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**Identification of Volatile Odour Compounds from Controlled Spoilage
Whitefish (*coregonus clupeaformis*) using a
Gas Chromatography – Olfactometry Static Headspace Method**

BY

Marilyn G. Klassen Magnusson

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of
Manitoba in partial fulfillment of the requirement of the degree
Of
MASTER OF SCIENCE**

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Submitted to the Faculty of Graduate Studies
University of Manitoba, in partial fulfillment
of the requirements for the degree of

Master of Science

Department of Human Nutritional Sciences
Winnipeg, Manitoba

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ABSTRACT

A need was identified for research toward the development of a model reference set of odourants which characterize the continuum of fish quality that that could be used in the sensory evaluation, calibration and training of Canadian Food Inspection Agency (CFIA) fish inspectors and by the fish processing and import industry quality assurance personnel who are required to apply CFIA standards and to meet CFIA-enforced regulations. Freshwater whitefish (*coregonus clupeaformis*) was selected for study due to the high annual catch volume and economic value in western Canada and past research history regarding the volatiles contributing to its odour. A controlled spoilage series of eight increments held on ice from two through 19 days were produced and analyzed for proximate and fatty acid composition and for traditional chemical indicators of spoilage, Freshness Index, Ethanol and TVB-N. The spoilage samples were also evaluated for quality using traditional sensory evaluation methods and by GCO.

Quality evaluation of the whitefish spoilage increments by a panel of four expert sensory assessors (CFIA fish inspectors) using traditional sensory evaluation methods was conducted using a universal quality line-scale. The pass or fail quality, mean panel line-score and odour description were determined for each increment and related to the GCO data. The results of the expert sensory panel indicated that the spoilage increments showed a progression in odour attributes associated with fresh to highly decomposed and identified the pass/fail quality cut-off for the whitefish samples at 12 days held on ice.

No significant differences were found for moisture, protein and fat content between spoilage increments. Notably, the fat content of the samples used in the study

was 3 - 4% higher than the published average of 8%. No significant differences were found among the spoilage increments for saturated, monounsaturated and total polyunsaturated fatty acid content. Overall, the fish samples contained a high level of total polyunsaturated fatty acids that likely contributed significantly to the odours associated with oxidation and rancidity that developed during storage.

Three chemical indicators of spoilage: Freshness Index (G-value), ethanol and TVB-N were used to benchmark the quality of each spoilage increment as determined by the expert sensory panel. G-value and ethanol concentration were shown to be good indicators of spoilage since both indices could be used to benchmark the borderline quality with a G-value equal to 2.0 and an ethanol concentration equal to 1 ppm. Although TVB-N correlated well with spoilage increments indicative of advanced spoilage it could not be used to benchmark borderline quality.

A gas chromatography-olfactometry (GCO) static headspace method was used to identify the key descriptive terms and quantify the intensity and concentrations of volatile chemical compounds with odour impact in the whitefish spoilage increments. The static headspace collection technique was unique in that it allowed for a larger headspace volume and increased volatile concentration while emulating the odours perceived during traditional sensory evaluation of fish products. A GCO panel of four expert assessors was used to rate the intensity of eluting volatiles in tandem with a flame ionizer detector (FID). The panel collectively used a total of 402 terms to characterize the odours they perceived across the thirty two GCO sessions conducted (4 panelists x 8 increments). The 402 terms were reduced to a list of 133 terms by removing all qualifying terms such as slight, moderate and strong and then reduced again to a final list of 37 Key Terms by

grouping synonyms. The mean retention times and intensity ratings were determined for the 37 key volatiles with odour impact selected from the results of the expert GCO panel. The key odour volatiles from the list of 37 were determined and summarized for each spoilage increment using a multivariate statistical model. The identification of the odour character and intensity of these key volatiles will be beneficial towards future development of a model set of odourants characterizing whitefish quality from fresh to highly decomposed.

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Chapter 1

Introduction

The Canadian Food Inspection Agency (CFIA) is responsible for enforcing the policies and standards related to food, set by Health Canada on behalf of the Government of Canada (CFIA, 2003e). The Fish, Seafood and Production Program (FSPP) are one of 14, food, plant and animal related inspection programs delivered by the CFIA across Canada. A key purpose of the FSPP under the Fish Inspection Act is to develop, promote and implement product standards related to the safety and quality of fish and seafood products and to provide reasonable assurance that the products comply with these standards (CFIA, 2003e). Therefore, a major activity of CFIA inspection staff is to monitor fish, seafood and seafood products for compliance with specific standards for safety and quality under the mandate of FSPP. Sensory evaluation is the major method used by inspectors to assess the quality of fish and seafood products. CFIA inspectors apply product standards to assess fish products on a pass or fail basis for distinct and persistent odours and/or flavours of taint, decomposition or unwholesomeness. Raw fish and seafood products are primarily assessed for odour. Cooked or “ready-to-eat” fish and seafood products may also be inspected for odour alone or also for flavour. Inspectors must distinguish the “fail” odours and flavors from “pass” odours and flavours unique to or characteristic of many different species of fish products presented in the marketplace.

Canada has been an international leader in the training of its inspectors in sensory evaluation theory and its application to routine inspection of fish and seafood products. The CFIA’s, National Sensory and Chemical Indicators Laboratory Group (NSCIG), part of the CFIA Science Branch, Laboratory Directorate is currently responsible for delivery

of national sensory workshop training and calibration, standard development and assessment of inspectors in sensory evaluation of odour and flavour of nine major fish and seafood product standards under the authority of FSPP. The majority of products being imported or produced domestically in Canada are included in the nine standards listed in Table 1.1. The procedures and criteria for the three types of sensory workshops conducted; training and calibration, standard development and assessment are documented in the CFIA, Fish Products Inspection Manual (CFIA, 2003c). Workshops for fish or seafood products that do not fit into the nine standards or those specific to certain regions in Canada are also conducted on as required. For example, lumpfish roe, produced almost exclusively in Newfoundland and Labrador has been a “specialty product” category that has been the topic of a region specific sensory training, calibration and assessment workshop for inspectors in that area. For all fish inspectors there is a requirement that they meet a minimum sensory assessment standard (“Analyst” status) in order to qualify to assess product from each specific product standard (CFIA, 2003c). Fish inspectors may also be recognized as an “Expert Analyst” when they exceed the minimum standard and meet the criteria also stipulated in the CFIA Fish Products Inspection Manual. The NSCIG laboratory conducts species specific and/or multi-species sensory workshops in each of the nine product standard categories on a national level. The NSCIG laboratory also performs chemical tests to determine levels of known chemical indicators of spoilage that are used as a tool to benchmark sensory evaluations in a training,

**Table 1.1: Product standard sensory workshops delivered by NSCIG Laboratory
(National Sensory and Chemical Indicators Group)**

Item #	Chapter	Chapter Title	Product Standard No.	Product Standard Title
1	2	Canned Products	Standard 1	Canned Tuna
2	2	Canned Products	Standard 3	Canned Shrimp or Prawn
3	2	Canned Products	Standard 4	Canned Crab
4	2	Canned Products	Standard 8	Canned Salmon
5	3	Fresh and Frozen Products	Standard 1	Fresh and Frozen Groundfish
6	3	Fresh and Frozen Products	Standard 2	Fresh and Frozen Shrimp or Prawn
7	3	Fresh and Frozen Products	Standard 3	General Fresh and Frozen Fish
8	3	Fresh and Frozen Products	Standard 4	Fresh and Frozen Scallop
9	5	Salted and/or Dried Products	Standard 1	Salted Fish

From: Fish Products Standards and Methods Manual, CFIA, 2003

calibration and assessment workshops setting or at the request of FSPP for specific regulatory issues related to routine inspections or public or industry concerns. These chemical indicators of spoilage are not tested for in routine fish and seafood product inspections where the sensory evaluations performed by qualified inspectors are the definitive quality decision.

The training, standard development and assessment workshops are delivered using controlled spoilage, commercially available and inspection fish and seafood samples. Each product category workshop exposes the inspector to a variety of fish species in a continuum of quality (pass to fail) from freshly caught or fresh-frozen to highly decomposed or tainted samples. The controlled spoilage samples are especially important for the purpose of providing a continuum of spoilage to which the commercial and inspection fish and seafood samples can be compared. The inspectors are trained and calibrated to consistently identify, describe and quantify, using a 100 mm, universal line scale, the sensory attributes present in the pass and fail samples. Reference odour samples consisting of food items, plants, other organic materials, chemicals or other readily available household items are also used to assist inspectors in identifying specific odour and flavour attributes in fish and seafood and in learning to use uniform and consistent descriptive terminology. The inspectors are then tested on three-digit coded, randomly presented fish samples to assess their ability to distinguish pass, borderline and fail samples as determined by a three person, "Expert" panel of their peers (CFIA, 2003c). Therefore, having a constant set of odour or flavour chemical reference samples available would be advantageous in bench-marking and maintaining the sensory evaluation skills of the inspectors in training and assessment workshops and in the field.

