.52μm film thickness x 0.32mm i.d. x 25m length, Hewlett Packard Co., Avondale, PA). The carrier gas was helium.

Some working conditions that remained constant for all runs were as follows: injector temperature, 240°C; detector temperature, 250°C; septum purge vent, 2.5 mL/min. The gas flowrates of the FID detector were 33 mL/min for hydrogen, 375 mL/min for air, and 30 mL/min for make-up helium. The GC oven was programmed to spend 5 min at the initial temperature and to reach 175°C at a rate of 10°C/min. The temperature of the valve, the 3 mL loop, and the transfer line of the headspace sampler was set 5°C above the incubation bath temperature. Other SHA conditions were: vent/fill loop time, 2 s; injection time, 30 s.

3. Examination of the GC factors

The following list presents the factors and the ranges at which they were studied: temperature of vial incubation (TPI), 35 - 125°C; time of vial incubation (TMI), 0.25 - 5.5 h; amount of meat in vial (AMV), 2.5 -

12.5 g; initial oven temperature (IOT), 35 - 100°C; column headpressure (CHP), 30 - 120 kPa; total helium flowrate (TFR), 2 - 200 mL/min.

The first three factors were investigated independently while the last three were simultaneously optimized using the random-centroid optimization program (RCO) written by Nakai (1989). The flowchart of the optimization program is shown in Figure 1. It initially consisted of cycles of eight to ten randomly selected experiments for 3-5 factors and a subsequent centroid search with two to three experiments. A mapping subroutine (Nakai et al., 1984) was then used to visualize the response surface and guide the reassignment of narrower search ranges. When the approximate optimum areas were localized, the cycle was not repeated. Instead, a simultaneous shift procedure with up to five experiments was carried out to complete the optimization process. The objective function used was based on Kaiser's concept of peak separation (Kaiser, 1960b) summed over all adjacent pairs of peaks. Symmetry of peaks were assumed, therefore facilitating the estimation of resolution. Peaks with area counts below 100 were not considered.

4. Gas chromatography-mass spectrometry (GC-MS)

Tentative volatile identification was performed by connecting the headspace sampler to a Hewlett Packard 5985B GC-MS system. The ULTRA 2 capillary column was directly interfaced to the ion source. The oven temperature was programmed for 20°C for 5 min and then ramped to 175°C at 10°C/min. The mass spectrometer was operated in the electron impact mode

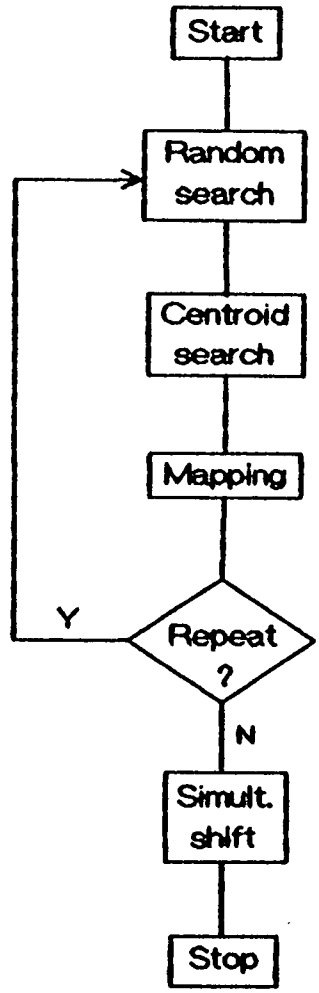

Figure 1. Flowchart of the random centroid optimization program.

with the following conditions: ion source temperature, 200°C; ionizing energy, 70 eV; scan range, 34-350 amu at 1 A/D measurement. The mass spectra were acquired with the data system (Rev G) and subsequent data reduction identified the peaks with base peak probability matching using the library EPA-NIH Mass Spectral Database (Eight Peak Index Mass Spectra). Confirmation of several compounds was accomplished by comparing their retention times (within 1.5 s range) with those of reference standards (Aldrich Chem. Co., Milwaukee, WI).

C. Results and discussion

Figure 2 shows the effect of flaked fish weight in vials on the total area of the chromatograms after 1 h incubation at 75°C. The total area increased in a slightly curvilinear fashion with the amount of meat between 2.5 g and 10 g. The means of total area (n_i = 5) from this line were linearly related to their standard deviations giving a constant coefficient of variation of 8.4% (range 8.1-8.9). This relationship reflects increased variability in flake compactness (or flake density) with fish weight in vials, which in turn, translated into increased variability in flake surfaces exposed to the headspace and therefore in internal pressure. A further increment from 10 g to 12.5 g did not increase the total area to a major extent while the coefficient of variation was near 10%.

The influence of incubation temperature on the chromatographic integrated area is illustrated in Figure 3. As the vials were incubated at higher temperatures, both overall peak intensities and number of peaks augmented and resulted in a quadratic increase in total area. When samples were subjected to incubation temperatures above 110°C for more than 1 h, septums tended to bulge due to internal pressure and leaks were encountered. Figure 4 shows that the total chromatographic area initially followed a linear relationship with incubation time (fish weight, 10 g; incubation temp., 75°C). After a period of 3 1/2 - 4 h, the total area reached a plateau indicating that the equilibrium state between the liquid coating on the fish flakes and the headspace was established. In simple

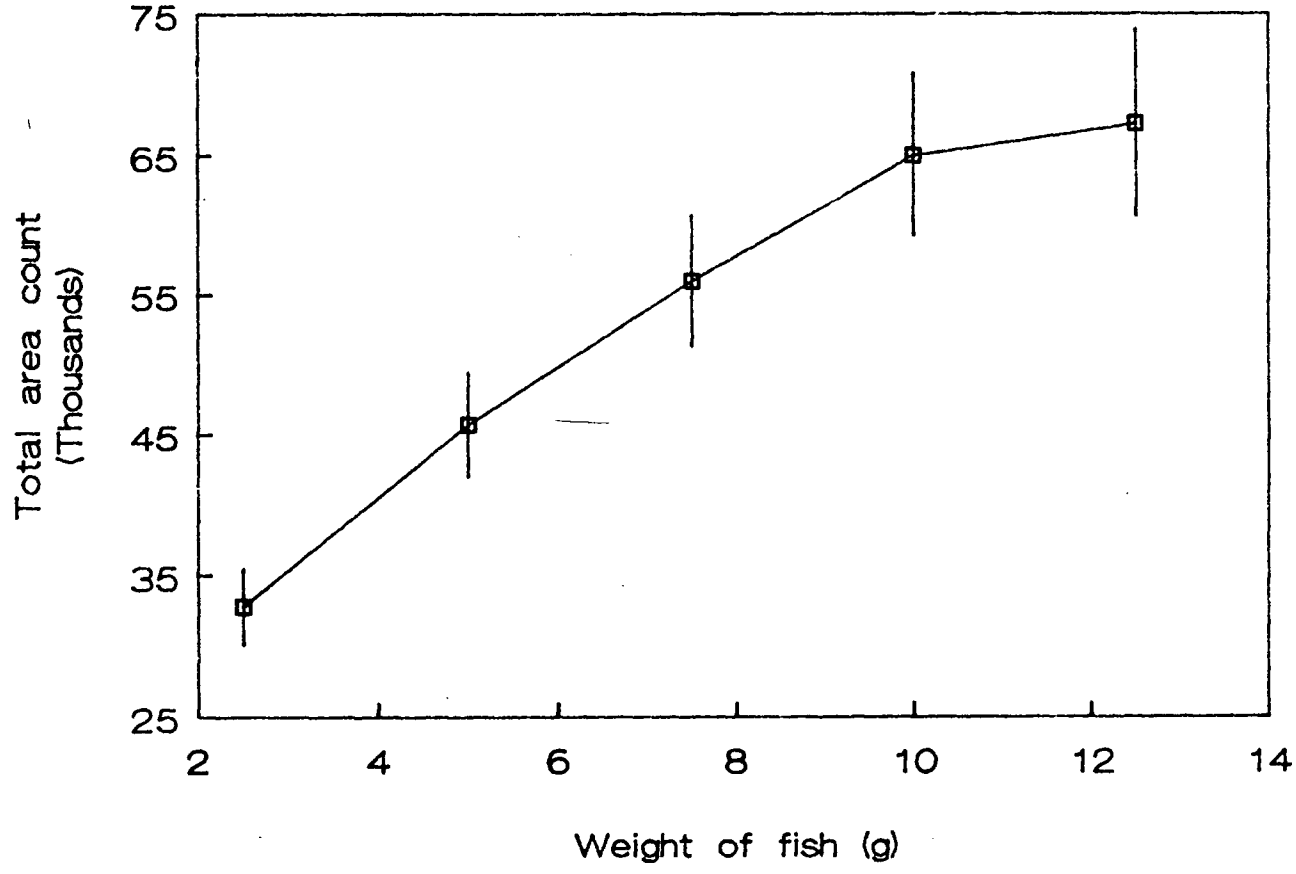

Figure 2. Effect of fish weight on total chromatographic area (temperature of vial incubation, 75°C; time of vial incubation, 1 h; column headpressure, 60 kPa; split ratio, 50:1).

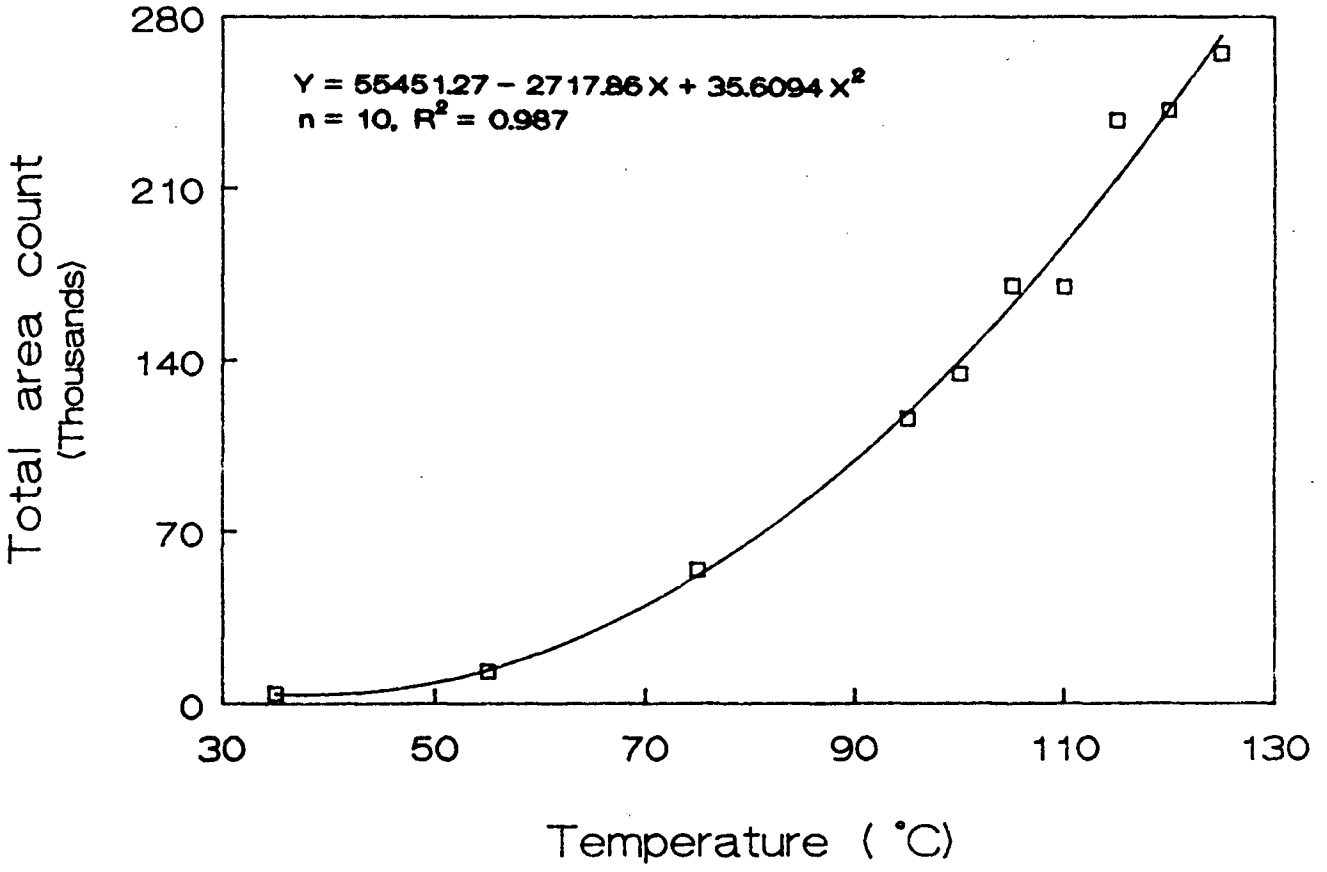

Figure 3. Effect of incubation temperature on total chromatographic area (time of vial incubation, 1 h; fish weight, 10g; column head-pressure, 60 kPa; split ratio, 50:1).

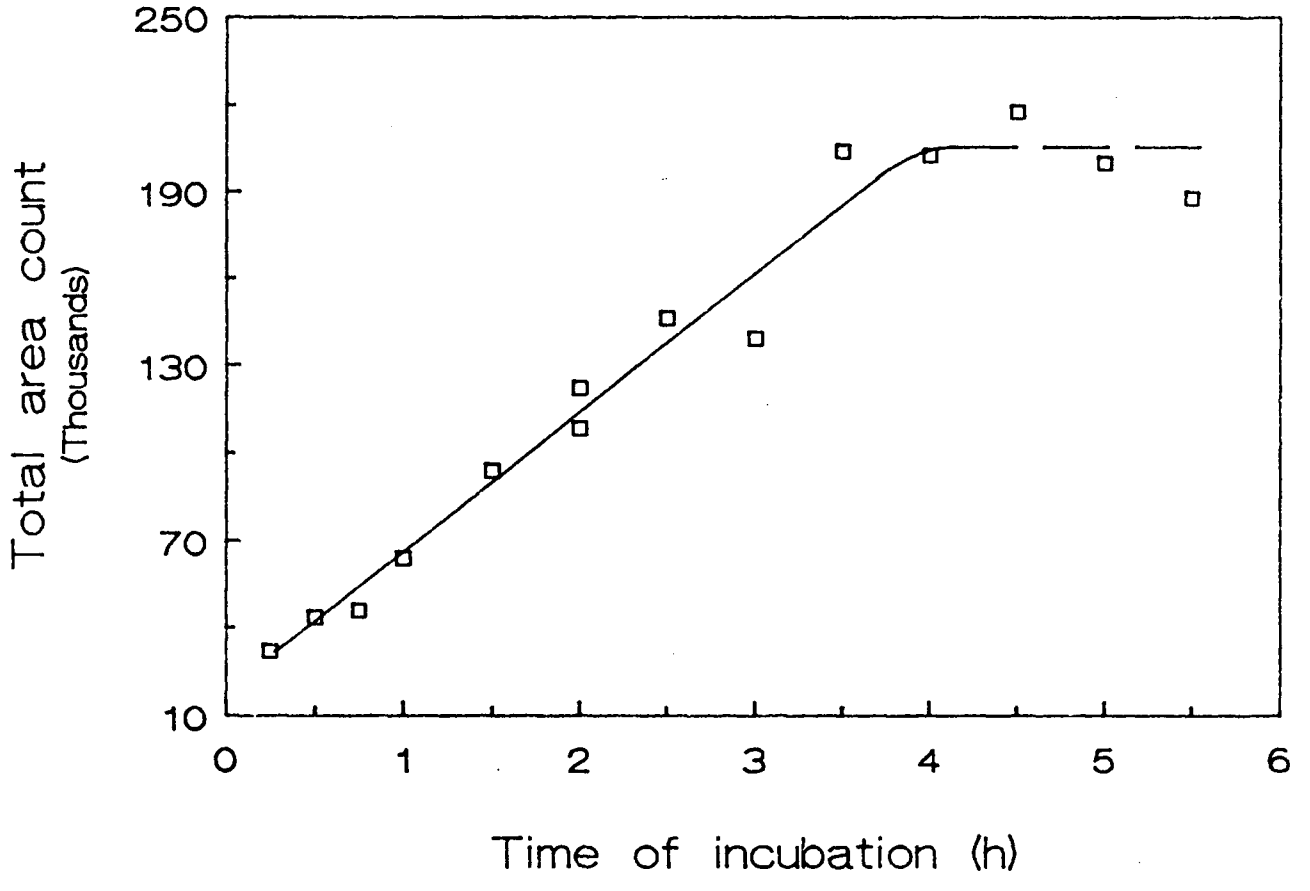

Figure 4. Effect of incubation time on total chromatographic area (temperature of vial incubation, 75°C; fish weight, 10g; column headpressure, 60 kPa; split ratio, 50:1).

situations such as dilute aqueous solutions of organic volatiles at constant temperature (25°C) and pressure (760 mm), equilibrium between gas and liquid phases has usually been attained within 30 min (Buttery et al., 1971). However, the factors controlling the equilibrium in food systems (e.g., salmon flesh) that contain a large number of embodied aqueous and organic constituents are very complex. These factors, in addition to high incubation temperature of salmon flakes which increased the amount of high boiling point volatiles in the headspace, seemed to increase the equilibrium time to 4 h. A period of 1 h was however considered more practical and increased sensitivity to a sufficient extent. This assessment was based on the original incubation temperature of 75 °C and was assumed to hold for the optimized temperature of 105°C.

Results from RCO on the initial oven temperature (IOT), column headpressure (CHP), and total flowrate (TFR) are shown in Figures 5, 6, and 7. After repeating the sequence of random simplex and centroid search followed by adjusting the search areas using the mapping procedure, the simultaneous shift process was carried out for final convergence. The lines drawn in these figures were derived from fitted quadratic equations to provide guidance toward localization of the optima. Among the 25 vertices explored, vertex 24 gave the best separation response (88.0) which corresponded to 35°C, 95.5 kPa, and 44 mL/min for IOT, CHP, and TRF respectively. The lowest IOT (35°C) was necessary since many low-boiling point compounds needed to be separated. The split ratio 11:1 was calculated from the optimal GC conditions and constituted the best compromise between the sensitivity required for detection of high-boiling

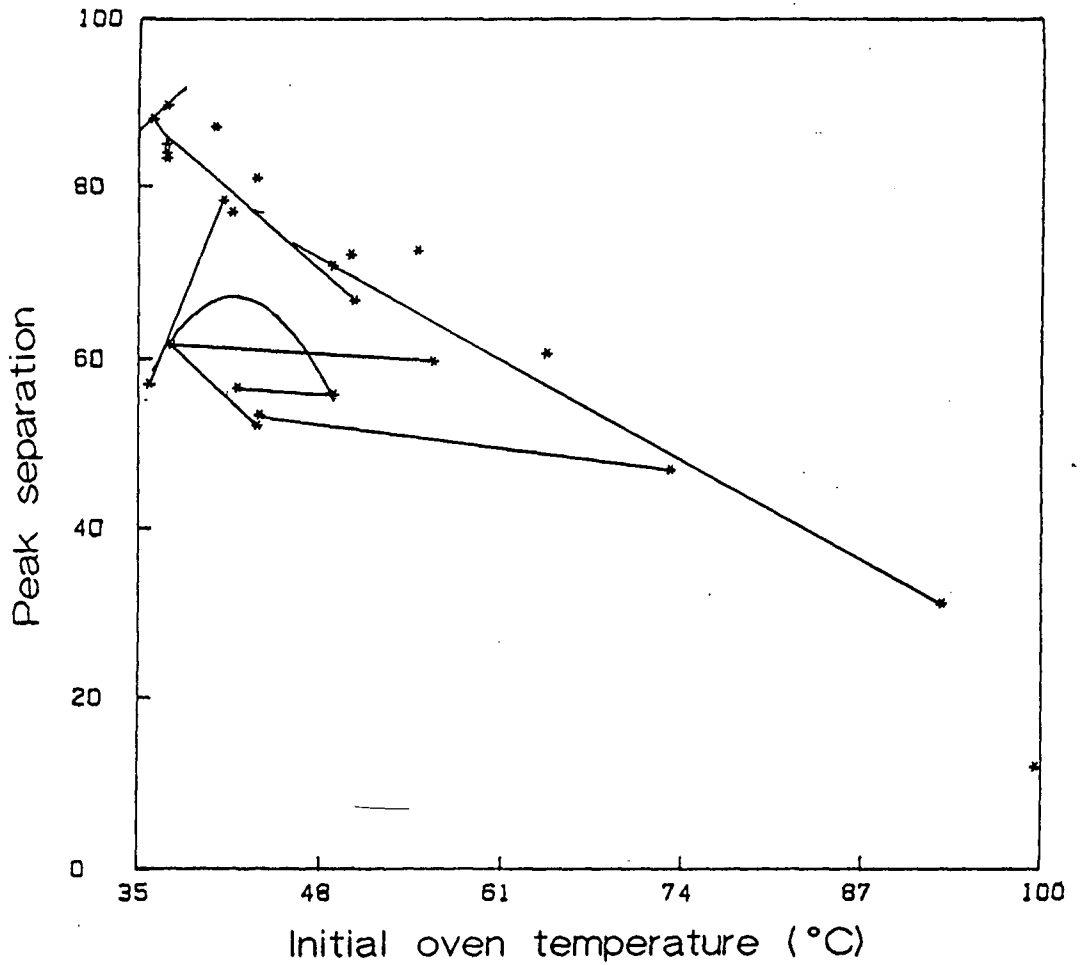

Figure 5. Optimization mapping results of peak separation as a function of initial oven temperature (temperature of vial incubation, 105°C; time of vial incubation, 1 h; fish weight, 10g).

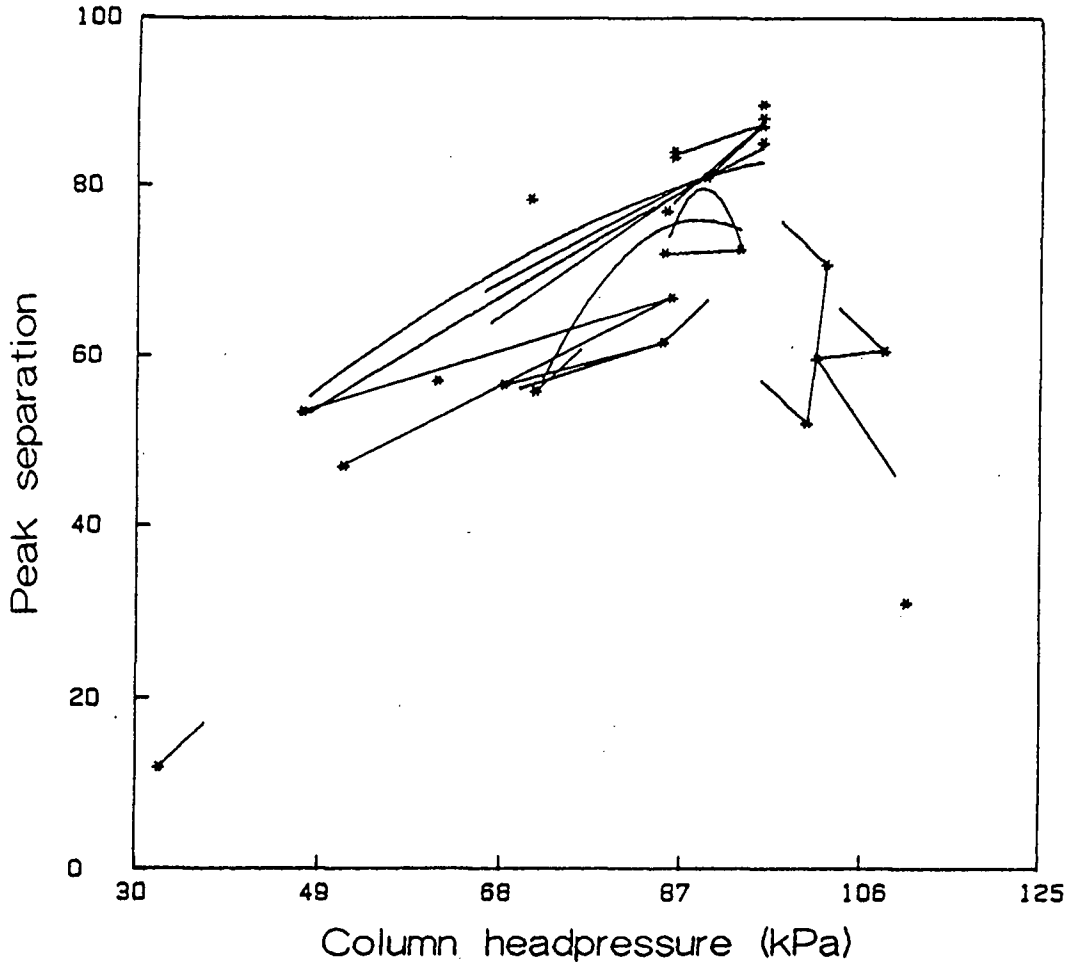

Figure 6. Optimization mapping results of peak separation as a function of column headpressure (temperature of vial incubation, 105°C; time of vial incubation, 1 h; fish weight, 10g).

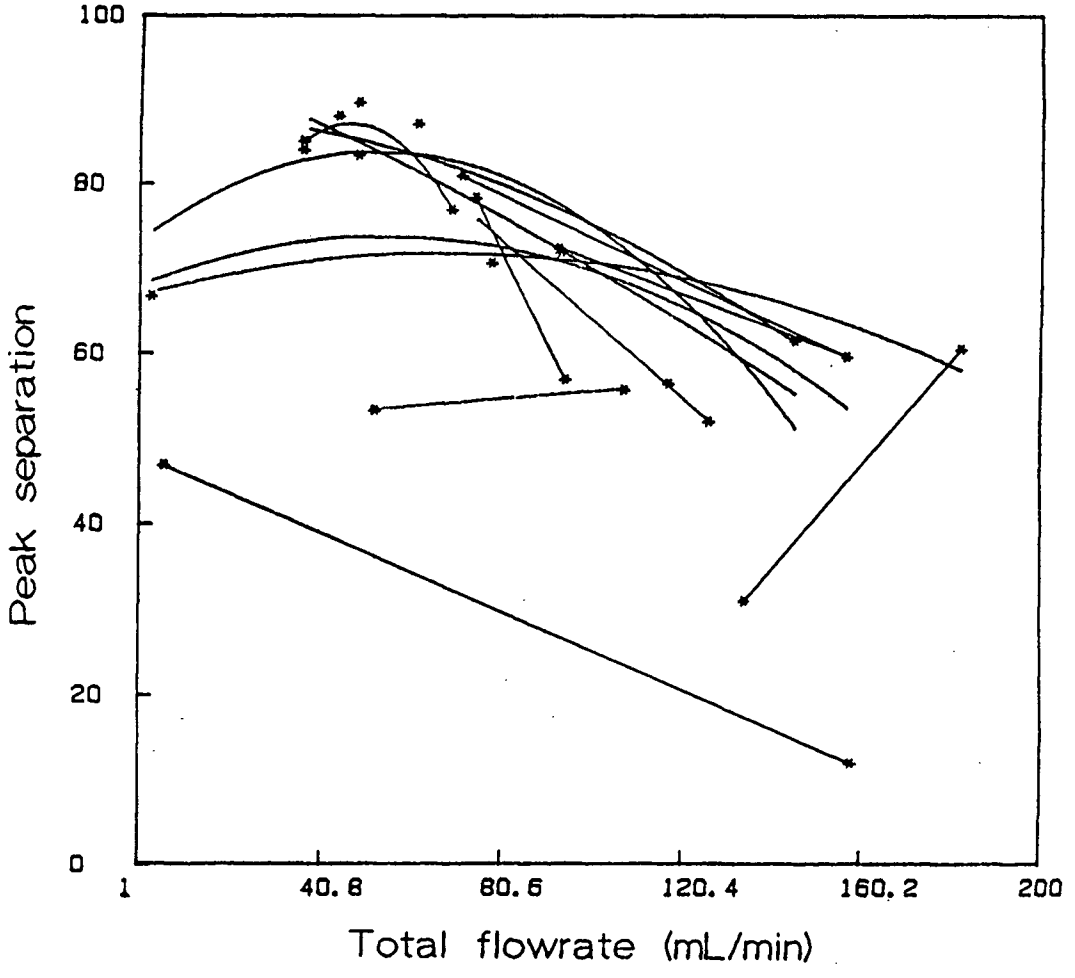

Figure 7. Optimization mapping results of peak separation as a function of total flowrate (tempearture of vial incubation, 105°C; time of vial incubation, 1 h; fish weight, 10g).

point components and resolution of early eluting volatiles.

Figure 8 is a typical chromatogram obtained under optimized conditions for 10 g fish and 1 h incubation at 105°C. Eighty peaks were detected with this method, using a capillary column and a flame ionization detector. For sake of comparison, a direct injection of a headspace volume drawn from a vial containing a liquor sample of canned salmon and equilibrated at room temperature would allow the detection of approx. 6 peaks on a packed column (Hollingworth and Throm, 1983). In contrast, the SHGC method considerably enlarged the spectrum of detectable volatile compounds. It was probable that some of the detected volatiles were a result of thermal and oxidative degradation due to incubation, even though canned salmon is a product which has undergone a sterilizing heat treatment. The ideal situation would be to perform the optimization on the integrator response for known compounds which respond only to the tested parameters. In applications where the food volatiles of interest were thermally labile, other means which could overcome this difficulty (e.g., salting out effect) should be used in the optimization. However, the usefulness of the SHGC method could certainly be extended to the analysis of volatiles from various thermally processed food products. A distinct advantage was that insoluble and cellular materials such as meat could be analyzed with minimum sample preparation and contamination. Another advantage concerns the high sample throughput. Apart from incubation time of vials, each sample analysis required approx. 30 min, offering the possibility of analyzing 13 to 15 samples a day.

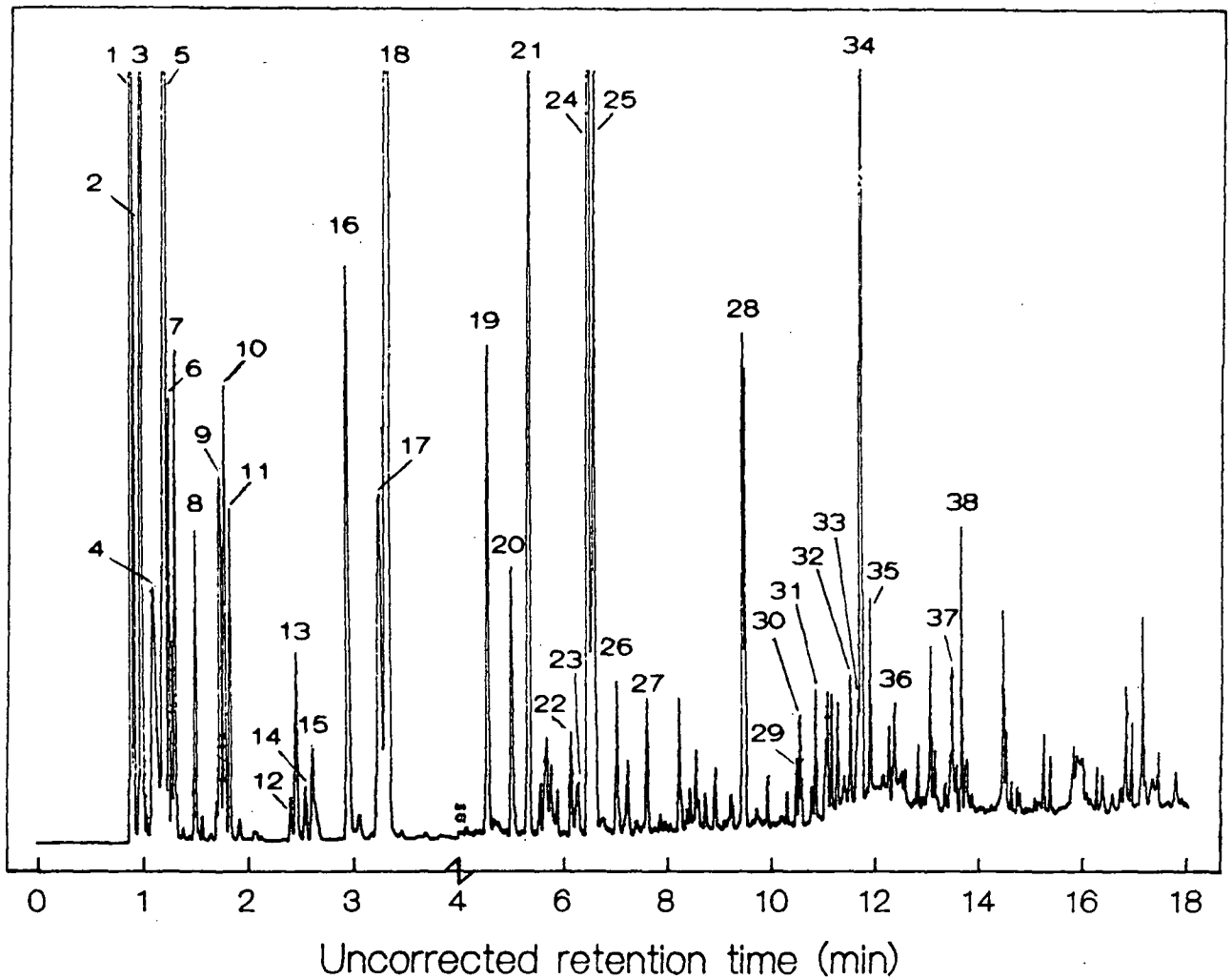

Figure 8. Chromatogram of volatiles from canned pink salmon (temperature of vial incubation, 105°C; time of vial incubation, 1 h; fish weight, 10g; initial oven temperature, 35°C; column headpressure, 95 kPa; total flowrate, 44 mL/min).

Table 1 lists the name of the compounds associated with the peak number found on the chromatogram of Fig. 8, the corresponding uncorrected retention time and percent peak area of a typical run, and the method used to identify the volatiles. Tentative identification was achieved by comparing mass spectrometry data with that of EPA/NIH library. Retention time, used as a first step confirmation, was checked with that of authentic standards. Compounds of various classes were found: eight aldehydes; six alkanes; two alkenes; five aromatic compounds; two ketones; three sulfur-containing compounds; one alcohol; one acid, and six miscellaneous compounds. Volatiles that produced large detector responses were hydrogen sulfide, acetaldehyde, methanethiol, butane, thiobis-methane, butanal, 1-penten-3-ol, heptane, 1,5-dimethyl-cyclopentene, methyl-benzene, 3-ethyl-2-methylpentane, and 4-ethyl-benzenemethanol. In addition, Table 1 compiles several references where more than half of the volatiles identified in this study were also found in other seafood products. Ethanol was one of the compound cross-referenced and has been used in the past as an indicator of decomposition in canned salmon (Hollingworth et al., 1986). In theory, the SHGC method could be used for the determination of ethanol. The origin of the remaining headspace volatiles as well as their contribution to perceived flavour is a speculative matter at this point and should be the object of further studies.

Table 1. Identification of volatile compounds detected in canned pink salmon.

Peak no.	Compound name	Uncorrected retention time (min)	Peak area (%)	ID[a]	Reference[b]
1	hydrogen sulfide	0.905	29.013	MS	3,6,10
2	acetaldehyde	0.935	2.817	MS,RT	5,6,10
3	methanethiol	1.003	15.003	MS	3,6
4	ethanol	1.135	0.962	MS,RT	2,4,5,6,10
5	butane	1.226	12.468	MS	
6	3-methyl-1-butene	1.292	0.985	MS,RT	
7	thiobis-methane	1.330	1.116	MS,RT	3,6,8,9,10
8	2-methyl-propanal	1.530	0.692	MS,RT	
9	hexane	1.773	0.779	MS,RT	
10	butanal	1.815	1.535	MS,RT	5,10
11	2-methyl-furan	1.869	0.707	MS	
12	3-methyl-butanal	2.453	0.175	MS	5,6,8,9
13	benzene	2.501	0.622	MS,RT	1,7,10
14	2-methyl-butanal	2.590	0.287	MS,RT	
15	2,2-dimethyl-propanal	2.656	0.543	MS	
16	1-penten-3-ol	3.000	1.952	MS,RT	4,5,8
17	heptane	3.305	1.330	MS,RT	6
18	1,5-dimethyl-cyclopentene	3.373	11.223	MS	
19	unknown 1	4.611	0.470	-	
20	acetic acid	5.104	0.340	MS,RT	6
21	methyl-benzene	5.414	1.881	MS,RT	1,6,7,8,10
22	3-hexanone	6.205	0.200	MS,RT	8
23	unknown 2	6.322	0.032	-	8
24	3-hexanol (standard)	6.522	5.372	-	
25	3-ethyl-2-methyl-pentane	6.653	1.528	MS	
26	ethylidene-cyclohexane	7.093	0.157	MS	
27	unknown 3	7.663	0.125	-	
28	nonane	9.475	0.373	MS,RT	9
29	1-propenyl cyclohexane	10.572	0.021	MS	
30	benzaldehyde	10.614	0.125	MS,RT	5,6,8,9
31	7-octen-4-ol	10.875	0.242	MS	
32	2-pentyl-furan	11.550	0.247	MS	8
33	decane	11.673	0.013	MS,RT	9
34	4-ethyl-benzenemethanol	11.750	1.869	MS	
35	octanal	11.944	0.257	MS,RT	
36	3-ethyl-1,4-hexadiene	12.295	0.105	MS	
37	2-nonanone	13.097	0.162	MS,RT	5,6,8,9
38	nonanal	13.700	0.270	MS,RT	5,6,8,9

[a] MS, tentatively identified by mass spectrometry; RT, retention time consistent with that of authentic compounds.

[b] 1, Easley et al. (1981); 2, Hollingworth et al. (1986); 3, Hughes (1964); 4, Human and Khayat (1981); 5, Kubota et al. (1982); 6, McGill et al. (1977); 7, Reinert et al. (1983); 8, Tanchotikul and Hsieh (1989); 9, Vejaphan et al. (1988); 10, Wong et al. (1967).

III. CANNED SALMON QUALITY CLASSIFICATION BY MULTIVARIATE ANALYSIS OF GAS CHROMATOGRAPHIC DATA

A. Introduction

Modern analytical techniques provide the potential to obtain large amounts of information from every experimental unit. On one hand, the data may originate from different methods measuring various parameters of interest. On the other hand, multiparameter methods, such as gas-liquid chromatography and sensory evaluation, can generate multidimensional bodies of data in a single analysis. Given the large number of factors contributing to the changes in the food properties being studied, multivariate analysis (MVA) constitutes a powerful tool to assist in interpretation of complex datasets.

MVA includes several procedures, all designed to describe the relationships that exist in a multivariate dataset, by isolating and identifying redundancies. These procedures offer advantages over univariate statistical methods in being able to deal with many variables simultaneously and thereby uncover relationships that could not be observed when examining the various random factors one by one. Although these multivariate techniques are similar in many ways, there are important differences with regard to the questions they are designed to address and the interpretation of their results.

Multivariate techniques can be divided in two groups, dependence and interdependence methods (Dillon and Goldstein, 1984). The first type

relates to the techniques dealing with the association between a set of criterion (predictant) variables and a set of predictor variables, i.e., linear discriminant analysis and canonical correlation analysis. The second type is less predictive in nature and centres on the mutual association across a set of given variables, i.e., principal component analysis, factor analysis, cluster analysis, and multidimensional scaling.

Principal component analysis (PCA) derives linear combinations (principal components) of the original variables, e.g.,

$$Z = WX \qquad (1)$$

where Z is a matrix of PC, X a matrix of original variables, and W a matrix of weights which have been computed to maximize the ratio of the variance of PC to the total variation. The successive linear combinations are extracted to produce uncorrelated principal components (PC) that consecutively account for smaller amounts of the total variance. The complexity of the data can be reduced by representing the numerous variables in a few selected components which comprise a large proportion of the original variance. Based on the uniqueness and exact solution of PCA, scores of selected PC can be used in later analyses in place of the original responses.

The interpretation of sensory characteristics has been facilitated by using PCA in the study of wine (Heyman and Noble, 1989), beer (Clapperton and Piggott, 1979), fish (Quarmby and Ratkowshy, 1988), frozen peas (Sanford et al., 1988), and selected food items (Syarief et al., 1985). PCA was applied to data obtained from a variety of physical,

chemical, biochemical, and other instrumental methods for analysis of rum (Herranz et al., 1990), durum wheat (Autran et al., 1986), whisky (Headley and Hardy, 1989), orange juice (Aries et al., 1986), cheese (Kwak et al., 1989), and wine (Moret et al., 1986).

The common factor-analytic model (CFA), or less precisely factor analysis, expresses the original variables in terms of newly generated common factors and a unique factor, e.g.,

$$X = WF + E \quad (2)$$

where X is a matrix of original variables, W a matrix of weights associating the common factors to the original variables, F a matrix of latent common factors, and E a matrix of unique factors. Its purpose consists of finding the part of the total variance that a particular variable shares with the other variables. In other words, CFA attempts to explain the correlations or covariances among a set of variables in terms of a limited number of unobservable, latent variables. Factor scores, which give the coordinates of each observation in the multidimensional space of the common factors, cannot be computed directly. They must instead be estimated. Because of this indeterminacy problem, the non-uniqueness of factor scores sometimes arouse criticisms of their use for subsequent analyses.

CFA has been used to determine relationships among instrumental methods and sensory qualities of cheese (Vangtal and Hammond, 1986), green beans (Powers et al., 1977), peanut beverage (Rubico et al., 1988), dry beans (Hosfield et al., 1984), and tomatoes (Resurreccion and Shewfelt,

1985). It was also applied in the study of frozen cod (Leblanc et al., 1988), cooked beef (Galt and MacLeod, 1983), and chicken patties (Lyon, 1988).

Rotation is a post hoc step which could be performed on both PCA and CFA solutions in order to improve the interpretability of the results. Two types of procedures for rotating axes are encountered, orthogonal and oblique. Orthogonal rotations restrict the factors to be mutually independent (uncorrelated) by preserving their perpendicular orientation, and oblique rotations allow the resultant factors to adopt various angles and therefore be correlated. Rotating a set of factors does not change the statistical explanatory power of the factors. However, a consequence of correlated factors as a result of using oblique rotation is that no single unambiguous measure of the importance of a factor can explain a variable.

Linear discriminant analysis (LDA) is a multivariate approach which handles the case of a qualitative, or categorical dependent variable and a set of independent variables. This technique aims at correctly classifying observations into mutually exclusive groups known a priori. Original independent variables are linearly assembled to form discriminant functions with the objective of minimizing the misclassification rate. This is accomplished under the specification that the between-group variance of the weighted predictor variable combinations relative to the within-group variance is maximal. LDA relies upon certain conditional assumptions. In particular, the independent variables must have

multivariate normal distributions and their variance-covariance matrix in each group should be considered homogeneous.

Discriminant analysis (DA) of GC volatile patterns have often been used to segregate different groups within various food commodities including wine (Montedero and Bertuccioli, 1983; Marais et al., 1982; Moret et al., 1986), beer (Helbert and Hoff, 1974; Hoff et al., 1975; Lindsay, 1977), milk (Leland et al., 1987; Smeyers-Verbeke et al., 1977), coffee and potato chips (Powers and Keith, 1968), cola beverage (Young et al., 1970), grape brandy (Schreier and Reiner, 1979), soy sauce (Aishima, 1979a; Aishima, 1979b; Aishima, 1983), cheese (Aishima and Nakai, 1987), grapefruit juice (Pino et al., 1986), and corn (Dravnieks et al., 1973; Dravnieks and Watson, 1973). Interpretation of data from other instrumental methods have also benefitted from the use of DA, e.g., rheological characteristics of cheese (Amantea et al., 1986), heavy metal characteristics of cereals (Stryjewska et al., 1987), high pressure liquid chromatography of cheese extract (Pham and Nakai, 1984; Mohler-Smith and Nakai, 1990), milk clotting activity of proteolytic enzymes (Aishima et al., 1986), meat protein functionality (Li-Chan et al., 1987), and pyrolysis mass spectrometry of orange juice (Aries et al., 1986). Discriminative relationships among sensory and instrumental data have been examined in canned blueberries (Powers et al., 1978), butter (Woo and Lindsay, 1983), and beer (Brown and Clapperton, 1978). The discriminative textural characterization of various physical properties was demonstrated in cooked fish by Hatae et al. (1984). Similarly, sensory properties of 18 common Atlantic species of fish were assessed for their usefulness in

species discrimination (Sawyer et al., 1988).

The SHGC method developed in Chapter II produced a large array of responses each time a sample of canned salmon was submitted for volatile analysis. The pattern of volatile compounds may reflect a number of quality attributes including species of Pacific salmon, stage of sexual maturity, and level of freshness. Therefore, the objectives of the second part of this research were: (1) to apply multivariate statistics to data from the SHGC method for classification of canned Pacific salmon in terms of species, stages of sexual maturity of chum salmon, and refrigerated decomposition of pink salmon, and (2) to study the relationships between the results from sensory evaluation and gas chromatography of fresh and canned pink salmon during refrigerated storage.

B. Materials and methods

1. Collection and canning of salmon

Pacific salmon of selected species were collected directly from commercial boats at a local fish processor in Vancouver. All fish were kept refrigerated at approximately 5°C for 2-3 h if they could not be cleaned and eviscerated immediately. Steaks of approx. 3.8 cm in width were cut and 215 g salmon flesh was placed in 307 x 115 two-piece cans with 2 g sodium chloride. The cans were then vacuum-sealed, retorted, and cooled according to practice of the commercial plant as described in Chapter II.

2. Investigated treatments

Table 2 shows the number of Pacific salmon that were cleaned and processed for the three treatments under investigation. These treatments were studied over a two year sampling period to account for possible year to year variation in the fish and analyses. The first treatment of interest covered four different species of Pacific salmon, namely, pink (Oncorhynchus gorbuscha Walbaum), sockeye (O. nerka Walbaum), coho (O. kisutch Walbaum), and chum (O. keta Walbaum). For all treatments but the second year of the species study, two samples per fish were analyzed to account for within-fish variation. All observations were however considered independent of each other in the statistical analyses.

Table 2. Number of Pacific salmon processed with respect to the species, stages of sexual maturity, and refrigerated decomposition studies for two sampling years.

Treatment	Year[a]	
	1	2
Species of Pacific salmon		
pink	6 (2)	6 (1)
sockeye	4 (2)	6 (1)
coho	6 (2)	6 (1)
chum	4 (2)	6 (1)
Sexual maturity of chum salmon		
silver-bright (stage 1)	6 (2)	11 (2)
semi-bright (stage 2)	12 (2)	6 (2)
commercial dark (stage 3)	6 (2)	6 (2)
Refrigerated storage of pink salmon	42 (2)	54 (2)

[a] The digit in parenthesis represents the number of cans per fish analyzed by SHGC.

The second treatment focussed on the aspect of sexual maturity of chum salmon. Fish were classified in three different stages of maturity based on external appearance using the Color Evaluation Guide for Pacific Salmon (Alaska Seafood Marketing Institute, Juneau, AK). Fish corresponding to illustrations A, B, and C were classified as stage 1 (silver-bright), D,E, and F as stage 2 (semi-bright), and G, H, and I as stage 3 (dark).

The third treatment was refrigeration storage time of pink salmon. Fish with good external appearance were taken from unloading commercial boats and stored between layers of ice in a cold room at 2-5°C. The fish were not eviscerated before storage in ice. About every two days for up to 13 and 21 days of refrigerated storage for years 1 and 2, respectively, six pink salmon were randomly sampled; the remaining fish held in the bogey were re-iced if needed. The two day sampling period was increased towards the end of storage to 3 or 4 days in order to extend the decomposition process.

3. Sensory assessment of raw and canned salmon

The quality of each fish selected was assessed before canning. Chum salmon of different stages of maturity were pre-categorized by commercial plant personnel and their classification based on the Colour Evaluation Guide for Pacific Salmon were subsequently determined. Pink salmon subjected to the refrigerated storage treatment were assessed by the author with the help of experienced personnel from a local fish processor

using the grading guide shown in Table 3. The procedure involved evaluating condition of the eyes, the gills, and the flesh texture for the external characteristics. Once the fish was opened and eviscerated, the integrity and odour of the belly cavity were then the main criteria examined.

Canned samples were evaluated by a trained and experienced Federal Fish Inspector using the 1986 Canned Pacific Salmon Grade Standard (Department of Fisheries and Oceans, Vancouver, BC). The canned pink salmon from the refrigerated storage studies were randomized amongst the cans of the other treatments. The vacuum of each can was measured with a Seaman Vacuum Gauge and most values were found to be between 11-12 in Hg. The cans were then opened and drained for 2-5 min. The colour grade was obtained by visual comparison with coloured porcelain enamel tiles (Department of Fisheries and Oceans, Vancouver, BC). Appearance of the canned salmon was examined for attributes such as watermarking (skin with uncharacteristic colour), belly-burn (reddened flesh), and curd. The final grade of the products was however based solely on odour and taste. The important flavour defects were characterized as decomposed, rancid, late (advanced sexual maturity), and overheating. If possible, the decomposed flavours were further qualified as fruity, sour, faecal, cheesy, ammoniacal, or by use of other relevant descriptors. Every criterion assessed was rated to describe its intensity, and samples were subsequently assigned scores integrating the ratings. Finally, scores were translated into grades corresponding to one of the following categories of quality: A, B, and reject (Table 4).

Table 3. Grading guide for whole raw Pacific salmon.

Criteria	Grade A	Grade B	Reject
External characteristics			
Eyes	clear, bright, convex	slightly sunken and dull	dull, sunken, and cloudy
Gills colour	bright red	pink	brown, grey
Gills odour	fresh, seaweed, or shellfish odour	neutral, slight but definite sour, faecal, or putrid odour	strong sour, putrid, putrid or faecal odour
Texture	firm and resilient, flesh springs back when thumb depression is released	moderately soft, thumb indentations may slowly fill out	very soft, thumb indentations may remain in flesh
Internal characteristics			
Belly cavity	transparent, intact peritoneal lining	moderate reddening, some ribs may protrude	extensive reddening, liquefaction of belly walls
Odour of belly	fresh and characteristic odour	slight but definite sour, faecal, or putrid odour	strong sour, faecal, or putrid odour

Table 4. Grading guide for canned Pacific salmon.

Criteria	Grade A	Grade B	Reject
Colour	0-2	3-4	-
Appearance[a]	0-1	2-3	-
Odour and taste[b,c]	0-2	3	4-9

[a] Cross-packing, belly-burn, watermarking

[b] Descriptive terms: decomposed (fruity, vegetable, sour, faecal, putrid), rancid, late, overheating, contamination, etc.

[c] Grade assignment:

Rating	Score	Grade
1	0	A
(1+1)	1	A
(1+1+1) etc.	2	A
2	2	A
3	4	B
4	8	R
5	16	R
6	32	R
7	64	R
8	128	R
9	256	R

4. Static headspace gas chromatography (SHGC)

GC analyses were performed on canned salmon that had been stored at room temperature for 3 to 12 months. Sample preparation for gas chromatography was done in the following manner. Each can was opened and the liquor was drained by tilting the can and its lid for 2 min. After flaking the white muscle 3-5 mm in size with a spatula, 10 g were transferred in 20 mL headspace vials (Hewlett Packard, Avondale, PA) avoiding dark meat, skin, and bones. One millilitre of 3-hexanol working solution (84.8 ppm) was added to the vials, which were then sealed with Teflon-faced silicone septums and aluminum caps.

Some of the raw salmon from the refrigerated storage of year 2 was also kept for SHGC analysis. Samples were inserted in plastic freezer bags (Baggies, Colgate-Palmolive Canada, Toronto, Canada), and after evacuating the air with a straw, the bags were closed with twist ties before freezing at -20°C for approx. 1 month. As needed, the frozen samples of raw salmon were thawed in water (20°C) for 3-4 hours. The thawed white muscles were then cut in pieces of 2-5 g with clean scissors and 12.5 g was put in headspace vials. Aliquots of 3-hexanol standard (84.8 ppm) were then added to the vials before sealing as described earlier for canned salmon.

The SHGC method used was derived from Chapter II. A HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a flame ionization detector (FID) was connected to a HP 19395A headspace sampler.

A HP 3396A integrator recorded the chromatograms and also transmitted the output to an IBM-compatible personal computer through a RS-232-C cable. The signal was handled by a program called FILE SERVER which allowed the host computer to serve as a remote external disk drive. Separation of volatiles was made on a HP ULTRA 2 fused silica capillary column (25m length x 0.32mm i.d. x 0.52μm film thickness). The operating conditions of the GC system are shown in Table 5.

5. Static headspace gas chromatography-mass spectrometry (SHGC-MS)

Tentative volatile identification was performed by connecting the headspace sampler to a Hewlett Packard 5985B GC-MS system. The conditions of operation and separation as well as mass spectra acquisition and confirmation by retention time comparison with reference standards were executed as described in Chapter II.

6. Preparation of standard solutions

Approx. 1 mL of 3-hexanol (Aldrich Chemical Co., Milwaukee, WI) was weighed in 1 L volumetric flask before diluting to volume with deionized distilled water (DDE). Ten millilitres of this stock solution was pipetted into a 100 mL vol. flask. The flask was brought up to volume with DDE and served as working solution (84.8 ppm).

Table 5. Operating conditions for the static headspace gas chromatographic method used to analyze volatiles in canned Pacific salmon.

Headspace sample conditions	
incubation temperature of vials	105°C
incubation time of vials	1 h
loop size	3 mL
valve temperature	110°C
vent/fill loop time	2 s
injection time	30 s
GC conditions	
carrier gas	helium
injector temperature	240°C
detector temperature	250°C
column headpressure	95 kPa
split vent	40 mL/min
septum purge vent	2.5 mL/min
Detector flowrates	
hydrogen	33 mL/min
air	375 mL/min
make-up gas (helium)	30 mL/min
Oven temperature program	
initial oven temperature	35°C
time at initial temperature	5 min
ramp rate	10°C/min
final temperature	175°C
Integrator conditions	
initial settings	
attenuation	0
chart speed	2
threshold	-1
peak width	0.03

7. Data handling and statistical analysis

A computer program was written in QuickBasic 4.5 (Microsoft Corporation) to preprocess the chromatographic data. Raw data were first extracted from the stored datafiles of the integrator reports and filtered to compile chromatographic area within the desired retention time intervals. Standardization was done by dividing each filtered chromatographic area with the peak area of the 3-hexanol standard and multiplying by 10000. All data were then accumulated in a spreadsheet format.

All duplicate and replicate analyses of both sampling years, which represented within fish, between fish, and year variations, respectively, were gathered and combined for the four species of Pacific salmon, three sexual maturity stages of keta salmon, and three decomposition levels of pink salmon with the goal of differentiating these specific groups. The Statistical Analysis System (SAS, 1989) was used for multivariate and regression analyses. Principal component analysis (PCA) and common factor analysis (CFA) with and without rotation were performed using the FACTOR procedure. Computations of linear, quadratic, and non-parametric discriminant analyses were carried out with the DISCRIM procedure. The REG procedure was applied to fit least-squares estimates to linear and multiple regression models. Estimated linear models were also obtained with the CATMOD procedure when the models involved categorical variables.

SYSTAT (Wilkinson, 1990) was another statistical package used for data analysis. The original spreadsheets of standardized data were first converted to compatible workfiles with the IMPORT command of the DATA module. The NPAR module was then used to compute the Kolmogorov-Smirnov (KS) statistic with the Lilliefors option for testing normality. Bartlett's test for homogeneity of variance was computed using the STATS module.

C. Results and discussion

1. MVA of volatiles from canned salmon of different species

For every sample of canned salmon analyzed, numerous volatiles were separated and constituted the chromatograms. A visual examination of the SHGC chromatograms indicated little qualitative differences in the patterns of volatiles between the species of Pacific salmon studied. The use of multivariate statistical methods was dictated by the complex array of responses obtained from each can. The initial step of data analysis involved the selection of peaks to build up complete matrices. Small peaks having actual area counts below 100 were not detected and/or integrated reproducibly and therefore were not considered. Forty-four peak responses were selected on the basis of consistent detection and integration of the same treatments, and most of them had a coefficient of variation between 4 and 12%. The 44 selected peaks are shown in Figure 9 and their identity in Table 6. On occasion, the first two peaks of some chromatograms were not adequately separated. For this reason, they were combined to provide one response and formed peak 1.

The first multivariate analysis performed was principal component analysis (PCA). In theory, the number of principal components that could be extracted from the data set of salmon species equals the rank of the correlation matrix. Of the 44 possible components, the first nine components followed Kaiser's rule of thumb (Kaiser, 1960a) which recommends to retain those components having eigenvalues (latent roots)

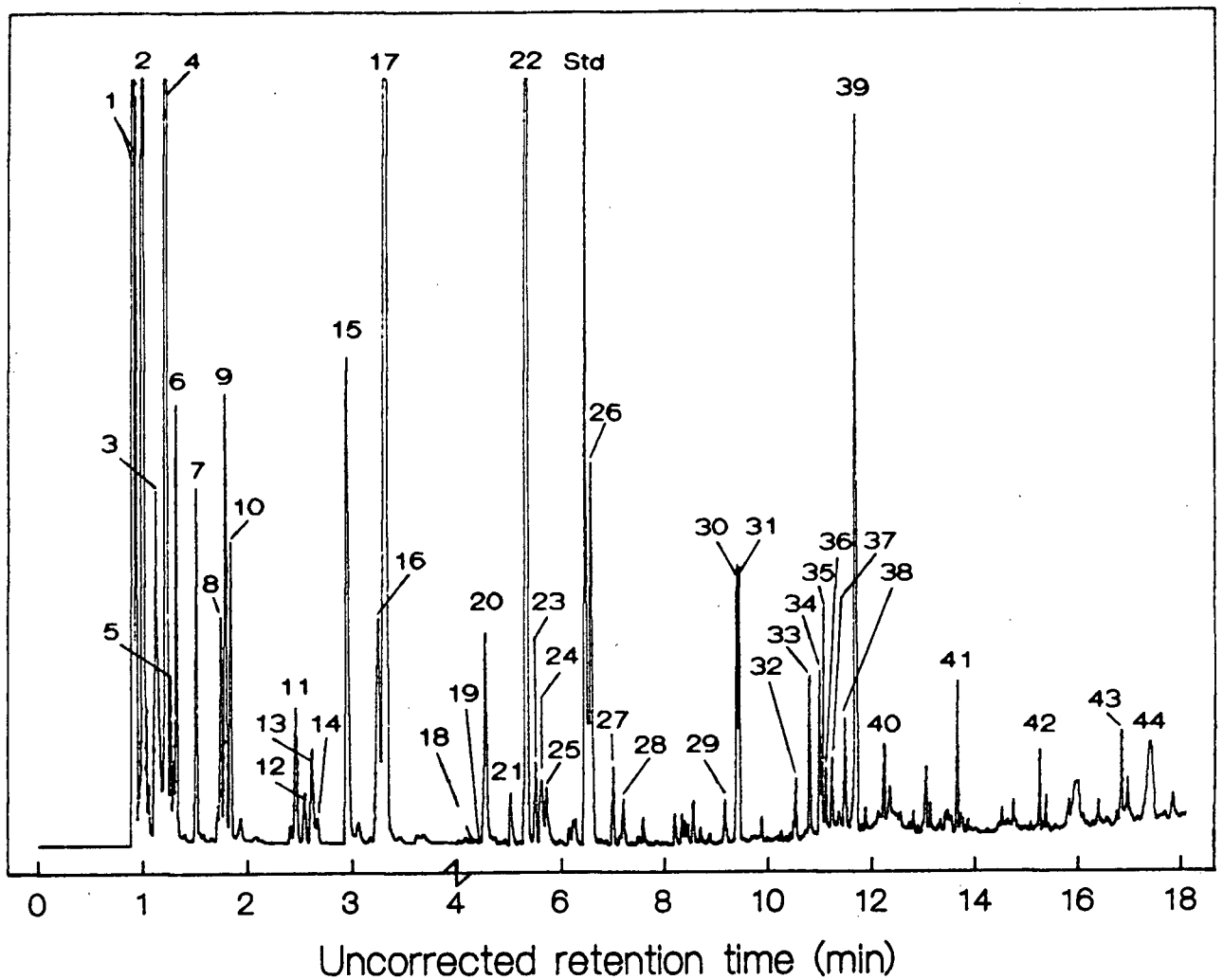

Figure 9. Chromatogram of volatiles from canned pink salmon selected to carry out multivariate statistical analyses.

Table 6. Identification of volatile compounds used in multivariate analysis of canned Pacific salmon.

Peak no.	Compound name	ID[a]
1	hydrogen sulfide	MS
1	acetaldehyde	MS,RT
2	methane thiol	MS
3	ethanol	MS,RT
4	butane	MS
5	3-methyl-1-butene	MS,RT
6	dimethyl sulfide	MS,RT
7	2-methyl propanal	MS,RT
8	hexane	MS,RT
9	butanal	MS,RT
10	2-methyl furan	MS
11	benzene	MS,RT
12	2-methyl butanal	MS,RT
13	2,2-dimethyl propanal	MS
14	unknown 1	-
15	1-penten-3-ol	MS,RT
16	heptane	MS,RT
17	1,5-dimethyl cyclopentene	MS
18	3-methyl-butanol	MS,RT
19	2-methyl-2-butenal	MS,RT
20	unknown 2	-
21	acetic acid	MS,RT
22	toluene	MS,RT
22	3-hexanone	MS,RT
23	unknown 3	-
24	unknown 4	-
25	unknown 5	-
26	3-ethyl-2-methyl-pentane	MS
27	ethylidene cyclohexane	MS
28	unknown 6	-
29	unknown 7	-
30	nonane	MS,RT
31	unknown 8	-
32	benzaldehyde	MS,RT
33	7-octen-4-ol	MS
34	unknown 9	-
35	unknown 10	-
36	unknown 11	-
37	unknown 12	-
38	2-pentyl furan	MS
39	4-ethyl benzene methanol	MS
40	3-ethyl-1,4-hexadiene	MS
41	nonanal	MS,RT
42	unknown 13	-
43	unknown 14	-
44	unknown 15	-

[a] MS, tentatively identified by mass spectrometry; RT, retention time consistent with that of authentic compounds.

greater than one (Table 7). But since the tenth component had a value approaching one, it was also included in the subsequent analyses.

Interpretation of principal components was facilitated by computing the component loadings. A component loading gives the product-moment correlation of each variable and the respective component. Table 7 presents these correlations for the peak area variables from the salmon species data. Based on those variable loadings highest on a given factor, the first principal component (PC1) was dominated by peaks 1 (hydrogen sulfide and acetaldehyde), 5 (3-methyl-1-butene), 16 (heptane), 17 (1,5-dimethyl cyclopentene), 29 (unknown 7), 30 (nonane), 31 (unknown 8), 32 (benzaldehyde), 37 (unknown 12), and 38 (2-pentyl furan). Peaks 9 (butanal) and 34 (unknown 9) had high negative correlations with principal component 2 (PC2) while peak 14 (unknown 1) had a dominant positive correlation. Because of the bipolarity in algebraic signs, PC2 contrasted the group of peaks 9 and 34 with peak 14. Similar interpretations could be done for the remaining of the components.

Nearly 40 percent of the variance was explained by the first principal component. Together, the ten principal components in Table 7 accounted for over 87 percent of the total variance. PCA effectively reduced the dimensionality of the data set indicating a high degree of intercorrelations among the original variables. By definition, PCA transforms the peak area factors into new latent variables (PC's) uncorrelated to each other. Therefore a multicolinearity problem due to high intercorrelations has been alleviated which otherwise would have

Table 7. Correlation (loadings) of gas chromatographic peak variables from canned Pacific salmon (chum, coho, pink, sockeye) with the first ten principal components.

Peak	Principal component									
	1	2	3	4	5	6	7	8	9	10
1	0.847	0.125	0.296	-0.119	-0.234	0.006	-0.044	0.089	0.203	-0.051
2	0.257	-0.354	0.661	-0.423	0.009	0.211	0.142	-0.166	0.063	0.049
3	-0.373	0.327	-0.591	0.000	0.074	0.339	-0.171	-0.028	0.050	0.175
4	0.526	-0.233	0.637	0.041	-0.078	-0.010	-0.032	-0.229	0.150	-0.168
5	0.810	0.371	0.145	0.062	-0.094	0.003	0.057	0.144	0.193	-0.162
6	0.682	0.312	0.113	0.278	-0.151	-0.119	-0.144	-0.102	-0.340	-0.089
7	0.539	-0.460	0.309	-0.485	-0.072	0.288	0.078	0.039	-0.075	-0.051
8	0.500	0.344	-0.307	-0.017	-0.552	0.159	0.087	-0.236	0.193	0.002
9	0.405	-0.740	0.363	0.042	0.057	0.167	-0.206	-0.048	-0.081	-0.045
10	0.744	0.321	0.093	0.130	0.017	0.003	0.040	0.039	-0.250	-0.150
11	0.677	-0.316	-0.138	-0.388	-0.312	0.110	0.050	0.170	-0.246	0.054
12	0.195	-0.231	0.007	-0.520	-0.195	-0.210	-0.505	0.031	0.196	0.044
13	0.454	-0.428	-0.339	-0.513	-0.182	0.134	0.062	0.116	-0.214	0.113
14	0.273	0.668	0.348	-0.181	-0.107	-0.006	0.084	0.022	0.033	0.140
15	0.783	-0.112	-0.023	0.313	-0.448	0.031	0.052	-0.074	0.037	0.173
16	0.800	-0.422	-0.085	0.191	-0.147	-0.094	-0.017	0.251	0.052	0.045
17	0.802	-0.053	-0.515	0.003	0.022	0.114	-0.039	-0.025	0.098	-0.043
18	0.143	0.476	-0.368	-0.344	-0.099	0.119	0.234	0.261	-0.076	-0.448
19	0.193	0.358	0.230	0.241	0.080	0.588	-0.483	0.102	-0.069	0.049
20	0.505	-0.562	0.316	0.186	0.147	-0.054	-0.053	-0.078	0.306	-0.240
21	0.481	-0.054	0.240	0.207	-0.552	0.061	0.201	-0.250	-0.170	0.097
22	0.003	-0.460	-0.679	-0.055	0.183	0.088	0.044	-0.265	-0.078	-0.025
23	0.598	0.093	0.262	0.066	0.347	-0.129	-0.302	-0.126	-0.412	0.128
24	0.589	-0.419	-0.075	0.247	-0.364	0.004	-0.017	0.285	0.012	0.221
25	0.708	0.338	-0.119	0.169	-0.130	-0.114	0.118	-0.202	0.291	0.143
26	0.551	-0.032	0.058	0.317	-0.146	-0.051	-0.082	-0.031	-0.208	-0.491
27	0.788	0.081	0.128	0.099	0.311	-0.187	-0.018	0.213	0.015	0.252
28	0.785	0.320	0.138	-0.047	0.287	-0.183	0.016	0.184	0.004	0.117
29	0.862	0.014	-0.125	-0.051	0.315	-0.137	-0.011	0.086	0.075	-0.026
30	0.821	-0.200	-0.431	0.115	0.084	-0.058	0.001	0.041	0.200	0.028
31	0.841	0.156	-0.274	0.173	-0.209	0.109	-0.033	-0.119	-0.162	-0.001
32	0.919	0.066	0.038	0.129	0.160	-0.041	-0.025	0.100	-0.037	0.029
33	0.602	-0.488	0.356	-0.271	0.179	0.056	0.091	-0.011	0.057	-0.036
34	0.056	-0.871	-0.125	0.252	0.174	-0.042	0.058	0.046	-0.058	-0.045
35	0.466	0.318	-0.089	-0.347	0.198	0.023	-0.130	-0.588	-0.070	0.043
36	0.783	0.383	0.047	-0.213	0.092	0.001	-0.033	-0.050	0.094	0.122
37	0.888	0.226	0.120	-0.043	0.014	-0.024	-0.055	0.042	-0.050	0.120
38	0.879	0.263	-0.083	-0.220	0.177	-0.029	0.112	0.080	-0.090	-0.003
39	0.679	-0.372	-0.520	-0.031	0.259	0.102	0.039	-0.085	0.023	-0.041
40	0.336	-0.011	0.227	0.181	0.396	0.047	0.610	-0.178	-0.078	0.131
41	0.556	-0.599	-0.489	0.180	0.074	0.029	-0.006	-0.096	-0.003	0.069
42	0.765	0.423	-0.251	-0.183	0.226	-0.015	0.115	0.067	-0.010	-0.118
43	0.662	0.174	-0.166	0.072	0.241	0.177	-0.206	-0.134	0.239	-0.183
44	-0.048	0.123	0.258	0.290	0.256	0.714	0.148	0.189	0.097	0.062
Latent root	17.178	5.945	4.276	2.448	2.383	1.480	1.351	1.228	1.109	0.982
Variance (%)	39.042	13.512	9.719	5.564	5.415	3.363	3.070	2.790	2.521	2.233

occurred during the use of the original variables in further statistical procedures.

Figure 10 shows a plot of the scores of the different salmon species for the first two principal components. There was an initial attempt to uncover differences between the species based on PC1. On the other axis, PC2 clearly contrasted the salmon species between the two sampling years. At least two major sources of variation could be put forward to explain this demarcation on PC2. Firstly, salmon of different years may produce variations in volatile patterns due to their differences in feed sources, water temperature changes, and other possible environmental factors. However, not all salmon of a given species were from the same runs, as they were sampled on different days during the fishing seasons. Since salmon of different runs, but collected the same year, responded similarly on PC2, biological explanations do not provide satisfactory answers. The two year-groups were analyzed approximately one year apart. Although the analyses were performed on the same GC column and on the same instrument, slight changes in factors such as detector sensitivity, column phase, and gas flow rates could contribute to variations in the GC responses in a consistent manner between years. A careful inspection of peaks 9, 14, and 34 which received high loadings on PC2 revealed that they had decreased in resolution between year 1 and year 2. To a large extent, PC2 may reflect the loss in chromatographic separation or increased measurement error for these peaks.

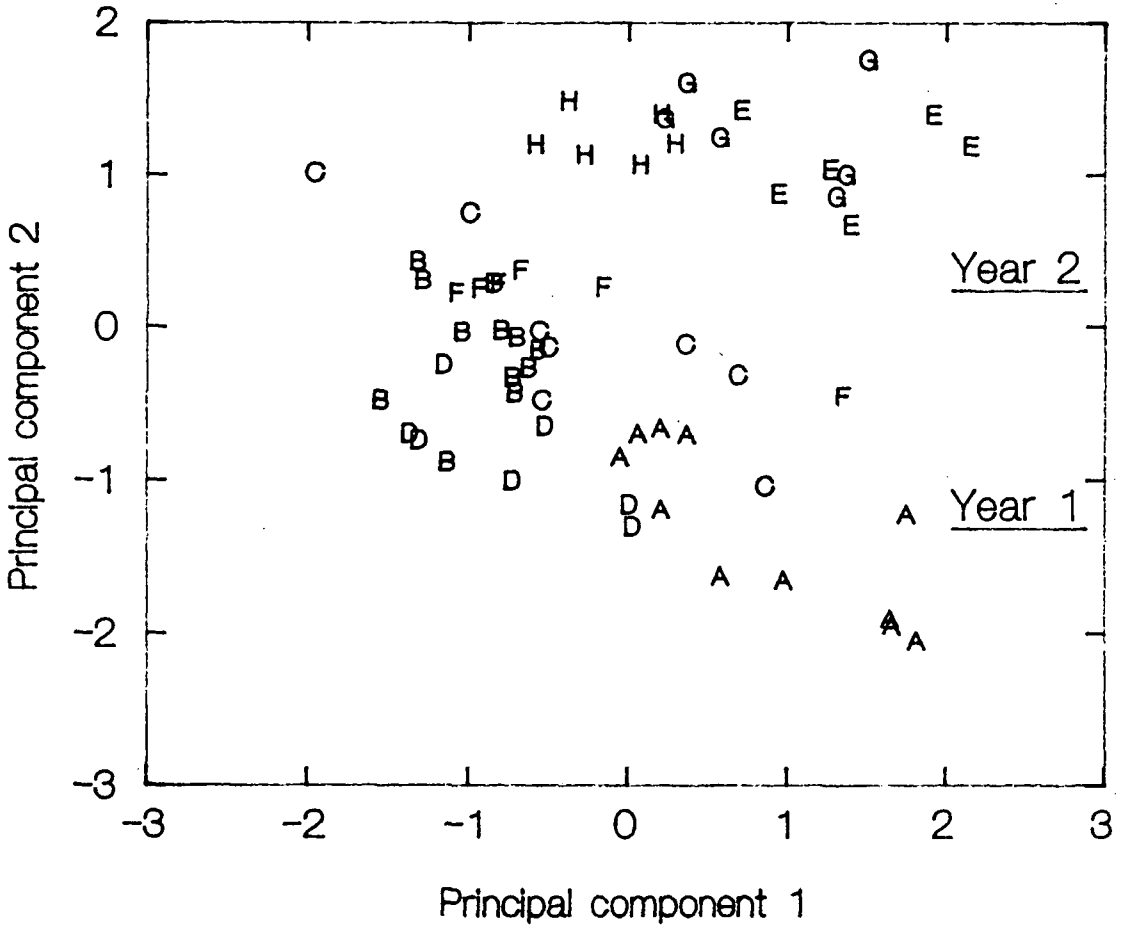

Figure 10. Plot of the first two principal component scores for the salmon species (A, pink-year 1; B, coho-year 1; C, chum-year 1; D, sockeye-year 1; E, pink-year 2; F, sockeye-year 2; G, coho-year 2; H, chum-year 2).

The data for both years were not subsequently analyzed separately as the number of observations was insufficient, and they therefore remained in a pooled format. Scores of the ten uncorrelated principal components were used to carry out discriminant analyses. Homogeneity of within-group variance-covariance was the first underlying assumption examined. Table 8 presents both univariate and multivariate statistics to test the null hypothesis regarding this aspect. When variances were taken separately for each variable, four PC's were significant at 0.05 level and one of them, namely PC6, was significant at 0.01 level. The multivariate test based on Bartlett's modification of the likelihood ratio was also found to be highly significant ($P<0.0001$). This indicates that the assumption of homogeneity of dispersion matrices between groups was not multivariately respected.

The next assumption examined was normality. Although multivariate normality was not tested, certain necessary conditions (e.g., univariate normality) can be verified in order for it to not be rejected. In this context, Kolmogorov-Smirnov tests based on Lilliefors probability distributions where means and standard deviations are not a priori assumed were carried out on the salmon species for each principal component. Results presented in Table 9 shows that sockeye was univariate normal in all but one principal component. On the other hand, coho groups behaved in a non-normal fashion in four instances of which three came out significant at the 0.01 level. Chum and pink salmon both were significant at the 0.05 level in two occasions.

Table 8. Bartlett's tests for homogeneity of within group variance-covariance between canned Pacific salmon (chum, coho, pink, sockeye) for the first ten principal components.

Variable	df	Chi-square
Univariate		
PC1	3	2.983
PC2	3	8.874 *
PC3	3	5.230
PC4	3	7.717 *
PC5	3	9.090 *
PC6	3	14.800 **
PC7	3	7.533
PC8	3	9.085 *
PC9	3	0.874
PC10	3	4.440
Multivariate		
PC1-10	165	520.476 ***

* Significant difference at 0.05.
** Significant difference at 0.01.
*** Significant difference at 0.0001.

Table 9. Kolmogorov-Smirnov normality test of canned Pacific salmon (chum, coho, pink, sockeye) within principal components.

Variable	Maximum difference			
	Coho (n=18)	Chum (n=14)	Pink (n=17)	Sockeye (n=13)
PC1	0.294 **	0.165	0.145	0.198
PC2	0.165	0.189	0.174	0.210
PC3	0.175	0.258 *	0.176	0.207
PC4	0.124	0.254 *	0.141	0.239 *
PC5	0.105	0.203	0.118	0.184
PC6	0.230 *	0.182	0.141	0.219
PC7	0.321 **	0.222	0.230 *	0.117
PC8	0.129	0.126	0.215 *	0.104
PC9	0.158	0.149	0.152	0.157
PC10	0.293 **	0.176	0.108	0.184

* Significant difference at 0.05 level based on Lilliefors probability.
** Significant difference at 0.01 level based on Lilliefors probability.

As the normality assumption was violated a number of times and the group dispersion structures were unequal, the conclusions that can be extracted from the tests of significance and estimated classification error rates which rely on parametric classification criteria may be biased. Caution should be exercised in the interpretation of the significance tests based on the parametric distributions since failure to meet the assumptions influences the estimates of variance and affects the probability of an hypothesis to be true or false.

Linear discriminant analysis (LDA) was performed with the prior probabilities set proportional to the unequal sample sizes. Table 10 summarizes both univariate and multivariate test statistics. Six of the ten principal components were significant at or beyond the 5% level, namely, PC1, PC2, PC3, PC4, PC6, and PC7. These principal components were important elements of variation between the salmon species, especially PC3, PC1, and PC4, based on their F ratios. Several volatile compounds had high correlations with the important discriminators PC1, PC3, and PC4. Methane thiol, ethanol, butane, toluene, 4-ethyl benzenemethanol, and nonanal gave large loadings to PC3 while 2-methyl propanal, 2-methyl butanal, and 2,2-dimethyl propanal provided loadings of substantial magnitude toward PC4. As mentioned earlier, at least ten volatiles dominated PC1 (see page 56). Although PC3 and PC1 regrouped large contributors having various functional groups, three aldehydes strongly correlated with PC4.

Table 10. Univariate and multivariate test statistics of discriminant analysis on the first ten principal components from the volatiles of canned Pacific salmon (chum, coho, pink, sockeye).

Variable	num df,den df	F
Univariate		
PC1	3,58	12.7976 ***
PC2	3,58	4.0722 *
PC3	3,58	43.0986 ***
PC4	3,58	10.1754 ***
PC5	3,58	1.0423
PC6	3,58	4.4860 **
PC7	3,58	3.5366 *
PC8	3,58	0.6748
PC9	3,58	0.8462
PC10	3,58	0.9877
Multivariate		
Wilk's lambda = 0.0230	30,144.5	12.591 ***
Pillai trace = 2.1258	30,153	12.402 ***
Hotelling-Lawley trace = 7.8011	30,143	12.395 ***
Roy's largest root = 3.5936	10,51	18.327 ***
Canonical		
LR_1 = 0.0230	30,144.5	12.5914 ***
LR_2 = 0.1058	18,100	11.5280 ***
LR_3 = 0.3728	8,51	10.7248 ***

* Significant difference at 0.05 level.
** Significant difference at 0.01 level.
*** Significant difference at 0.0001 level.
LR stands for Likelihood ratio.

The multivariate tests including Wilk's lambda, Pillai's trace, Hotelling-Lawley's trace, and Roy's greatest root were computed using the eigenvalues extracted from Wishart matrices (matrices of corrected sums of squares and products of assumed multinormal variates), and constitute various ways to test the hypothesis that the class means were equal in the population (Morrison, 1976). All highly significant ($P < 0.0001$), these tests clearly revealed underlying between-group differences involving the ten variables jointly. A plot of estimated density distributions for PC3, the largest contributor toward group separation, is shown in Figure 11. In this context, normality and homoscedasticity were modalities assumed to be respected. Among the resulting curves equivalent in form, only two groups were well separated in the PC3 dimension. The curve describing the sockeye group was located in the negative range of PC3 and intersected only marginally with the chum salmon curve situated in the positive end of the scale.

Canonical functions were derived to find uncorrelated composites of the 10 PC's that best summarize the differences among the salmon species. The approximate F statistic based on Rao's approximation of the likelihood ratio distribution tested the hypotheses that the canonical correlation associated with a particular discriminant function and all succeeding smaller ones were not different than zero (Rao, 1973). Canonical analysis indicated that all three canonical variates (CV) were highly statistically significant at the 0.0001 level (Table 10). In other words, CV1, CV2, and CV3 were all necessary to adequately describe differences between the four salmon species. The standardized coefficients or weights for the

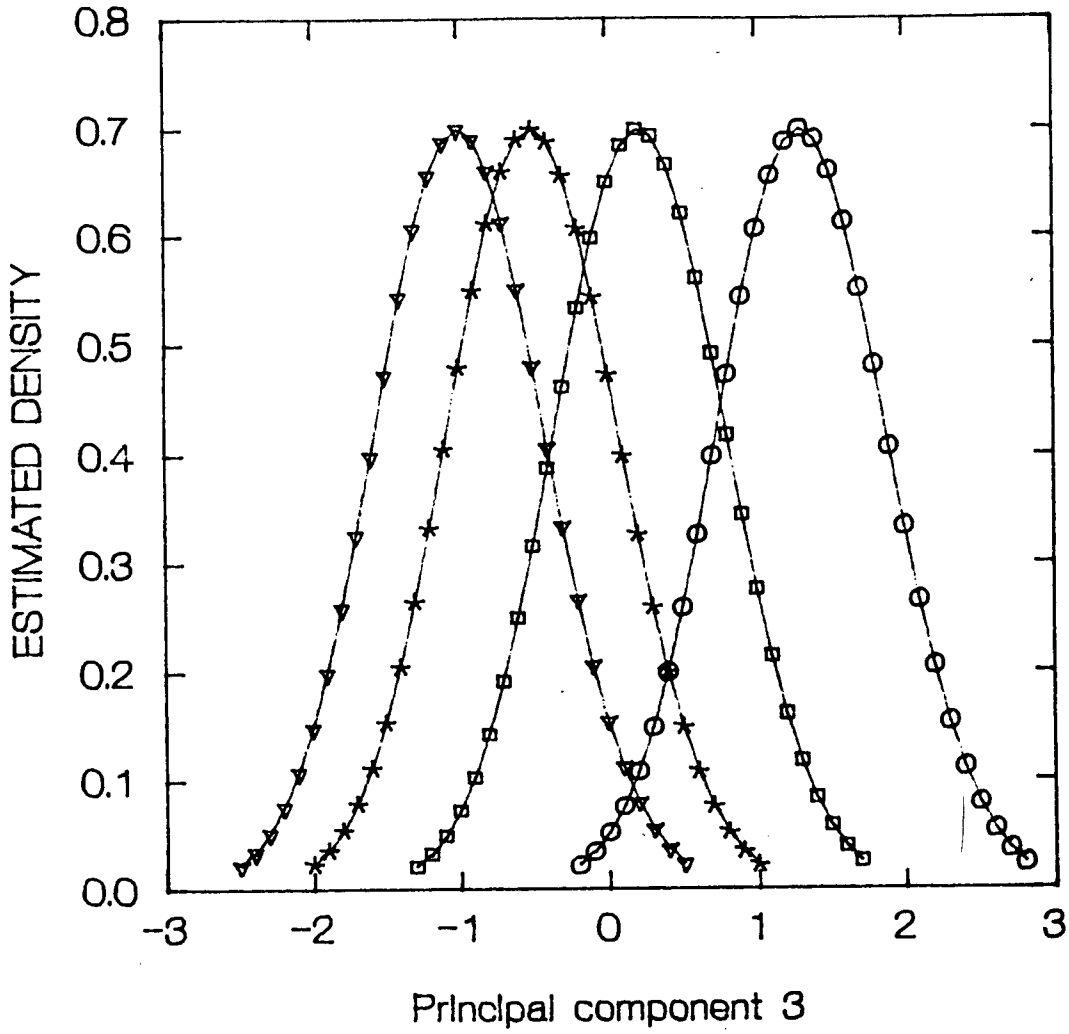

Figure 11. Estimated density distributions of canned Pacific salmon for principal component 3 based on linear discriminant analysis (▽, sockeye; □, pink; ○, chum; ★, coho).

canonical variates are presented in Table 11. PC3 with a coefficient of 1.1161 completely dominated the first canonical variate CV1. The largest coefficient of 1.0318 on CV2 was associated with PC4. PC7 and PC6 were also attributed relatively large coefficients on CV2. Due to opposite signs found on the weights of importance, CV2 was essentially a contrast between PC4 and PC7 with PC6. The first two principal components contributed largely toward CV3. In all, the large coefficients given to PC1, PC2, PC3, PC4, PC6, and PC7 in the three discriminant functions corroborated the results of the univariate statistics in Table 10.

The means of the four groups of salmon species are plotted in Figure 12 in a three dimensional canonical discriminant function space. The plots on the 3 facets of the graph are two-dimensional image perspectives brought onto the background planes at right angles. The centroids of the four groups were not equally distant from one another in the canonical function space, but appeared either near or far from each other depending on the observed plane. Graphically, all three canonical variates were needed to separate the groups effectively. CV1 primarily separated sockeye and coho from pink and chum, while CV2 essentially separated coho from the remaining groups. CV3 mainly segregated coho and pink from sockeye and chum. In addition to the depiction of distances between group means, Figure 12 shows isodensity contours of the bivariate normal distribution of the canonical variates. Because standardized discriminant function coefficients were used to compute scores whose pooled-group covariance matrix was an identity, the isodensity ellipses about the centroids are circles. The circles surrounding each centroid represent

Table 11. Standardized canonical variate coefficients for species discrimination of canned Pacific salmon (chum, coho, pink, sockeye).