Production of controlled spoilage samples and collection of commercial product is expensive and is not feasible beyond workshop sample requirements. Therefore a sensory reference tool modeling fish odours for a continuum of spoilage would be highly valuable. In addition, the fish and seafood industry is required to perform and document their own sensory assessments in compliance with the specific product standards and Fish Inspection Regulations under the requirements of the FSPP, Quality Management Program (QMP) and Quality Management for Importers (QMPI). The QMP program requires all federally registered fish processing plants to develop and implement a hazard analysis critical control point (HACCP) based, in-plant quality management program. In addition, the QMPI program requires that the fish and seafood products imported by licensed importers comply with CFIA process and product standards. Both of these HACCP based programs require that industry demonstrate that they are able to perform sensory evaluations according to the CFIA product standards. A sensory reference tool would therefore, also be valuable to this group in the design and maintenance of their QMP and QMPI programs.

Thus, a need has been identified for the development of a model reference set of odourants that could be used in calibration and training of inspectors and industry personnel that simulate actual fish sample odours. The reference set would be an effective training tool alongside controlled spoilage and commercially available fish samples as well as an effective means of maintaining the skills of trained inspectors. In addition, a reference set of odourants would provide industry personnel with an effective tool for monitoring their own fish products and meeting the requirements of QMP and QMPI. Finally, the capability to calibrate and train CFIA inspectors and industry

personnel with the same odour references would be an immense benefit from a regulatory stand-point. Therefore, a target fish species was selected to investigate and work toward the development of a chemical odour reference set.

Freshwater finfish are a valuable domestic product category. In particular, the Lake Whitefish (*Coregonus clupeaformis*) catch is a cornerstone of the domestic, inland freshwater fishery. In 2001, 23.9 million kg of freshwater finfish were purchased, processed and marketed by the Crown Corporation, Freshwater Fish Marketing Corporation (FFMC), which has exclusive rights to purchase fish from all registered commercial fisheries in Manitoba, Saskatchewan, Alberta, Northwest Territories and part of North Western Ontario. The total Lake Whitefish catch volume for the region was 6.5 million kg in 2001. This represents almost 30% of the total catch volume for all freshwater species and an economic value of 9.3 million dollars paid to commercial fisherman prior to value-added processing, sale and distribution to domestic and international markets (FFMC Annual Report, 2001). Lake Whitefish has already been the focus of two studies by Josephson and colleagues (1983, 1984) which identified volatile compounds contributing to the aroma of fresh and oxidized whitefish. The researchers however, did not report the sensory methods used and were limited by the chemistry technology of the time. Due to the high annual catch volume, significant economic value and past research history, Lake Whitefish was selected for further investigation to characterize, quantify the intensity and the relative contribution of individual volatiles with odour impact over a continuum of spoilage. Thus, the objectives of this study were:

- 1) To produce and determine the quality of an incremental spoilage series of whitefish samples using an expert sensory panel of four CFIA inspectors.
- 2) To measure traditional chemical indicators of spoilage, Freshness Index (Burns et al, 1985), Ethanol (McLachlan et al, 1999) and Total Volatile Base Nitrogen (Woyewoda et al, 1986) to benchmark sensory quality assessments and to assess uniformity within each sample increment.
- 3) To determine the proximate and fatty acid composition of the spoilage increments in order to evaluate the biological variability of the sample set in order to assess any possible influence on the sensory attributes perceived for each treatment.
- 4) To identify the key descriptive terms and quantify the intensity of individual volatile chemical compounds with odour impact in an incremental spoilage series of whitefish samples using a gas chromatography – olfactometry (GCO).
- 5) To examine the change in odour impact volatiles and their intensity between controlled spoilage whitefish samples of different quality levels.

Chapter 2

Review of Literature

2.1 Major Factors and Mechanisms Contributing to Fish Spoilage

Fish in general are composed of approximately 70-85% moisture, 13-24% protein, 1-26% fat and less than 1% carbohydrate which can vary between species, within species, with the season, the feeding environment and physiological health (CFIA, 2002). Liston (1982) states that the spoilage of fish held on ice is primarily due to bacterial action. Bacterial enzymes are produced and breakdown fat, protein and carbohydrate which results in the production of off-odours and flavours associated with decomposition. *Shewanella putrefaciens* and *Pseudomonas spp.* are the specific spoilage bacteria of fresh fish held on ice (Gram and Huss, 1996). The high moisture content and high availability of water in a free, unbound state make fish flesh an ideal environment for bacterial growth. Fish muscle is arranged in myotomes which give the flesh its flaky texture and zigzag appearance. The muscle structure of fish lacks connective tissue and allows bacteria to penetrate the fish flesh much more readily compared to the tissue of mammals. Mammal muscle tissue consists of muscle bundles called myofibrils connected by a tendinous system which are more resistant to the penetration of bacteria.

Autolytic spoilage occurs due to the action of enzymes already present in fish. The biochemical processes carried out by enzymes in the living fish continue after death but in an uncontrolled manner. For example, the digestive enzymes in the intestine continue to function and breakdown the intestinal wall, then the gut contents and finally the surrounding muscle tissue. Nucleotide degradation, that is the breakdown pathway of adenosine triphosphate (ATP) and the accumulation of its biproducts such as

hypoxanthine is also an example of autolytic spoilage (CFIA, 2002). The autolytic breakdown of fish flesh is primarily related to textural changes such as softening, but contribute to an environment where bacterial growth continues at an exponential rate and results in spoilage odours related to advanced decomposition (Liston, 1982).

Hydrolytic and oxidative rancidity of the fat component of fish are also responsible for the production off-odours and flavours (CFIA, 2002). Hydrolytic rancidity occurs when lipids come in contact with lipase enzymes which hydrolyze the ester bonds of triacylglycerol (esterase activity) which results in the production of free fatty acids, many of which are volatile and have objectionable odours and flavours (Goffman and Bergman, 2003). Hydrolytic rancidity continues on ice and in frozen storage but is temperature dependent. It will occur at a faster rate and at an increased level at -18°C as compared to -30°C . Oxidative rancidity is the most important type of lipid spoilage (CFIA, 2002). It is caused by the reaction of oxygen with unsaturated fatty acids side-chains of mono-unsaturated and poly-unsaturated fats which are the major components of the total fat content. The process known as auto-oxidation occurs spontaneously during exposure to air. Malodorous volatile chemicals such as aldehydes, ketones and alcohols are formed (Khayat, 1979, Khayat and Schwall, 1983, CFIA, 2002). Oxidative rancidity can be minimized by vacuum packaging or glazing fish samples and holding at -30°C rather than -18°C , but it will still occur over time in storage. Glazing fish involves rapidly freezing the whole, gutted fish or fillet sample then dipping the sample in a cold water bath which results in the formation of a thin layer of ice onto the fish sample. The procedure is typically repeated three times. The thin ice layers form a glaze that is less permeable to oxygen.

Thus the development of odours indicative of taint, decomposition and unwholesomeness (TDU) in raw fish can occur by a variety of pathways. It is critical that inspectors as sensory assessors and GCO methods are able to detect and are sensitive to the intensity of these various off-odours.

2.2 Methods Used to Evaluate Fish Quality

In order to define whitefish quality it is necessary to identify, describe and quantify the specific volatiles with odour impact throughout the continuum of spoilage increment samples from pass, borderline pass, borderline fail, and fail. This is best achieved using a variety of methods including traditional sensory evaluation, chemical indicators of spoilage and gas chromatography-olfactometry (GCO) techniques which combine sensory and chemical method. Traditional sensory and chemical indicators of spoilage are used routinely in the regulatory environment as outlined in the introduction.

The use of GCO to characterize key odourants in foods began shortly after gas chromatography (GC) was introduced by James and Martin in 1952 (Mistry et al, 1997). GCO has evolved from simply sniffing GC effluent after separation to the evolution of advanced GCO methods for the analysis of food volatiles. Such as development of sniff ports utilizing humidified make-up carrier gas and electronic intensity response systems.