Dependent variable	Canonical variate		
	1	2	3
PC1	0.3737	-0.0739	0.9584
PC2	-0.3046	0.4552	0.6058
PC3	1.1161	-0.0774	-0.0414
PC4	0.1530	1.0318	-0.1550
PC5	-0.3106	-0.2167	0.3107
PC6	-0.0001	-0.6570	-0.5279
PC7	0.1366	0.7845	-0.1030
PC8	0.1389	0.2123	-0.2935
PC9	-0.1904	-0.3118	0.2469
PC10	-0.0928	0.3946	-0.2370

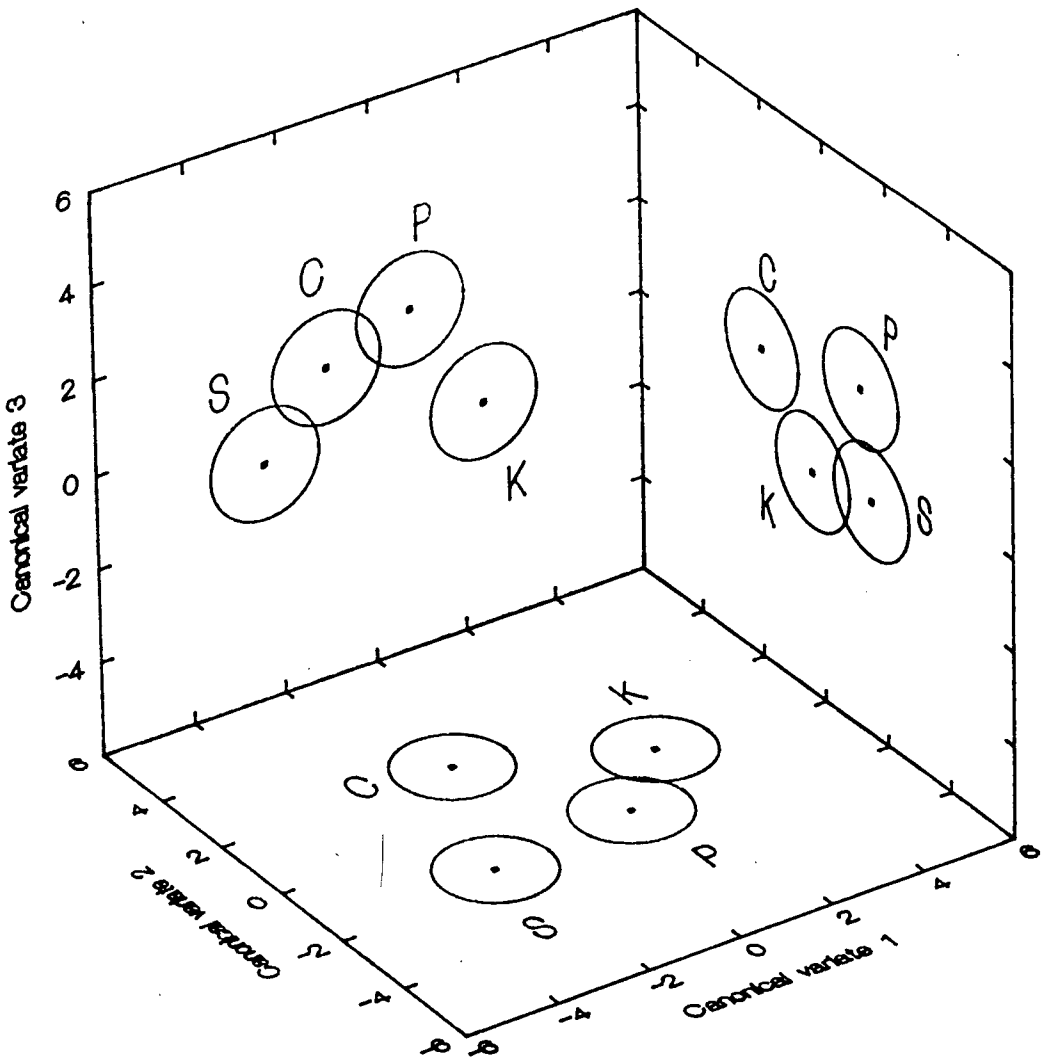

Figure 12. Canonical representation of centroid means and dispersions for canned Pacific salmon (C, coho; K, chum; P, pink; S, sockeye).

the bivariate dispersion of each group at a selected probability level of 0.50, that is, the circles are expected to contain approximately 50 percent of the observations in each group. Some intersections between circles indicated that groups were overlapping in the two dimensional discriminant space. However, the overlaps appeared to be minimal when considered among the three canonical dimensions, and therefore, misclassification errors were expected to remain low.

The Mahalanobis distances were computed using the canonical scores and the probabilities of group membership were then calculated from these distances. The closer an observation is to a particular group's location or centroid in the discriminant space, the more likely it is that it belongs to the group. The resubstitution method, which simply yields the direct sample proportions of misclassified observations (Hills, 1966) was used to cross-tabulate the actual and predicted group membership of salmon species (Table 12). Nearly all samples were correctly allocated to their respective species, except one pink salmon was categorized as coho. The apparent error rate generated was therefore 1.6%.

In view of the heterogeneity of variance-covariance previously tested, quadratic discriminant analysis was performed where the individual within-group covariance matrices were not pooled in calculating the generalized squared distances. Estimated densities from the quadratic functions were plotted against PC3 in Figure 13. The sockeye and chum salmon curves had wider spread distributions than those of coho and pink but remained relatively well detached from one another. Computations of

Table 12. Classification matrix for actual and predicted group membership of canned Pacific salmon (chum, coho, pink, sockeye) by linear discriminant analysis using the resubstitution method.

Actual group	Predicted group			
	Pink	Sockeye	Coho	Chum
Pink	16	0	1	0
Sockeye	0	13	0	0
Coho	0	0	18	0
Chum	0	0	0	14

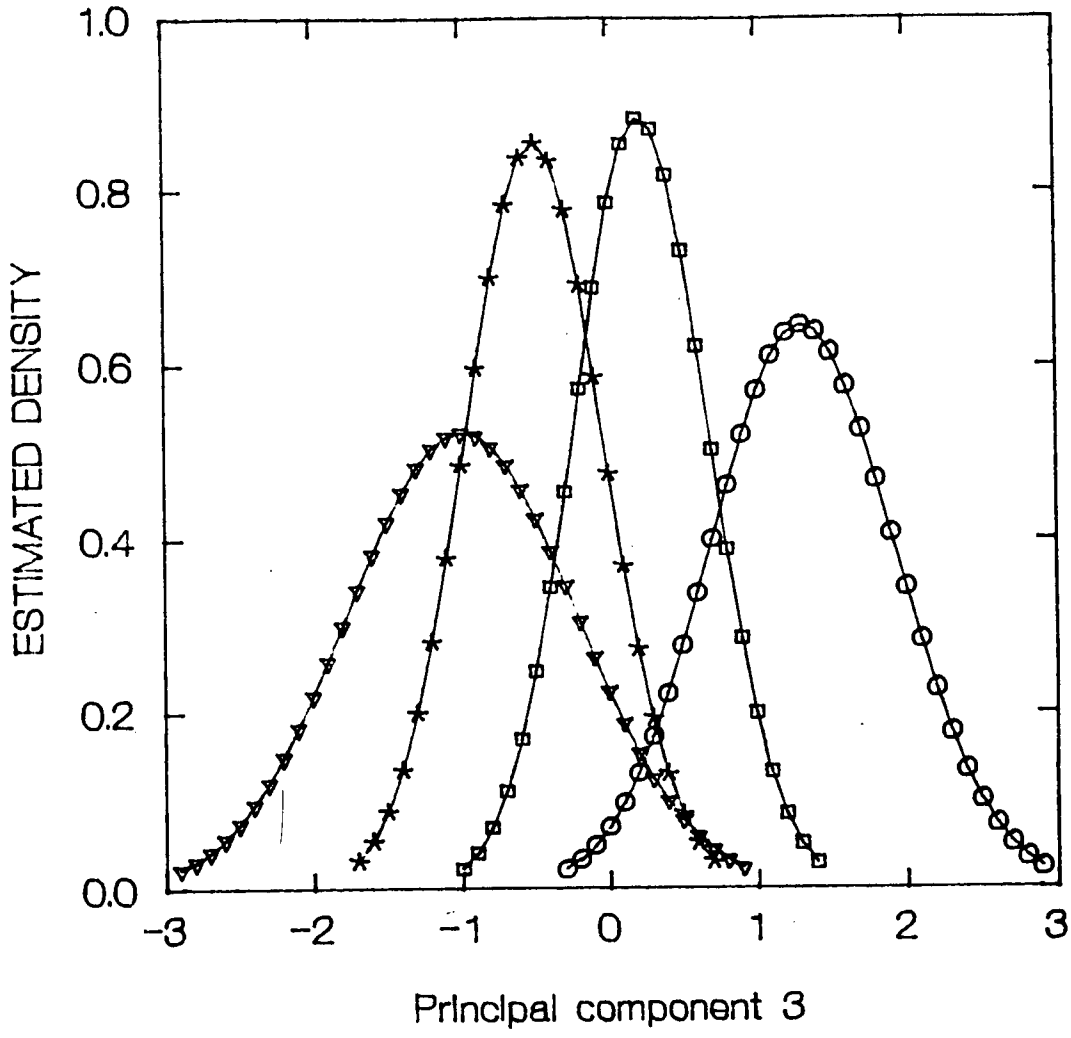

Figure 13. Estimated density distributions of canned Pacific salmon for principal component 3 based on quadratic discriminant analysis (▽, sockeye; □, pink; ○, chum; ✶, coho).

the quadratic rule on the ten principal components using the resubstitution method resulted in the assignment of all observations in their respective group, hence leading to a 100% of correct classification.

The influence of unequal dispersions on multivariate statistical tests has been studied in the past. Evidence indicated that the effects of heterogeneity of dispersions on the tests of significance depends on both the number of variables and the group sample sizes (Dillon and Goldstein, 1984). Under conditions of heteroscedasticity, sensitivity to rejecting the null hypothesis (mean vectors being equal) increases with increasing number of variables or with increasing disproportion between sample sizes. Although the apparent error rate of the linear and quadratic discriminant functions did not appear to be drastically different for the salmon species data, unequal dispersion matrices is a factor known to affect the classification rule (Marks and Dunn, 1974). Other factors influencing classification include the separation among groups, the number of variables, and sample sizes. Agreement between results obtained from linear and quadratic equations diminishes as the differences in group dispersion increase, the group means become closer, the number of variables increases, or the sample sizes decrease.

When the data within each group are not assumed to possess any specified distribution or are assumed to have distributions different from multivariate normal, nonparametric methods can be considered as alternatives. The nonparametric approach does not constrain the statistical distribution in question to fall in a given parametric family

with rigid predetermined assumptions of homoscedasticity and normality, but still relies on independently collected observations originating from identical distributions. Nonparametric discriminant methods are based on nonparametric estimates of group-specific probability densities. For the following analyses, the kernel estimator was chosen among the nonparametric methods available. The kernel method uses a fixed p-dimensional volume by specifying a radius, r, and estimate the group densities at each observation vector (Hand, 1982). The parameter r also has the property of determining the degree of irregularity or smoothness in the estimation of the density function. Small values of this smoothing parameter produce jagged contours while large values obscure and broaden the curve definition of kernel estimates. Although various methods for selecting the smoothing parameter have been suggested, there is as yet no simple solution to this problem. One way to choose the smoothing parameter is to plot classification error rates with different r values and select the estimate which minimizes the misclassification rate (Hand, 1982).

Over the years, a number of kernel functions have been developed to estimate the densities. Silverman (1986) compared the efficiencies of some established kernels and found very little difference between them as all obtained efficiency values close to one. The Epanechnikov kernel was applied to the principal components extracted from the salmon species dataset in this study. In Figure 14, the behaviour of the error count estimate was followed over a range of the smoothing parameter that spanned from 2 to 18. Based on the resubstitution method (straightforward

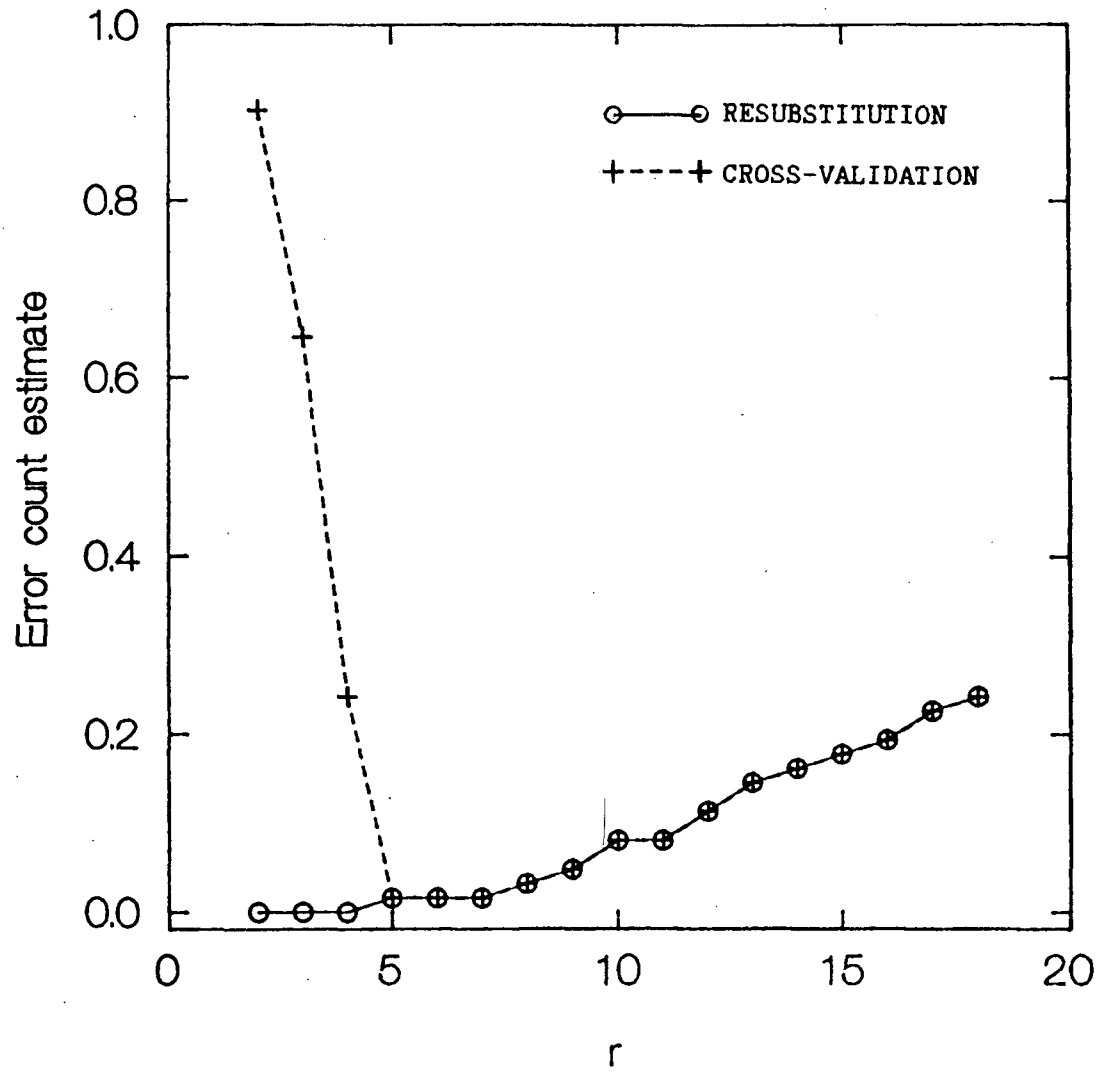

Figure 14. Comparison of two error count estimation methods against the smoothing parameter of the Epanechnikov kernel classifier on principal component scores of canned Pacific salmon (chum, coho, pink, sockeye) volatiles.

reclassification) to calculate the error rate, the three first r values generated no classification error. Although simple in nature, the apparent error rate computed by resubstitution has been criticized in the past as being over-optimistic (Hand, 1982). The bias originates from the fact that the classifier has been designed to optimize some function of the dataset and therefore performs better on the original observations than on other similarly generated datasets.

Another method for error rate estimation introduced by Lachenbruch (1967) is the cross-tabulation or U-method. Also self-explanatorily referred to as the "leave-one-out" method, it consists of holding out one observation at a time, estimating the discriminant function on n-1 observations, and classifying the held-out observation. This process is repeated for each observation, and achieves a nearly unbiased estimate but with a relatively large variance (Glick, 1978). When cross-validation was applied to evaluate the kernel performance, it was found that the three first smoothing parameters mentioned earlier (r = 2,3,4) yielded much larger error counts as opposed to those from resubstitution (Figure 14). On the other hand, the next three r values (r = 5,6,7) gave the lowest error rate of 0.0161 which was also the same as that obtained with resubstitution. In fact, the error rates associated with the smoothing parameters ranging from 5 to 18 were identical for both estimation methods. The estimated density distributions computed using the Epanechnikov kernel with r=6 for principal component 3 are shown in Figure 15. Compared to the previous results based on parametric discriminant analyses, the curves possess similar mean positions, a high degree of

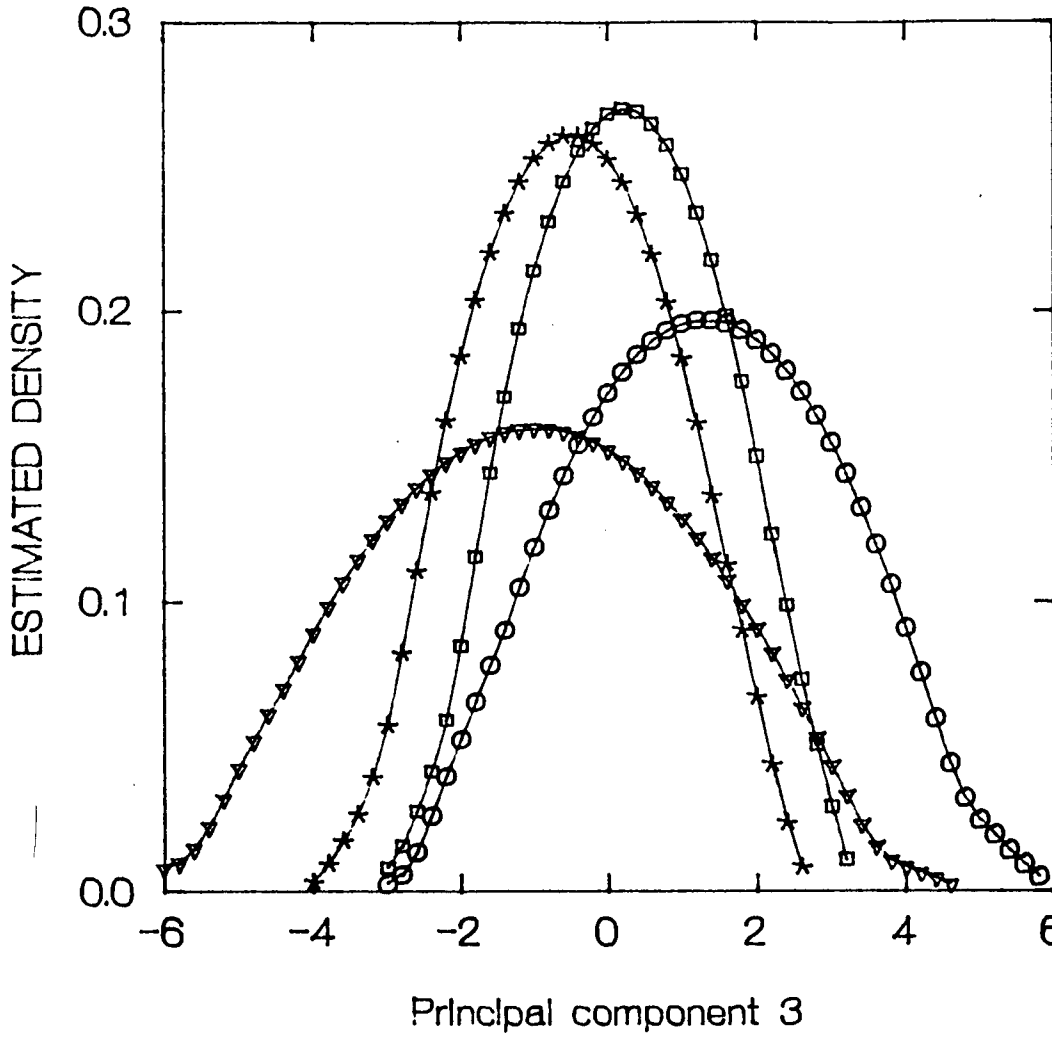

Figure 15. Estimated density distributions of canned Pacific salmon for principal component 3 based on non-parametric discriminant analysis (▽, sockeye; □, pink; ○, chum; ★, coho).

symmetry, but wider spread consequently causing more overlapping between all salmon groups. The species averages in terms of estimated density had the same ranking order (sockeye, chum, coho, and pink) for the quadratic and kernel methods. However, the Epanechnikov kernel produced rounder contours of the bell shape curves.

In order to make an appropriate comparison between the above three discriminant analyses, error count estimates were calculated by both resubstitution and cross-validation for the salmon species (Table 13). The cross-validation error estimate for linear discriminant analysis was 11.29% while it was 1.61% when computed using resubstitution. There was an even more drastic difference between cross-validation and resubstitution for quadratic discriminant analysis (19.3% and 0.0%, respectively). No difference was found for the Epanechnikov kernel method as was shown in Figure 14. Results clearly indicated the danger of relying on the resubstitution method for error rate calculation as it generally provides a biased level of confidence. The rank in increasing order on the basis of cross-validation error count estimate for the discriminant analysis methods was kernel, linear, and quadratic. This sequence is in accord with that of Van Ness and Simpson (1976) who studied the relationship between dimensionality, error rate, and sample size for different types of classifier. Besides the absence of the restrictive parametric assumptions, they also attributed the superiority of the kernel over linear discriminant methods to the stability of the kernel estimator in high dimensionality. The linear estimator performed relatively well despite the non-normality and heteroscedasticity of the

Table 13. Comparison of error count estimation methods for different discriminant analysis (DA) applied to the first ten principal components from canned Pacific salmon (chum, coho, pink, sockeye) volatiles.

DA function[a]	Error count estimate[b]				
	Coho (n=18)	Chum (n=14)	Pink (n=17)	Sockeye (n=13)	% Total (n=62)
Resubstitution					
linear	0.000 (0)	0.000 (0)	0.059 (1)	0.000 (0)	1.6
quadratic	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)	0.0
nonparametric	0.000 (0)	0.000 (0)	0.059 (1)	0.000 (0)	1.6
Cross-validation					
linear	0.167 (3)	0.000 (0)	0.176 (3)	0.077 (1)	11.3
quadratic	0.056 (1)	0.143 (2)	0.118 (2)	0.538 (7)	19.4
nonparametric	0.000 (0)	0.000 (0)	0.059 (1)	0.000 (0)	1.6
Prior prob.	0.290	0.226	0.274	0.210	

[a] The nonparametric method consists of the Epanechnikov kernel with r=6.
[b] The numbers in parentheses are the misclassifications associated with the error count estimates.

species data. Lachenbruch et al. (1973) investigated the performance of linear discriminant function applied to some multivariate non-normal distributions. They concluded that results in such situations can be misleading though the overall classification error rates were usually not severely affected. In particular, the individual group error rates were distorted in that error rates were generally much larger than the optimal value for one population group, and much smaller for the other. Attempts to adjust for unequal dispersion matrices by use of the quadratic rule proved to be ineffective. Sockeye salmon especially received a high rate of incorrect classification. The poor performance of the quadratic method could be attributed to sample covariance matrices being poor estimates in presence of large dimensionality/sample size ratios (Van Ness and Simpson, 1976). Therefore, larger sample sizes could improve the performance of the quadratic functions.

2. MVA of volatiles from canned chum salmon at three stages of sexual maturity

Data on the forty-four GC peaks identified earlier were gathered from samples of adult chum salmon in three different ranges of maturity during their spawning migration. Only quantitative differences in the GC patterns could be found between the samples as no appearance or disappearance of peaks were observed. This situation was comparable to the previous species study and therefore a similar multivariate approach was undertaken to analyze and extract information about the structure of the dataset.

Principal component analysis was carried out on the correlation matrix. Table 14 shows the factor loadings of the first eight PC's that had a latent root higher than 1.0. Thirteen peaks were allocated loadings of 0.8 and above on PC1. Of these thirteen peaks, eight of them had correspondingly high and positive loadings on the first principal component of the species dataset (Table 7). They were peaks 1 (hydrogen sulfide and acetaldehyde), 5 (3-methyl-1-butene), 16 (heptane), 17 (1,5-dimethyl cyclopentene), 29 (unknown 7), 30 (nonane), 32 (benzaldehyde), and 38 (2-pentyl furan). PC2 highly correlated with three peaks: 14 (unknown 1), 22 (3-hexanone) and 34 (unknown 9). Again, two of these peaks (14 and 34) loaded high coefficients on PC2 in the analysis of the salmon species data (Table 7). Compared to the earlier study, peak 14 also contrasted with peak 34 based on their opposite algebraic signs but in a reverse manner.

Forty-one percent of the total variance was explained by PC1 and did not differ markedly from the respective value of 39.0% found in the earlier analysis. The rate at which the variance explained by the succeeding principal components decreased, was also a common aspect between the species and sexual maturity studies. As a result, the variance cumulated over the first eight components was 84.2%, and corresponded well with the figure of 87.2% obtained previously. Notwithstanding the parallel drawn between the two studies, the similarities in component loadings were found mainly with the two first PC's and the remaining principal components showed more specificity to their respective dataset.

Table 14. Correlation (loadings) of gas chromatographic peak variables from canned chum salmon tested at three sexual maturity stages with the first eight principal components.

Peak	Principal component							
	1	2	3	4	5	6	7	8
1	0.829	-0.292	-0.052	-0.299	-0.086	-0.063	0.113	-0.170
2	0.596	0.394	0.083	-0.548	-0.260	-0.126	-0.049	0.055
3	-0.353	0.084	-0.049	0.341	0.486	0.478	0.218	0.015
4	0.779	0.068	-0.027	-0.532	0.067	-0.053	-0.031	0.046
5	0.808	-0.427	-0.089	-0.010	-0.088	-0.033	0.254	-0.078
6	0.245	0.269	-0.315	0.214	-0.562	0.214	-0.069	0.257
7	0.341	0.382	0.725	-0.301	-0.056	-0.044	0.180	0.124
8	0.479	-0.265	0.116	-0.186	0.265	0.581	0.098	0.023
9	0.790	0.316	0.106	-0.430	-0.085	-0.002	0.101	-0.067
10	0.641	0.325	0.177	0.126	-0.001	0.092	0.289	0.229
11	0.073	0.131	0.909	-0.044	0.159	-0.095	0.090	0.148
12	0.219	0.573	-0.100	0.155	-0.501	0.327	0.140	-0.147
13	0.012	0.527	0.483	0.212	0.549	-0.032	0.084	0.141
14	0.290	-0.672	0.349	-0.351	-0.237	0.191	0.084	0.155
15	0.767	-0.412	-0.318	-0.171	0.132	0.154	-0.029	0.079
16	0.937	0.030	-0.101	-0.117	-0.043	0.013	0.188	-0.067
17	0.819	0.148	0.015	0.363	0.247	-0.009	0.038	-0.095
18	0.112	0.097	0.154	0.173	-0.217	0.022	0.005	0.131
19	0.025	-0.038	0.544	-0.067	-0.124	-0.026	0.162	0.281
20	0.656	0.091	-0.309	-0.526	-0.061	-0.027	0.149	0.003
21	0.465	0.394	-0.027	-0.164	0.492	-0.001	-0.310	0.030
22	0.076	0.717	-0.022	0.164	-0.270	0.467	-0.003	-0.121
23	0.411	0.515	-0.116	-0.013	0.297	-0.078	0.195	0.039
24	0.696	0.180	-0.388	-0.237	0.322	0.023	-0.083	0.158
25	0.666	-0.274	-0.354	-0.126	0.236	-0.044	0.027	0.220
26	0.798	0.339	-0.147	-0.023	0.004	-0.027	-0.107	-0.093
27	0.886	0.016	-0.087	0.224	-0.174	-0.106	0.196	-0.109
28	0.916	0.033	-0.065	0.228	-0.115	-0.125	0.176	-0.110
29	0.840	0.191	-0.194	0.310	-0.054	-0.129	0.074	-0.113
30	0.889	0.051	-0.003	0.154	0.228	0.029	0.069	-0.168
31	0.763	-0.071	0.164	-0.149	0.347	0.190	-0.253	-0.050
32	0.911	-0.200	0.086	0.067	-0.183	-0.049	-0.023	-0.042
33	0.588	0.534	0.156	-0.040	-0.123	-0.093	-0.342	0.111
34	0.299	0.775	-0.327	-0.029	-0.089	-0.075	-0.073	-0.069
35	0.845	-0.050	-0.225	0.024	0.013	0.217	-0.147	0.197
36	0.709	-0.504	-0.083	0.352	-0.014	0.010	-0.116	0.176
37	0.711	-0.412	-0.139	0.338	-0.155	0.040	-0.188	0.225
38	0.808	-0.190	0.189	0.458	-0.084	-0.083	-0.098	0.038
39	0.739	0.379	0.171	0.382	0.200	-0.137	-0.062	-0.096
40	0.131	0.277	0.417	-0.056	-0.305	0.188	-0.340	0.013
41	0.836	-0.320	0.296	-0.059	-0.010	0.099	-0.155	-0.029
42	0.610	-0.567	0.359	0.138	-0.055	0.003	-0.041	-0.170
43	0.734	-0.184	0.387	0.371	-0.022	-0.122	-0.064	-0.103
44	-0.039	-0.197	0.535	-0.270	0.064	0.176	-0.146	-0.565
Latent root	18.060	5.522	4.461	3.051	2.510	1.315	1.066	1.059
Variance (%)	41.046	12.55	10.139	6.933	5.705	2.989	2.423	2.406

The scores of the second principal component were plotted against those from PC1 in Figure 16. No definite group separation could be distinguished along the PC1 axis. Beside an expression of general variation common to all groups, this dimension did not suggest any evident interpretation. On the other hand, PC2 distinctly segregated samples from year 1 and 2. This principal component, carrying high loadings for peaks 11, 14, and 34, reflected a form of year to year variability. As expressed in the species study, slight but notable decreases in peak resolution were also observed in the three mentioned GC areas. Although not disproving the explanation inherent to the biological nature of the chum salmon samples themselves, the separation of the year-groups along PC2 was likely due to chromatographic factors since a comparable situation emerged in all species (Figure 10).

Linear discriminant analysis was performed on the first eight principal components extracted from the chum salmon data. Prior probabilities were specified to be proportional to the sample sizes. Five out of eight principal components were found significant at the 5% level or below as indicated in the upper portion of Table 15 dealing with the univariate statistics. The four largest contributors toward group discrimination were PC3, PC5, PC6, and PC4 in decreasing order. Although they account for less variance of the dataset than the initial principal components, PC3-PC6 were selected over PC1-PC2 for group discrimination. This situation underlined the danger of rejecting components accounting for small amount of variation when they are to be used in subsequent data

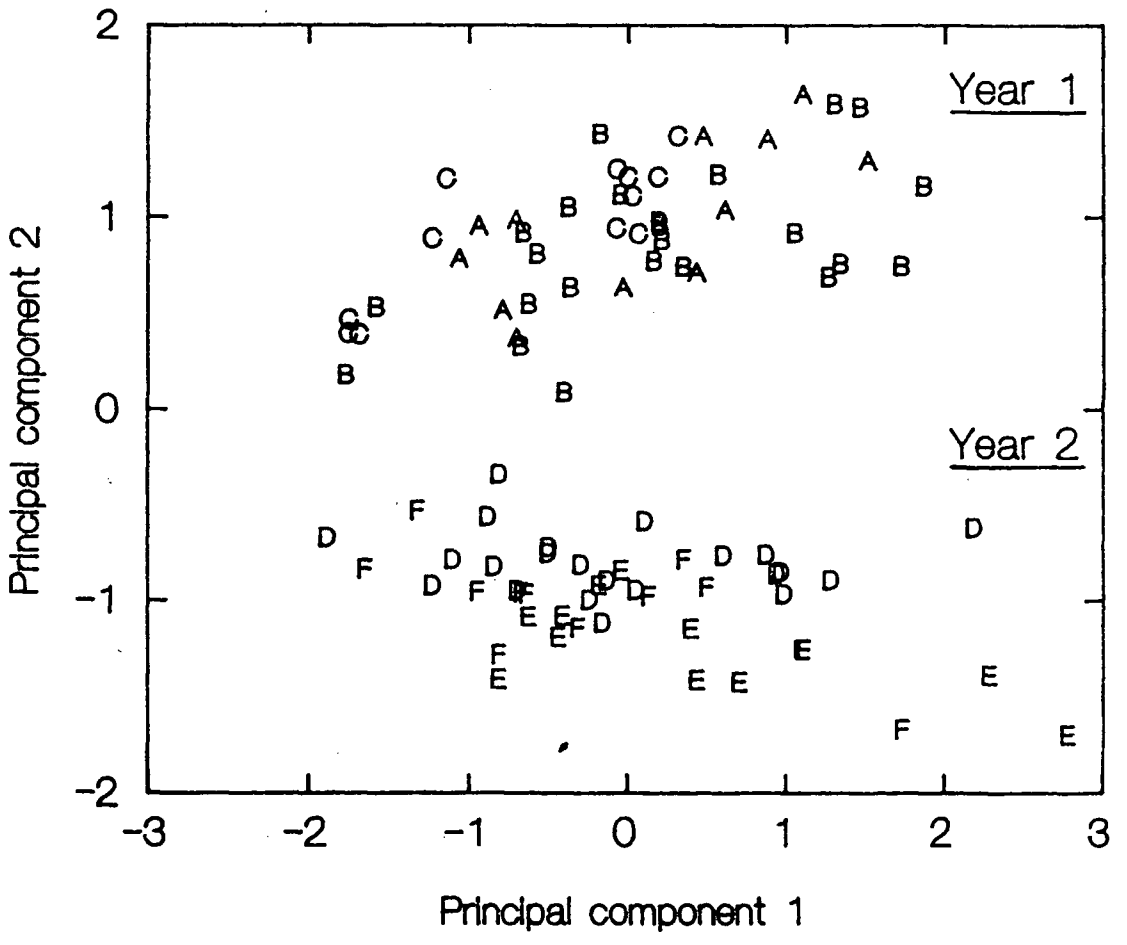

Figure 16. Plot of the first two principal component scores for the canned chum salmon tested at three sexual maturity stages (A, silver-bright/year 1; B, semi-bright/year 1; C, dark/year 1; D, silver-bright/year 2; E, semi-bright/year 2; F, dark/year 2).

Table 15. Univariate and multivariate test statistics of discriminant analysis on the first eight principal components from volatiles of canned chum salmon tested at three sexual maturity stages.

Variable	num df,den df	F
Univariate		
PC1	2,91	3.4606 *
PC2	2,91	1.1410
PC3	2,91	31.9029 ***
PC4	2,91	13.6433 **
PC5	2,91	23.1504 ***
PC6	2,91	16.6785 ***
PC7	2,91	0.1749
PC8	2,91	0.8634
Multivariate		
Wilk's lambda = 0.0726	16,168	28.4686 ***
Pillai trace = 1.3659	16,170	22.8872 ***
Hotelling-Lawley trace = 6.7338	16,166	34.9318 ***
Roy's largest root = 5.6683	80,85	60.2256 ***
Canonical		
LR_1 = 0.0726	16,168	28.4686 ***
LR_2 = 0.4841	7,85	12.9388 ***

* Significant difference at 0.05 level.
** Significant difference at 0.01 level.
*** Significant difference at 0.0001 level.
LR stands for Likelihood ratio.

treatments. Sometimes only the largest principal components are retained as predictors. However, as Dunteman (1984) pointed out, "There is no compelling reason to suspect that larger components are better predictors of some dependent variable than smaller components."

PC3 and PC5, the principal components with high F ratios, received high loadings from 2-methyl propanal and benzene, and from ethanol, dimethyl sulfide, 2-methyl butanal, 2,2-dimethyl propanal, and acetic acid, respectively. Ethanol also had a high loading with PC6 along with hexane and toluene while PC4 gathered correlations above 0.5 from methane thiol, butane, and unknown compound 2. The last three volatiles as well as two others from PC5 possessed negative loading values. Among these volatiles, methane thiol and dimethyl sulfide are compounds that could be generated from sulfur-containing amino acids. As spawning migration progresses, salmon are using their non-collagenous and cellular protein supplies and are not replenishing them (Love, 1970; Aksnes et al., 1986). The utilization of amino acids for metabolic processes could therefore decrease the content of sulfur-containing amino acids available for the production of methane thiol and dimethyl sulfide. Ethanol, used in the past as an indicator of decomposition, was also influenced by the changes of sexual maturity. As a consequence, sexual maturity of chum salmon is a factor that could bias the result of quality determination when established on the basis of ethanol quantification only. The other species of Pacific salmon should be investigated with this regard in order to ascertain the quality classification of canned salmon of progressing sexual maturity.

In terms of multivariate statistics of the linear discriminant analysis, all tests were significant at $P<0.0001$ (Table 15). They indicated that the principal components taken together affirmed the presence of between-group differences. Canonical analysis shown at the bottom part of Table 15 revealed that the two possible canonical functions were necessary to capture the differences among the three maturity stages of chum salmon as judged by the high levels of significance computed ($P<0.0001$). Table 16 presents the standardised coefficients allocated to each principal component for both canonical variates. Three of the important factors explaining the between-group variables, found in Table 15, i.e., PC3, PC4, and PC5, occupied predominant positions having large coefficients for CV1. On the other hand, the second canonical variate was monopolized by PC6. The graphical representation of the scores of the two canonical functions is shown in Figure 17. Scores of the three maturity stages, namely silver-bright, semi-bright, and dark, were distinctly regrouped. The centroids of each group were not plotted but the density circles about the centroids of the bivariate dispersions were drawn at the probability level of 0.50. Group memberships were computed from the Mahalanobis distances of the canonical scores. Two samples in each of the silver-bright and semi-bright maturity stages were misclassified using the resubstitution method to calculate the error count estimate. Thus, this linear discriminant analysis yielded an apparent error rate of 3.2%.

Table 16. Standardized canonical variate coefficients for discrimination of canned chum salmon tested at three sexual maturity stages.

Dependent variable	Canonical variate	
	1	2
PC1	-0.4833	-0.3971
PC2	0.4282	-0.0476
PC3	1.5820	0.5809
PC4	1.0707	-0.5645
PC5	1.5627	-0.2735
PC6	-0.0487	1.0246
PC7	0.0973	0.1009
PC8	0.0703	-0.2654

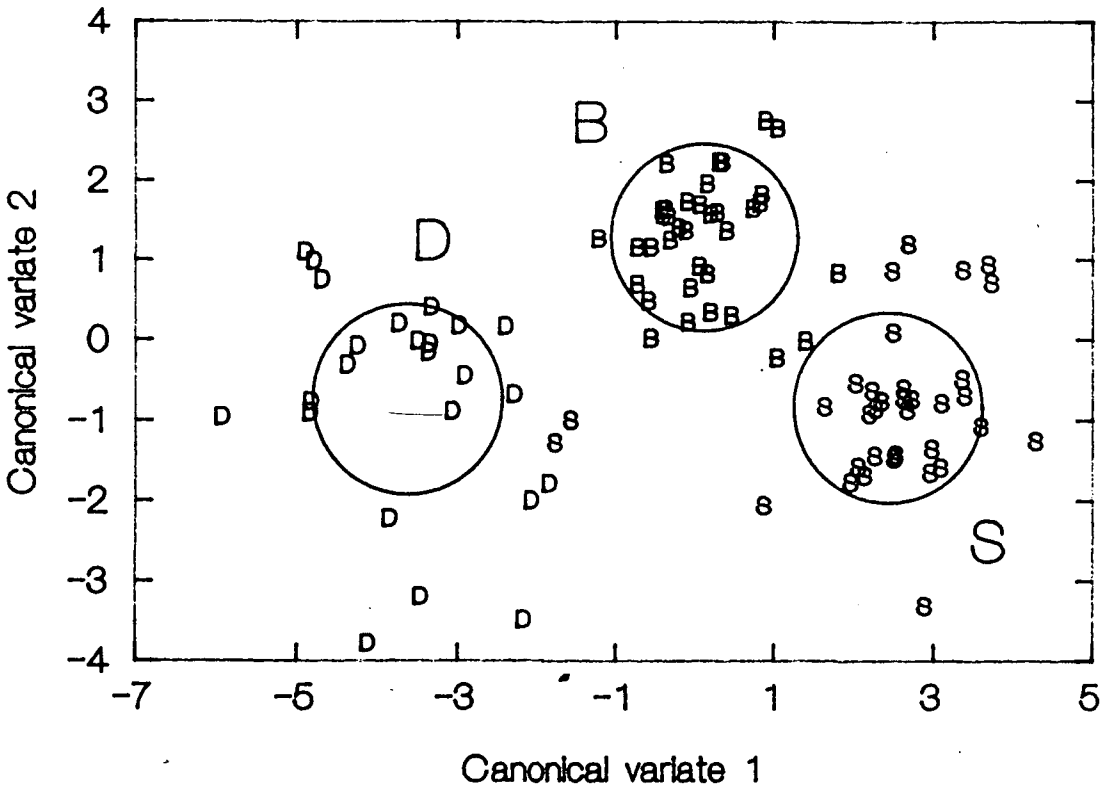

Figure 17. Plot of the two canonical variate scores for canned chum salmon tested at three sexual maturity stages (S, silver bright; B, semi-bright; D, commercial dark).

Figure 17 can also be interpreted from a different point of view. In addition to the three distinctive areas where the groups had dispersed, a U-shape pattern also emerged: silver-bright at the bottom-right, semi-bright at the top-centre, and dark at the bottom-left. During the spawning migration of salmon, which begins in the ocean and ends at the spawning beds, gradual physiological changes occur. This process of sexual maturation proceeds in a continuous manner. However, categorization of salmon in different stages based on physical appearance is currently done by processors to establish quality grading systems. In light of this, the results of Figure 17 can also be viewed as an uninterrupted sequence from the silver-bright stage to the commercial dark stage behaving according to a quadratic function with a maximum inversion point at the semi-bright stage.

Before carrying out discriminant analysis, Bartlett's tests for homogeneity of variance were computed on the first ten principal components of chum maturity data and results are found in Table 17. Five principal components out of eight did not show any statistical sign of heteroscedasticity across the maturity stages. However, the chi-square values associated with PC3 presented a significant difference at the 0.0001 level while PC2 and PC8 were significant at the 1% probability. The multivariate test was also highly significant ($P<0.0001$) indicating that the principal components, when considered in a joint fashion, were not able to satisfy the parametric assumption of homogeneity of variance.

Table 17. Bartlett's tests for homogeneity of within group variance-covariance between canned chum salmon tested at three sexual maturity stages for the first eight principal components.

Variable	df	Chi-square
Univariate		
PC1	2	0.290
PC2	2	10.872 **
PC3	2	30.027 ***
PC4	2	0.020
PC5	2	5.460
PC6	2	2.769
PC7	2	3.426
PC8	2	10.724 **
Multivariate		
PC1-10	72	302.937 ***

* Significant difference at 0.05.
** Significant difference at 0.01.
*** Significant difference at 0.0001.

Results of the Kolmogorov-Smirnov normality tests on the maturity data are shown in Table 18. Scores of four out of eight principal components departed from normality for silver-bright chum but only PC2 and PC3 were in a similar situation for semi-bright chum. In addition, the normality assumption was concurrently respected by all principal components for dark chum.

In order to respond to the heterogeneity of variance-covariance found between the chum salmon maturity stages, quadratic discriminant analysis was carried out on the same eight principal components. The few cases of non-conformity to the assumption of normality also prompted the re-analysis of the sexual maturity data with non-parametric discriminant analysis using the Epanechnikov kernel. Figure 18 compared the error count estimate methods, resubstitution and cross-validation, with regard to the smoothing parameter of the kernel estimator. In accordance to what was found in the section on the analysis of the species data, the resubstitution method underestimated the error rates for small values of r but eventually reached perfect agreement as the smoothing parameter increased. The lowest error rate estimate along the cross-validation curves was associated with a r of 5. At that point, however, there was a discrepancy between the two error estimation methods. Albeit a slight difference, it indicated that the error rates estimated by the two methods may not always be concordant at the optimal cross-validative conditions.

The error count results computed using both estimation methods for all three discriminant analyses were compiled in Table 19. Based on

Table 18. Kolmogorov-Smirnov normality test of canned chum salmon tested at three sexual maturity stages within the first eight principal components.

Variable	Maximum difference		
	Silver-bright (n=34)	Semi-bright (n=36)	Dark (n=24)
PC1	0.119	0.117	0.102
PC2	0.209 **	0.190 **	0.144
PC3	0.160 *	0.154 **	0.157
PC4	0.177 **	0.087	0.085
PC5	0.102	0.105	0.163
PC6	0.160 *	0.114	0.104
PC7	0.105	0.080	0.147
PC8	0.161	0.087	0.169

* Significant difference at 0.05 level based on Lilliefors probability.
** Significant difference at 0.01 level based on Lilliefors probability.