2.3 Early Chemical and Sensory Analysis of Whitefish Odour Volatiles

Josephson and colleagues (1983, 1984), were the first researchers to apply GCO techniques to whitefish. In two separate studies, they endeavoured to identify the volatile compounds characterizing the odour of fresh whitefish (Josephson et al, 1983) and

oxidized, frozen whitefish (Josephson et al, 1984) from the headspace of a prepared slime extract. The overall odour of freshly caught whitefish was characterized as distinct cucumber-like or melon-like (Josephson et al, 1983). The overall odour of oxidized whitefish which had been held for 1 year at -10°C was described as distinct paint-like and cod-liver-like (Josephson et al, 1984). The researchers reported that the odour of whitefish flesh and whole whitefish was very similar and contained the same volatiles. In addition, they reported that the whitefish slime and juices contained a higher concentration of odour compounds and were a more convenient odour volatile source than the muscle tissue. Therefore they analyzed whitefish slime extracts, prepared by removing the slime layer with a saturated salt (NaCl) solution and agitation. Then 1 ml of an internal standard, active amyl alcohol (2-methyl-1-butanol) was added to 99 ml of the slime extract in 100 ml round-bottom flask. The researchers reported that the concentrates used in each study exhibited the aroma of fresh whitefish flesh and oxidized whitefish flesh respectively. A dynamic headspace purge and trap method was employed which involved purging each sample flask with nitrogen for 2.5 h at 60 ml/min. at room temperature (21°C). The salt solution was also used to retard any bacterial activity during the purge and trap time period that could result in odour changes in the extract. Headspace volatiles were collected and concentrated on Tenax GC[®] traps and eluted with ethyl ether. The extracts were separated with a Varian 1740 GC equipped with an effluent splitter, 4:1 in favour of the exit port and a flame ionization detector (FID). The effluent splitter allowed for simultaneous FID detection and sensory odour evaluation at the exit port of eluting compounds. It is important to note that no sensory methods were reported and the sensory evaluation procedures followed by the person or persons

sniffing at the exit port were not described. Compounds were also identified by a separate gas chromatography, mass spectrometer (GC/MS) analysis using a Carbowax 20 M fused silica column, with matching of full or partial mass spectra and manual matching with published spectral data. The researchers reported identification of 47 volatile peaks by GC/MS in fresh whitefish and 59 in oxidized whitefish. They determined that 23 of the compounds in fresh whitefish had odour impact from the corresponding GC/FID analysis with simultaneous odour evaluation. The volatiles with odour impact identified by the researchers for fresh and oxidized whitefish are listed in Table 2.1. The chemical compound and the associated odour "quality" descriptor for fresh whitefish are listed. The compounds marked with an "X" were also present in the oxidized whitefish. The volatiles that were found only in the oxidized whitefish are also listed at the bottom of Table 2.1. The researchers did not report whether or not these compounds had odour impact.

The researchers reported a comprehensive list of the identity, structure and estimated concentration of odour volatiles, with and without odour impact found in the slime of fresh and oxidized whitefish. However, the procedure used to identify the chemical structure of the volatiles was problematic since not all volatiles with odour impact appear as discrete peaks. That is, the volatile with an odour impact may be a small peak hidden behind a large peak, may be two peaks co-eluting, or may be a small shoulder on a peak or may not be detected at all if at concentrations below the detection limit of the FID detector. In addition, the volatile elution order for the GC/MS and GC/FID can be different due to the use of different columns and GC operating conditions. Therefore, direct comparison of the volatiles with odour impact detected by

Table 2.1: Summary of Volatiles identified in Fresh and Oxidized Whitefish
adapted from Josephson et al, 1983, 1984.

Peak Number	Fresh Whitefish Volatile Compounds	Oxidized Whitefish Volatile Compounds	GC Effluent Odour Quality
1	2,3-butanedione		butter-like
2	hexanal	hexanal	green
4	3-heptanone		earthy
16	2-octanone	X	green,earthy, aldehyde
17	octanal	X	green,earthy, aldehyde
18	1-octen-3-one	X	boiled mushrooms
22	1,5-octadien-3-one	X	geranium leaves
24	nonanal	X	planty aldehyde
26	(Z)-2-octenal	X	fatty, heavy, green
27	acetic acid		fatty, heavy, green
28	1-octen-3-ol	X	raw mushroom
29	1,5-octadien-3-ol	X	earthy mushroom
30	benzaldehyde	X	sl. cucumber, green, vine-like
31	decanal	0	sl. cucumber, green, vine-like
32	pentadecane	X	sl. cucumber, green, vine-like
33	(E)-2-nonenal	X	cardboard-like cucumber
36	(E,Z)-2,6-nonadienal	X	cucumber rind, peeling
37	2-octen-3-ol	X	green, musty
38	hexadecane	0	green, musty
40	2,5-octadien-1-ol	X	fresh fish undertone
41	6-nonen-1-ol	0	above threshold
43	3,6-nonadien-1-ol	0	clean cucumber
47	unknown	0	green, cucumber-like
		pentanal	oxidation biproduct
		decane	no odour reported
		1-penten-3-one	oxidation biproduct
		2,3-pentanedione	no odour reported
		(E,Z)-2,4,-heptadienal	no odour reported
		(E,E)-2,4,-heptadienal	no odour reported
		(Z,E)-3,5,octadiene-2-one	no odour reported
		(E,E)-3,5,octadiene-2-one	no odour reported
		1-pentadecene	no odour reported
		(Z,E)-3,5,nonadien-2-one	no odour reported
		(E,E)-3,5,nonadien-2-one	no odour reported
		(E,Z)-2,4,-decadienal	cold storage
		(E,E)-2,4,-decadienal	cold storage

GC/FID and odour evaluation of the volatiles identified by GC/MS at the same or similar retention times are not possible without using co-chromatography of the same compounds on both systems. The interpretation of the chromatograms is also complicated by having to eliminate solvent peaks from the eluting volatiles. The studies by Josephson et al (1983, 1984) did not report the use of valid sensory methods which should include, the screening, selection and training criteria for the person or panel used to perform the sensory evaluations, the environmental conditions the sensory assessments were conducted in and a description of the sensory evaluation procedures used. In addition, statistics were not used or reported to support their findings for either the sensory results. Therefore, a GCO experiment using newer GCO techniques on whitefish tissue samples representing a continuum of quality using valid sensory methods was warranted. The GCO method used should include odour volatile concentrations techniques representative of fish tissue samples with supporting composition analysis, traditional quality indicator chemistry and statistical analysis.

2.4 Summary of Current GCO Methods

Currently, four main techniques have emerged for the analysis of food volatiles; GCO headspace analysis (dynamic and static), OSME a quantitative bioassay method, Combined hedonic aroma response Measurements Analysis (CharmAnalysis), and Aroma Extract Dilution Analysis (AEDA) (Blank, 1997, Mistry et al, 1997). CharmAnalysis, AEDA and OSME were not considered appropriate methods for the analysis of volatiles contributing to the odour of whitefish based on two critical factors. First and most importantly, none of the methods examine the volatiles contributing to the overall odour

of whitefish at concentrations that represent the physiological concentrations perceived when smelling actual fish tissue samples. That is, all three methods involve GCO analysis of a concentrated extract prepared from the food product being studied (Mistry et al, 1997, Guth and Grosch, 1994, McDaniel et al, 1989 and Abbott et al, 1993) rather than assessing the volatiles from the actual food matrix. The odour of an extract is inherently different from the physiological impression of the odour of the intact food sample because volatility of odour compounds in a food sample are dependent on their solubility in the food matrix and their interaction with other non-volatile constituents unique to the food product (Mistry et al, 1997). In addition, some extraction processes involving steam distillation or a direct solvent extraction result in the loss of low molecular weight odour impact volatiles (Pollien et al, 1997). For fish, these low molecular weight compounds are important odour impact components. Second, AEDA and CharmAnalysis are both considered to be screening methods based on odour detection threshold and do not indicate which volatile chemical compounds with odour impact have the greatest contribution to the overall food aroma (Mistry et al, 1997). Nonetheless, for the purpose of this literature review these methods will be reviewed briefly, while GCO headspace methods will be examined in more detail in the next section.

2.4.1 CharmAnalysis and AEDA

CharmAnalysis, developed by Acree and Bernard, (1984), Acree, (1993a) and Acree et al (1993b) and AEDA developed by Grosch and Preininger (1994) are the most widely published extract dilution techniques which endeavour to identify the key odour

impact compounds in a food system or matrix (Blank, 1997, Mistry et al, 1997). A key feature of both methods is that they attempt to rank the key odour impact compounds by evaluation of individual GC peaks by GCO (Mistry et al, 1997). In both methods, odour impact (also referred to as odour activity), OA of individual compounds) is evaluated by sniffing the GC effluent in a series of dilutions produced from an original aroma extract from a specific food product or odour/flavourcocktail (Blank, 1997). Since both methods are based on odour-detection threshold several injections are required in order to determine the dilution factor where no odour is detected (Blank, 1997, Mistry et al 1997). Extract preparation is unique for the specific food product being studied and extract dilution technique involves stepwise addition of odour inactive solvents appropriate to the food product (Mistry et al, 1997). Each dilution is evaluated by GCO in order to determine the odour detection threshold. Extract dilution techniques have several limitations. First, the techniques can be extremely time consuming because a GCO dilution study can take several days to analyze numerous dilutions. In addition, the time factor may prohibit performing replicate analyses to check reproducibility among GCO subjects (Mistry et al, 1997). AEDA and CharmAnalysis are both screening methods that do not definitively identify the volatile compounds that contribute the most to the overall odour of a sample (Mistry et al, 1997). It is important to recognize that the diluted extracts and their odour intensity do not necessarily represent the intensity of those same odour active compounds in an intact food sample (Blank, 1997). Mistry et al (1997) established that AEDA and CharmAnalysis differ primarily on interpretation of results. In CharmAnalysis, the duration of the odour perceived by GCO is recorded versus AEDA where only the maximum dilution value detected is reported. CharmAnalysis uses the

surface area of volatile peaks known as Charm Values while AEDA uses FD values, a relative measure related to the ratio of concentration to odour threshold and is essentially identical to the peak maximum of a Charm Value. A study by Abbott et al (1993) conducted on beer compared the data collected using the two methodologies and found that the order of potency of key odourants was different for most of the six panelists when the data were presented as FD factors versus Charm Values. They also found that it was difficult for the panelists involved to determine the end of an odour active peak and the beginning of another, due to rapid changes as the volatiles eluted. This problem however would be true for all GCO methods. Piggot (1990) also criticized the two detection threshold based methods because the intensity and odour character of volatiles at concentrations above threshold concentrations are not taken into account. Therefore, neither CharmAnalysis nor AEDA are considered appropriate for determining the volatiles with odour impact that most contribute to overall fish aroma at physiological concentrations perceived when sniffing the fish sample.