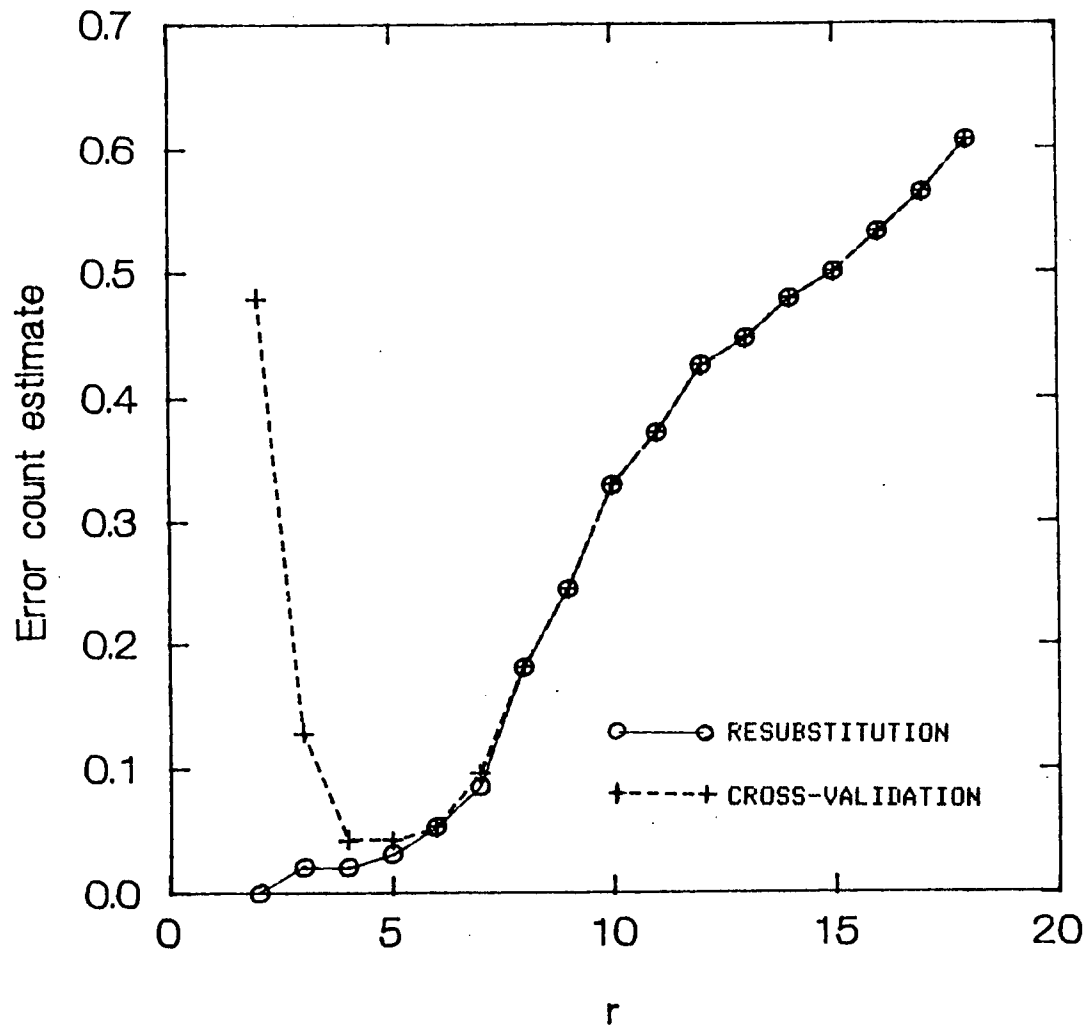

Figure 18. Comparison of error count estimate method against the smoothing parameter of the Epanechnikov kernel classifier on principal component scores of canned chum salmon tested at three sexual maturity stages.

Table 19. Comparison of error count estimation methods for different discriminant analysis (DA) functions applied to the first eight principal components from canned chum salmon tested at three sexual maturity stages.

DA function[a]	Error count estimate[b]			
	Silver-bright (n=34)	Semi-bright (n=36)	Dark (n=24)	% Total (n=94)
Resubstitution				
linear	0.059 (2)	0.056 (2)	0.000 (0)	4.3
quadratic	0.059 (2)	0.000 (0)	0.000 (0)	2.1
nonparametric	0.088 (3)	0.000 (0)	0.000 (0)	3.2
Cross-validation				
linear	0.059 (2)	0.083 (3)	0.000 (0)	5.3
quadratic	0.059 (2)	0.056 (2)	0.000 (0)	4.3
nonparametric	0.118 (4)	0.000 (0)	0.000 (0)	4.3
Prior prob.	0.362	0.383	0.255	

[a] The nonparametric method consists of the Epanechnikov kernel with r=5.
[b] The numbers in parentheses are the misclassifications associated with the error count estimates.

resubstitution, the quadratic and kernel analyses improved the error rates compared to those obtained by the linear functions, but the differences were small. All total error rates calculated with the cross-validation method were larger than that of resubstitution thereby bringing again forward the bias of the latter method. Yet the two sets of estimated error rates were not as disparate as those found with the species data (Table 13).

In terms of cross-validation, the total error rate was lower for the non-parametric kernel estimator and the quadratic than for the linear discriminant functions. The error counts nevertheless remained in the 4 to 5% region. These results therefore suggested that even though the parametric assumptions did not hold true, the misclassification rates could still be kept low. Besides dark chum and two instances for semi-bright chum, most cases of non-normality were found for silver-bright chum salmon data (Table 18). Since PC2 and PC8 were not involved in group separation for linear discriminant analysis as indicated by a nonsignificant univariate F ratio (Table 15), the strong non-normality of these components did not bear as much negative influence on the membership classification results as PC3, PC4 and PC6 seemingly could have. Hence, membership classification could have reflected a certain stability or robustness to a few non-normal anomalies. In addition, PC3 being the only major discriminator afflicted by heterogeneity of variance was expected to negatively affect the results of LDA (Table 17). On one hand, an explanation among others already discussed in the section on the salmon species data refers to the distance between group means. As the distances

between group centroids and their overlaps in the multivariate space increases, the effects of heteroscedasticity on misclassification rates diminishes (Dillon and Goldstein, 1984). In these circumstances, linear and quadratic discriminant functions may appear to be equally effective. On the other hand, the two samples of silver-bright chum that were categorized as dark chum (Figure 17) could have been outliers with characteristics uncommon to their group. If they were removed from the analysis, the number of rejections of the parametric assumptions could likely be decreased and thereby help to account for the small differences between discriminant functions.

3. MVA of volatiles from canned pink salmon during refrigerated decomposition

Unlike the previous SHGC chromatograms obtained for the species and sexual maturity studies, the patterns of volatiles from canned pink salmon during the storage periods possessed qualitative as well as quantitative differences. Figure 19 shows typical chromatograms from canned pink salmon at day 0, day 8, and day 13 of year 1 which respectively corresponded to grade A, B, and reject, as determined prior to canning. Peaks 3 (ethanol), 18 (3-methyl-1-butanol), and 19 (2-methyl-2-butenal) have been labelled and were some of the volatiles that storage time influenced. Their peak sizes increased with time, and the sensory grade associated with these typical samples sequentially progressed from grade A to grade reject. The peak areas of these three volatile compounds have been translated into actual concentrations using standard curves. The ranges of concentrations within which all measurements fell, are presented

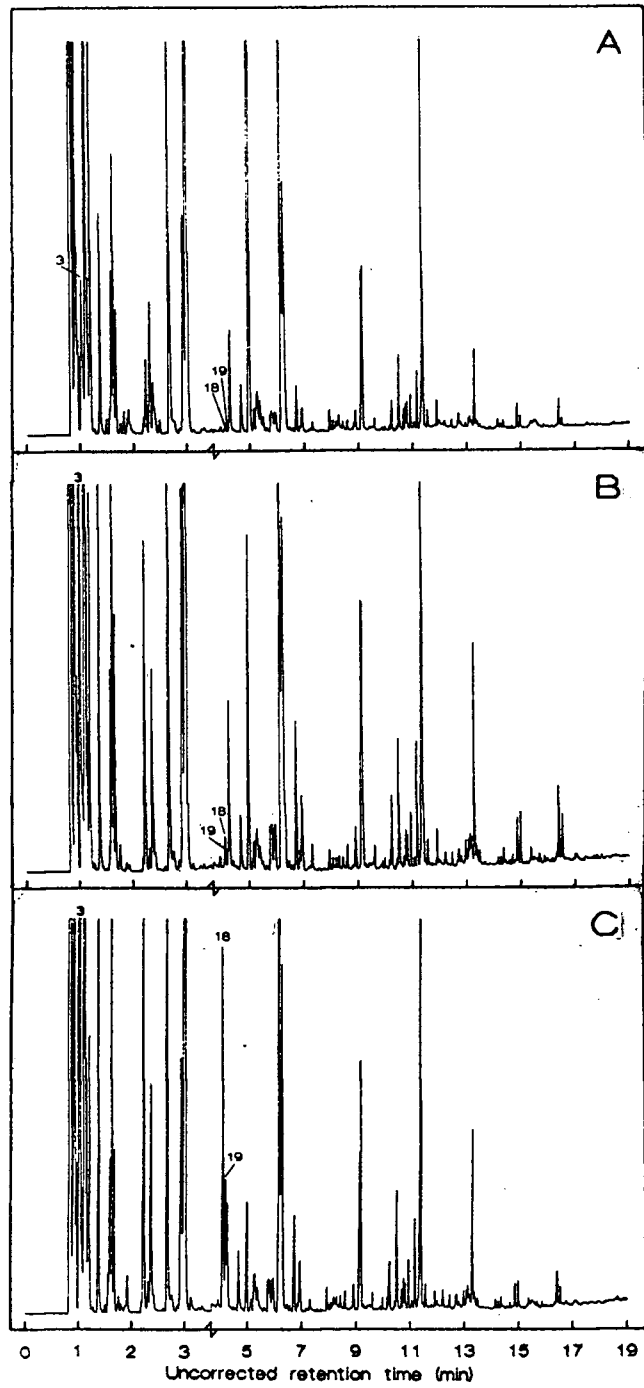

Figure 19. Chromatogram of volatiles from canned pink salmon during the refrigerated storage of year 1. (A, day 0/grade A; B, day 8/grade B; C, day 13/grade reject).

for each grade level in Table 20. With changing grade levels, both the increase in concentration and the spread of the ranges appeared to augment in a non-linear manner. Peak 18 (3-methyl-1-butanol) was the only one, among 44 analyzed volatiles, that was not detected in grade A canned pink salmon. Regarding the comparison of the obtained values for ethanol with those of Hollingworth et al. (1986), a 1000-fold factor in scale could be observed. The main explanation lies in the sample preparation and analysis. Results of this study were obtained using an automated method sampling headspace volatiles above heated salmon flakes while Hollingworth et al. (1986) manually injected headspaces of liquid samples equilibrated at room temperature.

Common factor analysis (CFA) with varimax rotation was the first multivariate method performed on the 44 volatile compounds analyzed throughout the refrigerated storage study. As Table 21 indicates, nine factors were allocated eigenvalues of 1.0 and over, explaining nearly 88% of the common variance. The volatiles outlined in Figure 19, i.e., peaks 3, 18, and 19, all received loadings above 0.9 for factor 3. The standardized peak area of these three volatiles were plotted in Figure 20 for both years of the refrigerated storage study. Constant values over a period of time, followed by an exponential increase was a pattern common to all peaks. Peak 18 (3-methyl-1-butanol) however was distinct in that it was undetected up to a certain point in time, day 8 for year 1 and day 13 for year 2. By the time peak 18 appeared on the chromatogram, the fish from which the samples originated had consistently been classified as grade B.

Table 20. Concentration ranges of three volatile compounds from canned pink salmon of different quality grades.[a]

Grade[b]	Concentration ($x10^{-3}$ppm)		
	Ethanol[c]	3-methyl-1-butanol[d]	2-methyl-2-butenal[e]
A	2.6 - 37.0	-	0.0 - 5.0
B	31.4 - 210.3	1.7 - 13.7	3.6 - 16.1
reject	263.7 - 878.9	18.7 - 128.0	19.3 - 138.7

[a] Measurements obtained using a headspace sampler with a 3 mL loop.
[b] Grade of canned salmon based on assessment prior to canning.
[c] FID response = 2920.2 x [ethanol (ng/mL)], r^2 = 0.997, n = 22, S err = 24.2.
[d] FID response = 1097.0 x [3-ME-1-butanol (ng/mL)], r^2 = 0.973, n = 15, S err = 28.2.
[e] FID response = 364.9 x [2-ME-2-butenal (ng/mL)], r^2 = 0.984, n = 21, S err = 6.2.

Table 21. Loadings of the first nine varimax rotated factors from factor analysis of canned pink salmon volatiles of the refrigerated storage study.

Peak	Factors								
	1	2	3	4	5	6	7	8	9
1	0.372	0.674	0.080	0.096	0.019	0.182	0.105	-0.143	-0.131
2	0.232	-0.032	-0.368	0.134	0.242	-0.072	-0.195	0.581	0.184
3	0.145	0.175	0.904	0.255	0.086	-0.027	0.057	-0.135	0.005
4	0.530	0.466	-0.041	0.248	0.430	0.180	0.013	0.097	0.022
5	0.297	0.826	0.243	0.258	-0.039	0.111	0.181	-0.065	-0.016
6	0.141	0.122	-0.066	-0.097	-0.191	-0.087	0.123	-0.015	0.003
7	0.285	0.270	0.156	0.868	0.069	0.007	-0.035	0.080	0.085
8	-0.053	0.115	-0.050	0.024	0.157	0.898	0.029	-0.007	-0.031
9	0.582	0.387	-0.022	0.387	0.301	-0.399	-0.079	0.219	0.029
10	0.585	0.222	0.264	0.346	0.306	-0.059	-0.030	0.220	0.029
11	0.168	0.173	0.306	0.876	0.187	-0.015	0.013	-0.110	0.048
12	0.013	0.057	-0.002	-0.052	0.163	-0.045	0.044	0.192	0.132
13	0.176	0.155	0.345	0.862	0.137	-0.029	0.004	0.007	0.039
14	0.039	0.144	0.205	0.152	-0.285	-0.071	0.546	-0.074	-0.198
15	0.360	0.374	0.217	0.040	0.542	0.166	0.501	-0.153	0.026
16	0.558	0.522	-0.008	0.143	0.277	0.083	-0.071	0.111	-0.018
17	0.796	0.084	-0.092	0.143	0.359	0.043	0.028	0.244	-0.013
18	0.059	0.111	0.963	0.171	0.010	-0.002	0.110	-0.071	-0.047
19	0.031	0.108	0.956	0.197	-0.016	-0.001	0.105	-0.065	-0.059
20	0.549	0.109	0.182	0.077	0.030	0.123	0.209	-0.012	-0.115
21	0.049	-0.003	-0.022	0.209	0.822	0.024	0.045	0.184	0.226
22	-0.059	-0.128	-0.148	-0.081	0.122	-0.124	-0.247	0.700	0.081
23	0.566	-0.163	-0.038	-0.103	0.078	-0.002	-0.041	0.111	-0.057
24	0.488	0.217	0.196	0.128	0.650	0.086	0.250	0.000	0.084
25	0.154	0.060	0.121	-0.126	0.172	0.146	0.783	-0.196	0.050
26	0.472	0.020	0.019	0.207	0.636	0.011	-0.163	0.280	0.080
27	0.421	0.761	0.256	0.297	0.076	-0.014	0.097	-0.044	0.063
28	0.549	0.704	0.268	0.277	0.074	0.002	0.086	-0.023	0.040
29	0.775	0.271	0.091	0.125	0.073	0.195	-0.118	-0.120	-0.113
30	0.570	0.467	0.004	0.006	-0.068	0.227	0.116	-0.076	0.003
31	0.614	0.061	0.213	0.233	0.477	-0.260	-0.023	-0.004	-0.013
32	0.858	0.288	0.184	0.182	0.129	0.003	0.046	-0.047	0.051
33	0.645	0.077	-0.028	0.435	0.266	-0.021	-0.149	0.280	0.230
34	0.074	-0.016	-0.069	0.112	0.143	-0.036	-0.013	0.134	0.726
35	0.358	0.063	0.031	-0.060	-0.164	0.794	0.106	-0.118	-0.023
36	0.657	0.238	0.085	-0.010	-0.221	0.072	0.462	-0.152	0.067
37	0.797	0.231	0.136	0.104	0.122	0.071	0.273	-0.168	0.241
38	0.936	0.168	0.070	0.166	0.026	-0.065	0.100	-0.030	0.130
39	0.856	0.064	-0.041	0.169	0.297	0.003	-0.059	0.206	0.019
40	0.181	0.140	0.021	-0.074	-0.472	0.353	0.257	-0.205	0.180
41	0.775	0.288	0.205	0.180	0.111	0.210	0.147	-0.098	-0.148
42	0.880	0.171	-0.041	-0.010	-0.112	0.093	0.166	-0.085	-0.008
43	0.858	0.180	0.005	0.032	-0.159	0.047	0.123	-0.113	-0.006
44	0.191	-0.017	0.123	-0.042	-0.273	0.015	0.046	-0.333	-0.211
Latent root	11.543	4.083	3.707	3.624	3.602	2.148	2.015	1.718	0.999
Variance (%)	30.371	10.743	9.755	9.535	9.476	5.652	5.303	4.520	2.630

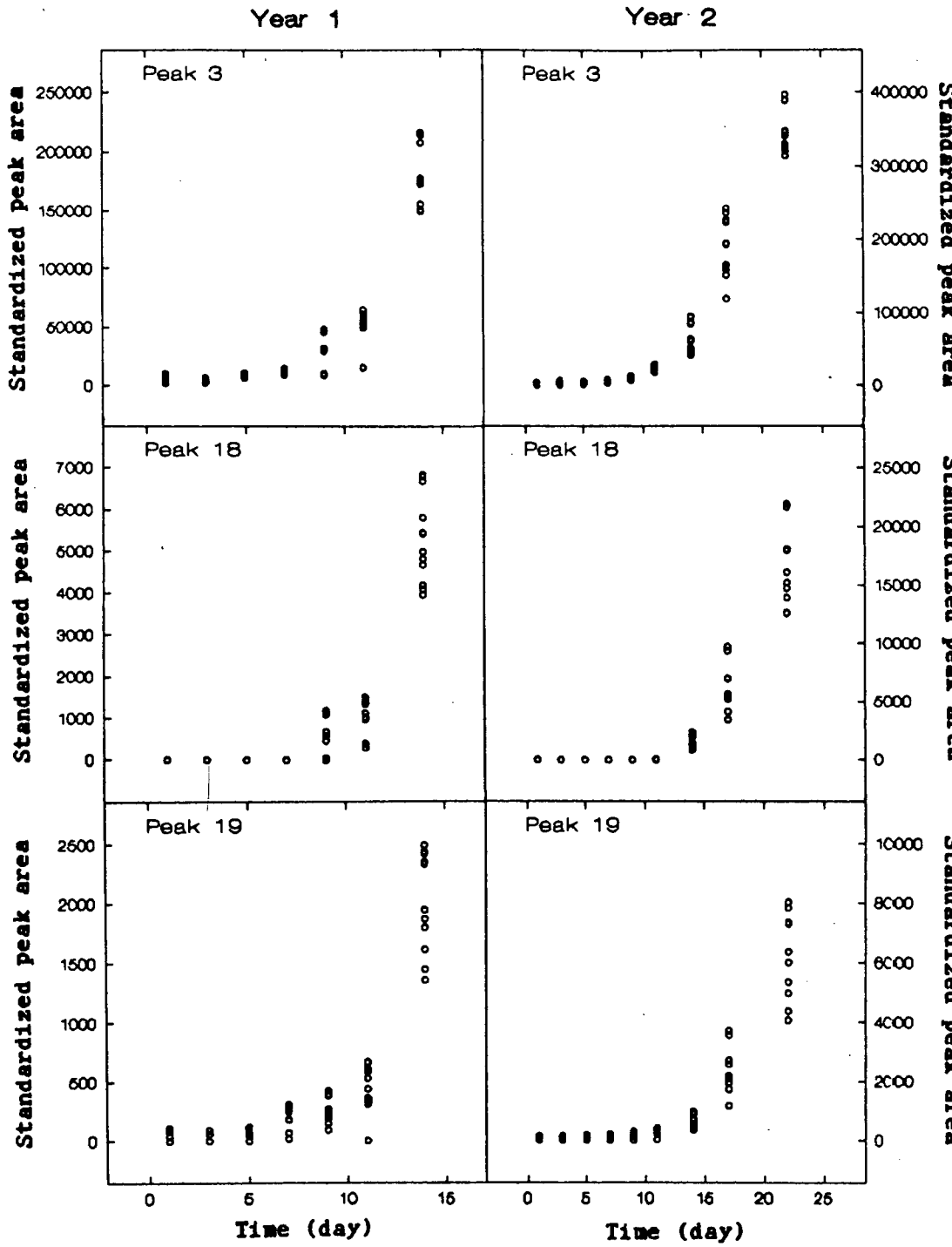

Figure 20. Plots of volatiles from canned pink salmon of the refrigerated storage study with high loadings for factor 3.

Upon further investigating the results of CFA in conjunction with refrigerated storage time, factor 4 also appeared to be influenced by storage. Based on Table 21, three volatile compounds, peak 7 (2-methyl propanal), peak 11 (benzene), and peak 13 (2,2-dimethyl propanal), were allocated high loadings by factor 4. As was expected of variables that were correlated to each other, peaks 7, 11, and 13 had similar trends (Figure 21); they increased in concentration near the beginning of storage, and levelled off towards the end of the experiments. In addition, it is worth noting that the spread in peak area increased with time for all six volatiles just discussed (Fig. 20 and 21).

Principal component analysis was also carried out with a varimax rotation on the same data matrix gathered from canned salmon of the refrigerated storage study. Figure 22 shows the 44 loadings as vectors in the subspace spanned by PC4 and PC5. Loadings with absolute values higher than 0.2 have been labelled. Peaks 3, 18, and 19, and peaks 7, 11, and 13 were found in the vicinity of each other and each group of peaks dominated PC4 and PC5, respectively. Figures 23 and 24 are plots of scores of PC4 and PC5 during the refrigerated storage for years 1 and 2. The trends found in these figures were clearly comparable to the sets of graphs in Fig. 20 and 21. PC4 and PC5 essentially contained the same information as factors 3 and 4.

The results of CFA and PCA were alike in terms of peak grouping and confirm the presence of intercorrelations among several of the variables in the original dataset. Although PCA could be viewed as another type of

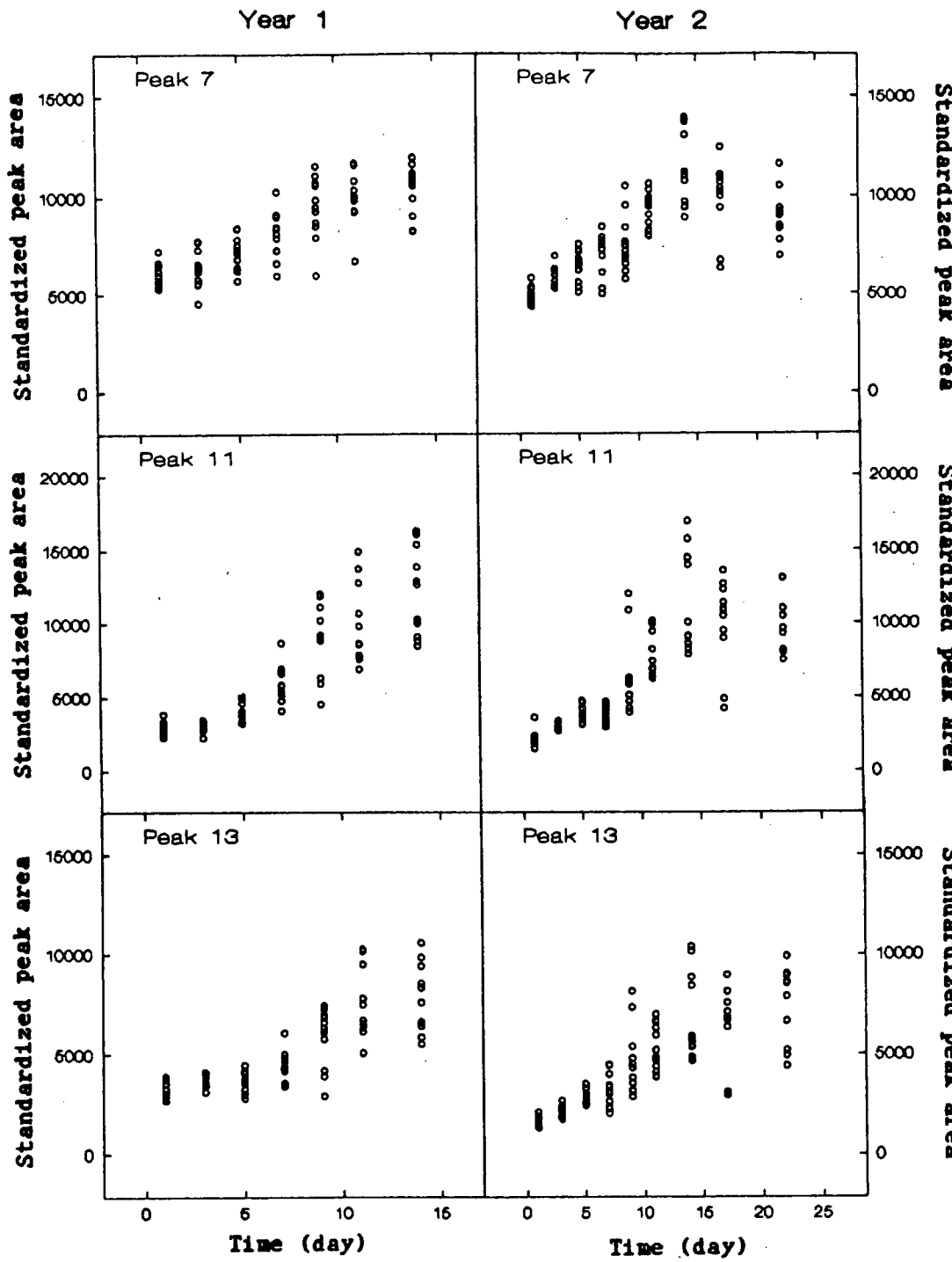

Figure 21. Plots of volatiles from canned pink salmon of the refrigerated storage study with high loadings for factor 4.

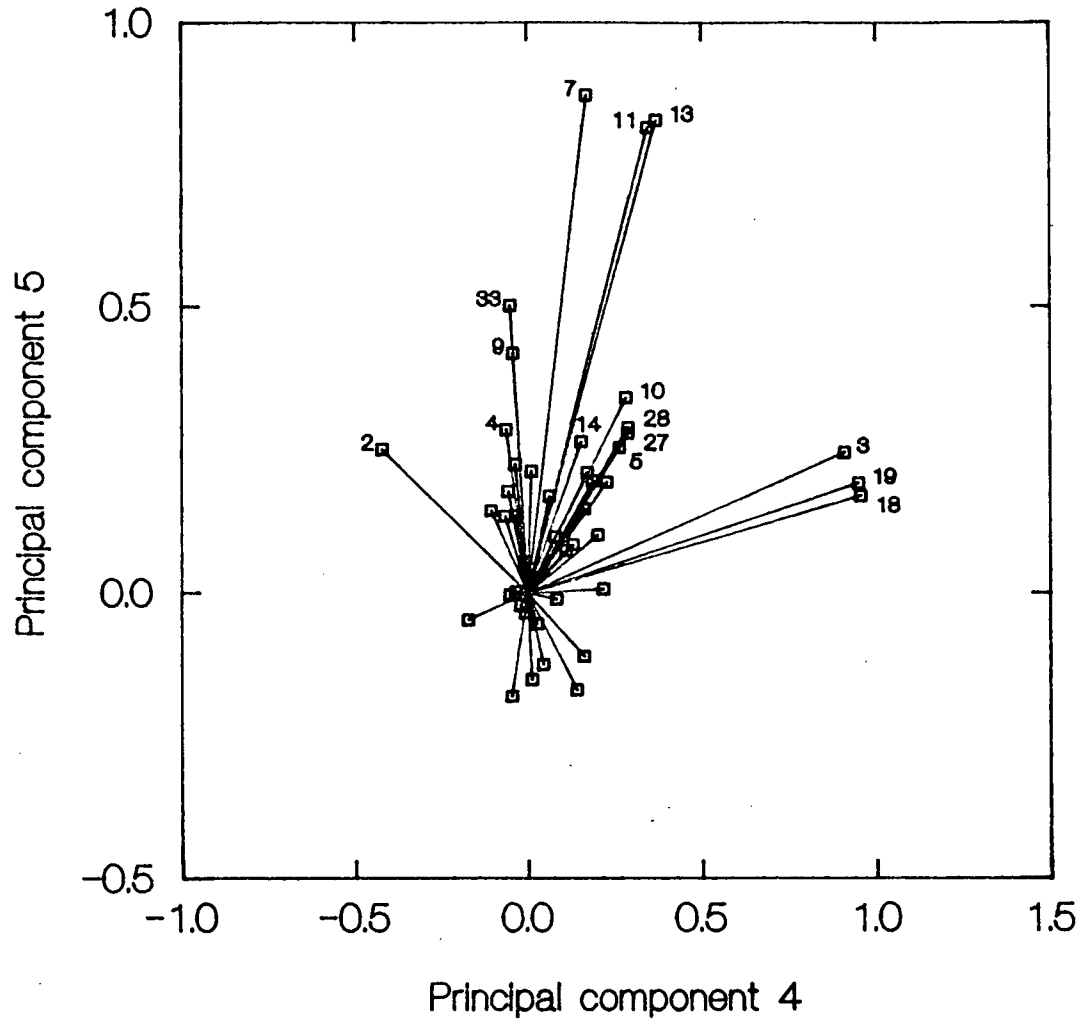

Figure 22. Projection of gas chromatographic peak variable loadings on principal components 4 and 5 for canned pink salmon of the refrigerated storage study.

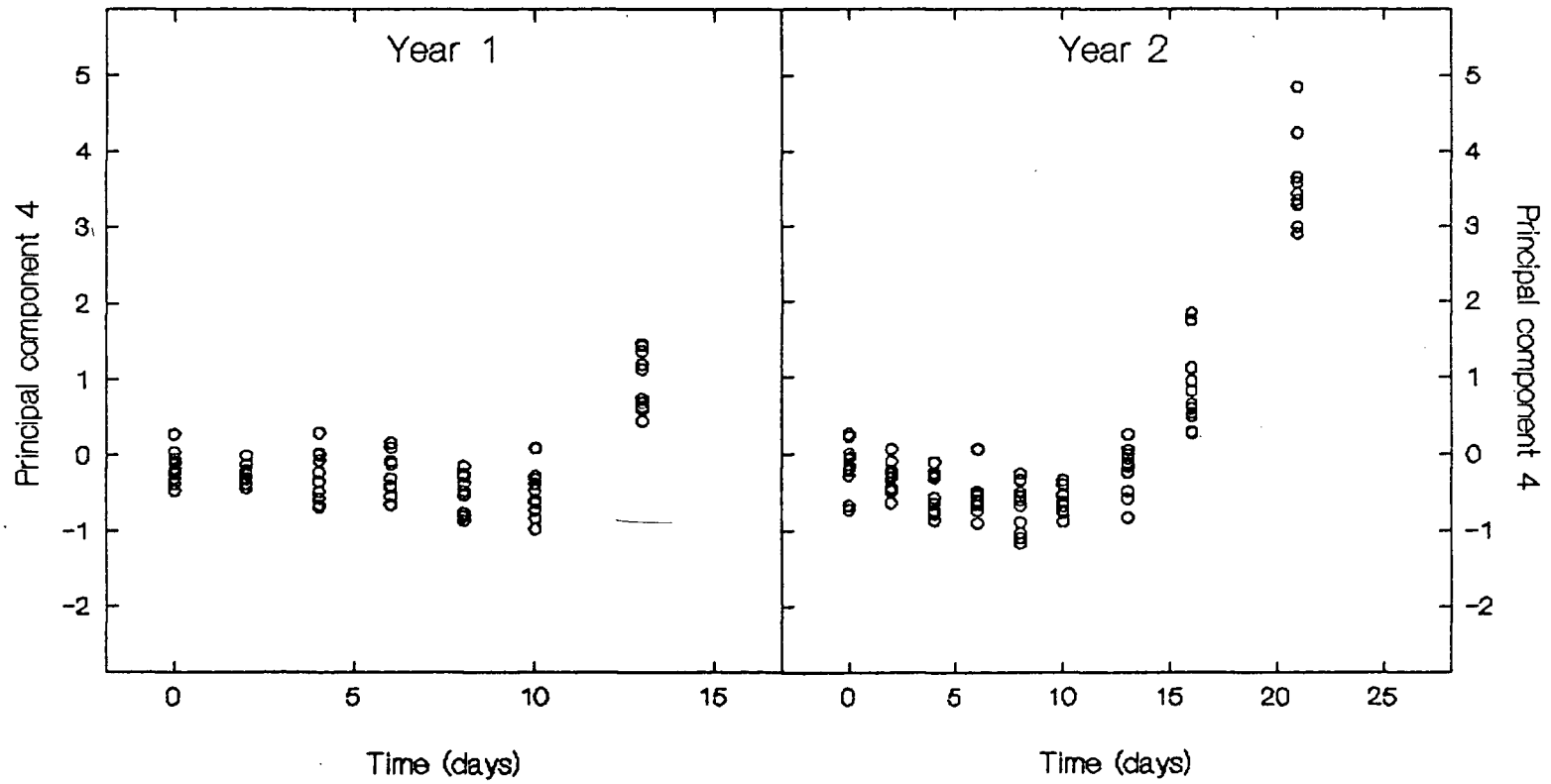

Figure 23. Plot of the scores of principal component 4 over refrigerated storage time for canned pink salmon.

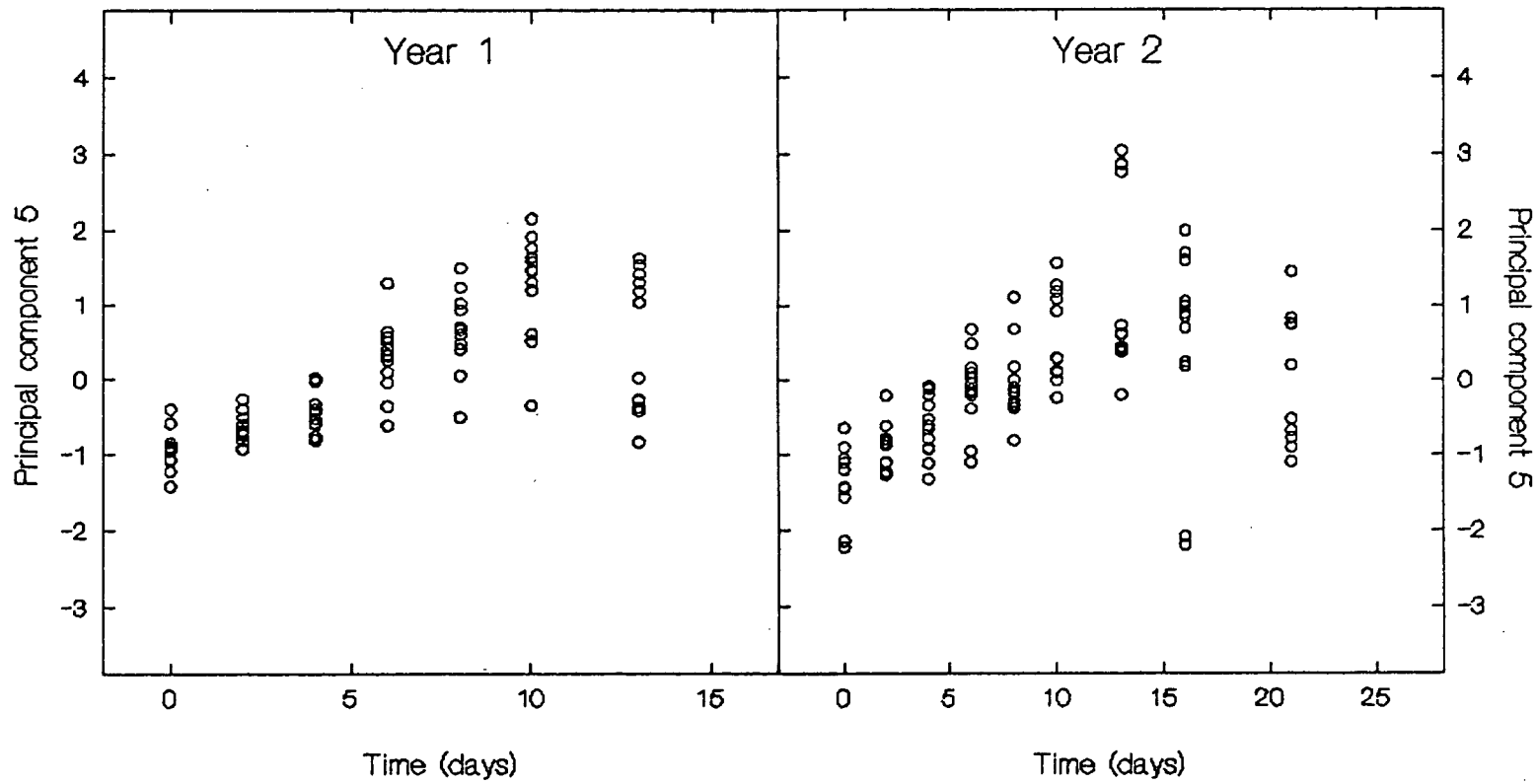

Figure 24. Plot of the scores of principal component 5 over refrigerated storage time for canned pink salmon.

factor analysis, there are theoretical differences between them. Nevertheless, these two techniques often yield solutions that are very similar (Dillon and Goldstein, 1984).

When PC4 and PC5 were plotted against each other, segregation of the 3 quality levels became apparent (Fig. 25). PC4 separated grade reject, found on the right side of the graph, from grade A and grade B located on the left. Referring back to Fig. 23, score values of PC4 remained relatively constant for some time but began to increase toward the end of the storage periods. These late changes were correlated with the increase in concentration of volatiles that received especially high loadings on PC4 (peaks 3, 18, and 19) and coincided with a shift of quality grade reject. Similarly, PC5 mainly served to vertically separate grades A and B. The increasing scores of PC5 from the start of storage up to a maximum point (Fig. 24) provided valuable information to discern samples of grade A from grade B (Fig. 25).

Table 22 regroups the results of univariate and multivariate Bartlett's tests for homogeneity of variance on peak variables and principal components involved in the above discriminant analyses. Important discriminating variables such as PC4, PC5, peaks 3 and 18 had significant Chi-square statistics at $P<0.05$. The multivariate probabilities that the peaks and principal components had equal variance-covariance matrices for all quality grades were very low ($P<0.0001$). Kolmogorov-Smirnov normality tests were also performed on the peaks and principal components (Table 23). Both sets of variables had instances

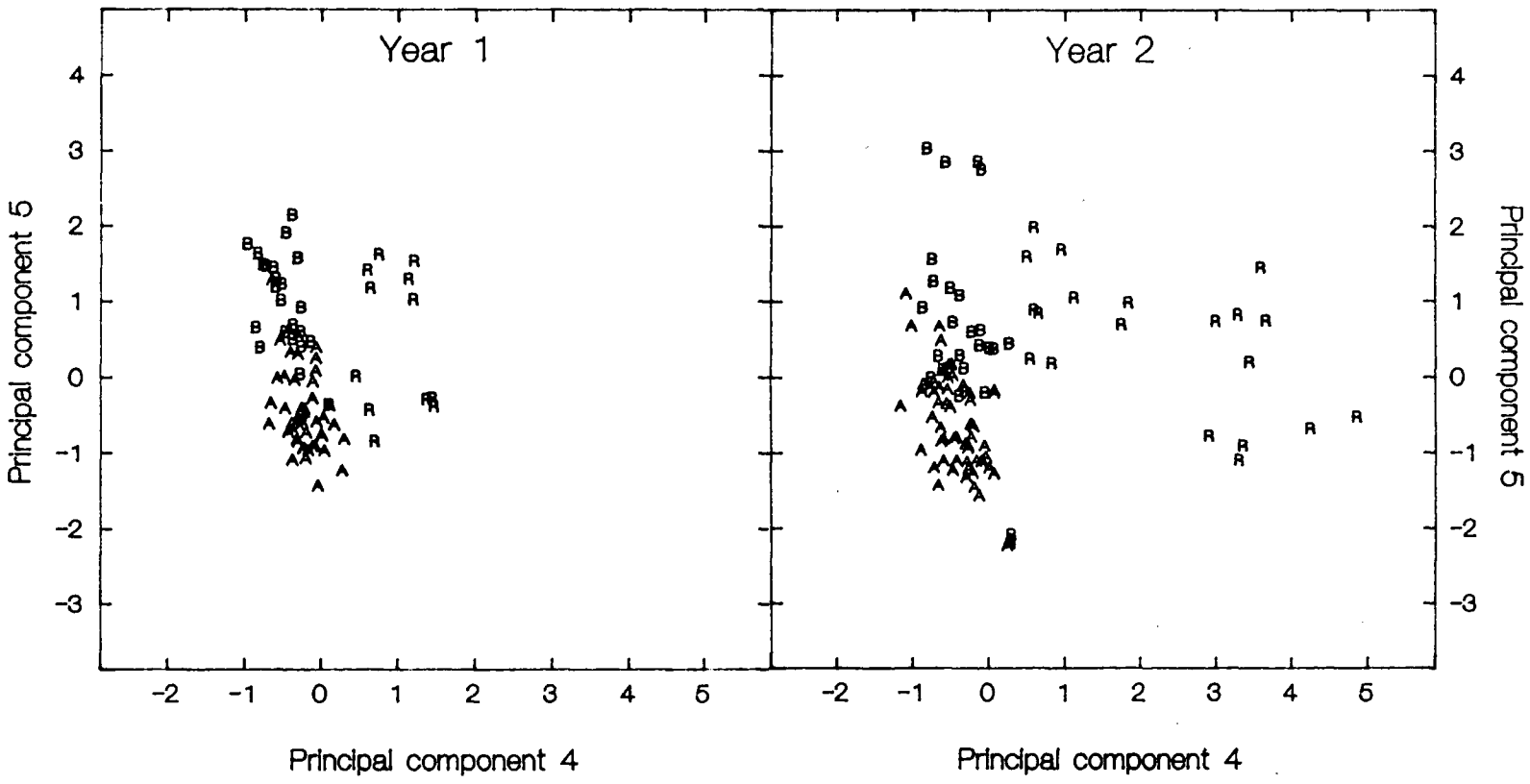

Figure 25. Plot of the scores of principal component 5 against 4 for canned pink salmon of the refrigerated storage study.

Table 22. Bartlett's tests for homogeneity of within group variance-covariance between quality grades of canned pink salmon for selected gas chromatographic peaks variables and the first ten principal components.

Variable	df	Chi-square
Univariate		
PC1	2	9.2574 **
PC2	2	16.8106 **
PC3	2	28.1296 ***
PC4	2	175.5999 ***
PC5	2	19.0639 **
PC6	2	2.3869
PC7	2	7.8339 *
PC8	2	21.9451 ***
PC9	2	3.4393
PC10	2	8.2366 *
Peak 3	2	503.4706 ***
Peak 7	2	5.6272
Peak 18	2	1504.9712 ***
Multivariate		
PC1-PC10	110	635.3260 ***
Peak 3,7,18	12	1867.8104 ***

* Significant difference at 0.05.
** Significant difference at 0.01.
*** Significant difference at 0.0001.