2.4.2 OSME

OSME is a Greek term meaning smell and is a quantitative bioassay (human sensory detection method often called a quantitative bioassay method in the literature. It is used to measure the perceived odour intensity of a volatile eluting from a GC-olfactometer (Miranda-Lopez et al, 1992a, 1992b). Like AEDA and Charm Analysis, OSME involves the preparation of an extract representative of the food odour being studied (Mistry et al, 1997). The method is different from AEDA or CharmAnalysis in that OSME is not based on odour detection thresholds and involves evaluation of only

one concentration of an extract (Miranda-Lopez et al, 1992a, 1992b). Data including odour retention times, time intensity ranges and corresponding odour descriptors are collected and registered directly into a computer data-base. A panel of three or four trained analysts is used to rate the intensity of an odour volatile using a slide-bar, time-intensity device which provides a computer generated odour peak, similar to the peak of a GC chromatogram, called an Osmegram (Mistry et al, 1997, McDaniel et al, 1989). The individual Osmegrams of several panelists detecting the same compounds at least 50% of the time for replicate samples are then averaged to produce a consensus Osmegram. OSME was founded on current psychophysical theories consistent with sensory descriptive analysis techniques such as using a trained panel and a 15 cm line intensity scale. In addition, OSME does not assign a relative importance to a compound to overall food odour based on its perceived intensity threshold (Mistry et al, 1997). OSME as a well published, academic and commercial method stands alone based on valid sensory relationships and quantitative data permitting statistical comparison between samples and subjects. The OSME technique has been used to investigate the aroma of Pinot noir wines and the conditions that result in aroma variation (McDaniel et al, 1989, Miranda-Lopez et al, 1992a, 1992b). However, because the method is based on analysis of an extract it is not appropriate for fish. The data generation and analysis components of the OSME technique which includes collection of odour retention times, intensity measurements for volatiles with odour impact, and collection of associated descriptive terms using trained sensory panelists to produce overall average Osmegrams are valuable data collection and analysis tools that can be applied to other GCO techniques.

2.5 GCO Headspace Analysis Techniques

GCO using volatile headspace sampling techniques (GCO-H) without the use of extracts better represent the physiological concentration perceived by the human when smelling food samples (Blank, 1997, Mistry et al, 1997). GCO-H techniques involve collection and concentration of the volatile vapour phase surrounding a sample in a sealed vessel (Wampler, 1997). Unique GCO-H sampling techniques have been developed to improve collection and concentration of odour impact volatiles (Chaintreau et al, 1995, Pollien et al, 1997, McLachlan, 1997). In addition, studies applying GCO-H have been published for an extensive range of food products including: stewed beef juice (Guth and Grosch, 1994), cheddar cheese (Arora et al, 1995), artisanal Spanish cheese (Fernandez-Garcia, 1996), cola beverages (Elmore et al, 1997), and yogurt (Ott et al, 1997). Two researchers have specifically applied GCO-H techniques to fish products including boiled cod (Milo and Grosch, 1995) and raw Atlantic scallops (Sims and McLachlan, 2000).

GCO-H sampling techniques are typically divided into two categories: static and dynamic/purge and trap (Wampler, 1997). In typical GCO static headspace sampling, a small food sample (5 g) is placed into a headspace vial, sealed and often warmed to increase vapourization of volatiles, then allowed to stand and equilibrate for a specific time period as volatiles pass from the sample matrix into the headspace. An aliquot of the headspace is then drawn and injected directly into the GC injector port (Wampler, 1997). The quantity of volatile compounds entering the headspace depends on several factors, including: the size/quantity of sample, the volatility of the compounds, the solubility of the compound in the sample, the temperature of the vessel and the time

allowed to equilibrate (Wampler, 1997). In addition, the amount of volatiles that are carried onto the GC column depends on the portion of the headspace that was injected which is typically 0.1 to 2.0 ml. Wampler (1997) identified three key advantages of static headspace sampling, which were: the technique has the ability to analyze low molecular weight volatiles without the use of a solvent, thereby simplifying the technique and increasing its sensitivity; the technique is easily automated and the technique is relatively inexpensive due to simplified preparation time and elimination of reagents. Also, from a sensory evaluation perspective, the static headspace better emulates odours perceived during traditional sensory analysis of a food product by an assessor. Despite this, Wampler (1997) also identified several disadvantages of conventional static headspace sampling. Firstly, only a fraction of the volatile compounds of interest are injected due to the small headspace volume. Secondly, the concentration of odour volatiles is decreased due to their equilibrium with the food sample. Thirdly, the method is only useful for compounds with low boiling points because the use of high temperatures to volatilize compounds with high boiling points could denature the food sample. Finally, volatile artifacts may be created from the food sample if heat is applied or enzymatic activity takes place due to lengthy equilibration time.

Dynamic headspace sampling involves the constant purging of volatiles above a food sample by the flow of a carrier gas (Wampler, 1997). Therefore, volatiles are not allowed to equilibrate above the food sample allowing for more volatiles to escape from the sample into the headspace. This allows an increase in the final headspace volume beyond the capacity of the headspace vial. The total volume typically collected may range from 100 ml to 1.0 L of headspace but may be even larger if collected over many

hours (Wampler, 1997). In order to transfer this entire volume to the GC, the dynamic headspace purge gas is diverted through a collection trap where the volatiles are retained and concentrated (Wampler, 1997). A collection trap may involve cold trapping by condensing or freezing or adsorption of volatiles onto a sorbent material such as the commonly used porous polymer Tenax (Wampler, 1997). The collection trap is then either desorbed directly onto the GC column or eluted with a solvent and the solvent extract is injected onto the column. The advantage of the dynamic headspace trapping stage is the increased volume collected and subsequent concentration of volatiles which results in increased sensitivity, allowing volatile detection at levels at part per billion (ppb) and even parts per trillion (ppt) in the original food matrix if careful scrutiny is applied to control for contaminants or instrument background noise. Wampler (1997) also identified several disadvantages in dynamic headspace sampling. Firstly, the purge and trap instrumentation is more complex due to required instrumentation monitoring at several steps, such as valves, heat zones and carrier gas flow rates. Secondly, although relatively less expensive than extraction methods it is still more expensive than static headspace methods (Wampler, 1997). Finally, theoretically dynamic headspace collection could take longer than static methods, however, sample preparation may take place while the GC is still analyzing the previous sample and equilibration time is eliminated. Chaintreau et al (1995) also identified that dynamic headspace was not suitable for the quantification of headspace volatiles because the release of volatiles into the vapour phase is dependent on their interaction with the matrix. Therefore some volatiles may not be released into the vapour phase due to the constant purge.

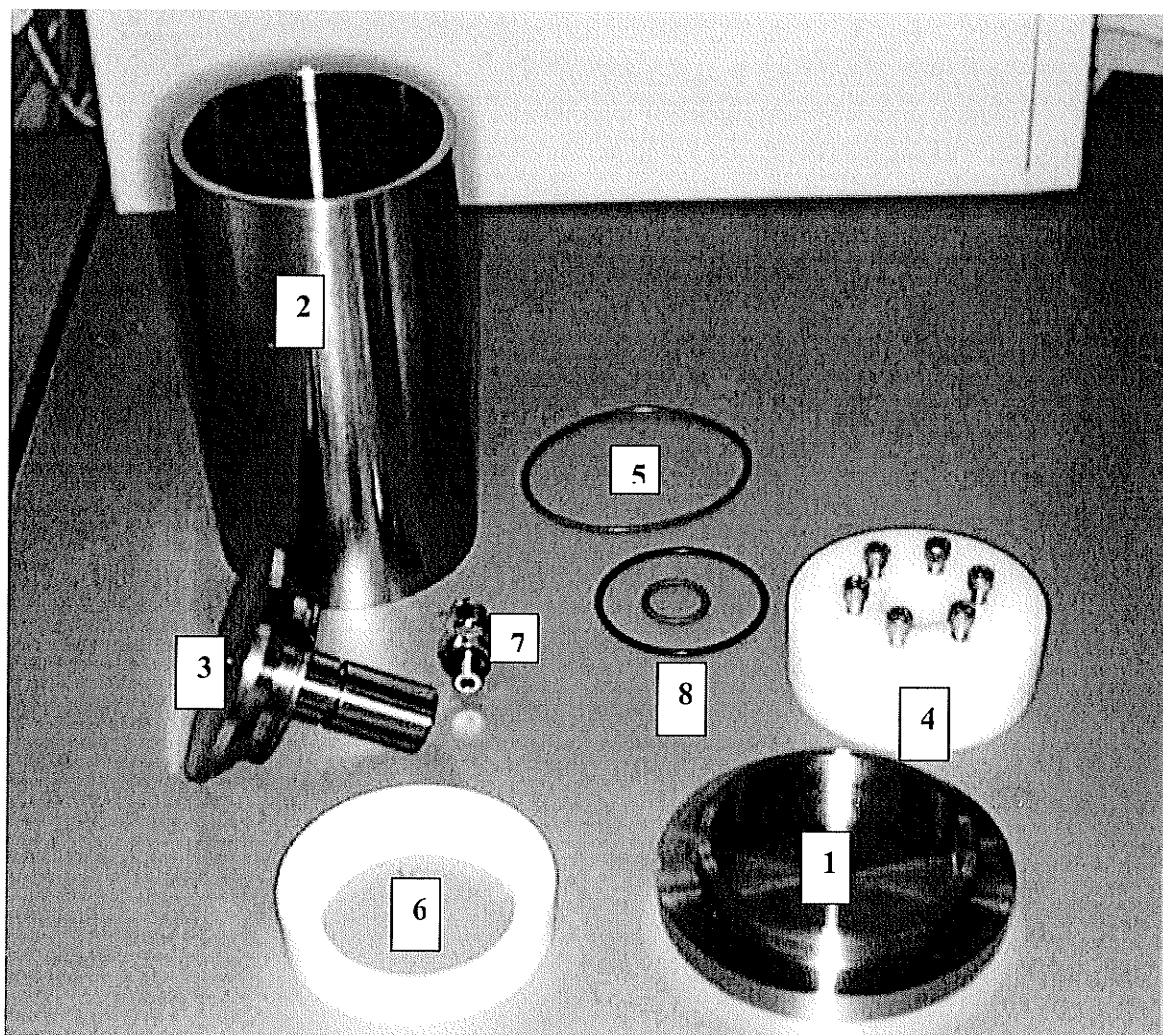
A modified static headspace method, incorporating features typically used in dynamic headspace analysis, was developed by Chaintreau et al (1995) to examine partition coefficients for different food-matrices and not specifically for GCO. They developed a headspace, sampling cell consisting of a 5 cm x 15 cm glass tube fitted with a bottom end piece and piston made from Teflon with Viton seals. The bottom was fitted with a removable, glass reservoir able to hold up to 2 ml of test sample. The test sample was allowed to equilibrate within the cylinder like a static headspace method. A Tenax trap was attached to the piston, which captured the equilibrated headspace volatiles when the piston was depressed and is therefore similar to dynamic headspace, purge and trap techniques. The headspace, sampling cell was tested using a liquid, model of a mixture of food flavors with three different solvents, including water, oil and an emulsion. The partition coefficients were determined for each solvent mixture. Pollien et al (1997) employed the combined static and dynamic headspace sampling technique of Chaintreau et al (1995) for GCO analysis of a flavour cocktail and animal dejecta (excrement) samples. Pollien et al (1997) used the headspace method of Chaintreau et al (1995) due to the qualitative rather than quantitative information provided by extract dilution techniques used for many GCO studies published prior to their work. Samples of the flavour cocktail (0.5 g) or animal dejecta (9 g) were placed in the sample reservoir of the headspace cell and equilibrated in a 30°C water bath for 0.5 h for the flavour cocktail and 2 h for the animal dejecta. As the cell piston was depressed, the volume of the cell was displaced through a trap containing 250 mg of Tenax. Volatiles were then thermally desorbed from the Tenax at 250°C, then refocused in an internal cold trap (-30°C) and desorbed into a Hewlett Packard GC at 260°C. Helium was used as the carrier gas at a

flow rate of 8 ml/min. The temperature program started with an oven temperature at 20°C that increased at a rate of 4°C /min up to 200°C and was held for 10 min. The column outlet was connected to either the FID detector or the sniff port equipped with humidified air. The sniffing procedure involved six untrained panelists randomly selected from a pool of colleagues. Each person participated in a 25 min GC effluent sniffing session that was divided into two segments to avoid fatigue. Indication of volatile odour perception was recorded by pressing a button for the duration of a specific eluting odour volatile and the corresponding odour descriptor was recorded on tape. GCO “aromagrams”, resembling GC chromatograms were produced using the Hewlett Packard Pascal workstation. The six individual aromagrams for each of the respective flavour cocktails and dejecta were summed and averaged to produce one average aromagram per sample type. Peak height and peak area were determined for each volatile. The researchers defined peak height as nasal impact frequency (NIF) and peak area as surface of nasal impact frequency (SNIF). NIF's were normalized to a height scale of 100%. Using NIF and SNIF values the researchers tested whether the peaks of two aromagrams could be directly compared with experimental confidence. They first evaluated the reproducibility of the sniffing procedures of untrained panelists using a simplified flavour cocktail. Three sniff runs were carried out using two separate, untrained panels of six people. Panel A performed one sniff series and panel B performed two sniff series. Intra-panel comparison of the two sniff series performed by panel B and inter-panel comparison of the sniff series performed by panel A compared to panel B were made using NIF and SNIF standard deviation calculations. The researchers concluded that the NIF values were not more reproducible than the SNIF values because

the average relative standard deviation (RSD) did not differ significantly for NIF and SNIF (RSD Range = 13.8 - 15.7%). Similar results were found using the animal dejecta samples with a RSD of 20%. In addition, they concluded that the two independent, untrained panels were able to generate similar aromagrams because intra-panel reproducibility appeared to be comparable to inter-panel repeatability according to standard deviation scores. The researchers also suggested that their method was more reproducible compared to another study by Guth and Grosch (1994) that used a trained GCO panel to evaluate stewed beef juice and which reported higher standard deviation scores (30.9%) for their intensity scores. This conclusion is misleading because direct comparison of the two data sets is not valid since collection of intensity scores by a trained panel is different from measuring presence and duration of a volatile with an untrained panel. The researchers conceded that NIFs and SNIFS were not direct measurements of perceived odour intensity but concluded that because both measures increase with volatile concentration they also increase with odour intensity. Mistry et al (1997) identified this reasoning as a classic error made by chemists in relation to GCO experiments. Sensory psychophysical experiments have demonstrated that perceived intensity is not directly proportional to concentrations in solutions or in volatized form (Mistry et al, 1997). The researchers did demonstrate that the GCO sensory evaluation method was reproducible but the information collected only determined that an odour was perceived, not its intensity. Therefore it is not possible to extrapolate to what degree a particular odour volatile contributed to the original sample headspace odour. Despite the limitations of the Pollien et al (1997) study, the modified static headspace sampling technique was successful in the collection, trapping and purging of the headspace odour

of a food at a physiological concentration representative of the overall odour of the samples.

McLachlan (1997) developed a modified GCO method that used a sampling technique built on the headspace sampling technique employed by Chaintreau et al (1995) and Pollien et al (1997). It combined the core principles of static headspace generation with the increased sample volume and volatile concentration techniques of dynamic headspace. A custom-made stainless steel static headspace chamber with a volume of 850 ml was machined based on the headspace cell developed by Chaintreau et al (1995). The chamber consisted of a stainless steel cylinder wall, a base containing a sample well which threads onto the cylinder with an air-tight seal and a stainless steel piston with a Teflon beveled ring that forms an air-tight seal within the cylinder (Figure 2.1). An airtight Swagelok fitting on the top of the piston allowed for the attachment of a Tenax cartridge. The well of each base piece may be filled with liquid or solid material. Upon assembly of the apparatus, a standardized sample was inserted into the cylinder and allowed to equilibrate. The entire volume of the cylinder was displaced onto the Tenax cartridge without disturbing the equilibrium between the vapour phase and food matrix, unlike dynamic purging. The larger headspace chamber allowed for a much increased volatile concentration as compared to the headspace cell developed by Chaintreau et al (1995). McLachlan (1997) method is particularly unique at the GCO level. The Tenax cartridge volatiles are desorbed using an external thermal desorption device onto the GC column via a heated transfer line. The column eluent flow is split between the FID detector and a human sniff port. Hydrated air was used as a make-up gas to carry the volatiles from the sniff port to the trained GCO panelist who provides the odour character