Table 23. Kolmogorov-Smirnov normality test of quality grades of canned pink salmon for selected gas chromatographic peaks and the first principal components.

Variable	Maximum difference		
	Grade A (n=107)	Grade B (n=48)	Reject (n=34)
PC1	0.0710	0.1380 *	0.1667 *
PC2	0.0843	0.1004	0.1116
PC3	0.0620	0.1134	0.1295 **
PC4	0.0822	0.0694	0.2108 *
PC5	0.0645	0.1320 *	0.1562
PC6	0.0754	0.0923	0.1238
PC7	0.1981 ***	0.1773 **	0.1281
PC8	0.1136 **	0.0889	0.1535 *
PC9	0.0768	0.0987	0.1254
PC10	0.1341 **	0.0864	0.1455
Peak 3	0.104 **	0.161 **	0.184 **
Peak 7	0.064	0.094	0.126
Peak 18	0.529 ***	0.174 **	0.281 ***

* Significant difference at 0.05 level based on Lilliefors probability.
** Significant difference at 0.01 level based on Lilliefors probability.
*** Significant difference at 0.0001 level based on Lilliefors probability.

where the assumption of normality was not respected in grade A, B, and reject. However, while peaks 3 and 18 showed non-normal behaviour in all grades, the distributions of PC4 and PC5 were statistically different from a normal one for only grade reject and grade B, respectively.

The scores of the rotated principal components of canned pink salmon from the storage study were submitted to linear discriminant analysis. The univariate tests on the ten PC's having eigenvalues above 1.0 showed that the first five were significant at either the 0.0001 or 0.05 levels (Table 24). The two largest F ratios were assigned to PC4 and PC5, and confirmed their substantial involvement in grade categorization. The joint contribution of the PC's also proved to be highly significant ($P<0.0001$) in discriminating the 3 quality grades as analyzed by the multivariate tests. Table 24 indicates the need for using both possible canonical functions for the computation of optimal distances between group centroids based on the canonical analysis. The total error rate, calculated using the cross-validation method, generated by the linear discriminant functions was 6.9%.

Going back to the results of principal component analysis, peak 18 (3-methyl-1-butanol) was previously shown to be strongly correlated to peaks 3 and 19, and all three were major contributors to the linear relationship making up PC3. When the behaviour of these three peaks were compared, peak 18 was found to be the only compound that remained undetected for the initial period of refrigeration storage. This peculiarity was not taken into account when carrying out tandem PCA-LDA.

Table 24. Univariate and multivariate test statistics of linear discriminant analysis on the first ten varimax rotated principal components from canned pink salmon during the refrigerated storage study.

Variable	num df,den df	F
Univariate		
PC1	2,186	3.8697 *
PC2	2,186	9.9110 ***
PC3	2,186	4.1739 *
PC4	2,186	154.0639 ***
PC5	2,186	61.4829 ***
PC6	2,186	2.5676
PC7	2,186	0.1830
PC8	2,186	2.5589
PC9	2,186	2.6270
PC10	2,186	1.2413
Multivariate		
Wilk's lambda = 0.07785	20,354	45.7376 ***
Pillai trace = 1.29703	20,356	32.8421 ***
Hotelling-Lawley trace = 7.02994	20,352	61.8635 ***
Roy's largest root = 6.26080	10,178	111.4423 ***
Canonical		
LR_1 = 0.07785	20,354	45.7376 ***
LR_2 = 0.56525	9,178	15.2118 ***

* Significant difference at 0.05 level.
** Significant difference at 0.01 level.
*** Significant difference at 0.0001 level.
LR stands for Likelihood ratio.

To investigate the potential usefulness of this information, a form of stepwise discriminant analysis was performed on selected peak variables (Table 25). Functions based only on peak 3 (ethanol) provided a cross-validated total error rate of 19.6%, the majority of the error made on classifying grade B to grade A samples. When peak 18 was combined with peak 3 for the analysis, the error rate for grade B was reduced from 75% to about 42%. This underlined the fact that, even though an increased amount of ethanol signalled a shift from grade A to grade B with or without the presence of 3-methyl-1-butanol, peak 18 was consistently associated with samples of grade B. Peak 7, having the highest correlation with PC5, helped achieve a further decrease in error rate to approx. 17% for grade B whether or not peak 19 was put in the model. Peak 19 was redundant due to inter-correlations with peaks 3 and 18. Peaks 11 (benzene), 5 (3-methyl-1-butene), 21 (acetic acid), and 38 (2-pentyl furan), which were all given high loadings onto PC5, PC2, PC3, and PC1, respectively, did not improve the classification rate of 93% established by peaks 3, 18, and 7.

In view of the heteroscedasticity and non-normality cases (Table 23), the investigation of quadratic and non-parametric discriminant analyses was justified and pursued for the classification of canned salmon in the 3 quality grades. Error counts estimated by the cross-validation method were computed for the three types of discriminant functions and are reported in Table 26. As previously discussed, the total error rates were comparable for the two LDA's. Out of the 3 quality levels, grade B was the one where the linear functions performed the least successfully (12.6%

Table 25. Cross-validated error count estimates of linear discriminant functions carried out on selected gas chromatographic peaks from canned pink salmon of the refrigerated storage study.

Peak variable	Error count estimate[a]			
	Grade A (n=107)	Grade B (n=48)	Reject (n=34)	% Total (n=189)
3	0.000 (0)	0.750 (36)	0.029 (1)	19.6
3,18	0.000 (0)	0.417 (20)	0.029 (1)	11.1
3,18,19,7	0.037 (4)	0.167 (8)	0.029 (1)	6.9
3,18,7	0.028 (3)	0.167 (8)	0.029 (1)	6.4
3,18,7,11	0.028 (3)	0.188 (9)	0.029 (1)	6.9
3,18,7,5	0.028 (3)	0.188 (9)	0.029 (1)	6.9
3,18,7,21	0.028 (3)	0.208 (10)	0.029 (1)	7.4
3,18,7,38	0.028 (3)	0.167 (8)	0.029 (1)	6.4

[a] The numbers in parentheses are the misclassifications associated with the error count estimates.

Table 26. Comparison of cross-validated error count estimates for different discriminant analysis functions (DA) of selected gas chromatographic peaks and the first ten principal components from canned pink salmon of the refrigerated storage study.

DA function[a]	Error count estimate[b]			
	Grade A (n=107)	Grade B (n=48)	Reject (n=34)	% Total (n=189)
Principal components				
linear	0.047 (5)	0.125 (6)	0.059 (2)	6.9
quadratic	0.028 (3)	0.083 (4)	0.000 (0)	3.7
non-parametric (r=4)	0.019 (2)	0.104 (5)	0.000 (0)	3.7
Three peaks (3, 7, 18)				
linear	0.028 (3)	0.167 (8)	0.029 (1)	6.4
quadratic	0.009 (1)	0.062 (3)	0.000 (0)	2.1
non-parametric (r=3)	0.009 (1)	0.042 (2)	0.000 (0)	1.6
Prior prob.	0.566	0.254	0.180	

[a] The nonparametric method consists of the Epanechnikov kernel.
[b] The numbers in parentheses are the misclassifications associated with the error count estimates.

and 16.7%). The quadratic and non-parametric kernel methods were found to be equally effective in bringing down the error count of all grades; but grade B samples benefitted most from their implementation. Although rates of correct classification were heightened to levels between 96% and 98%, non-parametric discriminant functions should be regarded as more stable due to the considerations encompassing the non-normal behaviour of some of the variables involved. Furthermore, the use of the non-parametric Epanechnikov kernel DA based on the three peak variables had the advantage of computing the functions directly on the peak areas themselves. In this manner, principal component analysis was by-passed, the number of variables handled was considerably reduced, thereby lessening the amount of computation.

4. Performance of sensory evaluation and MVA

The pink salmon that had been subjected to refrigerated storage were graded before canning. The grades obtained prior to canning served as reference grades in the multivariate discriminant techniques used on the SHGC chromatograms of volatiles. Cans from each of the pink salmon were also submitted to a trained federal inspector for sensory quality assessment. Figure 26 presents the sensory classification of canned salmon plotted against the grades determined before canning. While the post-canning results for grade A were in perfect agreement with pre-canning quality assessment, some divergence was observed for a number of grade reject samples. The largest discrepancy occured with grade B samples, of which a notable portion were classified as grade A. The

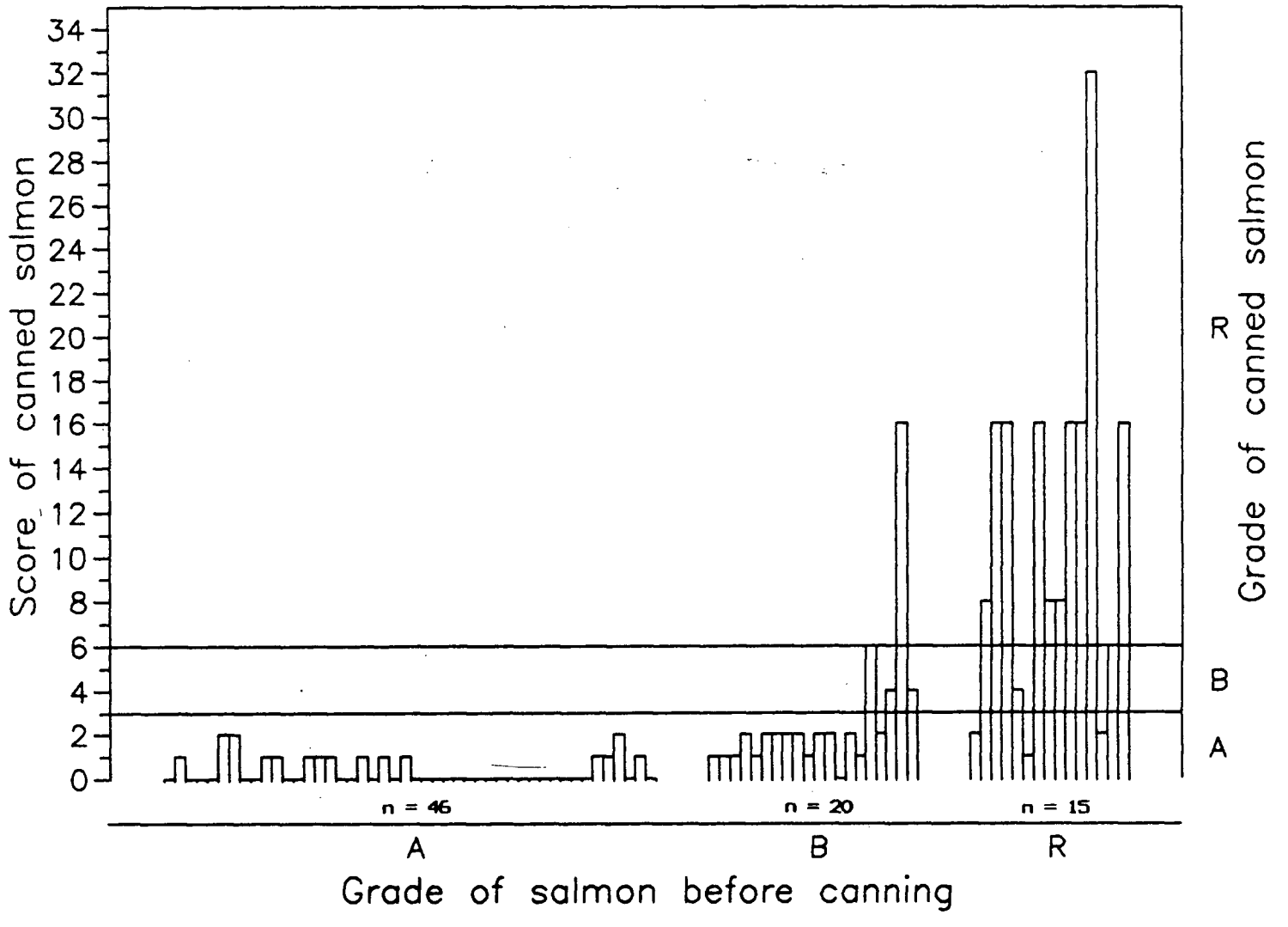

Figure 26. Sensory classification before and after canning pink salmon of the refrigerated storage study.

present post-canning grading scheme grants relatively small ranges of scores for grade A and B compared to grade reject. Samples of grade B were expected to be in greater numbers at the onset of decomposition. The experiments were carried out at refrigerated temperatures which were designed to allow time for transition from one grade to another. While grade B targeted samples with slight but definitely persistent and perceptible odours of decomposition, samples grouped in grade reject possessed a strong odour of decomposition as evidence of advanced decay. Figure 26 indicates the tendency of sensory evaluators to segregate samples of canned salmon into two categories, grade A and grade reject, and consequently underlined the difficulty of consistently detecting early signs of decomposition. This problem could be linked to sensory fatigue due to the evaluation of numerous cans of salmon consecutively.

The comparison of error rates between instrumental analysis and sensory evaluation of canned salmon is shown in Table 27. The data of categorical nature (correct or incorrect classification) were analyzed with the SAS Catmod procedure which fits linear models to functions of categorical variables. Because the performance of the classification methods varied with grades, that is the interaction was significant ($P<0.01$), weighted least squares estimations were carried out for each grade. The error rates for instrumental and sensory analyses were obtained directly from Table 26 and Fig. 26. Both non-parametric discriminant functions and sensory grading kept the error rates for grade A to a minimum. While there was no error obtained by the discriminant analyses for grade reject, sensory grading had an error rate significantly

Table 27. Comparison of error rates for non-parametric discriminant functions (NPAR-DA) and sensory grading of canned pink salmon of the refrigerated storage study.

Method[g]	Error rate[h]		
	Grade A	Grade B	Reject
NPAR-DA			
5 PC's (r=4)	0.019[a] (2/107)	0.104[b] (5/48)	0.000[e] (0/34)
3 Peaks (r=3)	0.009[a] (1/107)	0.042[c] (2/48)	0.000[e] (0/34)
Sensory grading	0.000[a] (0/46)	0.900[d] (18/20)	0.267[f] (4/15)

[a-f] Error count estimates in columns with the same letters are not significantly different from each other (P<0.05).

[g] The nonparametric method consists of the Epanechnikov kernel.

[h] The numbers in parentheses are the misclassification ratios associated with the error count estimates.

greater (26.7%). The rate for non-parametric DA based on 5 principal components was significantly higher than that based on the 3 peak variables, but both rates were found to be between 4 to 10%. The most striking feature of Table 27 was the 90% error rate of misclassification of grade B samples by sensory evaluation.

Sensory grading of whole salmon relies on a larger number of observable variables than does sensory grading of canned salmon. Still, odour is among the criteria used to evaluate a raw fish, and samples categorized in grade B usually possessed a perceivable odour of decomposition. Spoilage, thermal processing, and their interaction can affect the aroma of a canned seafood in various ways. In some instances, thermal processing causes an increase in off-odour. For example, TMAO is partially degraded to TMA and DMA during the canning process of fish (Hebard et al., 1982). Microbial metabolism of meat components can change the composition of non-volatile precursors resulting in a reduction or loss of the characteristic meat flavour. Decomposition products can also serve as reactants in the complex chemical pool of flavour precursors, and be involved in the formation of new volatiles during heating. Pokorny (1980) reported that the reaction of proteins with rancid fish oils at elevated temperatures resulted in the development of baked or roasted fish flavours. It is therefore possible that components contributing to off-odours decrease in concentration, or are masked by other thermally-generated volatiles produced in canned salmon but absent in raw fish. This could happen particularly during the initial stages of spoilage.

In part, the success of the instrumental method may be due to the early detection of concentration changes to which sensory evaluation remains insensitive. For example, the odour threshold for ethanol has been reported to vary between 100 ppm (Guadagni et al., 1963) and 900 ppm (Mulders, 1973) depending whether the method involved squeezable plastic flasks or smelling over open beakers. Hollingworth and Throm (1983) provided suggested ranges of ethanol concentration associated with quality levels of canned salmon: sensory class 1 (grade A), 0-24 ppm ethanol; sensory class 2 (grade B), 25-74 ppm ethanol; and sensory class 3 (grade reject), 75 ppm ethanol and above. Therefore, changes of concentration below or around high threshold values similar to those of ethanol could pass undetected by sensory evaluation. It is very likely that the reverse is also true. Experienced inspectors can rely on the perception of compounds with low sensory threshold values that are not detected by the SHGC method. But it follows from Figure 26 and Table 27 that the accuracy with which these 'character-impact' odours were detected for grade B pink salmon in particular, did not provide results that corroborated well with those obtained from sensory grading prior to canning, whereas the instrumental SHGC method was successful in this regard.

The nature of the sensory and SHGC-MVA results accumulated thus far allowed further computations of regression equations in order to investigate their relationships during the refrigerated storage time. Plots of the sensory grading of canned salmon for years 1 and 2 are presented in Figure 27. Because the data to be plotted were categorical, digits instead of points were used in the graphs to represent the number

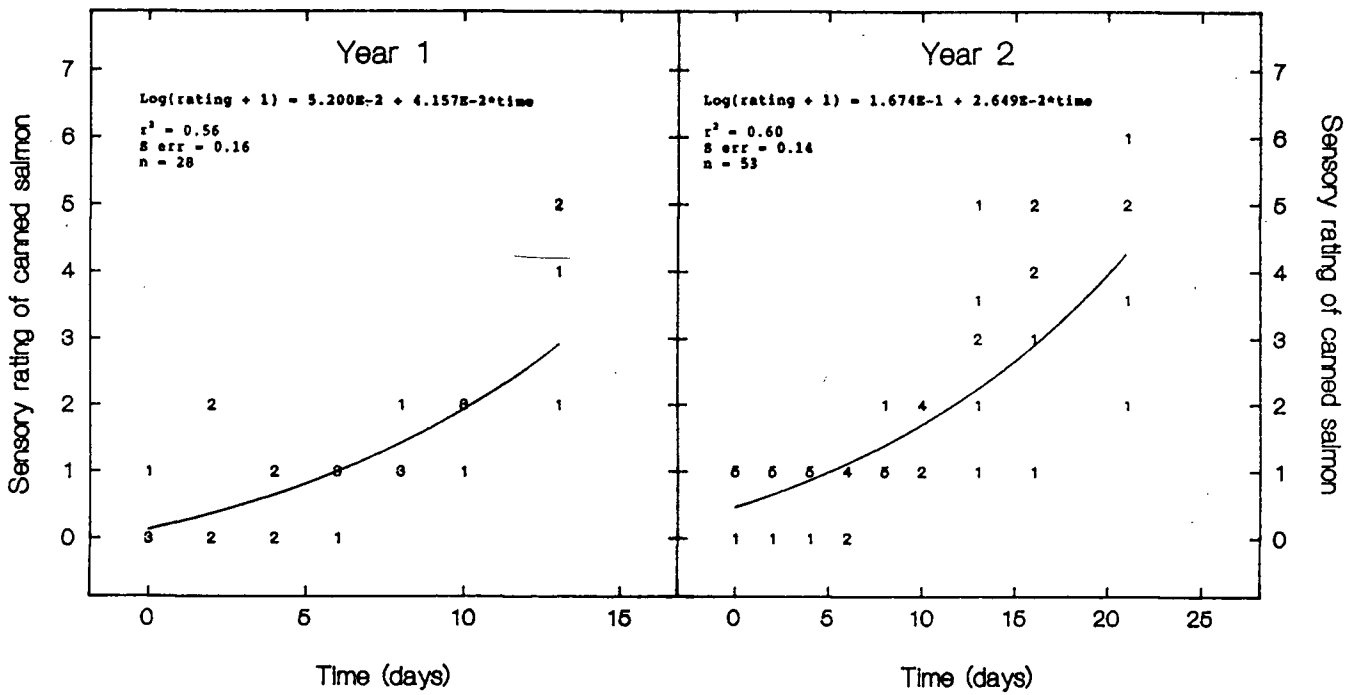

Figure 27. Logarithmic relationships of sensory rating of canned salmon with refrigerated storage time. Digits represent the number of times a rating was encountered for each day of refrigerated storage.

of times a rating was encountered for each selected day of refrigerated storage. The changes in ratings were assumed to occur at an exponential rate. As variation in ratings increased towards the end of the storage period, especially for year 2, a logarithmic transformation not only improved the fit of the model but helped to diminish the heteroscedasticity in the data. Although the data is of categorical nature, the strength of the relationship may be assessed if the sensory ratings are taken to behave as continuous variables. The correlation index (r^2) was used to measure the relationship in a way similar to a correlation analysis context (Zar, 1984). For the curves of years 1 and 2, the correlation indices describing the amount of variability in logarithmically-transformed sensory rating of canned salmon accounted for by correlation with refrigeration storage were 0.55 and 0.60, respectively.

Among the multivariate results, the scores of the two most discriminating principal components, PC4 and PC5, served to calculate a second degree polynomial quality function (Z). All terms but $PC4^2$ were significant in the model ($P<0.0001$). This PC quality function without the $PC4^2$ term is plotted against refrigerated storage time in Figure 28. A coefficient of determination of 0.80 ($P<0.0001$) was generated by the obtained linearized function. Similar quality functions based on peaks 3 (P3), 7 (P7), and 18 (P18) were computed for years 1 and 2 (Figure 29). Peaks 3 and 18 previously appeared to behave exponentially and therefore were logarithmically transformed before carrying out stepwise regressions by backward elimination. Three of the second degree terms of the

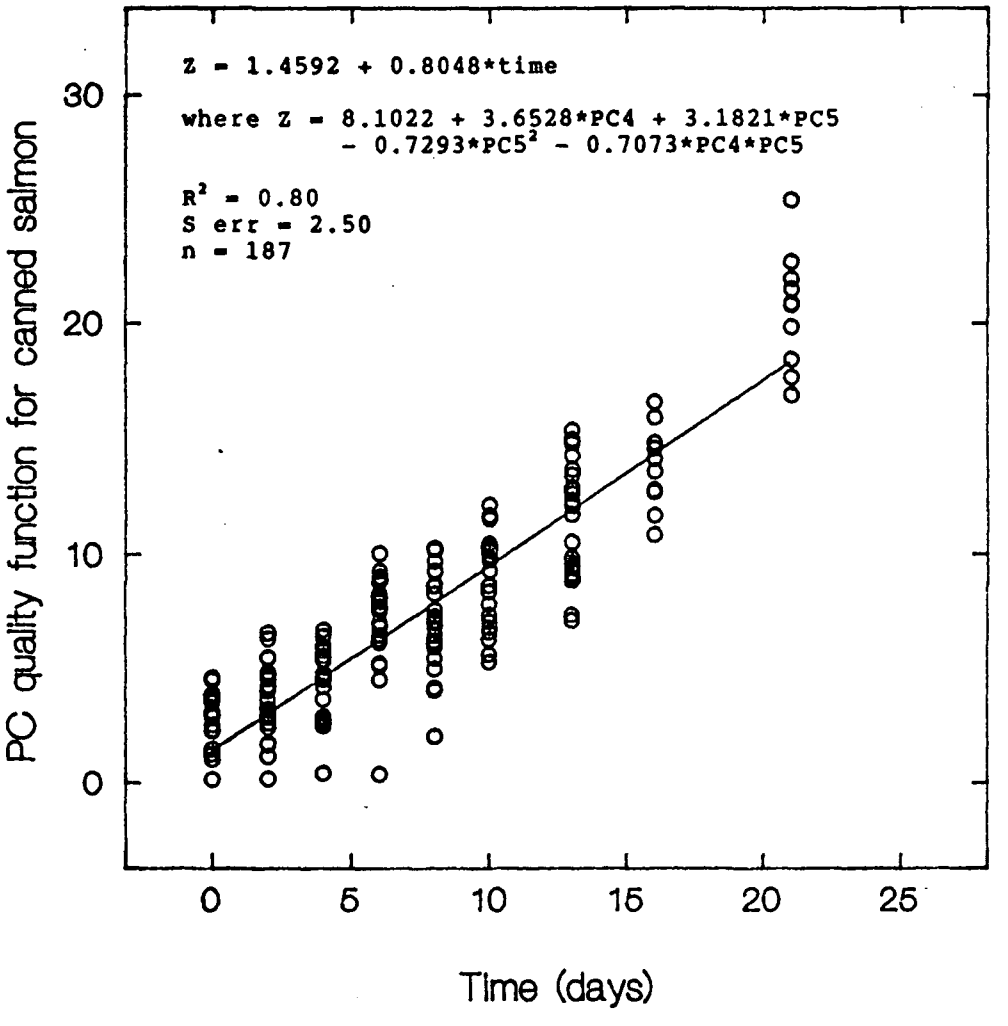

Figure 28. Linearized relationship of the polynomial quality function using principal components 4 (PC4) and 5 (PC5) over refrigerated storage time.

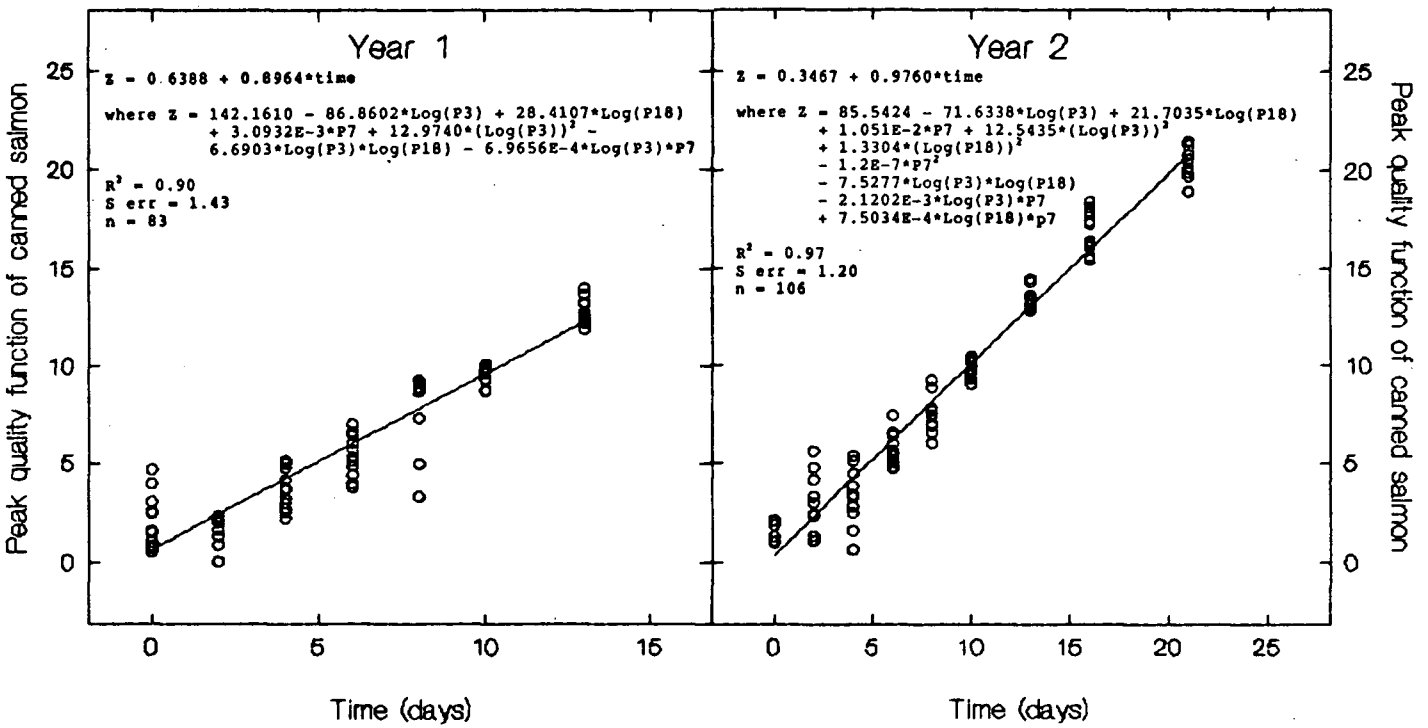

Figure 29. Linearized relationship of the polynomial quality function using peaks 3, 7, and 18 over refrigerated storage time.

polynomial equation for year 1 were found to be non-significant ($P>0.05$) but none of the terms in the equation of year 2 were rejected. The highest coefficients of determination, 0.90 and 0.97, yet obtained from all above regressions were associated with these last two equations for years 1 and 2 ($P<0.0001$), respectively. In addition to better curve fittings, the linearized functions calculated from SHGC data had lower coefficients of variation, i.e., 33.5%, 21.2%, and 13.8% for PC's and peaks quality functions of years 1 and 2, respectively, compared to 35.2% and 53.1% for the previous functions of years 1 and 2 applied on sensory data. All above functions were attempts to express the decomposition behaviour of canned salmon during refrigerated storage periods. The differing degrees of precision embodied in the various equations partially depended on the nature of the methods themselves. They were also tied to many other factors that have not been considered but which influenced the rate of decomposition, e.g., the nature, diversity, and prominence of the microbial flora. The intent of the regressions at this point is more descriptive than predictive.

5. MVA of volatiles from raw pink salmon during refrigerated decomposition

The SHGC method established in Chapter II was optimized to analyze volatiles from samples of canned salmon and provided important information that was used in multivariate analysis. The grading of raw salmon might also gain from similar instrumentally-generated data treatments. The SHGC method was thus applied on samples of raw salmon from the refrigerated storage study of year 2. Figure 30 presents typical chromatograms of raw

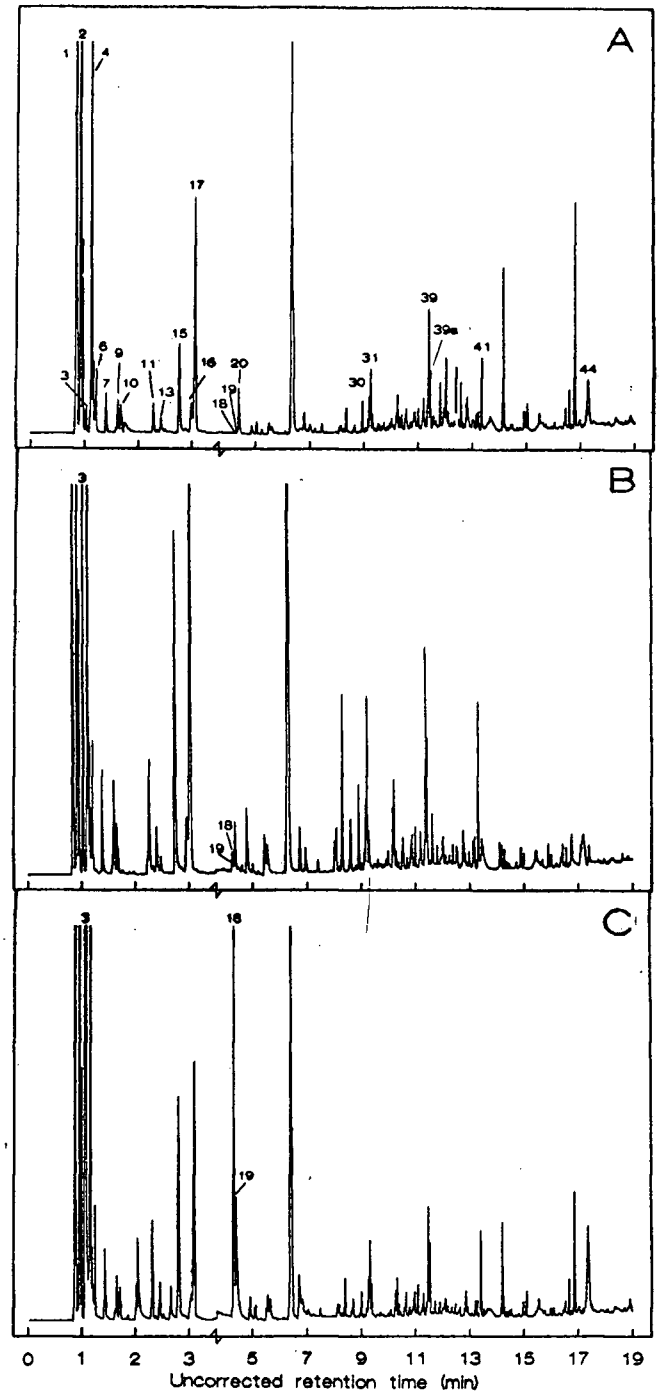

Figure 30. Chromatograms of volatiles from raw pink salmon during the refrigerated storage of year 2. (A, day 0/grade A, day 10/grade B, day 21/grade reject).

salmon after 0 day (A), 10 days (B), and 21 days (C) of refrigerated storage after sampling. The salmon, from which the samples were taken, were evaluated as grade A, B, or reject, respectively. The peak areas from volatiles of raw salmon were in general not as intense as with samples of canned salmon. The nature of the samples themselves was likely the reason. The pieces of raw salmon introduced in headspace vials received a thermal treatment for 1 h at an incubation temperature of 105°C. During the course of this treatment, the myofibrillar proteins denatured to form matrices entrapping food components including volatiles. In addition, raw salmon pieces did not provide as much surface exposure as canned salmon flakes from which volatiles could escape and build up partial pressures in the vial headspace.

Peaks 3 (ethanol), 18 (3-methyl-1-butanol), and 19 (2-methyl-2-butenal) were labelled on the three graphs of Fig. 30 and their behaviours were found to bear a resemblance to those of canned salmon samples in Fig. 19. A number of new volatiles with relatively low FID responses were detected, particularly in the second half of the chromatograms. Twenty two peaks having coefficients of variation near 12% or less were selected for multivariate analysis. These volatiles are numerically indicated in graph A of Fig. 30. On the basis of similar retention time, all volatiles except peak 39a were previously reported and are so labelled in Table 6.

Principal component analysis with varimax rotation was performed on the standardized integrated areas of the 22 peaks; the resulting peak-principal component correlations are shown in Table 28. Nine peaks had

Table 28. Loadings of the first four varimax rotated principal components of volatile compounds from raw pink salmon of the refrigerated storage study (year 2).

Peak	Principal components			
	1	2	3	4
1	0.780	0.236	0.199	0.195
2	0.692	-0.221	0.281	0.157
3	0.098	0.969	-0.066	-0.027
5	0.373	0.100	0.842	0.163
6	0.308	0.088	0.206	0.918
7	0.480	0.701	0.201	0.184
9	0.883	0.248	0.244	0.129
10	0.493	0.302	0.207	0.095
11	0.351	0.817	0.163	0.138
13	0.363	0.776	0.187	0.152
15	0.833	0.376	0.214	0.009
16	0.855	0.221	0.264	0.165
17	0.909	0.232	0.199	0.095
18	0.116	0.983	0.042	-0.003
19	0.148	0.978	0.050	0.030
20	0.349	0.092	0.828	0.142
30	0.698	0.362	0.234	0.241
31	0.955	0.108	0.129	0.051
39	0.900	0.176	0.177	0.076
39a	0.922	0.181	0.073	0.165
41	0.894	0.165	0.091	0.144
44	-0.104	-0.086	0.013	-0.034
Latent root	13.061	3.657	1.267	1.039
Variance (%)	59.369	16.621	5.758	4.721

loadings of approx. 0.8 and above for PC1: peak 1 (hydrogen sulfide and acetaldehyde), peak 9 (butanal), peak 15 (1-penten-3-ol), peak 16 (heptane), peak 17 (1,5-dimethyl cyclopentene), peak 31 (unknown), peak 39 (4-ethyl benzenemethanol), peak 39a (unknown), and peak 41 (nonanal). PC1 expressed correlated variations in a diversity of volatile compounds. Due to their loadings above 0.7, peak 3 (ethanol), peak 7 (2-methyl propanal), peak 11 (benzene), peak 13 (2,2-dimethyl propanal), peak 18 (3-methyl-1-butanol), and peak 19 (2-methyl-2-butenal) were more strongly associated with PC2 than the other volatiles detected. This last principal component regrouped the same volatile compounds as the important discriminators, PC4 and PC5, from canned salmon. PC2 accounted for 16.6% of variation while the first 4 PC's with latent roots above 1.0 explained 86.5% of the total variance.

Scores of PC2 plotted against that of PC1 are shown in Fig. 31. Quality levels were segregated by PC2 while each grade was spread along the PC1 dimension. Low values for PC2 were found in grade A samples. Scores increased as grades changed from A to B, and eventually to reject. Samples of grade reject occupied a large part of the PC2 scale compared to samples from grades A and B, hinting at an heteroscedasticity problem. In any case, PC2 could be considered as the dimension representing the decomposition process during refrigeration.

The changes in standardized area of the three selected peaks analyzed from raw pink salmon of the refrigerated storage study, previously used in the discriminant analyses for canned salmon (peaks 3,

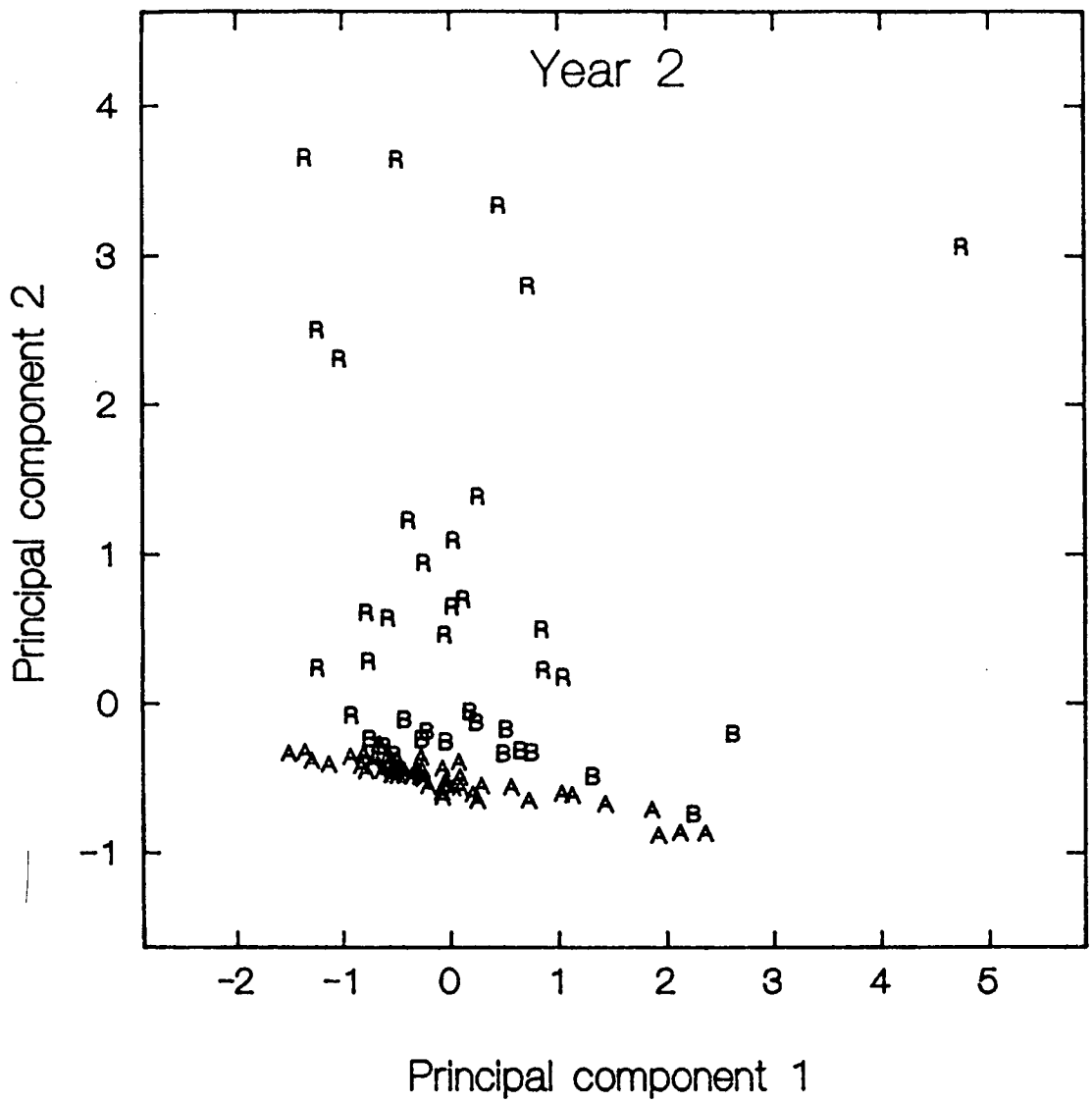

Figure 31. Plot of the scores of principal component 1 against 2 for raw pink salmon of the refrigerated storage study of year 2 (A, grade A; B, grade B; R, grade reject).

7, and 18), are shown in Fig. 32. The non-linear relationship of peaks 3 and 18 was very similar. Peak 18 (3-methyl-1-butanol) was not detected in the fish at the beginning of the storage period. Peak 3 (ethanol) was, however, detected in all samples. Peak 7 (2-methyl-2-butenal) had a different relationship to storage time than was observed with canned salmon. Instead of increasing soon after the initiation of the refrigerated storage, its concentration remained relatively low until the end, when its variance abruptly increased. The visual difference observed between peaks 3 and 18, and peak 7 (Fig. 32) was reflected in a lower loading for peak 7 on PC2 (Table 28).

Subsequent multivariate analyses were carried out on peaks 3, 7, and 18 only. The homogeneity of variance and normality assumptions were tested for the peak variables. Univariate and multivariate Bartlett's tests were all significant ($P<0.0001$) and cases of non-normality, assessed by the Kolmogorov-Smirnov test, were found for the three peaks ($P<0.01$). Consequently, non-parametric DA functions, based on the Epanechnikov kernel, were computed. As peak 7 showed some signs of weaker discriminant power than peaks 3 and 18 (Fig. 32 and Table 28), its involvement was investigated by testing whether its presence improved the cross-validated error rate. Functions obtained which relied on peaks 3 and 18, were as effective with or without peak 7, resulting in a same total classification rate of 95.6%. Classification rates for each quality level determined by the non-parametric DA based on peaks 3 and 18 are shown in Table 29. While all 53 samples of grade A were correctly classified by the functions, samples were misclassified three times and one time for grades

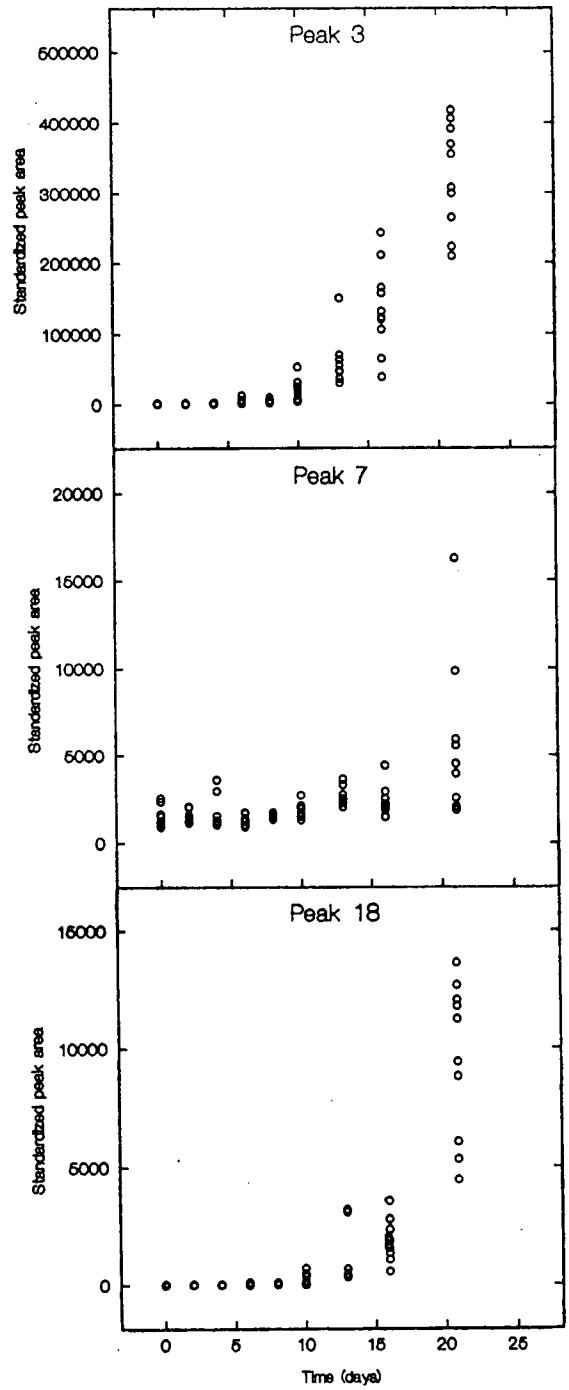

Figure 32. Plots of volatiles from raw pink salmon of the refrigerated storage study of year 2 with high loadings for principal component 2.