1 = cylinder base

5 = base plate viton ring

2 = cylinder wall

6 = Teflon beveled ring

3 = piston head stainless hub

7 = Swagelok stainless fitting

4 = nylon guide bushing

8 = hub viton o-rings

identification and intensity rating simultaneously with FID detection. The GCO odour intensity and associated retention times were provided by the assessor using a slide wire which generated an electrical signal to the Varian Star workstation producing an aromagram similar to the FID chromatogram. The slide wire would be positioned to the far left or "zero" when no odour was perceived and then moved to the right as an odour was detected and became more intense. The wire was moved back to the left as the odour intensity decreased or disappeared. The final steps were to describe the volatiles with odour impact, to determine the intensity and proportion of the odour impact volatiles to each other and to work toward the simulation of the odour in a final odour-recombination stage. The key advantage of the method was that the benefits of using a static headspace sampling procedure were fulfilled, combined with the advantages of dynamic headspace which involves trapping and concentration of volatiles. In addition, the static headspace volume emulated the odour perceived when inspecting fish using traditional sensory evaluation procedures. The sensory panelists used for GCO analysis were expert level fish inspectors who had already been established in an elite category of sensory assessors. The panel was also introduced to and trained in the GCO sniffing technique using a chemical cocktail with volatiles that eluted distinctly across the GCO run. This allowed the experimenter to insure all analysts could perceive a variety of volatiles with different odour character. In addition, it allowed the panelists to familiarize themselves and become comfortable using a rapid breathing technique similar to that described by Hanoaka et al (2001). The breathing technique involved inhaling through the mouth and exhaling through the nose at a higher rate than normal for each assessor in order to minimize the possibility of missing a volatile with odour impact

when inhaling. The breathing rate was based on individual comfort, to ensure that the assessor did not hyperventilate. The GCO sensory environment was also controlled for noise, distractions and temperature, using a portable air conditioner due to heat produced by the GCO equipment. A fume exhaust hood and air exchange system was used to exhaust volatiles accumulating in the GCO laboratory and to draw in fresh air (three exchanges per h). In addition, comfort of the analysts was insured by adjustment of the GCO nose piece for each person while in a seated position.

Sims and McLachlan (2000) applied the method developed by McLachlan (1997) to study the odour volatiles of raw sea scallops (*Placopecten magellanicus*). A controlled spoilage series of shucked scallop meats held on ice (-0.5 to -1.5°C) over time and a series held at ambient temperature (14°C) over time were produced from commercially harvested scallops. The samples were utilized in consultative workshops between the Canadian Food Inspection Agency (CFIA) and scallop industry representatives. During the workshops, spoilage increments representative of specific scallop sensory attributes of interest at four quality levels (Day 0, 7, 11, and 17) were selected for GCO analysis. The original method of McLachlan (1997) was used but modified by the addition of a GC/MS compound identification process. The GCO instrumentation was fitted with a “selective-programmable active splitter” which allowed the GCO analyst to divert selected compounds in the effluent stream to a secondary Tenax TA thermal desorption trap. The trapped odour active compounds were then re-chromatographed in high resolution on a HP 5890 GC/MS on a 0.25 mm x 60 m Carbowax column. The mass spectrometer allowed for positive identification of some odour active volatile compounds by mass spectra comparisons of the identified

compounds and their retention times to authentic standards. The researchers identified forty compounds that had at least some odour impact within the controlled spoilage samples and found unique odour impact volatile patterns for the various scallop increments. Some odour components were identified in all increments but at varying concentrations. They concluded that these compounds are likely always present but that the relative concentration may change relative to product quality. Other components were reported to have lower incidence in occurrence and appeared to be largely responsible for changes in overall odour associated with scallop quality. From traditional sensory evaluation results, day 0 represented the best quality, day 7 was neutral and acceptable, day 11 was borderline fail and day 17 was severely decomposed. Peaks were categorized as odour volatiles associated with either high quality sample or with fail quality. Interestingly, GCO results for the ambient controlled spoilage samples were missing odour volatile peaks that were present in the iced spoilage series. The researchers suggest that this may imply that the decomposition pathways are temperature dependent resulting in different volatile odour impact products. The odour impact intensity data were used to identify which odour impact compounds were most important in contributing to the odour of scallop quality from freshest to advanced decomposition.

Milo and Grosch (1995) applied a static GCO-H method to examine the differences in the odour volatiles of boiled cod after the raw material had been stored under vacuum and in the presence of air for 26 weeks at -60°C and -13°C respectively. Skin-free commercially purchased Atlantic cod fillets (3 kg) were minced in a meat grinder. The minced cod was divided into 20, 150 gram portions, 10 portions were vacuum packaged in polyethylene bags (sample A) and stored at -60°C (ideal storage

conditions). The other 10 samples were sealed in the presence of air (sample B) and stored at -13°C (sub-standard storage conditions). After the storage period, the overall odour of sample A was characterized as a pleasant mild fishy odour and sample B as putrid, fishy and malty. The samples were thawed at 6°C , sub-divided into 50 gram portions and boiled for 10 min in aluminum foil packets. The samples were allowed to cool at room temperature for 5 min, then unwrapped and placed in a 240 ml flask with sealed septum and held in a 40°C water bath. The headspace was allowed to equilibrate for 1 to 1.5 h. Initially 20 ml of headspace was drawn by a gastight syringe and injected into the GC purge system and swept into the nitrogen cooled trap with helium carrier gas and onto the column for GCO-H analysis. The GC effluent was split into a FID and sniff port. GC-MS analysis was also performed on 20 ml headspace volumes for identification of volatiles contributing to the odour of the boiled cod samples. The researchers indicated that 10 odourants were found for sample A and 15 for sample B. Successively decreasing headspace volumes (10, 4, 1 and 0.5ml) were then drawn and injected to show the most potent odour impact volatiles for boiled cod samples A and B (Milo and Grosch, 1995). The assumption being that only the most potent odour volatiles would be perceived in the lowest injected volumes. Therefore, for sample A the researchers reported a cabbage-like, putrid odour associated with dimethyl trisulfide determined by GC-MS as the most potent odourant because it alone appeared in the 1 ml volume headspace injection. While for sample B, they reported that a malty odour, associated with 3-methylbutanal was the most potent odourant because it was the sole compound sniffed in the 0.5 ml volume headspace injection. In addition, the presence of an amine-like odour (trimethylamine), a buttery-like odour (butane-2,3-dione), a second malty

odour (2-methylbutanal) and a mushroom-like odour (unknown) all were perceived at lower headspace volumes for sample B as compared to sample A. Therefore they concluded that these compounds were responsible in the change from the pleasant mild fishy odour of sample A to the putrid, fishy and malty odour of sample B. It is quite likely in this experiment that many odour impact volatiles were not collected and trapped due to the relatively low headspace volume, even for the highest injection volume of 20 ml. The study was successful in showing some of the most highly volatile odourants contributing to the off-odours formed during inappropriate storage. Conclusive identification of volatiles by GC-MS however, is questionable based on the different GC conditions used during the respective GCO-H and GC-MS analyses. From the work of others (Chaintreau et al, 1995, Pollien et al, 1997 and Sims and McLachlan, 2000) it is clear that the headspace volatile collection and concentration method used by Milo and Grosch (1995) was not as effective since the concentration of some odour impact volatiles was probably too low to be detected by the FID or by the human. In addition, the application of recognized sensory methods, description of the number or training level of the sensory assessors used in their study was not reported making it difficult to assess the reliability of the sensory data collected.

2.6 Screening, Selection & Training of Expert Sensory Assessors

A highly trained sensory assessor or panel is an analytical instrument that must be calibrated and tested to produce objective, precise and reproducible measurements (Poste et al, 1991). Therefore it is necessary to select and screen candidates based on their ability to detect odour and flavour differences in food products (Amerine et al, 1965).

This concept also extends to fish inspectors who are highly trained expert assessors and to GCO assessments where the detection, description and intensity ratings of individual odour volatiles are required. The American Society for Testing Materials (ASTM, 1981) recommends three important tests for screening basic sensory discriminative ability. These are: to determine the assessor's ability to differentiate the basic tastes at above threshold concentration; to determine the assessor's aptitude for identifying and describing different odourants; and to test the assessor's ability to rank basic taste samples in order of increasing intensity. The results of these tests determine whether acceptable candidates have a normal or superior ability to detect, discriminate and describe the basic tastes of sweet, sour, salty, bitter and umami and to perceive and describe common odours (Poste, et al, 1991). Umami is a Japanese term that loosely translates to the English term savory. Codex (1999), has also recommended the use of these screening tests in their published guidelines for the sensory evaluation of fish and shellfish in the laboratory setting. CFIA inspectors have undergone this form of screening as part of a formal sensory theory training program or as new recruits during the hiring process.

The expert sensory assessor is considered to be in an elite category defined by their extensive training, product knowledge and years of experience evaluating a particular product category (Poste et al, 1991, Reilly and York, 1993, York and Sereda, 1994, York, 1995). As described in the introduction, in Canada, sensory training, standard development and assessment workshops procedures for fish and seafood products have been formalized and incorporated into the CFIA's Fish Inspection Manual (2003c). CFIA fish inspectors must meet a minimum sensory assessment standard ("Analyst" status) in order to qualify to assess product from each specific product

standard (CFIA, 2003c). The format of a CFIA sensory training and calibration workshop consists of three main components, snapshot, demonstration and discussion and practice sets. Each workshop typically involves a maximum of 12 trainees, a panel of three experts, a sensory facilitator and computer data analysis facilitator. A training workshop is typically 2.5 days in length, one half day for the snapshot component and one half day assigned to each product category addressed in the workshop. The snapshot component is typically comprised of a group of three sets, each consisting of 15 to 20 three-digit coded, randomly assigned samples. The samples are representative of the controlled spoilage and commercial samples that will be used for training. The snapshot allows for the assessment of individual inspector's sensory skills prior to training. This allows for the comparison of pre and post-training skills and permits the measurement of training effectiveness. The demonstration and discussion component consists of samples presented in order, representative of a wide range of quality from fresh to highly decomposed. Controlled spoilage samples demonstrating a continuum of spoilage along with commercial and inspection samples are also displayed together. During the demonstration, trainees initially evaluate the samples independently, recording the descriptive terms they associate with each sample and using a 100mm universal scale, record the quality of the product from pass to fail. The 50 mm mark is considered the borderline between pass and fail samples and is not used, i.e. a conclusive pass or fail decision must be indicated. A line-score of 45-49 mm is considered a borderline pass sensory decision and 51-55 mm is considered to be a borderline fail sensory decision. It is at this borderline quality region that inspectors must learn to distinguish slight but distinct and persistent odours of taint, decomposition or unwholesomeness (TDU)

indicative of fail quality product. In depth discussion of the samples with the expert panel follows. The expert panel works together to describe their perceptions, provide line-scores and point out unique product features or potential causes related to odour attributes associated with TDU. The workshop facilitators work alongside the expert panel to summarize conclusions, chart results and provide information regarding a chemical indicator of spoilage, used to benchmark sensory decisions. In addition, the facilitator provides odour reference materials to assist the experts and trainees to precisely describe their sensory perceptions and to assist identifying specific components contributing to the overall odour of a pass or fail sample. A practice set of 15 to 20, three-digit, blind coded samples, randomly distributed in individual booths, are then presented to allow the trainees to practice the skills obtained during the demonstration and discussion set. The practice set is also used to provide further product experience, reinforce pass or fail decisions made earlier and to gather and confirm the use of consistent terminology. The demonstration and discussion session and practice session format is repeated for each product category being examined in a workshop. Trainees must achieve a minimum score of 80% correct overall and 75% for both pass and fail samples compared to the expert panel in order to qualify as an "Analyst". CFIA fish inspectors may be recognized as an "Expert Analyst" when they exceed the minimum standard and achieve a score of 85% overall and 80% for both pass and fail samples compared to the expert panel. It should be noted that both "Analyst" and "Expert" levels are still considered to be expert sensory assessors but the classification of "Analyst" and "Expert Analyst" recognizes a distinction in their scores achieved during the workshops and allows "Expert Analysts" to be used as trainers. The CFIA's internal standard

distinguishes "Expert Analysts" so that inspectors with this designation are used as trainers in the workshop setting and form the three-person panel against which the workshop participants are calibrated against. The "Expert Analysts" must also have completed a recognized course in sensory evaluation techniques, have at least two years of experience in the workshop product category and inspect the product on a regular basis at the operational level. This training program has served as a model for other international regulatory bodies and has been influential in the guidelines developed by Codex (CAC-GL31-199), American Society for Testing Materials (ASTM) and the International Organization for Standardization (ISO, 1993,1994). Canada and the United States (U.S.) have also worked cooperatively in the standardization of sensory training of fish inspectors as part of the North American Free Trade Agreement (NAFTA). The CFIA , U.S. Food and Drug Administration (USFDA) and U.S. Department of Commerce, National Marine Fisheries Service (USDC-NMFS) co-host sensory workshops on an annual or semi-annual basis to harmonize inspectors from both countries in the sensory attributes and pass or fail quality cut-offs for a diverse range of fish and seafood products as described by Reilly and York (1993). In addition, Codex, ASTM and ISO have incorporated the methods to screen, select and train assessors, already used by Canada and the U.S. regulatory agencies into their standards (Codex, 1999; ASTM, 1981; ISO, 1993, 1994). The sensory training requirements of CFIA fish inspectors also makes them ideal candidates for GCO analysis because they already have extensive descriptive training in identifying a diverse range of odour attributes and are familiar with using a universal line scale to rate their perceptions.

2.7 Control of Psychological Error and Physiological Fatigue in Sensory Assessments

In addition to appropriate screening and training criteria there is a need to control for psychological errors and fatigue that may be experienced by assessors during sensory evaluations. Most psychological errors are associated with naive or untrained assessors, however, two key errors, proximity error and contrast and convergence error must be controlled for the trained assessor (Poste et al, 1991, Stone & Sidel, 1985). Proximity error occurs when adjacent characteristics tend to be rated as more similar than those that are far apart. This error is controlled by random sample presentation, experience in evaluation of the product, the ability to focus on individual samples, and by rating sample replicates. Therefore as part of routine inspections, inspectors are required to draw a random, statistically determined number of representative sample units or fillets from a product lot (CFIA, 2003b). The final inspection decision is based on the number of passed or failed units within the representative sample. In addition, in CFIA training, standard development and assessment workshops, sample sets are comprised of randomly presented, three-digit blind-coded samples, with replicates to control for this effect.

Contrast and convergence error are probably the most difficult errors to control for (Stone & Sidel, 1985). These errors often occur simultaneously and are product related. Contrast error is characterized by the exaggeration of perceived differences between two samples. For example, a highly decomposed sample presented before a slightly decomposed sample results in the assessor perceiving the slightly decomposed sample to be much better than it is. Convergence error is the opposite of contrast error in which large differences between two or more samples may mask small differences

between other samples. These errors may be controlled by random presentation of samples and by sniffing a control odour sample between test samples to recalibrate the assessor. Extensive training and experience of sensory assessors is also highly effective in controlling for this type of error (Stone and Sidel, 1985).

Sensory fatigue at the physiological level is a problem encountered by both trained and untrained panelists (Poste et al, 1991, Blank, 1997, Mistry et al, 1997). The effects of sensory fatigue can be prevented or controlled by taking regular breaks to allow the sensory systems to “re-zero” and by using rinsing aids such as sniffing water, coffee beans or eating an unsalted cracker to remove odour or flavour molecules that are tightly bound to the odour receptors (Poste et al, 1991). Controlled random presentation of odour volatiles is impossible for GCO sessions, however the use of standardized sniffing techniques, calibration of panelists prior to GCO evaluation and adequate rest for panelists between GCO sessions are used to control for sensory fatigue.

2.8 Importance of Reference Standards & Descriptive Terminology

Rainey (1986) defines a reference standard as any chemical, spice, ingredient or product that can be used to characterize or identify an attribute or attribute intensity found in any type of product, food or non-food, being evaluated by a trained sensory analyst. Reference standards should be simple, reproducible and be defined by one descriptive term. Ideally, they can be diluted without changing character, for example, a sodium chloride solution for the attribute of salty. Rainey (1986) described the importance of using reference standards in a multitude of training and practical settings. Firstly, the use of reference standards can facilitate the development of terminology used

to describe the attributes in the product being evaluated. This is achieved by expediting the process of terminology development and making the task easier and more accurate. Reference samples are also useful in resolving panel disagreements regarding ambiguous characteristics or those not apparent to the entire panel. Secondly, reference standards can assist in determining attribute intensities and be used to anchor end-points of attribute intensity scales. These eliminate the use of subjective terms such as weak or strong and allow one to make relative comparisons between products varying in intensities of the same attribute. Thirdly, reference standards can be used to demonstrate the function of product ingredients. For example, salt content can greatly affect a product and having references for varying salt levels could assist in product development or improvement work. Fourthly, documented reference standards, their attributes and related descriptive terminology provides a reliable tool for future use. It is important to consider that the use of processed food reference standards may also undergo formulation changes over time. Therefore it is necessary to reassess these standards for the attribute they represent before using them for new panels. Finally, reference standards can be a great asset to industry. They may represent a quality standard or check point for a quality assurance program, representing acceptable and unacceptable limits for a product. They may also assist in demonstration of small differences between products with similar characteristics. For example, assistance in identifying small differences in fish samples produced in a controlled spoilage sample series.

The need for reference standards has lead to the development of a number of tools. The Aroma and FlavourLexicon for Sensory Evaluation (Civille & Lyons, 1996) is a comprehensive computer database and manual that contains descriptive terminology,

definitions and references listed alphabetically. It provides instructions and methods for mixing specific solutions. The database is expandable, allowing one to add new references and related terminology pertinent to a specific product, in the same format. The Atlas of Odour Character Profiles (Dravnieks, 1985) is a comprehensive list of odour chemicals and mixtures and their associated odour character profiles compiled on behalf of the American Society for Testing and Materials (ASTM). Tools such as these also facilitate standardization of terminology used between different researchers and can be used in the development of government standards.