Table 29. Classification by non-parametric discriminant analysis functions (NPAR-DA) of selected peaks (3 and 18) from the gas chromatographic analysis of raw pink salmon of the refrigerated storage study.

Assigned grade	Predicted grade by NPAR-DA[a]			Prior Prob.
	A	B	Reject	
A (n=53)	1.000 (53)	0.000 (0)	0.000 (0)	0.582
B (n=16)	0.125 (2)	0.812 (13)	0.063 (1)	0.176
Reject (n=22)	0.0000 (0)	0.045 (1)	0.956 (21)	0.242

[a] The nonparametric method consists of the Epanechnikov kernel, r=3.

B and reject, respectively. These results indicated that the NPAR-DA produced low error rates and demonstrated a compatibility with the sensory grading prior to canning. The different behaviour of peaks 7, 11, and 13, between raw and canned salmon, indicated that canning and the subsequent thermal processing could be an influencing factor.

Ethanol and 3-methyl-1-butanol are known microbial catabolic products. These short-chain alcohols are derived from the metabolic degradation of carbohydrates and amino acids, respectively (Brock, 1979). Ethanol has been suggested as potential index of spoilage in raw (Hillig, 1958; Iida et al., 1981a, b; Human and Khayat, 1981; Kelleher and Zall, 1983; Ahamed and Matches, 1983) and canned fish (Holaday, 1939; Lerke and Huck, 1977; Crosgrove, 1978; Khayat, 1979; Iida et al., 1982; Tokunaga et al., 1982; Hollingworth et al., 1986). The concentration of 3-methyl-1-butanol has also been reported to increase during the course of spoilage in fish (Miller et al., 1973b; Kamiya and Ose, 1984; Ahamed and Matches, 1983) and mussels (Yasuhara, 1987). Production of ethanol in substantial amounts, followed by increases in 3-methyl-1-butanol, was previously observed in refrigerated fish (Lindsay et al., 1987). Based on a study by Ahamed and Matches (1983) on bacterial isolates from king salmon (Oncorhynchus tshawytscha) and rainbow trout (Salmo irrideus), test organisms appeared to prefer 5 and 6 carbon sugars as initial energy sources and then began to utilize free amino acids which are the precursors of various products such as alcohols. Indices of fish spoilage, such as some of the proposed discriminant functions, that rely on these two metabolic aspects, reflect the stages of decomposition that

fish may undergo. Another advantage in using alcohols as indicator of microbial spoilage in fresh and canned fish products is their thermal stability during processing.

More research should therefore be carried out to investigate the factors that affect the generation of these volatiles. More work should be performed to increase the number of samples analyzed particularly for grades B and reject thereby enhancing the accuracy of their population estimates in multidimensional discriminant space.

IV. DYNAMIC HEADSPACE ANALYSIS OF VOLATILE FLAVOUR COMPONENTS IN CANNED SALMON

A. Introduction

Volatile aroma components are generally regarded as the most important parameters of food flavour quality. Due to both economic importance and academic interest, research has been carried out on the identification of volatile flavour compounds in various foods including fish. Over the years, volatile analysis by dynamic headspace techniques has received substantial attention from researchers. It is based on a dynamic process in which the sample volatiles are transferred with a stream of an inert gas to a trap and then desorbed by various means into a GC. The dynamically purged headspace volatiles of tuna (Khayat, 1979; Human and Khayat, 1981) and herring (Hughes, 1964) were condensed by cryogenic trapping while adsorbent trapping was used for pickled smelt (Josephson et al., 1987), north sea fish oil (Christensen et al., 1981), crayfish (Vejaphan et al., 1988; Tanchotikul and Hsieh, 1989), Atlantic and Pacific oysters (Josephson et al., 1985), prawns and sand-lobsters (Whitfield et al., 1982), and various species of fish (Easley et al., 1981; Reinert et al., 1983; Josephson et al., 1984a).

Fresh Pacific salmon, either pan-fried, baked, or roasted, possesses a typical flavour, distinctive from other seafood, that is recognized by consumers. Canned salmon releases, upon opening, an aroma which appears similar but is yet different from salmon processed by other means. The volatiles responsible for these aromas have not been elucidated.

Josephson (1987) found that salmon carotenoids may either serve as direct precursor compounds or modulate chemical reactions which convert fatty acids or other lipid precursors to salmon-loaf-like aroma compounds.

The orange pigmentation of the flesh of adult wild Pacific salmon is due to carotenoids, especially astaxanthin, in a free form. Kitahara (1983) showed that the levels of astaxanthin in muscles decreased markedly when salmon were engaged in their spawning migration. It was transported to the skin and gonads via the blood serum. During anadromous migrations of salmon, the lipids that depleted from the body stores were almost entirely triacylglycerols. The amount of triacylglycerols in dorsal muscle of chum salmon decreased from 2.1g/100g tissue in males and 3g/100g in females, to less than 0.2g/100g in both sexes, between feeding in the sea and spawning (Ando et al., 1985a; 1985b). The sexual maturation process also brings about a stimulation of mucus secretion, and a darkening and thickening of the skin resulting in the scales becoming more deeply embedded. The biochemical regulation of mucus secretion in late spawning-condition Pacific salmon was further conceptually linked with increases of 8- and 9-carbon volatiles which contributed distinct cucumber or melon-like aromas (Josephson, 1987).

Odour changes in fish may also arise from spoilage as a consequence of microbial metabolism. Although numerous volatile components have been identified in spoiled fish, the direct association of specific compounds to particular odours remains a great challenge. Early efforts to relate trimethylamine to fishy odour were reported by Davies and Gill (1936) and

Stansby (1962). Fruity odours were considered by Miller et al. (1973a) to be due to the presence of ethyl esters of acetate, butyrate, and hexanoate. Methyl-3-isopropyl pyrazine was determined to be responsible for the potato-like off-odour produced by Pseudomonas perolens (Miller et al., 1973c). The studies of Herbert and Shewan (1975, 1976) were instrumental in attributing the sulfurous odour to hydrogen sulfide, methanethiol, and dimethyl sulfide as well as uncovering their origin.

The factors mentioned above, e.g., thermal processing, spawning migration, and spoiling during cold storage, have unknown effects on the volatile patterns of canned salmon. Therefore, the objectives of the third part of this research were: (1) the separation and identification of volatile compounds in canned pink salmon of good quality (grade A), canned pink salmon of advanced decomposition (grade reject), and canned chum salmon of advanced sexual maturity (spawning dark) by using sorbent trap sampling/GC/MS, and (2) the evaluation of the sensory characteristics of cryofocussed GC effluents from these 3 different types of canned salmon.

B. Materials and methods

1. Collection and canning of salmon

Canned pink salmon (Oncorhynchus gorbuscha Walbaum) were taken among those processed for the second year of the refrigerated decomposition study of Chapter III. Pink salmon of grade A and reject had undergone a cold storage of 2 and 17 days, respectively. "Late-run" chum salmon (Oncorhynchus keta Walbaum) were obtained from the Chilliwack (B.C.) hatchery during the month of October. Very dark green or brown barring of a thick skin with a heavy slime, eroded fins, hooked jaws, very thin belly flaps and corpulence, pale greyish flesh colour, and strong "late" odour of the flesh and skin were external signs indicative of advanced sexual maturity.

Approximately 215g of chum salmon flesh and 2g sodium chloride were put in 307 x 113 three-piece cans. The samples were then vacuum-sealed and thermally processed in a FMC 500W Universal Sterilizer (F.M.C. Corporation, Santa Clara, CA). A retort temperature of 120°C was used with a process time of 65 min. These conditions gave a lethality (F_o) of approx. 7.6 min based on data from Collins (1989).

2. Tenax trap sampling/gas chromatography/mass spectrometry (TTS/GC/MS)

Forty mL of drained liquid from canned salmon were placed in a headspace sampling tube of an HP 7675A Purge and Trap sampler. The

samples were purged with purified helium (Zero Gas, Medigas, Vancouver, BC) at a rate of 20 mL/min while the sampling tube was immersed in a water-bath held at 50°C. After a 30 min sparging time, the porous polymer Tenax TA™ (0.30g, 60-80 mesh, Hewlett Packard) sorbent trap (8.89cm x 0.64cm o.d.) was dry-purged for 1 h with helium.

Volatiles were then thermally desorbed (185°C) and brought at a flowrate of 2 mL/min on an Ultra 2 capillary column (30m x 0.32mm x 0.52 µm film thickness) installed in a HP 5840A gas chromatograph/HP 5985B mass spectrometer. For volatile separation from good quality canned pink salmon, the oven temperature was programmed from 20°C to 220°C at a rate of 5°C/min with an initial holding time of 1 min. Runs for samples of the two other treatments, e.g., canned pink salmon of poor quality and canned late-run chum salmon, were programmed to hold the initial temperature at 20°C for 1 min, increase to 120°C by 5°C/min, and subsequently reach 220°C at a rate of 10°C/min. Mass spectra acquisition, tentative identification based on mass spectral library and confirmation by retention time comparison with reference standards was done as described in Chapter II.

3. Cryofocussing concentration sampling/gas chromatography/odour evaluation (CCS/GC/OE)

In order to evaluate the sensory characteristics of the GC effluent, the volatiles were concentrated by cryofocussing. The sample loop of a headspace sampler was replaced with a stainless steel 4-way ball valve (Whitey Co., Highland Hts., OH) and a fixture assembled by coupling two can piercers (Alltech Associates Inc., Deerfield, IL) connected altogether

with stainless steel tubing of 1.59 mm o.d. (Figure 33). A can was tightened in the piercer and immersed in a water bath held at 50°C. After 30 min, the ball valve was opened and the volatiles were swept with helium at a flowrate of 10 mL/min. Cryofocussing occurred in the 40 cm section of the loop lowered into a bath of dry ice-acetone (-78°C).

Thirty min later, the flowrate was reduced to 4 mL/min before the ball valve was switched to by-pass the can. The oven temperature program illustrated in Figure 34 was initiated, and the desorption process was started by immersing the loop in an ice-water bath. The loop was subsequently dipped in boiling water when the oven temperature reached -20°C. It remained in the boiling water bath until an oven temperature of 0°C was obtained. The volatiles travelled through the transfer line (125°C) of the headspace sampler and reached the injector port. Two lengths of deactivated vitreous silica tubing (30 cm x 0.22 mm i.d.) were connected to the end of an Ultra 2 capillary column (30m x 0.32mm x 0.52 m film thickness) with a 1:1 ratio splitter. One tube was directed to an FID maintained at 250°C while the second tubing led to a sniffing port heated at 175°C. The part of the effluent for odour evaluation was mixed with air (300 mL/min) introduced via the gas supply of the second FID base and travelled through a Teflon tube (20 cm x 0.32 cm o.d. x 0.16 cm i.d.) connected with a stainless steel Swagelok union (0.32 cm x .32 cm) to an adaptor fitting the FID exit. Two analysts having experience with canned salmon assessed the aroma of the GC effluent by noting the retention time and a description of the perceived sensation.

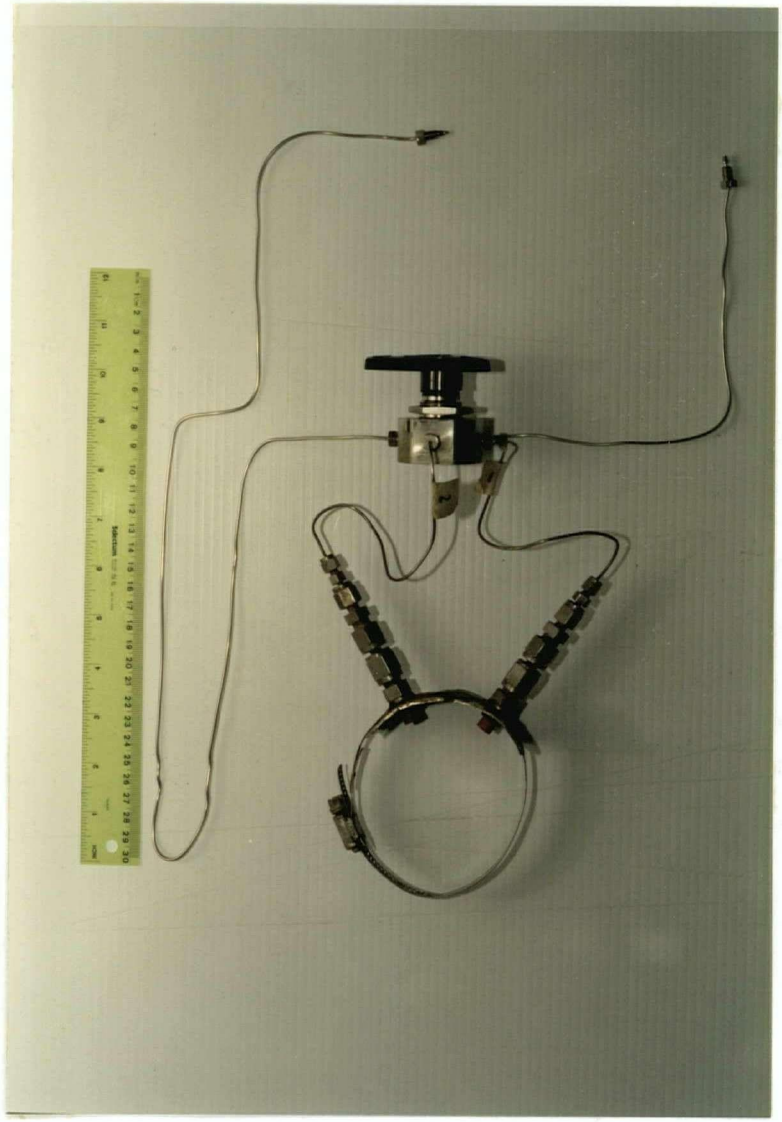

Figure 33. Can piercer fixture, valve, and stainless steel tubing assembled to concentrate canned salmon volatiles by cryofocussing (CCS).

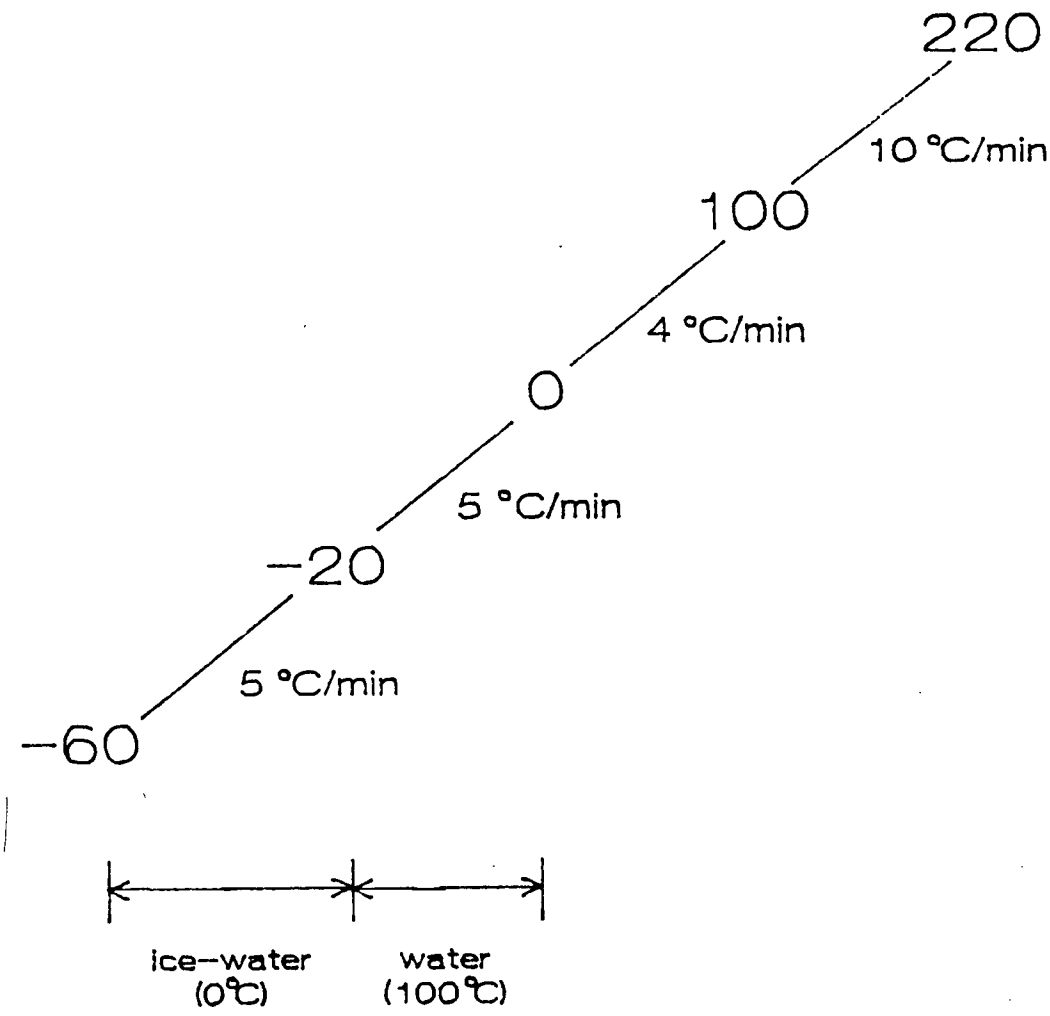

Figure 34. Schematic representation of the volatile desorption steps and the oven temperature program for cryofocussing concentration sampling/gas chromatography/odour evaluation (CCS/GC/OE).

C. Results and discussion

Total ion chromatograms obtained for canned pink salmon of good quality, canned pink salmon of grade reject, and canned late run chum salmon are shown in Figures 35, 36, and 37, respectively. Volatiles at the beginning of these chromatograms were not as well resolved as those eluting later on. With hindsight, the use of cryofocussing subsequent to the TTS procedure would have been beneficial, to increase the sharpness of the first compounds of lower boiling point. However, the information provided by the mass spectra was efficaciously deciphered at the data handling stage.

Table 30 lists the names of the compounds identified, the type of samples where the volatiles were found, the means of identification, and the mass spectral data. Identifications were made by comparison with library mass spectra and retention times of authentic compounds (MS,RT) or by deduction from the mass spectra alone (MS). The latter cases must be regarded as tentative identifications. There were instances where different peaks bore the same chemical identity indicating the difficulty in distinguishing isomers based on their mass spectra. Although a number of compounds were present in only one type of sample, many volatile components were found in two or all three types of canned salmon. Among the 130 compounds that have been analyzed, the number of components detected for canned pink salmon of grade reject (83) were higher than that of canned pink salmon of grade A and canned late run chum salmon (60 and 66, respectively). The classes of volatile compounds included

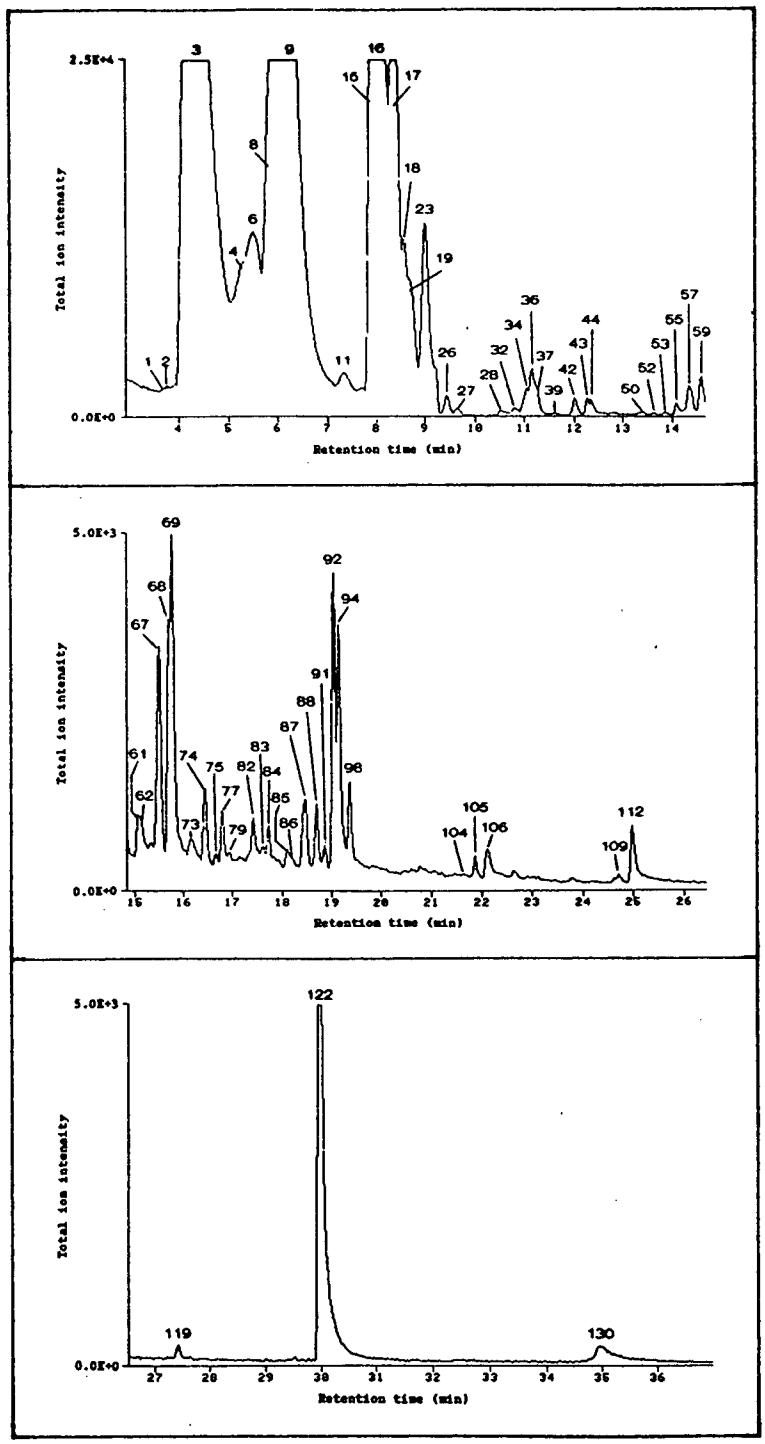

Figure 35. Total ion chromatogram obtained by gas chromatography/mass spectrometry (GC/MS) of headspace volatile components from canned pink salmon of good quality (grade A) concentrated using Tenax trap sampling (TTS). Compounds are identified by peak numbers shown in Table 30.

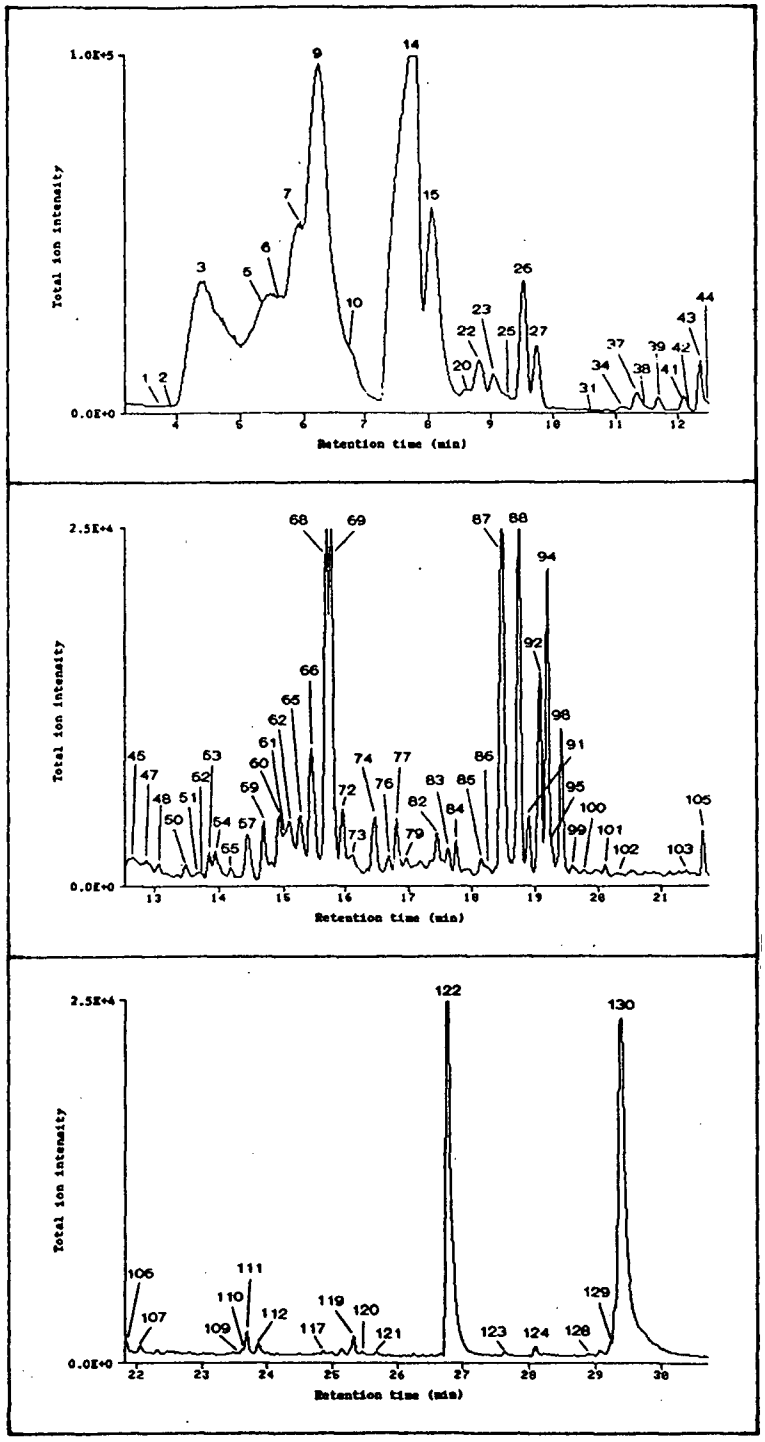

Figure 36. Total ion chromatogram obtained by gas chromatography/mass spectrometry (GC-MS) of headspace volatile components from canned pink salmon of advanced decomposition (grade reject) concentrated using Tenax trap sampling (TTS). Compounds are identified by peak numbers shown in Table 30.

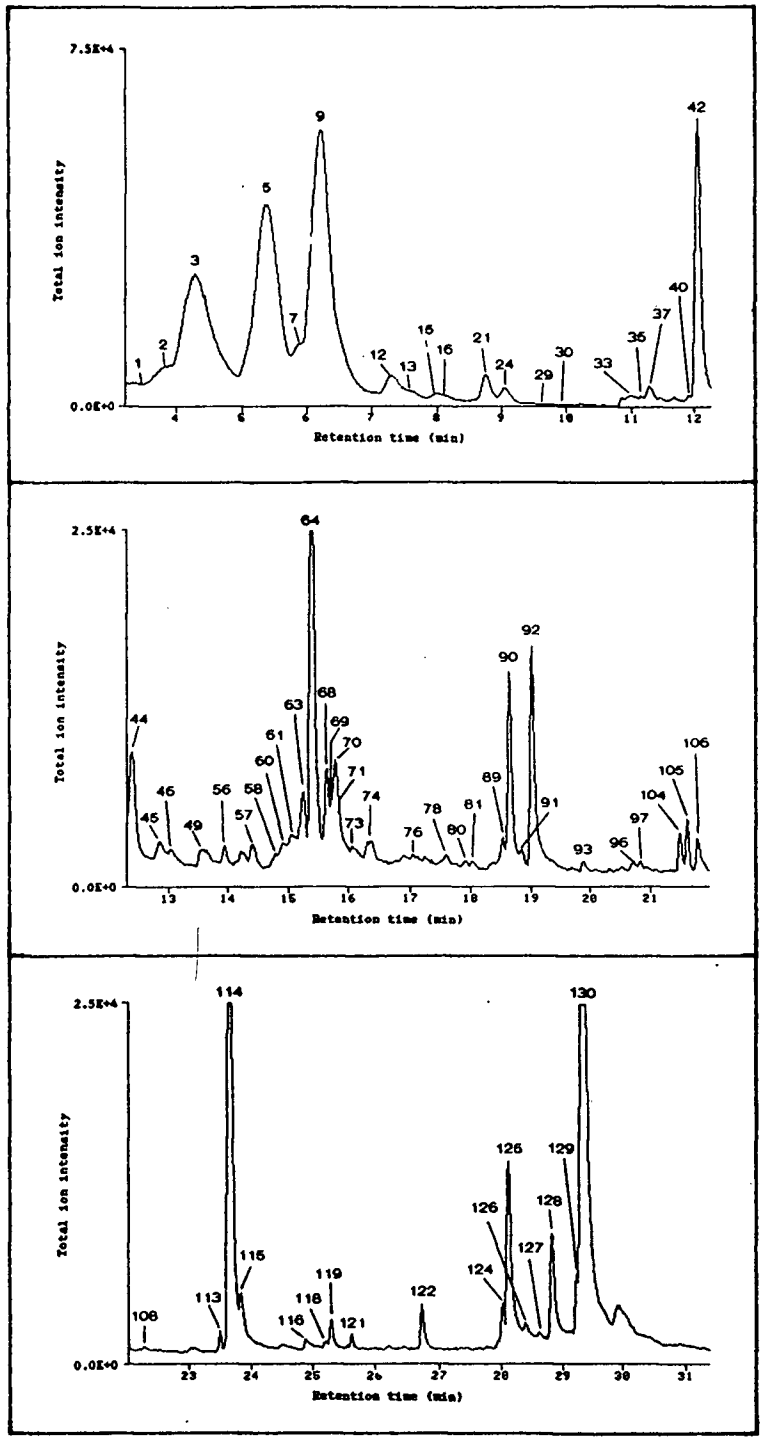

Figure 37. Total ion chromatogram obtained by gas chromatography/mass spectrometry (GC-MS) of headspace volatile components from canned chum salmon of advanced sexual maturity (spawning dark) concentrated using Tenax trap sampling (TTS). Compounds are identified by peak numbers shown in Table 30.

Table 30. Volatile compounds tentatively identified in canned pink salmon of grades A and reject, and canned late run chum salmon by Tenax trap sampling/gas chromatography/mass spectrometry (TTS/GC/MS).

Peak no.	Compound name	Sample[a]	ID[b]	Mass Spectral Data: mass to charge ratio (abundance)
1	acetone	G,P,L	MS,RT	43(100), 58(51), 41(36), 57(34), 56(25), 59(24), 42(24), 86(8)
2	hexane	G,P,L	MS,RT	57(100), 41(60), 43(58), 56(52), 42(32), 29(30), 86(11), 39(9)
3	2-butanone	G,P,L	MS,RT	43(100), 72(21), 57(8), 42(6), 44(5), 82(2), 53(1), 41(1)
4	cyclohexane	G	MS	56(100), 84(93), 41(66), 55(38), 69(28), 58(23), 57(20), 42(12)
5	2-methyl-butanal	P,L	MS,RT	57(100), 41(92), 58(90), 44(29), 86(17), 78(14), 84(12), 71(11)
6	benzene	G,P	MS,RT	78(100), 77(20), 51(19), 52(16), 50(14), 39(11), 79(10), 76(4)
7	methoxy-ethane	P,L	MS	45(100), 29(47), 60(27), 15(24), 27(19), 31(19), 59(11), 26(7)
8	1-penten-3-ol	G	MS,RT	57(100), 86(20), 81(14), 59(6), 96(4), 53(4), 41(1), 42(1)
9	3-pentanone	G,P,L	MS,RT	57(100), 86(17), 43(12), 81(9), 56(6), 41(4), 55(3), 96(3)
10	3-hydroxy-2-butanone	P	MS,RT	45(100), 43(76), 59(31), 57(25), 81(16), 88(12), 42(10), 86(8)
11	dimethyl disulfide	G	MS,RT	94(100), 79(50), 45(28), 80(15), 46(15), 81(12), 47(11), 55(10)
12	4-methyl-2-pentanone	L	MS,RT	43(100), 58(56), 57(36), 41(26), 55(23), 85(19), 40(15),100(14)
13	3,3-dimethyl-2-butanone	L	MS,RT	57(100), 43(72), 41(31), 58(20), 56(17), 59(14),100(11), 70(10)
14	3-methyl-1-butanol	P	MS,RT	55(100), 70(89), 41(70), 42(59), 57(58), 56(47), 43(46), 45(13)
15	methyl-benzene	G,P,L	MS,RT	91(100), 92(68), 65(15), 39(10), 63(7), 51(5), 93(4), 45(4)
16	2-methyl-thiophene	G,L	MS	91(100), 92(57), 65(11), 63(7), 51(6), 50(4), 89(4), 94(4)
17	2-methyl-2,4-hexadiene	G	MS	81(100), 96(49), 79(46), 53(21), 41(18), 67(15), 55(15), 54(10)
18	3-hexanone	G	MS,RT	57(100), 43(79), 71(62),100(31), 44(5), 72(4), 42(3), 58(2)
19	unknown	G	MS	55(100), 84(42), 41(36), 69(27), 56(25), 42(14), 70(4), 58(4)
20	cyclopentanol	P	MS,RT	57(100), 42(35), 41(30), 55(30), 70(20), 44(20), 43(19), 68(7)

Table 30. Volatile compounds tentatively identified in canned pink salmon of grades A and reject, and canned late run chum salmon by TTS/GC/MS (cont.d).

Peak no.	Compound name	Sample[a]	ID[b]	Mass Spectral Data: mass to charge ratio (abundance)
21	2-hexanone	L	MS,RT	43(100), 58(67), 57(27), 41(18), 44(14),100(12), 71(7), 55(6)
22	unknown	P	MS	41(100), 69(83), 81(56), 55(54), 67(47), 84(26), 68(22), 56(17)
23	octane	G,P	MS,RT	43(100), 56(84), 57(73), 44(73), 41(67), 85(31), 71(29), 72(19)
24	tetrahydro-2,5-dimethyl-furan	L	MS	56(100), 44(87), 57(63), 41(61), 43(21), 55(19), 72(16), 45(14)
25	2-octene	P	MS	56(100), 55(94), 41(83), 57(66), 95(58), 70(57), 42(34), 59(21)
26	3-methyl-1,4-heptadiene	G,P	MS	81(100), 67(71), 68(63), 55(30), 79(28),110(24), 53(22), 41(21)
27	3-ethyl-1,4-heptadiene	G,P	MS	81(100), 67(94), 68(78), 79(46), 55(40),110(36), 53(37), 41(36)
28	unknown	G	MS	44(100), 55(56), 69(52), 43(51), 41(43), 56(40), 42(37), 57(31)
29	4-pyridinamine	L	MS	94(100), 6 (47), 53(17), 52(9), 93(8), 95(7), 66(6), 41(5)
30	5-methyl-3-hexanone	L	MS	57(100), 44(33), 85(24), 43(23), 41(10),114(8), 72(6), 45(4)
31	methyl-pyrazine	P	MS,RT	94(100), 67(36), 43(19), 53(8), 42(8), 45(7), 55(5), 41(5)
32	2,3-dimethyl-1,4-hexadiene	G	MS	95(100),41 (50), 55(45), 67(44), 69(29), 57(27),110(25), 83(21)
33	3-ethyl-thiophene	L	MS,RT	44(100), 97(49),112(18), 84(6), 59(5),111(4), 71(4), 67(4)
34	(E,E)-1,3,6-octatriene	G,P	MS	91(100), 79(92), 77(51), 93(42), 108(22), 78(13), 66(8), 94(5)
35	ethyl-benzene	L	MS,RT	91(100), 58(37), 43(31),106(17), 44(16), 57(9), 73(8), 92(8)
36	2,3,3-trimethyl-1,4-pentadiene	G	MS	95(100), 67(49),110(31), 55(19), 41(14), 53(8), 81(7), 97(5)
37	dimethyl-benzene	G,P,L	MS	91(100),106(43),105(14), 77(11), 95(8), 51(7), 44(7), 79(7)
38	1,3-cyclopentanedione	P	MS	98(100), 41(32), 43(27), 42(16), 70(16), 69(15), 53(14), 81(13)
39	1,3-cyclooctadiene	G,P	MS	79(100), 77(40),108(29), 93(15), 80(15), 44(15), 66(15), 78(15)
40	3-heptanone	L	MS,RT	57(100), 85(21), 41(12), 72(12), 43(8),114(7), 58(4), 95(4)

Table 30. Volatile compounds tentatively identified in canned pink salmon of grades A and reject, and canned late run chum salmon by TTS/GC/MS (cont.d).

Peak no.	Compound name	Sample[a]	ID[b]	Mass Spectral Data: mass to charge ratio (abundance)
41	2,5-diethyl furan	P	MS	109(100),124(20),104(12), 56(10), 110(6), 94(5), 57(5), 67(5)
42	2-heptanone	G,P,L	MS,RT	43(100), 91(87), 58(73),106(38), 44(31), 71(17),105(14), 77(14)
43	nonane	G,P	MS,RT	42(100), 57(89), 85(30), 56(16), 71(12), 84(10), 41(7), 99(6)
44	4-methyl-hexanal	G,P,L	MS	44(100), 70(88), 43(83), 55(53), 57(49), 41(47), 41(32), 71(23)
45	2,6-dimethyl-pyrazine	P,L	MS,RT	108(100), 42(64), 43(27), 71(23), 57(23), 56(8), 41(7), 81(7)
46	2,3-dimethyl-pyrazine	L	MS,RT	67(100),108(47), 44(37), 41(28), 59(9), 66(8), 52(8), 98(6)
47	4-ethyl-phenol	P	MS,RT	107(100),122(38), 77(15),108(10), 91(5), 39(5), 65(3), 53(3)
48	trimethyl-benzene	P	MS	57(100), 71(42),105(36), 45(33), 107(26), 41(26), 70(25),108(21)
49	4,5-dimethyl-thiazole	L	MS,RT	113(100), 71(59), 85(19), 86(18), 45(13), 59(8), 58(6), 57(6)
50	unknown	G,P	MS	80(100), 68(63), 69(59), 56(53), 95(51), 70(42), 57(31), 97(23)
51	unknown	P	MS	64(100), 41(39), 57(31), 71(28), 111(26),113(22), 63(22), 95(11)
52	unknown	G,P	MS	95(100),126(32), 43(29), 57(26), 41(22), 67(18), 65(19), 44(18)
53	unknown	G,P	MS	79(100), 54(75), 81(69), 55(63), 72(51), 53(50), 82(37), 85(37)
54	unknown	P	MS	81(100), 79(92),124(70), 43(44), 41(34), 54(21), 55(20), 67(19)
55	propyl-benzene	G,P	MS,RT	91(100), 43(31), 57(27), 44(20), 120(14), 56(12), 41(12), 85(11)
56	unknown	L	MS	54(100), 55(64), 71(37), 41(30), 83(24), 56(10), 68(10), 42(10)
57	ethylmethyl-benzene	G,P,L	MS	105(100),120(29), 77(11),101(9), 91(6), 51(5), 57(5), 65(4)
58	2,4-dimethyl-hexanone	L	MS	57(100), 43(37), 55(25), 71(23), 70(21), 41(21), 69(12), 85(11)
59	dimethyl trisulfide	G,P	MS	126(100), 79(53), 45(25),105(24), 111(11), 64(11), 47(11),120(9)
60	3,5,5-trimethyl-2-hexene	P,L	MS	57(100), 70(45), 55(31), 41(24), 69(23), 43(8), 93(7), 42(6)

Table 30. Volatile compounds tentatively identified in canned pink salmon of grades A and reject, and canned late run chum salmon by TTS/GC/MS (cont.d).

Peak no.	Compound name	Sample[a]	ID[b]	Mass Spectral Data: mass to charge ratio (abundance)
61	ethylmethyl-benzene	G,P,L	MS	105(100), 57(24),120(24), 44(22), 91(14), 55(11), 70(11), 77(11)
62	unknown	G,P	MS	116(100), 46(65), 74(31), 41(22), 42(20), 43(11), 73(10), 45(9)
63	3-octanone	L	MS,RT	43(100), 57(59), 72(47), 99(41), 71(36), 41(25), 55(22), 68(18)
64	2-octanone	L	MS,RT	58(100), 43(91), 71(21), 59(18), 41(14), 81(13), 57(12), 85(8)
65	7-octen-2-one	P	MS	43(100), 68(48), 41(27), 67(19), 55(19), 69(10), 71(8), 97(7)
66	2-pentyl-furan	P	MS	81(100), 82(22),138(14), 43(13), 105(12), 53(12), 41(9), 57(9)
67	trimethyl-benzene	G	MS	105(100),120(48), 77(8),119(8), 91(7), 81(6), 79(6),106(5)
68	decane	G,P,L	MS,RT	57(100), 43(81), 71(38), 41(30), 85(24), 56(15), 81(11), 70(11)
69	unknown	G,P,L	MS	68(100),107(88),136(78), 94(74), 79(71), 81(61), 77(58), 53(30)
70	octanal	L	MS,RT	42(100), 56(46), 43(44), 84(40), 57(35), 44(35), 55(32), 41(32)
71	trimethyl-pyrazine	L	MS,RT	122(100), 42(77), 81(9),123(7), 54(5), 52(4), 49(2), 50(2)
72	(s)-2,3-dihydro-4-(1-methylpropyl)-furan	P	MS	97(100),126(13), 45(10), 44(5), 43(5), 98(4), 42(4), 41(4)
73	unknown	G,P,L	MS	81(100), 53(16),110(13), 67(8), 41(8), 55(7), 78(6), 69(5)
74	trimethyl-benzene	G,P,L	MS	57(100),105(89),120(27), 56(27), 43(17), 71(14),119(11), 41(8)
75	(E)-2-hepten-1-ol	G	MS	57(100), 56(55), 41(21), 55(17), 93(14), 69(11), 67(10), 54(9)
76	unknown	P,L	MS	57(100), 56(30), 43(29), 44(19), 41(18), 45(14), 55(13), 93(12)
77	6-ethyl-2-methyl-octane	G,P	MS	57(100), 71(60), 85(26), 43(19), 70(16), 41(13), 55(6), 58(5)
78	1-decene	L	MS	43(100), 55(79), 57(67), 70(43), 41(36), 82(32), 97(30), 83(27)
79	trimethyl-octane	G,P	MS	57(100), 56(25), 43(15), 71(14), 70(7), 58(6), 85(4),113(4)
80	1-phenyl-ethanone	L	MS	105(100), 77(87),120(48), 57(33), 43(26), 51(19), 85(17), 71(13)

Table 30. Volatile compounds tentatively identified in canned pink salmon of grades A and reject, and canned late run chum salmon by TTS/GC/MS (cont.d).

Peak no.	Compound name	Sample[a]	ID[b]	Mass Spectral Data: mass to charge ratio (abundance)
81	unknown	L	MS	43(100), 71(72), 95(60), 57(36, 99(26),124(24), 58(24), 69(22)
82	trimethyl-octane	G,P	MS	57(100), 56(19), 71(18), 43(9, 85(8), 41(7),114(4), 57(3)
83	dimethylethyl-benzene	G,P	MS	119(100), 57(67),134(26), 56(24), 120(17), 71(14),105(11),124(10)
84	trimethyl-octane	G,P	MS	57(100), 56(44), 71(36), 43(31), 41(11), 70(8), 85(6), 55(6)
85	trimethyl-octane	G,P	MS	71(100), 57(83), 43(61), 85(27), 70(24), 41(15), 56(15), 55(13)
86	dimethylethyl-benzene	G,P	MS	119(100),134(29), 91(8),120(7), 77(6),115(5),117(4),105(4)
87	unknown	G,P	MS	79(100), 81(48), 67(41), 54(40), 58(36), 80(32), 96(29), 43(29)
88	unknown	G,P	MS	79(100), 67(55), 81(52), 54(50), 80(34), 91(26), 77(24), 71(23)
89	tetramethyl-pyrazine	L	MS,RT	136(100), 54(82), 42(42),135(40), 53(11), 55(7),137(7), 81(6)
90	2-nonanone	L	MS,RT	58(100), 43(65), 57(24), 59(21), 71(21), 41(10), 55(8),142(5)
91	undecane	G,P,L	MS,RT	57(100), 43(53), 71(33), 85(20), 41(14), 56(13), 69(11), 70(11)
92	nonanal	G,P,L	MS,RT	57(100), 43(57), 56(54), 41(52), 55(45), 44(45), 70(37), 98(26)
93	2,2,5-trimethyl-3,4-hexadione	L	MS	43(100), 57(38), 71(22), 58(21), 69(15), 98(12), 41(10), 85(7)
94	6-nonynoic acid	G,P	MS	79(100), 94(49), 67(44), 81(38), 55(22), 41(17), 93(19), 77(16)
95	1,4-undecadiene	P	MS	81(100), 67(89), 68(69), 55(48), 79(42), 43(32), 95(28), 41(19)
96	unknown	L	MS	95(100), 55(64), 69(61), 58(58), 41(55), 83(47), 57(39), 70(36)
97	2-pentyl-thiophene	L	MS	97(100), 83(54), 55(34), 56(33), 98(26), 84(20), 57(19), 41(17)
98	6-nonynoic acid	G,P	MS	79(100), 67(30), 94(24), 81(21), 93(20), 41(18), 55(17),108(15)
99	tetramethyl-benzene	P	MS	119(100),134(33), 79(13), 91(11), 67(10),120(8), 77(7),133(7)
100	decahydro-2-methyl-naphthalene	P	MS	152(100), 95(83), 82(82), 81(66), 67(62), 96(60),137(55), 55(55)

Table 30. Volatile compounds tentatively identified in canned pink salmon of grades A and reject, and canned late run chum salmon by TTS/GC/MS (cont.d).

Peak no.	Compound name	Sample[a]	ID[b]	Mass Spectral Data: mass to charge ratio (abundance)
101	methyl-cyclodecane	P	MS	69(100), 68(88), 55(75), 41(58), 83(55), 70(48), 97(39), 56(35)
102	diethylmethyl-benzene	P	MS	133(100),119(96),148(32), 43(28), 91(24),120(18), 45(16), 57(15)
103	naphtalene	P	MS,RT	128(100),137(82), 79(64), 91(28), 57(17),119(11),129(11), 63(11)
104	2-decanone	G,L	MS,RT	58(100), 43(64), 59(30), 71(28), 57(15), 41(9),156(9), 55(8)
105	dodecane	G,P,L	MS,RT	57(100), 43(62), 71(40), 85(25), 41(24), 56(21), 55(17), 70(14)
106	decanal	G,P,L	MS,RT	57(100), 43(92), 41(79), 55(62), 44(56), 70(51), 82(50), 71(48)
107	unknown	P	MS	81(100), 53(8),160(3), 82(2), 137(2), 57(2),111(1),119(1)
108	2-hexyl-thiophene	L	MS	97(100), 41(36), 98(34), 43(33), 70(31), 57(31), 69(29), 83(29)
109	unknown	G,P	MS	43(100), 57(90), 81(90), 79(89), 108(80), 91(60), 77(51),120(41)
110	2-undecanone	P	MS,RT	43(100), 58(91), 71(41), 59(36), 81(15), 55(10), 57(8), 85(8)
111	unknown	P	MS	57(100), 95(76), 43(74), 71(40), 79(38),108(35),109(23),120(21)
112	tridecane	G,P	MS,RT	57(100), 71(62), 43(56), 85(41), 41(27), 56(19), 55(17), 70(15)
113	1-tridecene	L	MS	57(100), 55(86), 43(81), 69(55), 70(46), 56(40), 41(40), 83(39)
114	undecanal	L	MS,RT	43(100), 57(94), 41(72), 55(69), 82(54), 56(44), 44(44), 29(43)
115	unknown (C12H130)	L	MS	55(100), 44(95), 56(82), 82(77), 68(55), 41(43), 81(43), 69(41)
116	6-tridecene	L	MS	97(100), 43(87), 44(71), 41(68), 71(53), 70(48), 98(47), 69(40)
117	unknown (C12H18)	P	MS	91(100), 80(99), 67(81), 41(79), 69(76),105(68), 92(63), 93(43)
118	3-dodecanone	L	MS	57(100), 72(68), 43(23),155(23), 85(22), 55(22), 71(13), 41(12)
119	tetradecane	G,P,L	MS,RT	57(100), 43(73), 71(44), 85(21), 41(11), 55(9), 56(8), 79(6)
120	unknown	P	MS	79(100), 55(47), 93(39), 80(36), 67(36), 91(33), 41(31), 77(27)

Table 30. Volatile compounds tentatively identified in canned pink salmon of grades A and reject, and canned late run chum salmon by TTS/GC/MS (cont.d).

Peak no.	Compound name	Sample[a]	ID[b]	Mass Spectral Data: mass to charge ratio (abundance)
121	unknown	P,L	MS	105(100), 93(81), 91(64), 77(63), 79(63), 69(63),107(58), 55(58)
122	pentadecane	G,P,L	MS,RT	57(100), 72(70), 43(62), 85(42), 41(25), 55(17), 56(14), 70(12)
123	unknown	P	MS	43(100), 57(62), 71(36), 85(17), 56(15), 55(11), 99(9), 69(9)
124	hexadecane	G,P,L	MS,RT	57(100), 43(82), 71(46), 85(35), 41(15), 70(11), 56(10), 55(10)
125	unknown	L	MS	71(100), 43(31), 41(7),159(7), 56(6), 55(5), 57(4), 69(4)
126	unknown	L	MS	57(100), 45(58), 69(44), 83(42), 43(41), 56(38), 97(31), 55(30)
127	2,2,4,4,6,8,8-heptamethyl-nonane	L	MS	57(100), 43(28), 44(22), 85(13), 41(10), 56(8), 55(6),113(6)
128	heptadecane	P,L	MS,RT	57(100), 71(77), 43(61), 85(56), 41(28), 70(23), 69(15), 99(8)
129	2,6,10,14-tetramethyl-pentadecane	P,L	MS	57(100), 71(92), 43(46), 85(31), 56(20), 55(18),113(17), 41(16)
130	nonadecane	P,L	MS,RT	57(100), 71(81), 43(47), 85(32), 56(19), 55(18), 41(15),113(14)

[a] G, canned pink salmon of grade A; P, canned pink salmon of grade reject; L, canned chum salmon of advanced sexual maturity.

[b] MS, tentatively identified by mass spectrometry; RT, retention time consistent with that of authentic compounds.

hydrocarbons (50), ketones (22), sulfur-containing compounds (6), nitrogen-containing compounds (6), aldehydes (6), alcohols (5), acids (2), and miscellaneous compounds (6).

Figures 38, 39, and 40 are FID chromatograms of CCS volatiles from samples of canned pink salmon of grade A, canned pink salmon of grade reject, and canned late run chum salmon, respectively. Based on the retention time of authentic standards and the pattern of elution, several peaks in these figures have been associated with compounds listed in Table 30. Also associated with these figures are Tables 31, 32, and 33 which list the odour attribute, the retention time and the possible identity of the volatile that caused the sensation. The CCS samples of canned pink salmon of poor quality included substantial concentrations of ethanol. To avoid overloading the column, an initial purging was performed for 5 min before cryofocussing. Although CCS allowed a non-discriminatory collection of volatiles which were subsequently evaluated for their sensory characteristics, the retention times of the early eluting volatiles tended to vary due to the presence of water collected during purging. As opposed to FID, mass spectrometers are sensitive to large quantities of eluted water. Therefore this CCS means could not be used in conjunction with mass spectrometry.

The FID chromatograms obtained using CCS measured only half of the volatiles concentrated since a 1:1 splitter was used to gather the aromagrams. In spite of this, comparison with both sets of chromatograms (Figures 35, 36, 37 vs 38, 39, 40) shows that, in general, higher

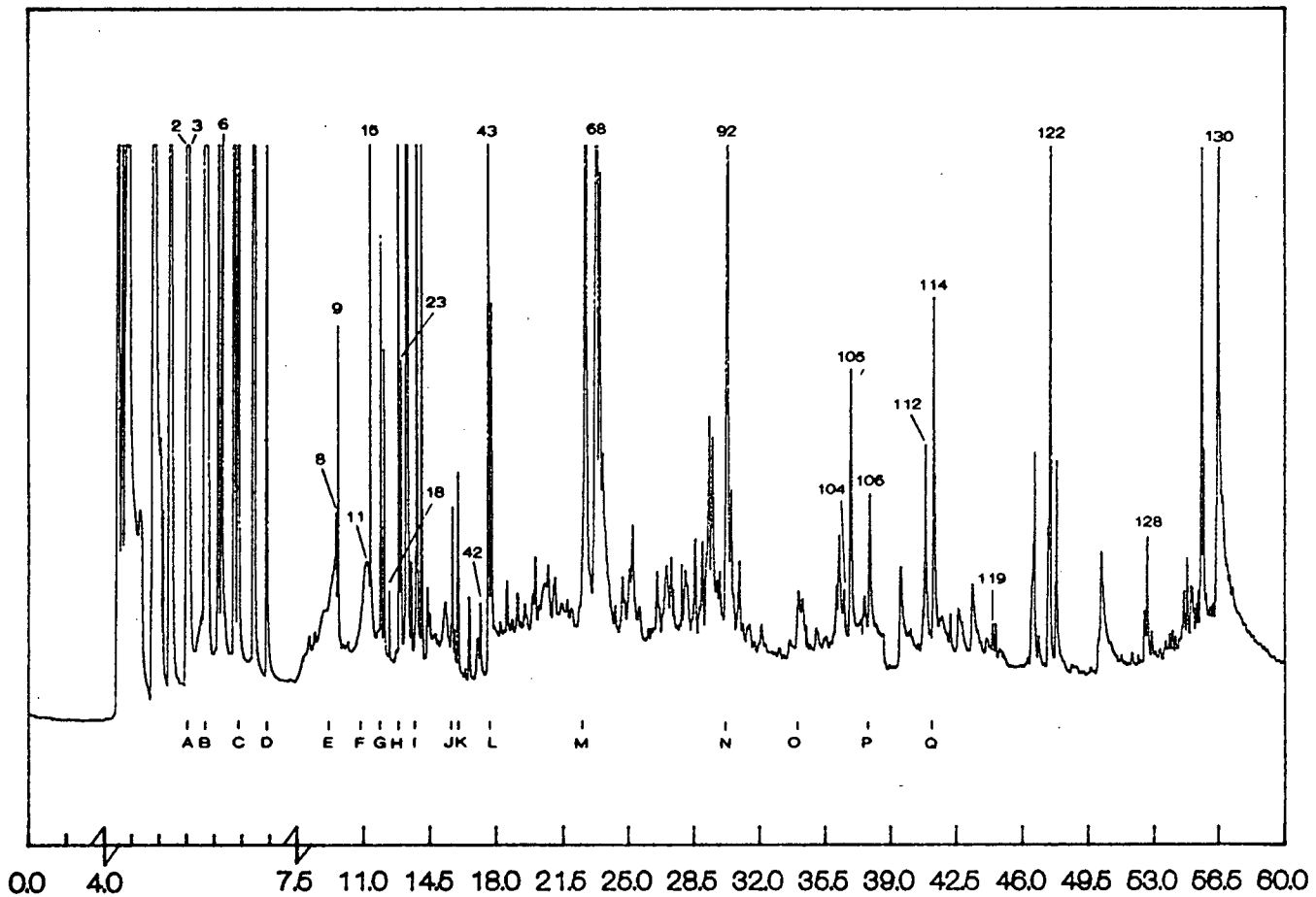

Figure 38. Chromatogram obtained by gas chromatography/flame ionization detection (GC/FID) of headspace volatile components from canned pink salmon of good quality (grade A) concentrated using cryofocussing. Compounds are identified by peak numbers shown in Table 30 and letters refer to Table 31.

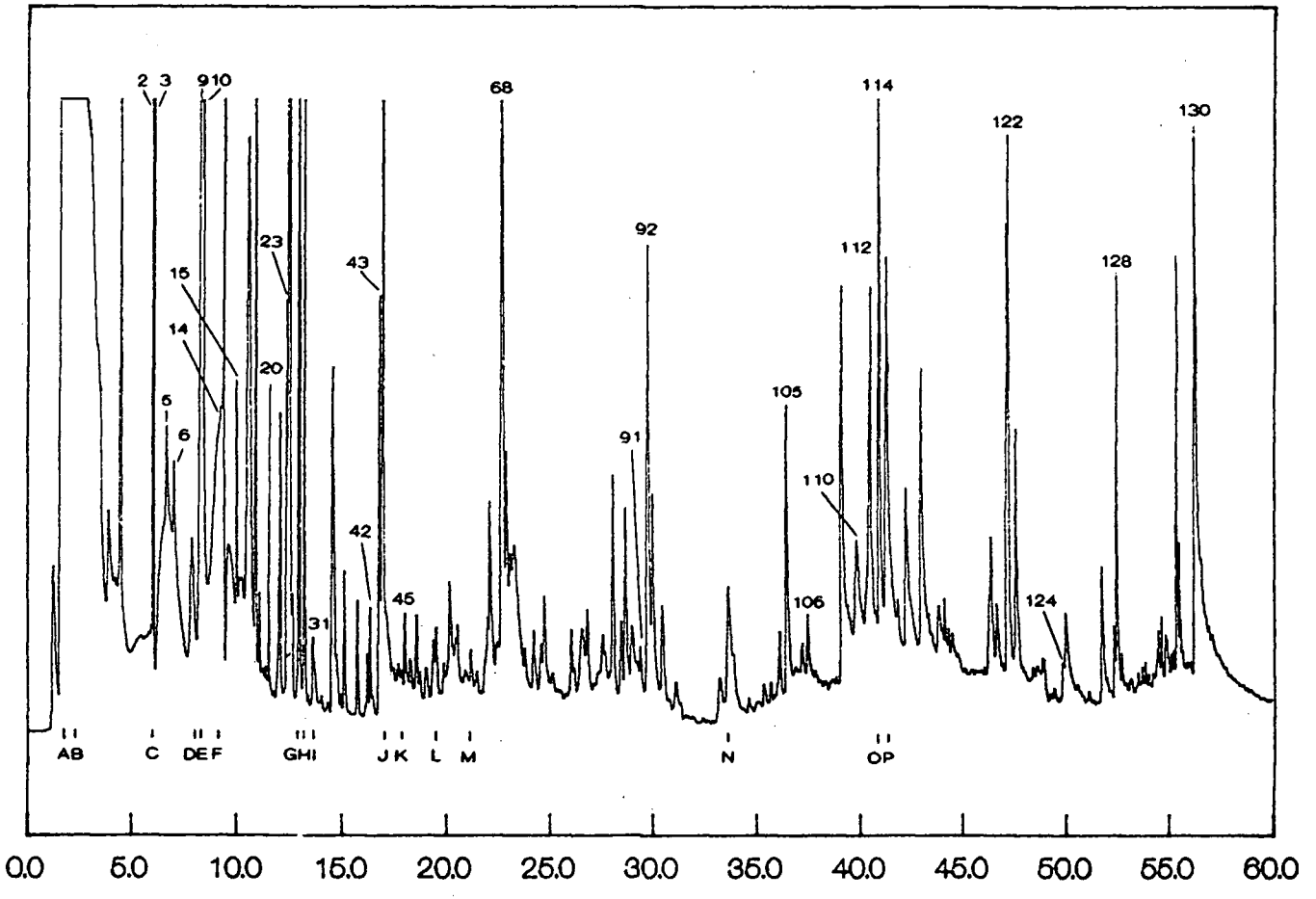

Figure 39. Chromatogram obtained by gas chromatography/flame ionization detection (GC/FID) of headspace volatile components from canned pink salmon of advanced decomposition (grade reject) concentrated using cryofocussing. Compounds are identified by peak numbers shown in Table 30 and letters refer to Table 32.

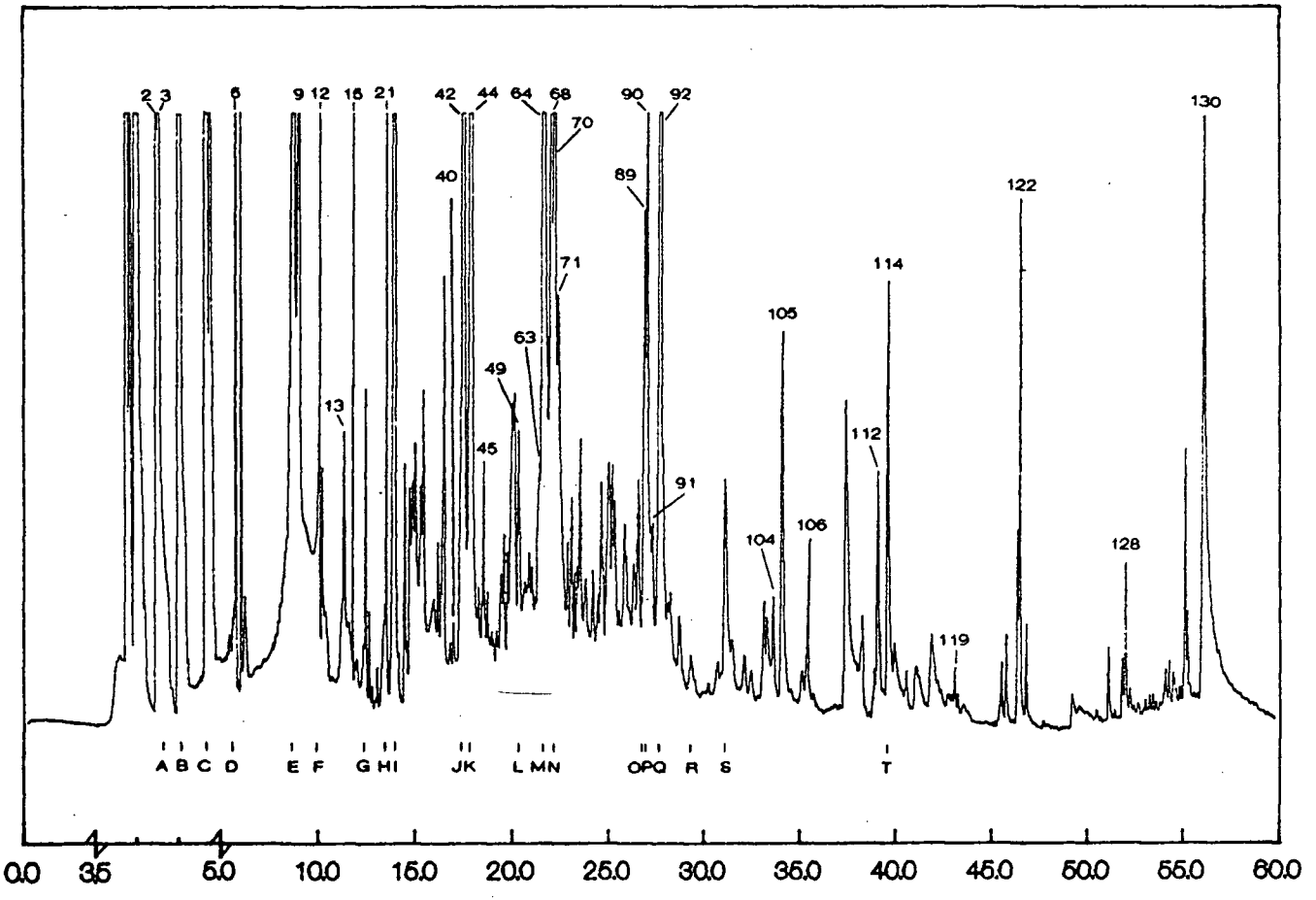

Figure 40. Chromatogram obtained by gas chromatography/flame ionization detection (GC-FID) of headspace volatile components from canned chum salmon of advanced sexual maturity (spawning dark) concentrated using cryofocussing. Compounds are identified by peak numbers shown in Table 30 and letters refer to Table 33.

Table 31. Cryofocussing concentration sampling/gas chromatography/odour evaluation (CCS/GC/OE) of volatile components from canned pink salmon of good quality (grade A).

ID	RT (min)	Possible compound	Odour attribute
A	5.4	2-butanone	green, raw, nutty
B	5.8	unknown	burnt
C	6.4	unknown	burnt
D	6.9	unknown	chlorine-like
E	9.1	1-penten-3-ol plus unknown	rancid, malodorant
F	9.9	dimethyl disulfide	sulfurous
G	11.6	2-methyl thiophene	green, gasoline-like
H	12.6	unknown	fishy
I	13.5	3-methyl-1,4-heptadiene	burnt, medicinal, stinky
J	15.3	1,3,6-octatriene	chlorine-like
K	15.7	2,3,3-trimethyl-1,4-pentadiene and dimethyl benzene	pungent, stinky
L	17.4	4-methyl hexanal	fish skin
M	22.3	trimethyl benzene	concrete, petroleum-like
N	29.6	nonanal	aldehydic, spicy, green
O	33.3	unknown	cucumber
P	37.2	decanal	aldehydic, fishy
Q	40.5	undecanal	green, waxy

Table 32. Cryofocussing concentration sampling/gas chromatography/odour evaluation (CCS/GC/OE) of volatile components from canned pink salmon of advanced decomposition (grade reject).

ID	RT (min)	Possible compound	Odour attribute
A	1.7	ethanol	alcoholic
B	2.2	unknown	burnt
C	5.8	2-butanone	green, nutty
D	7.9	3-pentanone	chlorine-like
E	8.1	3-hydroxy-2-butanone	buttery, toast-like
F	8.9	3-methyl-1-butanol	alcoholic, solvent-like
G	12.6	3-methyl-1,4-heptadiene	fishy
H	12.9	3-methyl-1,4-heptadiene	fishy
I	13.4	methyl pyrazine	roasted, baking
J	16.6	4-methyl hexanal	sour, sea-like
K	17.4	2,6-dimethyl pyrazine	roasted, nutty
L	19.0	dimethyl trisulfide	burnt hair
M	20.7	unknown	wet dog
N	32.9	unknown	spring smell, meaty
O	40.1	undecanal	green, waxy, flowery
P	40.5	unknown	cooked rice

Table 33. Cryofocussing concentration sampling/gas chromatography/odour evaluation (CCS/GC/OE) of volatile components from canned chum salmon of advanced sexual maturity (spawning dark).

ID	RT (min)	Possible compound	Odour attribute
A	1.7	ethanol	alcoholic
A	4.3	2-butanone	green, nutty, raw
B	4.5	unknown	strong odour of hay, straw, cooked malt, cereal, fishy
C	4.8	unknown	hay, straw-like
D	5.5	2-methyl-butanal	slight odour of hay, straw-like
E	8.5	unknown	chlorine, swimming pool
F	9.8	4-methyl-2-pentanone	chlorine-like, rubber, quinine
G	12.1	2-methyl thiophene	slightly fishy, green, sweet
H	13.3	2-hexanone	nutty, green
I	13.8	tetrahydro-2,5-dimethyl furan	burnt, ethereal
J	17.2	2-heptanone	sweat-like
K	17.6	4-methyl-hexanal	fishy, cardboard, straw-like
L	20.1	4,5-dimethyl thiazole	slightly meaty
M	21.3	2-octanone	earthy, soil, mushroom, herbal
N	21.9	octanal	pungent, green
O	26.5	tetramethyl pyrazine	nutty, grassy, roasted
P	26.6	nonanone	green, flower, cream
Q	27.3	nonanal	spicy, green
R	29.0	2,2,5-trimethyl-3,4-hexadione	very faint butter-like
S	30.7	unknown	spring smell, sweet
T	34.1	undecanal	waxy, flowery

concentrations of volatiles are achieved with the CCS method than with the TTS method. This could be observed particularly in the first 10 min of volatile elution. Tenax possesses a low capacity for the pre-concentration of highly volatile organic molecules. In addition, dry purging for 1 h before releasing the volatiles was necessary to remove most of the moisture accumulated in the Tenax trap. Compounds of molecular weight lower than about 5 carbons, including aliphatic alcohols and aliphatic carboxylic acids, are not quantitatively retained at room temperature (Nunez and Gonzalez, 1984).

The hydrocarbons identified included a homologous series of n-hydrocarbons ranging from C6 to C19 (Table 30). Watanabe and Sato (1971) suggested that saturated alkanes could result from decarboxylation and splitting of carbon-carbon chains of higher fatty acids. When heating tristearin in air, the presence of n-hydrocarbons among the volatiles was interpreted by Selke et al. (1975) to arise from the reaction of alkyl-free radicals, which were the product of thermally decomposed hydroperoxides with free hydrogen radicals. No defined aroma pertaining to alkanes were directly identified in the present study. Alkanes detected in beef (Larick and Turner, 1990), crayfish tail meat (Vejaphan et al., 1988), and crabmeat (Matiella and Hsieh, 1990) did not contribute significantly to aroma as they possess weak odour. In the past, alkanes from C11 to C15 have been reported to possess sensory characteristics which resembled aliphatic alcohols (Ohloff et al., 1985).

Seven branched alkanes, two cyclic alkanes, six straight chain alkenes, six branched alkenes, and one cyclic alkene were identified. 2,6,10,14-Tetramethylpentadecane, identified in this study, was also found in cooked Antarctic krill (Kubota et al., 1982) and crayfish waste (Tanchotikul and Hsieh, 1989). A chlorine-like smell was associated with 1,3,6-octatriene. A pungent, unpleasant aromatic sensation was perceived at retention times corresponding to the co-elutions of 2,3,3-trimethyl-1,4-pentadiene and dimethyl benzene. 3-Methyl-1,4-heptadiene was characterized as having a burnt, medicinal, disagreeable odour in canned pink salmon of grade A while it was associated with a fishy smell in canned pink salmon of grade reject. Although unsaturated and aromatic hydrocarbons are known to contribute to marine flavours of shellfish and seaweed (Ohloff et al., 1985), it is possible that an unidentified compound responsible for the odour in grade A canned pink salmon might have co-eluted with 3-methyl-1,4-heptadiene. Although the origin of most of these compounds remains uncertain, it is possible that some of the branched and cyclic hydrocarbons are secondary reaction products from the thermal oxidation of carotenoids and other unsaturated lipids (Ohloff et al., 1985).

Sixteen aromatic hydrocarbons, including 14 alkylbenzenes and 2 naphthalenes, were identified in canned salmon headspaces. Although they are often found as artefacts (from solvent residues or from Tenax degradation), the experimental method did not directly contribute to these benzene derivative peaks since the initial backgrounds were examined under comparable experimental conditions before sample analysis. Several of

these alkylbenzenes are known oxidation products of lipids and have been found in tea (Habu et al., 1985), corn (Buttery et al., 1978), nuts (Crain and Tang, 1975; Walradt et al., 1971), meat (Shahidi et al., 1986), and many heated foods (Forss, 1972). Even if none of these compounds have a meat or fish odour, they may play a role in the overall flavour (Min et al., 1979). Watanabe and Sato (1971) reported the formation of various alkylbenzenes from beef fats during heating. Carotenoids have been proposed to be precursors of toluene, xylene, and benzene derivatives found in chicken, beef, butter, and fish (Borenstein and Bunnell, 1966; Pippen et al., 1969). Possible routes to the aromatic compounds include the oxidation of unsaturated hydrocarbons (Min et al., 1977) or other products of fatty acid autoxidation (Nonaka et al., 1967). However, some of these hydrocarbons may have come from the aquatic environment. If present in polluted waters, they can be bioaccumulated by marine fish and shellfish through the skin, gills, and ingestion of contaminated food (Connell and Miller, 1981). In a study done by Neff et al. (1976), aromatic hydrocarbons were accumulated to a greater extent and were retained longer than alkanes by fish.

Straight chain and branched aldehydes were all found to be saturated (Table 30). Nonanal was the only aldehyde observed in all three types of canned salmon in relatively high concentration (Figures 35, 36, 37). Besides nonanal, canned late run chum salmon had high levels of 2-methyl-butanal, 4-methyl-hexanal, and undecanal. Four volatile straight chain aldehydes containing 8 to 11 carbons were identified. They contributed spicy, green, waxy notes. A sour, fishy, sea-like, cardboard sensation

was associated with 4-hexanal (Fig. 38, 39, and 40). A hay, straw-like aroma reminiscent of late run chum odour was noticed at a retention time corresponding to 2-methyl-butanal. This compound was, however, not the only one involved in the characteristic late run odour; two other occurrences similarly characterized as cooked malt, hay, straw, and fish-like were found to elute before 2-methyl-butanal (Table 33). Although detected by the CCS method, these two compounds were part of the breakthrough volume of the tenax material, since a late run odour came out of the trap during purging, and were therefore not identified by mass spectrometry. Straight chain aldehydes as well as 2-methyl-butanal have been reported in beef (MacLeod and Ames, 1986), cod (McGill et al., 1977) and crayfish (Vejaphan et al., 1988). The formation of alkanals can be attributed to thermal decomposition of hydroperoxides and peroxy radicals proposed to be initial products of thermally-oxidized fats (Sink, 1973). In some cases, they can originate from the Strecker degradation of amino acids; for example, 2-methyl-butanal may be derived from isoleucine (Dwivedi, 1975).

Due to their low threshold values, aldehydes and ketones are important aroma compounds in foodstuffs. On the one hand, they contribute to desirable aroma, but on the other hand, they are responsible for rancid odour and flavour during spoilage of fats and fatty foods (Forss, 1972). Wilson and Katz (1972) concluded that saturated and unsaturated aldehydes and ketones were important components of the desirable aroma of cooked chicken. However, a number of aldehydes and ketones in spoiled fish were identified by Wong et al. (1967) and Miller et al. (1972b). McGill et al.

(1974; 1977) also showed that aldehydes including 4-cis-heptenal were responsible for the typical off-odours of frozen cold stored cod.

Ketones were the second largest class of volatile components found in canned salmon. A larger number of ketones compared to aldehydes have also been reported as flavour volatiles in shrimp (Kubota et al., 1986). Ketones were the major volatile components in crayfish waste and tail meat (Vejaphan et al., 1988). One aromatic, one unsaturated, and seventeen saturated ketones as well as two alkadiones and one hydroxy ketone were identified in the present investigation. The most abundant components in the three types of canned salmon were 2-butanone and 3-pentanone. A green, nutty odour originated from 2-butanone and a chlorine-like, rubber odour was associated with 3-pentanone and 4-methyl-2-pentanone. 4-Methyl-2-pentanone found in canned chum salmon of advanced sexual maturity was shown to be present in beef (MacLeod and Ames, 1986), crabmeat (Matiella and Hsieh, 1990), and crayfish waste (Tanchotikul and Hsieh, 1989). 2,3-Butanedione (diacetyl) and 3-hydroxy-2-butanone (acetoin) are constituents of many food aromas and provide a buttery flavour (Arctander, 1969). A toast-like, buttery odour associated with acetoin was particularly noted in canned pink salmon of advanced decomposition (Table 32). Both diacetyl and acetoin have been identified in chicken (Minor et al., 1965) as well as in cooked beef and pork (Mottram et al., 1982).

In addition, a series of methyl and ethyl ketones (C4 to C12) and a number of branched methyl ketones were detected in the canned salmon samples. A number of them were prevalent in canned late run chum salmon

(Table 30) and were perceived to have various aromas. 2-Butanone and 2-hexanone were labelled with a green, nutty sensation compared to 2-heptanone and 2-octanone which respectively evoked sweat-like and earthy, soil odours. Autoxidation of fatty acids, particularly unsaturates (via hydroperoxides), has been proposed as a mechanism for the formation of methyl ketones (Thomas et al., 1971). Selke et al. (1975) reported the formation of a homologous series of methyl ketones from heated tristearin and concluded that they could be the result of ß-oxidation (from the carbonyl end) of the carbon chain followed by decarboxylation. Similarly, various other ketones could possibly be derived from distinct secondary degradation reactions involving diverse substances from the lipid fraction during heating.

Among the 5 alcohols detected by the TTS method, two were found in high concentration: 1-penten-3-ol in canned pink salmon of grade A and 3-methyl-1-butanol in canned pink salmon of grade reject. Because of their relatively high threshold concentrations, alcohols are generally minor odour contributors unless present at high concentrations or unless they contain unsaturated bonds. In canned grade A pink salmon, a rancid, malodorant aroma was perceived when 1-penten-3-ol co-eluted with an unknown compound (Table 31). 1-Penten-3-ol has been recognized in Atlantic and Pacific oysters (Josephson et al., 1985), roasted and boiled shrimp (Kubota et al., 1986), pickled smelt (Josephson et al., 1987), fresh and oxidized frozen whitefish (Josephson et al., 1983; Josephson et al., 1984b), and crayfish waste (Tanchotikul and Hsieh, 1989). Alcohols may be formed by decomposition of secondary hydroperoxides of fatty acids.

In a similar pathway to that involved in the generation of 1,5-octadien-3-ol (Wurzenburger and Grosch, 1986), a rearrangement and cleavage of hydroperoxides from linoleic or arachidonic acids could yield 1-penten-3-ol. Cyclopentanol, which has also been identified in Atlantic and Pacific oysters, can be formed from the cyclization of 1-penten-3-ol by a mechanism analogous to that proposed for the production of 1-octen-3-ol to cyclooctanol (MacLeod and Panchasara, 1983).

Two alcoholic odours corresponding to ethanol and 3-methyl-1-butanol were smelled during elution of volatiles from canned pink salmon of advanced decomposition. Ethanol was not detected by the TTS method but its presence was deduced based on previous analyses with the static headspace method developed in Chapter II. Along with ethanol and other volatiles, 3-methyl-1-butanol was found in Chapter III to be an important discriminator for quality classification of canned pink salmon. Aliphatic alcohols occur largely in fruits, vegetables, and fermented foods. During the period of refrigerated storage, known psychrotrophic spoilage microorganisms of fish, such as Gram-negative bacteria of the genera Pseudomonas and Achromobacter, were assumed to have proliferated and produced alcohols and other possible compounds. Ethanol can be derived from a variety of fermentable substrates, most sugars, many amino acids, certain organic acids, purines, pyrimidines and other miscellaneous substances whose catabolism leads to the pyruvate-acetaldehyde-ethanol transformation (Brock, 1979). Miller et al. (1973b, c) showed that S. putrefaciens, P. fluorescens, P. perolens, and Achromobacter produced 3-methyl-1-butanol during growth on fish muscle. The presence of this

compound has also been observed in yeast fermentations and metabolic by-products of fruit cells. Four and five-carbon methyl-branched alcohols were found to be generated by Saccharomyces cerevisae from amino acid precursors (Sentheshanmuganathan, 1960). Labelling experiments using postclimacteric banana tissue slices and labelled amino acids demonstrated the transformation of (U-^{14}C)-leucine to 3-methyl-1-butanol (Myers et al., 1969; 1970) possibly through transamination and decarboxylation.

The only phenolic substance detected in this investigation was 4-ethylphenol. This compound has previously been found in cod (McGill et al., 1977) and crayfish waste (Tanchotikul and Hsieh, 1989). It was, however, detected in trace amounts and did not seem to play an important role in advanced spoilage.

During purging of the canned pink salmon of grade A and reject, the effluent going through the cryogenic trap and exiting through the sniffing port had a sulfurous odour. This odour was not perceived in canned late run chum salmon. The compound responsible for the aroma was believed to be hydrogen sulfide based on its low boiling point and its prior identification among the volatiles found in Chapter II. Two straight chain sulfur-containing compounds, dimethyl sulfide and dimethyl trisulfide, were identified by GC-MS of the volatiles concentrated by the TTS method (Table 30). A sulfurous odour corresponding to dimethyl disulfide was perceived from canned pink salmon of good quality while dimethyl trisulfide gave a burnt hair smell to the effluent of canned pink salmon of grade reject (Tables 31 and 32). Due to their very low sensory

threshold values, volatile organic sulfur compounds are an important fraction of aroma in numerous foods. For example, when sulfur compounds were removed from the distillate during the cooking of chicken, the typical meat aroma also disappeared (Minor et al, 1965). These compounds have previously been reported in raw and thermally processed fish and crustaceans (Sipos and Ackman, 1964; Josephson et al., 1985; Hughes, 1964; Ronald and Thompson, 1964; Whitfield et al., 1981a,b; and Vejaphan et al., 1988). The numbers and concentrations of low molecular weight sulfur compounds were characteristic of the off-odours arising during cold storage of spoiling cod (Herbert et Shewan, 1976; McGill et al., 1977). They can be formed principally during heat treatments from the free, peptidic, and proteinic sulfur-amino acids as well as the glutathione pool in fish tissue. In refrigerated spoiling fish, they may also be of microbial origin derived from bacteria such as Pseudomonas and Achromobacter (Kadota and Ishida, 1972; Miller et al., 1973b, c). Using radioactively-labelled precursors, Herbert and Shewan (1975, 1976) conducted studies in which hydrogen sulfide was found to come from cysteine while methionine served as the precursor of methanethiol. Polysulfides may subsequently form as oxidation products of methanethiol (Maruyama, 1970; Christensen et al., 1981).

Four 2-alkylthiophenes and one alkylthiazole were identified (Table 30). A green, sweet, gasoline-like odour was associated with 2-methyl thiophene and noticed in canned pink salmon of good quality and in canned late run chum salmon (Fors, 1983). Thiophenes are important volatiles in the flavour of cooked meat as they can also contribute a mild sulfurous

odour. 2-Methylthiophene has been identified in boiled and pasteurized crabmeat (Matiella and Hsieh, 1990), boiled crayfish tail meat (Vejaphan et al., 1988), and roasted shrimp (Kubota et al., 1986). A meaty aroma compound, 4,5-dimethyl thiazole, was recognized in canned late run chum salmon. Fors (1983) also reported this compound to possess a braised, roasted, and meaty flavour. A large number of substituted heterocyclic thiophenes and thiazoles have been reported in thermally processed meat such as beef, pork and chicken (Shahidi et al., 1986). The sulfur in thiophenes and thiazoles may be derived from amino acids (cysteine, cystine, methionine) or from vitamin B_1. It has been suggested that thiophenes are formed by the action of hydrogen sulfide on sugar degradation products such as dehydroreductones and furans (Vernin and Parkanyi, 1982), furfural (Shibamoto, 1977), and furanones (van der Ouwelend and Peer, 1975) during the course of the Maillard reaction. Phospholipid oxidation products such as 1,4-ketoaldehydes or unsaturated aldehydes have also been suggested to interact with ammonia and hydrogen sulfide, derived from the Strecker degradation of cysteine, to give 2-alkylthiophenes (Mottram and Whitfield, 1987). Similar pools of Maillard reactants allow for the inclusion of nitrogen in the heterocycles to form derivatives such as thiazoles.

Traces of 3 furan derivatives were identified as volatile components of canned pink salmon of grade reject while one furanoid compound, tetrahydro-2,5-dimethyl furan, was found in canned chum salmon of advanced sexual maturity (Table 30). A burnt, ethereal odour was associated with this last compound. The volatile 2-pentyl furan, separated and identified

in this study, has been reported to impart reversion, beany, grassy, and licorice-like flavours in soybean oil, and can be produced by oxidation of fatty acids (Taylor and Mottram, 1990; Smouse and Chang, 1967). Furans can be found in dehydrated and thermally degraded condensates of carbohydrate, or formed by Amadori rearrangement pathways (Whistler and Daniel, 1985). A number of furanoid volatiles have previously been found in brewed coffee (Shimoda and Shibamoto, 1990), fried bacon (Ho et al., 1983), roast beef (Min et al., 1979), and chicken (Nonaka et al., 1967).

Besides the alkylthiazole, a total of 5 nitrogen-containing compounds including 4 alkylpyrazines and 1 pyridinamine were detected in the canned late-run chum salmon while 2 alkylpyrazines were found in canned pink salmon of poor quality (Table 30). The alkylpyrazines all contributed nutty, roasted odorous notes (Tables 32 and 33). Pyrazines are known to result from the classic Maillard browning reaction involving a combination of NH_3 or amino-containing compounds with sugars or other carbonyl compounds (Fors, 1983). Several alkylpyrazines also derive from pyrolysis of hydroxyamino compounds such as threonine, serine, ethanolamine, and glucosamine (Kato et al., 1970). They have been identified in roasted lamb fat (Buttery et al., 1977), cocoa butter (Rizzi, 1967), coffee (Shimoda and Shibamoto, 1990), cooked pork liver (Mussinan, and Walradt, 1974), fried chicken (Tang et al., 1983), and pressure cooked beef (Mussinan et al., 1973). Pyrazines are recognized as important contributors to the flavours of all roasted, toasted, or similarly heated foods (Maga, 1982).

Although a variety of compounds appear to have contributed towards the total flavour, no single odour impact compound was found responsible for the characteristic canned salmon aroma. As has been the case with meat in the past, this typical aroma more likely results from the sum of the sensory effects of a complex array of volatiles generated during thermal processing. In addition, various components such as trimethylamine and esters, that have previously been detected in decomposed fish, and were implicated in undesirable aromas, were not identified. More work is needed to complement the exploratory data accumulated thus far and to investigate the interactions of the important aroma components in order to elucidate canned salmon flavour.

V. CONCLUSIONS

All six factors studied using the static headspace gas chromatographic technique affected the sensitivity and/or resolution of the chromatograms, and needed to be optimized. Increases in weight of fish flakes, incubation temperature and time brought about significant chromatographic improvement measured by the increase in total area count. RCO allowed the completion of several treatment runs at a time in contrast to previous single-step sequence optimization programs. This optimization method responded to the need of performing several runs consecutively, and was effective at reaching the optima of initial oven temperature, column headpressure, and total flowrate. At the optimized conditions obtained, the capacity of detection was raised to 80 peaks, of which 34 were identified by retention time matching of reference standards and/or by GC-MS.

Multivariate interdependence methods such as PC and CFA were useful to decipher the underlying quantitative data structures of the 44 volatiles accumulated from the developed SHGC method and allowed a simplification of the number of variables for further statistical use. Subsequent discriminant analyses applied on the PC scores from canned salmon volatiles segregated the species of Pacific salmon, the stages of sexual maturity of chum salmon, and the quality grades of pink salmon with high rates of correct classification. Similar effectiveness was obtained when the same SHGC and statistical methods were performed on fresh salmon of different quality grades. Use of the appropriate error estimation

method coupled with the testing of statistical assumptions were required to obtain reliable and stable functions. The remedial use of non-parametric discriminant functions was found necessary in all treatments investigated and provided equivalent or superior results. Judicious interpretation of the PC's scores was also beneficial at extracting meaningful functions. The instrumental method was advantageous for helping sensory evaluation at classifying grade B canned pink salmon samples since the early stages of decomposition were very difficult to determine by flavour evaluation alone. The results of the refrigerated storage highlighted the important role of ethanol and 3-methyl-1-butanol among other possible compounds in the development of decomposition indices for fresh and canned salmon. These deserve more attention in future research.

On a practical basis, the fish processing industry may use the SHGC method for quality assurance purposes. In combination with sensory evaluation, it can serve as a screening tool by regulatory agencies to assess and confirm the quality of sampled lots of canned Pacific salmon, especially those of marginal grades. The method can also assist in the standardization of the sensory grading process by relating grades to volatile concentrations and consequently helping the training of inspectors. In addition, the growing aquaculture industry could possibly utilize this technique to assess the impact of various combinations of diets on the flavour of farmed raised Pacific salmon.

Use of dynamic headspace analysis showed certain groups of compounds to be important contributors to canned salmon flavours. Hydrocarbons, carbonyls, sulfides, and heterocyclic compounds were detected and associated with various flavour compounds. No single volatile constituents had a typical canned salmon aroma, indicating that a combination of compounds may be involved in this characteristic sensory perception. In addition, a few odour active compounds were noted using the odour evaluation technique but were not detected by the GC-MS method used. Refrigerated spoilage of salmon prior to canning caused the occurrence of additional compounds, particularly hydrocarbons, alcohols, ketones, furans, and pyrazines. A number of these compounds were correlated with aroma attributes. Furthermore, several alkylpyrazines and alkylthiophenes were present in the canned chum salmon samples. Large peak areas of several ketones and aldehydes were analyzed in canned late run chum salmon and were important contributors to the overall odour. 2-Methyl-butanal and two other unknown low-boiling point volatiles had hay, cooked malt, and straw-like characteristics typical of canned late run chum salmon. Based on the aroma characteristics and the retention times, it is be postulated that these two unknown compounds may also be aldehydes of molecular weight equal to or lower than 2-methyl-butanal.

REFERENCES

Ackman, R.G. and McLeod, C. 1988. Total lipids and nutritionally important fatty acids of some Nova Scotia fish and shellfish food products. Can. Inst. Food Sci. Technol. J. 21:390.

Ahamed, A. and Matches, J.R. 1983. Alcohol production by fish spoilage bacteria. J. Food Prot. 46:1055.

Aishima, T. 1979a. Classification of soy sauce on principal components in GC profiles. Agric. Biol. Chem. 43:1905.

Aishima, T. 1979b. Objective evaluation of soy sauce by statistical analysis of GC profiles. Agric. Biol. Chem. 43:1935.

Aishima, T. 1983. Relationships between gas chromatographic profiles of soy sauce volatiles and organoleptic characteristics based on multivariate analysis. In "Instrumental analysis of foods," ed. G. Charalambous, Vol. 1, p. 37. Academic Press, London.

Aishima, T. and Nakai, S. 1987. Pattern recognition of GC profiles for classification of cheese variety. J. Food Sci. 52:939.

Aishima, T., Yada, R.Y., Mohler-Smith, A., Nakai, S. 1986. Multivariate analysis of structure-related data to explain milk clotting activity of proteolytic enzymes. J. Agric. Food Chem. 34:675.

Aksnes, A., Gjerde, B., and Roald, S.O. 1986. Biological, chemical, and organoleptic changes during maturation of farmed Atlantic salmon, Salmo salar. Aquaculture 53:7.

Alford, J.A., Smith, J.L., and Lilly, H.D. 1971. Relationship of microbial activity to changes in lipids of food. J. Appl. Bacteriol. 34:133.

Amantea, G.F., Skura, B.J., and Nakai, S. 1986. Culture effect on ripening characteristics and rheological behavior of Cheddar cheese. J. Food Sci. 51:912.

Ando, S., Hatano, M., and Zama, K. 1985a. Deterioration of chum salmon (Oncorhynchus keta) muscle during spawning migration - I. Changes in proximate composition of chum salmon muscle during spawning migration. Comp. Biochem. Physiol. 80B:303.

Ando, S., Hatano, M., and Zama, K. 1985b. A consumption of muscle lipid during spawning migration of chum salmon Oncorhynchus keta. Bull. Jpn. Soc. Sci. Fish. 51:1817.

Apgar, M.E. and Hultin, H.O. 1982. Lipid peroxidation in fish muscle microsomes in the frozen state. Cryobiol. 19:154.

Aquaculture and Commercial Fisheries Branch, 1989. Fisheries production statistics of British Columbia. Commercial Fisheries Section, Ministry of Agriculture and Fisheries, BC.

Aries, R.E., Gutteridge, C.S., and Evans, R. 1986. Rapid characterization of orange juice by pyrolysis mass spectrometry. J. Food Sci. 51:1183.

Arctander, S. 1969. "Perfume and Flavor Chemicals (Aroma chemicals)," Vol. 1-2. Steffen Arctander, Publisher. NJ.

Autran, J.C., Abecassis, J., and Feillet, P. 1986. Statistical evaluation of different technological and biochemical tests for quality assessment in durum wheats. Cereal Chem. 63:390.

Barber, K., Weintraub, S.E., and Vanderstoep, J. 1987. Nutritional analysis of British Columbia canned salmon. Unpublished report. Fisheries Council of British Columbia, Vancouver, BC.

Barber, K., Weintraub, S.E., and Vanderstoep, J. 1988. Nutritional analysis of British Columbia fresh/frozen and cooked salmon. Unpublished report. Fisheries Council of British Columbia, Vancouver, BC.

Borenstein, B. and Bunnell, R.H. 1966. Carotenoids: properties, occurence and utilization in foods. Food Res. 15:195.

Botta, J.R., Lauder, J.T., and Jewer, M.A. 1984. Effect of methodology on total volatile basic nitrgen (TVB-N) determination as an index of quality of fresh Atlantic cod. J. Food Sci. 49:734.

Braddock, R.J. and Dugan L.R.Jr. 1972. Phospholipid changes in muscle from frozen stored Lake Michigan Coho salmon. J. Food Sci. 37:426.

Brock, T.D. (Ed.) 1979. "Biology of Microorganisms," 3rd ed. Prentice-Hall Inc., Englewood Cliffs, NJ.

Brown, D.G.W. and Clapperton, J.F. 1978. Discriminant analysis of sensory and instrumental data on beer. J. Inst. Brewing 84:318.

Buttery, R.G., Ling, L.C., Teranishi, R., and Mon, T.R. 1977. Roasted lamb fat: basic volatile compoents. J. Agric. Food Chem. 25:1227.

Buttery, R.G., Ling, L.C., and Chang, B.G. 1978. Volatiles of corn kernels and husks: possible corn ear worm attractants. J. Agric. Food Chem. 26:866.

Buttery, R.G., Bomben, J.L., Guadagni, D.G., and Ling, L.C. 1971. Some considerations of the volatilities of organic flavor compounds in foods. J. Agric. Food Chem. 19:1045.

Chang, S.S., and Peterson, R.J. 1977. Symposium: the basis of quality in muscle foods. Recent developments in the flavor of meat. J. Food Sci. 42:298.

Chen, H.M., Meyers, S.P., Hardy, R.W., and Biede, S.L. 1984. Color stability of astaxanthin pigmented rainbow trout under various packaging conditions. J. Food Sci. 49:1337.

Childerhose, R.J. and Trim, M. 1981. Pacific Salmon. Douglas and McIntyre Ltd., Vancouver, BC.

Christensen, B.W., Kjaer, A., and Madsen, J.O. 1981. Volatile sulfur compounds and other headspace constituents of north sea fish oils. J. Am. Oil. Chem. Soc. 58:1053.

Chung, J.R. 1968. Postmortem degradation of fish muscle proteins: the role of proteolytic Pseudomonas spp. and their mechanism of action. Ph.D. Dissertation, University of Washington, Seattle, WA.

Clapperton, J.F. and Piggott, J.R. 1979. Differentiation of ale and lager flavors by principal components analysis of flavor characterization data. J. Inst. Brewing 85:271.

Collins, L.S. 1989. Quality enhancement of canned late-run chum salmon (Oncorhynchus keta). M.Sc. Thesis, Univ. of British Columbia, Vancouver, BC.

Connell, J.J. (Ed.) 1980. Control of Fish Quality. 2nd ed. Fishing News Ltd., Surrey, Endland.

Connell, D.W. and Miller, G.J. 1981. Petroleum hydrocarbons in aquatic ecosystems - Behavior and effects of sublethal concentrations: Part 2. XI Tainting. CRC Crit. Rev. Environ. Control, 11:104.

Cowey, C.B., Daisley, K.W., and Parry, G. 1962. Amino acids free or as components of protein, and some B vitamins in the tissues of the Atlantic salmon during spawning and migration. Comp. Biochem. Physiol. 7:29-38.

Crain, W.O. and Tang, C.S. 1975. Volatile components of roasted macadamia nuts. J. Food Sci. 40:207.

Crosgrove, D.M. 1978. A rapid method for estimating ethanol in canned salmon. J. Food Sci. 43:641.

Davies, W.L. and Gill, E. 1936. Investigations on fishy flavors. Chem. Ind. (London) 55:1415.

Davis, P.L. and Chace, W.G.Jr. 1969. Determination of alcohol in citrus juice by gas chromatographic analysis of headspace. HortSci. 4:117.

Department of Fisheries and Oceans, 1988. Annual report 1987-1988. Communications Directorate, Ottawa, Ontario.

Department of Fisheries and Oceans, 1989. Quality Grade Standard for Frozen Gutted Pacific Salmon. Inspection Division, Field Services Branch, Department of Fisheries and Oceans, Vancouver, BC.

Dillon, W.R. and Goldstein, M. (Ed.) 1984. Multivariate Analysis. John Wiley and Sons, New York, NY.

Dravnieks, A., Reilich, H.G., Whitfield, J., and Watson, C.A. 1973. Classification of corn odor by statistical analysis of gas chromatographic patterns of headspace volatiles. J. Food Sci. 38:34.

Dravnieks, A. and Watson, C.A. 1973. Corn odor classification from low-resolution gas chromatographic profiles of head space volatiles. J. Food Sci. 38:1024.

Dunteman, G.H. (Ed.). 1984. "Introduction to Multivariate Analysis," Sage Publications Ltd, London, England.

Dwivedi, B.K. 1975. Meat flavor. CRC Crit. Rev. Food Technol. 5:487.

Easley, D.M., Kleopfer, R.D., and Carasea, A.M. 1981. Gas chromatographic-mass spectrometric determination of volatile organic compounds in fish. J. Assoc. Off. Anal. Chem. 64:653.

Ehira, S., and Uchiyama, H. 1987. Determination of fish freshness using the K-value and comments on some other biochemical changes in relation to freshness. In "Seafood Quality Determination," D.E. Kramer and J. Liston (Ed.), p.185. Elsevier Science Publishing Co., New York, NY.

Eitenmiller, R.R., Orr, J.H., and Wallis, W.W. 1982. Histamine formation in fish: microbiological and biochemical conditions. In "Chemistry and Biochemistry of Marine Food Products." R.E. Martin, G.G. Flick, C.E. Hebard, and D.R. Ward (Ed.), p. 39. AVI Publishing Co., Westport, CN.

Entz, R.C. and Hollifield, H.C. 1982. Headspace gas chromatographic analysis of foods for volatile halocarbons. J. Agric. Food Chem. 30:84.

Fors, S. 1983. Sensory properties of volatile Maillard reaction products and related compounds. In "The Maillard Reaction in Foods and Nutrition," G.R. Waller and M.S. Feather (Ed.), p. 185. ACS Symp. Series No. 215, American Chemical Society, Washington, DC.

Forss, D.A. 1972. Odor and flavor compounds from lipids. In "Progress in the Chemistry of Fats and other Lipids," R.T. Holman (Ed.), Pergamon Press, New York, NY.

Frazier, W.C., and Westhoff, D.C. (Eds.). 1978. Food microbiology. 3rd ed. McGraw-Hill, New York, NY.

Galt, A.M. and MacLeod, G. 1983. The application of factor analysis to cooked beef aroma descriptors. J. Food Sci. 48:1354.

Glick, N. 1978. Additive estimators for probabilities of correct classification. Pattern Recogn., 10:211.

Greene, C.W. 1926. The physiology of spawning migration. Physiol. Rev. 6:201.

Grosch, W. 1984. Neuere Vorstellungen uber die lipoxidation. Lebensm. Gerichtl. Chem. 38:81.

Guadagni, D.G., Buttery, R.G., and Okano, S. 1963. Odour threshold of some organic compounds associated with food flavours. J. Sci. Food Agric. 14:761.

Gunnar, F. 1982. Enzymatic ammonia production in penaied shrimp held on ice. In "Chemistry and Biochemistry of Marine Food Products," R.E. Martin, G.G. Flick, C.E. Hebard, and D.R. Ward (Ed.), p. 323. AVI Publishing Co., Westport, CN.

Habu, T., Flath, R.A., Mon, T.R., and Morton, J.F. 1985. Volatile components of rooibos tea (Aspalathus linearia). J. Agric. Food Chem. 33:249.

Hand, D.J. 1982. Kernel Discriminant Analysis. Research Studies Press, New York, NY.

Hardy, R., McGill, A.S., and Gunstone, F.D. 1979. Lipid and autoxidative changes in cold stored cod (Gadus morhua). J. Sci. Food Agric. 30:999.

Hatae, K., Yoshimatsu, F., and Matsumoto, J.J. 1984. Discriminative characterization of different texture profiles of various cooked fish muscles. J. Food Sci. 49:721.

Headley, L.M. and Hardy, J.K. 1989. Classification of whiskies by principal component analysis. J. Food Sci. 54:1351.

Hearn, T.L., Sgoutas, S.A, Hearn, J.A., and Sgoutas, D.S. 1987. Polyunsaturated fatty acids and fat in fish flesh for selected species for health benefits. J. Food Sci. 52:1209.

Heath H.B. and Reineccius, G. (Ed.). 1986. Flavor and its study. Ch. 1. In "Flavor Chemistry and Technology," p. 3. AVI Publishing Co., Westport, CN.

Hebard, C.E., Flick, G.J., and Martin, R.E. 1982. Occurence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In "Chemistry and Biochemistry of Marine Food Products," R.E. Martin, G.G. Flick, C.E. Hebard, and D.R. Ward (Ed.), p.149. AVI Publishing Co., Westport, CN.

Helbert, J.R. and Hoff, J.T. 1974. Discriminant analysis - a powerful tool for brewery research. Proc. Amer. Soc. Brew. Chem. 32:43.

Herbert, R.A. and Shewan, J.M. 1975. Precursors of the volatile sulphides in spoiling North Sea cod (Gadus morhua). J. Sci. Food Agric. 26:1195.

Herbert, R.A. and Shewan, J.M. 1976. Roles played by bacterial and autolytic enzymes in the production of volatile sulphides in spoiling North Sea cod (Gadus morhua). J. Sci. Food Agric. 27:89.

Herranz, A., de la Serna, P., and Barro, C. 1990. Multivariate statistical methods applied to the differentiation of rum brands. J. Sci. Food Agric. 51:555.

Heyman, H. and Noble, A.C. 1989. Comparison of canonical variate and principal component analyses of wine descriptive analysis data. J. Food Sci. 54:1355.

Hillig, F. 1958. Determination of alcohol in fish and egg products. J. Assoc. Off. Anal. Chem. 41:776.

Hills, M. 1966. Allocation rules and their error rates. J. Royal Stat. Soc., B28:1.

Ho, C., Lee, K., and Jin, Q.Z. 1983. Isolation and identification of volatile flavor compounds in fried bacon. J. Agric. Food Chem. 31:336.

Hoff, J.T., Helbert, J.R., and Chicoye, E. 1975. Classification of lager beers by computer analysis of volatile profiles. Tech. Q., Master Brew. Assoc. Amer. 12:209.

Hoff, J.T. and Herwig, W.C. 1976. Correlation of triangular taste test panel results with statistical differences between headspace volatile profiles. J. Amer. Soc. Brew. Chem. 34:1.

Holaday, D.A. 1939. The alcohols as a measure of spoilage in canned fish. J. Assoc. Off. Anal. Chem. 22:418.

Hollingworth, T.A.Jr. and Throm, H.R. 1983. A headspace gas chromatographic method for the rapid analysis of ethanol in canned salmon. J. Food Sci. 48:290.

Hollingworth, T.A.Jr., Throm, H.R., Wekell, M., Trager, W.F., and O'Donnell, M.W.Jr., 1986. Headspace gas chromatographic method for determination of ethanol in canned salmon: collaborative study. J. Assoc. Off. Anal. Chem. 69:524.

Hosfield, G.L., Ghaderp, A., and Uebersax, M.A. 1984. A factor analysis of yield and sensory and physico-chemical data from tests used to measure culinary quality in dry edible beans. Can. J. Plant Sci. 64:285.

Hughes, R.B. 1964. Chemical studies on the herring (Clupea harengus). IX.- Preliminary gas-chromatographic study of volatile sulphur compounds produced during the cooking of herring. J. Sci. Food Agric. 15:290.

Human, J. and Khayat, A. 1981. Quality evaluation of raw tuna by gas chromatography and sensory evaluation. J. Food Sci. 46:868.

Iida, H., Nakamura, K., Tokunaga, T., Sato, K., Hoshino, C., Terano, S., and Furukawa, T. 1982. Study on the several chemical tests for estimating the quality of canned product. II. On the canned sardine and masu-salmon. Bull. Tokai Reg. Fish. Res. Lab. 104:11.

Iida, H., Tokunta, T., and Nakamura, K. 1981a. Usefulness of ethanol as a quality index of fish and fish products - I. Bull. Tokai Reg. Fish. Res. Lab. 104:77.

Iida, H., Tokunta, T., Nakamura, K., and Oota, Y. 1981b. Usefulness of ethanol as a quality index of fish and fish products - II. Bull. Tokai Reg. Fish. Res. Lab. 104:83.

Jahns, F.D., Howe, J.L., Coduri, R.J., and Rand, A.G. 1976. A rapid enzyme test to assess fish freshness. Food Technol. 30(7):27.

Jones, N.R. 1969. Fish as raw material for freezing: factors influencing the quality of products frozen at sea. In "Freezing and Irradiation of Fish," R. Kreuzer (Ed.), p. 31. FAO, Fishing News Ltd, London, UK.

Josephson, D.B. 1987. Mechanisms for the formation of volatiles in fresh seafood s. Ph.D. Dissertation, University of Wisconsin-Madison, Madison, WI.

Josephson, D.B., Lindsay, R.C., and Struiber, D.A. 1983. Identification of compounds characterizing the aroma of fresh whitefish (Coregonus clupeaformis). J. Agric. Food Chem. 31:326.

Josephson, D.B., Lindsay, R.C., and Stuiber, D.A. 1984a. Variations in the occurences of enzymatically-derived volatile aroma compounds in salt- and freshwater fish. J. Agric. Food Chem. 32:1344.

Josephson, D.B., Lindsay, R.C., and Struiber, D.A. 1984b. Identification of volatile aroma compounds from oxidized frozen whitefish (Coregonus clupeaformis). Can. Inst. Food Sci. Technol. J. 17:178.

Josephson, D.B., Lindsay, R.C., and Struiber, D.A. 1985. Volatile compounds characterizing the aroma of fresh Atlantic and Pacific oysters. J. Food Sci. 50:5.

Josephson, D.B., Lindsay, R.C., and Struiber, D.A. 1987. Influence of processing on the volatile compounds characterizing the flavor of pickled fish. J. Food Sci. 52:10.

Kadota, H. and Ishida, Y. 1972. Production of volatile sulfur compounds by microorganisms. Ann. Rev. Microbiol. 26:127.

Kaiser, H.F. 1960a. The application of electronic computers to factor analysis. Educ. Psychol. Measurement 20:141.

Kaiser, R.E. 1960b. "Gas Chromatographie," Geest and Portig, Leipzig.

Kamiya, A. and Ose, Y. 1984. Study on the odorous compounds produced by putrefaction of foods, V: Fatty acids, sulfur compounds, and amines. J. Chromatogr. 292:383.

Kaneko, T., Takeuchi, M., Ishii, S., Higashi, H. and Kikuchi, T. 1966. Effect of dietary lipids on fish under cultivation. IV. Changes in fatty acid composition in flesh lipids in rainbow trout on non-feeding. Bull. Jpn. Soc. Sci. Fish. 33:56.

Kato, S., Kurata, T., Ishitsuka, R., and Fujimaki, M. 1970. Pyrolysis of p-hydroxy amino acids, especially L-serine. Agric. Biol. Chem. 36:951.

Ke, P.J., Linke, B.A., and Ackman, R.G. 1978. Acceleration of lipid oxidation in frozen mackerel fillet by pretreatment with microwave heating. J. Food Sci. 43:38.

Kelleher, S. and Zall, R.R. 1983. Ethanol accumulations in muscle tissue as a chemical indicator of fish spoilage. J. Food Biochem. 7:78.

Khayat, A. 1979. Correlation of off-odor scores of canned tuna with gas chromatographic data. J. Food Sci. 44:37.

Kitahara, T. 1983. Behavior of carotenoids in the chum salmon (Oncorhynchus keta) during anadromous migration. Comp. Biochem. Physiol. 76B:97.

Krzynowek, J. and Murphy, J. 1987. Proximate composition, energy, fatty acid, sodium, and cholesterol content of finfish, shellfish, and their products. U.S. Department of Commerce, NOAA, Seattle.

Kubota, K., Kobayashi, A., and Yamanishi, T. 1982. Basic and neutral compounds in the cooked odor from antarctic krill. Agric. Biol. Chem. 46:2835.

Kubota, K., Shijimaya, H., and Kobayashi, A. 1986. Volatile components of roasted shrimp. Agric. Biol. Chem. 50:2867.

Kwak, H.S., Jeon, I.J., and Perng, S.K. 1989. Statistical patterns of lipase activities on the release of short-chain fatty acids in Cheddar cheese slurries. J. Food Sci. 54:1559.

Lachenbruch, P.A. 1967. An almost unbiased method of obtaining confidence intervals for the probability of misclassification in discriminant analysis. Biometrics 23:639.

Lachenbruch, P.A., Sneeringer, C., and Revo, L.T. 1973. Robustness of the linear and quadratic discriminant function to certain types of non-normality. Commun. Stat. 1:39.

Larick, D.K. and Turner, B.E. 1990. Headspace volatiles and sensory characteristics of ground beef from forage- and grain-fed heifers. J. Food Sci. 54:649.

Leblanc, R.J., and Gill, T.A. 1984. Ammonia as an objective quality index in squid. Can. Inst. Food Sci. Technol. J. 17:734.

Leblanc, E.L., Leblanc, R.J., and Blum, I.E. 1988. Prediction of quality in frozen cod (Gadus morhua) fillets. J. Food Sci. 53:328.

Lerke, P.A. and Huck, R.W. 1977. Objective determination of canned tuna quality: identification of ethanol as a potentially useful index. J. Food Sci. 42:755.

Leland, J.V., Reineccius, G.A., and Lahiff, M. 1987. Eavaluation of copper-induced oxidized flavor in milk by discriminant analysis of capillary gas chromatographic profiles. J. Dairy Sci. 70:524.

Li-Chan, E., Nakai, S., and Wood, D.F. 1987. Muscle protein structure-function relationships and discrimination of functionality by multivariate analysis. J. Food Sci. 52:31.

Lindsay, R.C. 1977. Objective measurement of the flavour quality of beer. Brewers' Dig. 52:44.

Lindsay, R.C., Josephson, D.B., and Olafsdottir, G. 1986. Chemical and biochemical indices for assessing the quality of fish packaged in controlled atmospheres. In "Seafood Quality Determination," D.E. Kramer and J. Liston (Eds.), p.221. Elsevier Science Publishing Co., New York, NY.

Liston, J. 1982. Recent advances in the chemistry of iced fish spoilage. In "Chemistry and Biochemistry of Marine Food Products," R.E. Martin, G.G. Flick, C.E. Hebard, and D.R. Ward (Ed.), p. 27. AVI Publishing Co., Westport, CN.

Love, R.M. 1970. Maturity and Spawning. In "The Chemical Biology of Fishes I." Academic Press, London, UK.

Love, R.M. 1980. Maturation. In "The Chemical Biology of Fishes II." Academic Press, London, UK.

Lovern, J.A. 1934. Fat metabolism in fishes. Mobilization of depot fat in the salmon. Biochem. J. 28:1955.

Lyon, B.G. 1988. Descriptive profile analysis of cooked, stored, and reheated chicken patties. J. Food Sci. 53:1086.

MacLeod, G. and Ames, J.M. 1986. Capillary gas chromatography-mass spectometric analysis of cooked ground beef aroma. J. Food Sci. 51:1427.

MacLeod, A.J. and Panchasara, S.D. 1983. Volatile aroma components, particularly glucosinolate products, of cooked edible mushrooms (Agaricus bisporus) and cooked dried mushrooms. Phytochem. 22:705.

Maga, J.A. 1982. Pyrazines in flavour. In "Food Flavours," Part A, I.D. Morton and A.J. MacLeod (Eds.), p. 283. Elsevier Science Publishing Co., New York, NY.

Marais, J., van Rooyen, P.C., and du Plessi, C.S. 1982. Classification of white cultivar wines by origin using volatile aroma components. 1973-1981. Agric. Res., p.46.

Marks, S. and Dunn, O.J. 1974. Discriminant functions when covariance matrices are unequal. J. Amer. Stat. Assoc. 69:555.

Maruyama, F.T. 1970. Identification of dimethyl trisulfide as a major aroma component of cooked Brassicaceous vegetables. J. Food Sci. 35:540.

Matiella, J.E. and Hsieh, T.C.-Y. 1990. Analysis of crabmeat volatile compounds. J. Food Sci. 55:962.

McGill, A.S., Hardy, R., Burt, J.R., and Gunstone F.D. 1974. Hept-cis-4-enal and its contribution to the off-flavor in cold stored cod. J. Sci. Food Agric. 25:1477.

McGill, A.S., Hardy, R., and Gunstone, F.D. 1977. Further analysis of the volatile components of frozen cold stored cod and the influence of these on flavour. J. Sci. Food Agric. 28:200.

Mietz, J.L., and Karmas, E. 1978. Polyamine and histamine content of rockfish, salmon, lobster, and shrimp as an indicator of decomposition. J. Assoc. Off. Anal. Chem. 61:139.

Miller III, A., Scanlan, R.A., Lee, J.S., and Libbey, L.M. 1972a. Quantitative and selective gas chromatographic analysis of dimethyl- and trimethylamine in fish. J. Agric. Food Chem. 20:709.

Miller III, A., Scanlan, R.A., Lee, J.S., and Libbey, L.M. 1972b. Volatile compounds produced in ground muscle tissue of canary rockfish (Sebastes pinniger) stored on ice. J. Fish. Res. Bd. Can. 29:1125.

Miller III, A., Scanlan, R.A., Lee, J.S., and Libbey, L.M. 1973a. Identification of volatile compounds produced in sterile fish muscle (Sebastes melanops) by Pseudomonas fragi. Appl. Microbiol. 25:952.

Miller III, A., Scanlan, R.A., Lee, J.S., and Libbey, L.M. 1973b. Volatile compounds produced in sterile fish muscle (Sebastes melanops) by Pseudomonas perolens, Pseudomonas fluorescens and an Achromobacter species. Appl. Microbiol. 26:18.

Miller III, A., Scanlan, R.A., Lee, J.S., Libbey, L.M., and Morgan, M.E. 1973c. Volatile compounds produced in sterile fish muscle (Sebastes melanops) by Pseudomonas perolens. Appl. Microbiol. 25:257.

Min, D.B.S., Ina, K., Peterson, R.J., and Chang, S.S. 1977. The alkylbenzenes in roast beef. J. Food Sci.42:503.

Min, D.B., Ina, K., Peterson, R.J., and Chang, S.S. 1979. Preliminary identification of volatile flavor compounds in the neutral fraction of roast beef. J. Food Sci. 44:639.

Minor, L.J., Pearson, A.M., Dawson, L.E., and Shweigert, B.S. 1965. Chicken flavor: the identification of some chemical components and the importance of sulfur compounds in the cooked volatile fraction. J. Food Sci. 30:686.

Mohler-Smith, A. and Nakai, S. 1990. Classification of cheese varieties by multivariate analysis of HPLC profiles. Can. Inst. Food Sci. Technol. J. 23:53.

Montedero G. and Bertuccioli, M. 1983. Application of GC and HPLC in the predictive quality evaluation of wines. In "Instrumental Analysis of Foods," ed. G. Chalarambous, Vol. 2, p. 409. Academic Press, London, UK.

Moret, I., Capodaglio, G., and Scarponi, G. 1986. Statistical evaluation of the group structures of five Venetian wines from chemical measurements. Anal. Chim. Acta 191:331.

Morrison, D.F. 1976. Multivariate Statistical Methods. 2nd ed. McGraw-Hill Book Co., New York, NY.

Mottram, D., Edwards, R., and MacFie, J. 1982. A comparison of the flavor volatiles from cooked beef and pork meat systems. J. Sci. Food Agric. 33:934.

Mottram, D.S. and Whitfield, F.B. 1987. The effect of lipid on the formation of volatile heterocyclic compounds in the Maillard reaction. In "Flavour Science and Technology," M. Martens, G.A. Dalen, and H. Russwurm jr. (Ed.), p.29. John Wiley and Sons Ltd., New York, NY.

Mulders, E.J. 1973. The odour of white bread. IV. Quantitative determination of constituents in the vapour and their odour values. Z. Lebensm.-Unters. Forsch. 151:310.

Mussinan, C.J. and Walradt, J.P. 1974. Constituents of pressure cooked pork liver. J. Agric. Food Chem. 22:827.

Mussinan, C.J., Wilson, R.A., and Katz, I. 1973. Isolation and identification of pyrazines present in pressure cooked beef. J. Agric. Food Chem. 21:871.

Myers, M.J., Issenberg, P., and Wick, E.L. 1969. Vapor analysis of the production by banana fruit of certain volatile constituents. J. Food Sci. 34:504.

Myers, M.J., Issenberg, P., and Wick, E.L. 1970. L-Leucine as a precursor of isoamyl alcohol and isoamylacetate volatile aroma constituents of banana fruit discs. Phytochem. 9:1693.

Nakai, S. 1989. A new random-centroid optimization for food research and processing. Paper No. 302, presented at 50th Annual Meeting of Inst. of Food Technologists, Chicago, IL, June 25-29.

Nakai, S., Koide, K., and Eugster, K. 1984. A new mapping super-simplex optimization for food product and process development. J. Food Sci. 49:1143.

Nawar, W.W. 1985. Lipids. Ch. 4. In "Food Chemistry," O. Fennema (Ed.), p.139. Marcel Dekker Inc., New York, NY.

Neff, J.M., Cox, B.A., Dixit, D., and Anderson, J.W. 1976. Accumulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Marine Biol. 38:279.

Noble, A.C., Flath, R.A., and Ralph, R.R. 1980. Wine headspace analysis, reproducibility and application to varietal classification. J. Agric. Food Chem. 28:346.

Nonaka, M., Black, D.R., and Pippen, E.L. 1967. Gas chromatographic and mass spectral analysis of cooked chicken meat volatiles. J. Agric. Food Chem. 15:713.

Nunez, A.J. and Gonzalez L.F. 1984. Pre-concentration of headspace volatiles for trace organic analysis by gas chromatography. J. Chromtogr. 300:127.

Ohloff, G., Flament, I., and Pickenhagen, W. 1985. Flavor Chemistry. Food Rev. Internat. 1:99.

Parliment, T.H. 1986. Sample preparation techniques for gas-liquid chromatographic analysis of biologically derived aromas. Ch. 4. In "Biogeneration of Aromas," T.H. Parliment and R. Croteau (Ed.), p. 34. American Chemical Society, Symposium Series 317, Washington, DC.

Pesek, C.A., Wilson, L.A., and Hammond E.G. 1985. Spice quality: effect of cryogenic and ambient grinding on volatiles. J. Food Sci. 50:599.

Pham, A.M. and Nakai, S. 1984. Application of stepwise discriminant analysis to high pressure liquid chromatography profiles of water extract for judging ripening of Cheddar cheese. J. Dairy Sci. 67:1390.

Pino, J.A., Torricella, R.G., Orsi, F., and Figueras, L. 1986. Application of multivariate statistics for the quality classification of single-strength grapefruit juice. J. Food Qual. 9:205.

Pippen, E.L., Mecchi, E.P., and Nonaka, M. 1969. Origin and nature of aroma in fat of cooked poultry. J. Food Sci. 34:436.

Pokorny, J. 1980. Effect of browning on the formation of flavor substances. Nahrung 24:115.

Powers, J.J., Godwin, D.R., and Bargmann, R.E. 1977. Relations between sensory and objective measurements for quality evaluation of green beans. In "Flavor Quality: Objective Measurement," R.A. Scanlan (Ed.) Symp. Series 51. Am. Chem. Soc., Washington, DC.

Powers, J.J. and Keith, E.S. 1968. Stepwise discriminant analysis of gas chromatographic data as an aid in classifying the flavor quality of foods. J. Food Sci. 33:207.

Powers, J.J., Smit, C.J.B., and Godwin, D.R. 1978. Relations among sensory and objective attributes of canned rabbiteye (Vaccinium ashei Reade) blueberries. II. Cluster and discriminant analysis examination. Lebensmit.-Wissens. Technol. 11:279.

Powrie, W.D. 1984. Chemical effects during storage of frozen foods. J. Chem. Education. 61:340.

Quarmby, A.R. and Ratkowshy, D.A. 1988. Free-choice flavour and odour profiling of fish spoilage: does it achieve its objective? J. Sci. Food Agric. 44:89.

Rao, C.R. 1973. Linear Statistical Inference and its Application. 2nd ed. John Wiley and Sons Inc., New York, NY.

Reinert, K.H., Hunter, J.V., and Sabatino, T. 1983. Dynamic heated headspace analyses of volatile organic compounds present in fish tissue samples. J. Agric. Food Chem. 31:1057.

Resurreccion, A.V.A. and Shewfelt, R.L. 1985. Relationships between sensory attributes and objective measurements of postharvest quality of tomatoes. J. Food Sci. 50:1242.

Rizzi, G.P. 1967. The occurence of simple alkyl pyrazines in cocoa butter. J. Agric. Food Chem. 15:549.

Ronald, A.P. and Thomson, W.A.B. 1964. The volatile sulphur compounds of oysters. J. Fish Res. Bd. Canada. 21:1481.

Rubico, S.M., Resurreccion, A.V.A., and Beuchat, L.R. 1988. Comparison of sensory properties and headspace volatiles of a peanut beverage processed at different temperature and time conditions. J.Food Sci. 53:176.

Sanford, K.A., Gullett, E.A., and Roth, V.J. 1988. Optimization of the sensory properties of frozen peas by principal components analysis and multiple regression. Can. Inst. Food Sci. Technol. J. 21:174.

Sapers, G.M., Sullivan, J.F., and Talley, F.B. 1970. Flavor quality in explosion puffed dehydrated potato. 1. A gas chromatographic method for the determination of aldehydes associated with flavor quality. J. Food Sci. 35:728.

SAS Institute Inc. 1989. SAS User's Guide: Statistics, version 5 edition, release 6.03. Cary, NC.

Sawyer, F.M., Cardello, A.V., and Prell, P.A. 1988. Consumer evaluation of the sensory properties of fish. J. Food Sci. 53:12.

Schreier, P. and Reiner, L. 1979. Characterization and differentiation of grape brandies by multiple discriminant analysis. J. Sci. Food Agric. 30:319.

Seki, N. 1971. Nucleotides in aquatic animals and seaweeds. Bull. Jpn. Soc. Sci. Fish. 37:777.

Selke, E., Rohwedder, W.K., and Dutton, H.J. 1975. Volatile components from tristearin heated in air. J. Amer. Oil Chem. Soc. 52:232.

Sentheshanmuganathan, S. 1960. The mechanism of the formation of higher alcohols from amino acids by Saccharomyces cerevisiae. Biochem. J. 74:568.

Shahidi, F. Rubin, L.J., and D'Souza, L.A. 1986. Meat flavor volatiles: a review of the composition, techniques of analysis, and sensory evaluation. CRC Crit. Rev. Food Sci. Nutr. 24:141.

Shibamoto, T. 1977. Formation of sulfur- and nitrogen-containing compounds from the reaction of furfural with hydrogen sulfide and ammonia. J. Agric. Food Chem. 25:206.

Shimoda, M. and Shibamoto, T. 1990. Isolation and identification of headspace volatiles from brewed coffee with an on-column GC/MS method. J. Agric. Food Chem. 38:802.

Silverman, B.W. 1986. Density Estimation for Statistics and Data Analysis. Chapman and Hall, New York, NY.

Simpson, K.L. 1982. Carotenoid pigments in seafood. In "Chemistry and Biochemistry of Marine Food Products," R.E. Martin, G.G. Flick, C.E. Hebard, and D.R. Ward (Ed.), p. 115. AVI Publishing Co., Westport, CN.

Sink, J.D. 1973. Lipid soluble components of meat , odor and their biochemical origin. J. Amer. Oil Chem. Soc. 50:470.

Sipos, J.C. and Ackman, R.G. 1964. Association of dimethylsulphide with the "blackberry" problem in cod from the Labrador area. J. Fish. Res. Bd. Canada 21:423.

Skrede, G., and Storebakken, T. 1986. Characteristics of color in raw, baked and smoked wild and pen-reared atlantic salmon. J. Food Sci. 51:804.

Smeyers-Verbeke, J., Massart, D.L., and Coomans, D. 1977. Application of linear discriminant analysis to the differentiation of pure milk from different species and mixtures. J. Assoc. Off. Anal. Chem. 60:1382.

Smouse, T.H. and Chang, S.S. 1967. A systematic characterization of the reversion flavor of soybean oil. J. Amer. Oil Chem. Soc. 44:509.

Stansby, M.E. 1962. Speculations on fishy odors and flavors. Food Technol. 16(4):28.

Stryjewska, E, Rubel, S., Henrion, A, and Henrion, G. 1987. Statistical estimation of the influence of mineralization methods on the results of heavy metals determination in cereals by the DPASV method. Fresenius Z. Anal. Chem. 327:679.

Syarief, H., Hamann, D.D., Giesbrecht, F.G., Young, C.T., and Monroe, R.J. 1985. Interdependency and underlying dimensions of sensory flavor characteristics of selected foods. J. Food Sci. 50:631.

Tang, J., Jin, Q.Z., Shen, G.H., Ho, C.T., and Chang, S.S. 1983. Isolation and identification of volatile compounds from fried chicken. J. Agric. Food Chem. 31:1287.

Tanchotikul, U. and Hsieh, T.C.-Y. 1989. Volatile flavor components in crayfish waste. J. Food Sci. 54:1515.

Taylor, A.J. and Mottram, D.S. 1990. Composition and odour of volatiles from autoxidized methyl arachidonate. J. Sci. Food Agric. 50:407.

Thomas, C.P., Dimick, D.S., and McNeil, J.H. 1971. Sources of flavor in poultry skin. Food Technol. 25(4):109.

Tokunaga, T., Iida, H., Nakamura, K., Terrano, S., Furukawa, K., and Hoshino, C. 1982. Study on the several chemical tests for estimating the quality of canned product - I. On the canned mackerel. Bull. Tokai Reg. Res. Lab. 107:1.

van der Ouwelend, G.A.M. and Peer, H.G. 1975. Components contributing to beef flavor. Volatile compounds produced by the reaction of 4-hydroxy-5-methyl-3(2H)-furanone and its thio analog with hydrogen sulfide. J. Agric. Food Chem. 23:501.

Vangtal, A. and Hammond, E.G. 1986. Correlation of the flavor characteristics of Swiss-type cheeses with chemical parameters. J. Dairy Sci. 69:2892.

Van Ness, J.W. and Simpson, C. 1976. On the effects of dimension in discriminant analysis. Technometrics 18:175.

Vejaphan, W., Hsieh, T.C.-Y., and Williams, S.S. 1988. Volatile flavor components from boiled crayfish (Procambarus clarkii) tail meat. J. Food Sci. 53:1666.

Vernin, G. and Parkanyi, C. 1982. "Chemistry of Heterocyclic Compounds in Flavours and Aromas," G. Vernin and E. Horwood (Ed.). Halsted press, Chichester, West Sussex.

Walradt, J.P., Pittet, A.O., Kinlin, T.E., Muralidhara, R., and Sanderson, A. 1971. Volatile components of roasted peanuts. J. Agric. Food Chem. 19:972.

Watanabe, K. and Sato, Y. 1971. Gas chromatographic and mass spectral analyses of heated flavor compounds of beef fats. Agric. Biol. Chem. 35:756.

Wheaton, F.W., and Lawson, T.B. (Eds.) 1985. In "Processing Aquatic Food Products." John Wiley and Sons, N.Y.

Whistler, R.L. and Daniel, J.R. 1985. Carbohydrates. Ch. 3. In "Food Chemistry," O. Fennema (Ed.), p. 69. Marcel Dekker Inc., New York, NY.

Whitfield, F.B., Freeman, D.J., and Bannister, P.A. 1981a. Dimethyl trisulphide: an important off-flavour component of the royal red prawn (Hymenopenaeus sibogae). Chem. Ind. (London) October, p. 692.

Whitfield, F.B., Freeman, D.J., Last, J.H., and Bannister, P.A. 1981b. Bis-(methylthio)-methane: an important off-flavour component of prawns and sand lobsters. Chem. Ind. (London) March, p. 158.

Whitfield, F.B., Freeman, D.J., Last, J.H., Bannister, P.A., and Kennett, B.H. 1982. Oct-1-en-3-ol and (5Z)-octa-1,5-dien-3-ol, compounds important in the flavour of prawns and sand-lobsters. Aust. J. Chem. 35:373.

Wilkinson, L. 1990. SYSTAT: The System for Statistics, version 4.2. Evanston, IL.

Wilson, R.A. and Katz, J. 1972. Review of literature on chicken flavor and report of isolation of several new chicken flavor components from aqueous cooked chicken. J. Agric. Food Chem. 30:741.

Wong, N.P., Damico, J.N.P., and Slawin, H. 1967. Investigation of volatile compounds in cod fish by gas chromatography and mass spectrometry. J. Assoc. Off. Anal. Chem. 50:8.

Woo, A.H. and Lindsay, R.C. 1983. Statistical correlation of quantitative flavor intensity assessment and individual free fatty acid measurements for routine detection and prediction of hydrolytic rancidity off-flavors in butter. J. Food Sci. 48:1761.

Wurzenburger, M. and Grosch, W. 1986. Enzymic oxidation of linolenic acid to 1,Z5-octadien-3-ol, Z2, Z5-octadien-1-ol and 10-oxo-E-8-decenoic acid by a protein fraction from mushrooms (Psalliota bispora). Lipids 21:261.

Yasuhara, A. 1987. Comparison of volatile components between fresh and rotten mussels by gas chromatography-mass spectrometry. J. Chromatogr. 409:251.

Young, C.T. and Hovis, A.R. 1990. A method for the rapid analysis of headspace volatiles of raw and roasted peanuts. J. Food Sci. 55:279.

Young, L.L., Bargmann, R.E., and Powers, J.J. 1970. Correlation between gas chromatographic patterns and flavor evaluation of chemical mixtures and of cola beverages. J. Food Sci. 35:219.

Zar, J.H. (Ed.) 1984. "Biostatistical Analysis," 2nd ed. Prentice-Hall Inc., Englewood Cliffs, NJ