Munoz and Civille (1998) stress the importance of developing standardized practices or a common frame of reference for any descriptive training program. They identified both qualitative frames of reference such as the development of a common lexicon and language and quantitative frames of reference, which refer to scaling methods to rate attribute intensities. Ultimately, a well trained panel has solid common qualitative and quantitative frames of reference that are used in product or odour volatile evaluations. Munoz and Civille (1998) found that low panel variability was obtained when both frames of reference are successfully implemented and used. Trained panels are often used in descriptive analysis of food products, requiring them to assess their overall perception of a product in addition to its many varying attributes. Munoz and Civille (1998) described a universal scale approach as a philosophy that attribute intensities are rated on an absolute and universal basis rather than using a separate scale for each attribute. The universal scale is typically composed of an 100mm to 150 mm, unstructured, horizontal line, the ends of which may be anchored by intensity terms such a “not present” being equal to zero and “most extreme” being equal to 100. They felt

that, a universal scale is an appropriate tool allowing the assessor to make direct comparisons of odour intensity for different attributes on one scale or to rate an overall impression of different samples. Thus, in the case of odour assessment, all odour categories are considered and a highest intensity reference point is selected to represent the absolute strongest or most extreme odour. In addition, a lowest reference point and a midpoint reference point may be selected to assist panelists in scaling attribute intensities or overall quality assessments. The universal scale may be applied in two ways, comparison of products across product categories or comparison of similar products within a product category (Munoz and Civille, 1998). Munoz and Civille (1998) identified several advantages to using a universal scale. It allows for the inter-comparison of attribute intensities or overall quality within a specific product, across products within a product category and across products of several product categories. For industry, it provides a system by which products from different companies, sources and processes can be compared. Use of the universal scale also provides a system by which a panel can evaluate a variety of product or sample categories using only one scale and one set of reference points. Training using universal scaling is an easier and cheaper program to establish when several product categories are to be evaluated compared to product specific programs that would require retraining a panel for different products and different scales. Finally, the universal scale provides enough space for intensity or the quality continuum to address product reformulations or new compounds. Some disadvantages have been identified by Munoz and Civille (1998) for the universal scale. Firstly, similar attribute ratings may only occupy narrow ranges of the entire scale, that is, the entire scale might not be used for subtle, less intense odours or subtle quality

differences. In addition, implementing a universal training program is time consuming and expensive in the short term even though it is cheaper in the long run. Based on the criteria identified by Munoz and Civille (1998) the universal scale is an appropriate tool for GCO because GCO descriptive panels require the skills to verbally describe a diverse range of odour attributes and to rate the intensity of those odours relative to a standard scale in order to draw direct comparisons between samples. In addition, the universal scale concept translates ideally to the use of an electronic slide bar mechanism employed to collect the assessor's intensity response to an odour impact volatile being perceived.

Chapter 3

Methods and Materials

3.1 Whitefish Catch, Initial Handling and Transport

Ninety medium (0.7 – 1.10 kg) whitefish were net caught from Pakwa Lake, Manitoba, dressed and iced on board the fishing vessel and landed at Wabodon Handling Station on June 25, 2000. The fish were transferred into eight insulated boxes, layered in crushed ice and transported overnight to the Freshwater Fish Marketing Corporation (FFMC) in Winnipeg, Manitoba by refrigerated truck maintained at 4-6°C. The fish were received at FFMC on June 26, 2000, re-iced and placed in refrigerated storage (4°C) until the next day.

3.2 Controlled Spoilage Equipment and Set-up

On June 27, 2000, each fish was individually examined and was found to still be in rigor. Ten fish were randomly selected from the lot and removed for processing as the first spoilage increment samples. The remaining 80 fish were divided between two, 1.5 m³ insulated tote boxes. The bottom of each tote was layered with 30 cm of crushed ice. Ten fish alternating head to tail and not touching the adjacent fish were distributed across the ice layer. Approximately 15 cm of crushed ice was spread evenly across the fish followed by another layer of fish. This process was repeated until each tote held four layers of fish alternated with a layer of crushed ice. The final top layer of fish in each tote was covered with 12 cm of ice. The totes were sealed with insulated lids and stored

in a secure location in the FFMC processing area with an approximate room temperature of 15°C.

3.3 Controlled Spoilage Sample Production, Preparation, Handling and Storage

The fish samples were held on ice over a 19 d period from the time of catch with samples taken on days 2, 5, 9, 12, 15, 17 and 19. The controlled spoilage increments produced were labeled as D-2, D-5, D-9, D-12, D-15, D-17 and D-19. Ice levels were monitored and replenished on a daily basis. A layer of 10 fish was removed and processed at each sample period, alternating between tote one and tote two. As noted in section 3.2, the first set of 10 fish at two days from catch was processed immediately following the equipment set up. At each sample period, the preliminary sensory attributes of the fish samples were documented prior to processing into sample units. The visual attributes associated with the gills, eyes, skin, slime layer and body cavity were documented. The textural attributes of the skin and flesh and the odour attributes of the slime layer, gills and flesh were also noted and documented. The findings of the preliminary assessment were typical for fish in this state of spoilage for each sample increment. Each fish was hand-filleted with removal of the head, skeleton, fins and entire belly-flap, leaving the skin-side intact. The pair of fillets from each fish were kept together and placed in an insulated, portable cooler, covered in crushed ice and transported a short distance to the Freshwater Institute (FWI) laboratory in Winnipeg for final preparation, packaging, freezing and storage. Upon arrival at the FWI laboratory each pair of fillets was rinsed under cold, running water to remove any remaining blood or viscera, and cut in half crosswise to produce four sample units per fish. The four

samples from each of the 10 fish were given a code number which also identified the number of days storage from catch. Each half fillet was vacuum packaged in EVOH plastic barrier bags (UniPac Packaging Products Ltd, Edmonton, AB) using a Bizerba single chamber vacuum machine model 350D (Bizerba Canada Inc., Mississauga, ON). The packaged samples were then blast frozen using the Thermocel Plate Freezer (Dole Refrigerating Products Ltd., Oakville, ON) at -30°C for 20 min. The samples were stored at -20°C in a monitored walk-in freezer in the Winnipeg laboratory until June 2001.

3.4 Final Experimental Sample Inventory & Transport to Dartmouth Laboratory

The final sample inventory consisted of eight increments, seven increments of controlled spoilage samples produced in July 2000 and an additional fresh commercial sample, referred to as COMM-2. The fish for the fresh commercial sample were caught and processed 2 d after catch as for increment D-2, in May 2001, 10 months after production of the controlled spoilage series. Ten fish were caught, handled and processed according to the procedures described for the first increment of the controlled spoilage samples. The fresh commercial samples were produced to ensure that the unique attributes of freshly caught whitefish odour would be captured by the study. Past research has demonstrated that the odour attributes associated with freshly caught fish are lost in extended frozen storage, even under ideal packaging and storage temperature conditions (Nielson et al, 1999). Half of the final sample inventory was then shipped to the Canadian Food Inspection Agency laboratory (CFIA) in Dartmouth Nova Scotia for sensory evaluation by an expert panel and for GCO assessment using the customized Gas

Chromatography-Olfactometry (GCO) system located there. The samples were air shipped by guaranteed frozen storage along with DeltaTrak, Flashlink electronic data loggers. The data loggers recorded the temperature within the shipping coolers every 5 min. after leaving the FWI laboratory until being received at the Dartmouth laboratory. The temperature of the samples remained below -4°C for the twenty-four hour shipping period. The samples were placed in -25°C frozen storage when they reached the Dartmouth laboratory until experimental testing took place starting two weeks later.

3.5 Sensory Evaluation of Whitefish Samples by Expert Sensory Panel

The samples were presented in the Dartmouth sensory laboratory on June 12, 2001. The laboratory was equipped with a 100 % air exchange system, with three exchanges per hour. The laboratory was also equipped with conventional full spectrum, fluorescent light bulbs. The expert panel consisted of four, expert level fish inspectors who were trained, calibrated and assessed following the CFIA's, Fish Products Inspection Manual (2003c). The expert panel examined three sets of samples, one calibration set and two experimental sets consisting of the spoilage increment samples prepared for the study. All sample sets were evaluated on the same day, within a 4 h period. The calibration set began at 13:00 hr and lasted 1.5 h. The panel was given a 30 min break prior to the evaluation of the first experimental set and then a 15 min break prior to the evaluation of second test set. Each experimental set took approximately 35 min to complete.

3.6 Calibration of Expert Sensory Panel

The expert panel was calibrated prior to experimental testing by evaluating the controlled spoilage samples and fresh commercial sample in order of spoilage increment. The samples units were individually, vacuum packed half fillets. The fillets were thawed overnight, in a single layer, in a walk-in refrigerator (4°C) and allowed to equilibrate to room temperature (20-22°C) for 30 min prior to evaluation. The samples were presented on individual styrofoam trays, labeled A through H and placed in order, on stainless steel tables for evaluation by the panel. The inspectors were informed that the samples were in order of spoilage increment. First the inspectors evaluated the odour of each sample independently, using the ballot presented in Figure 3.1 to record their pass or fail decision, overall quality on a 100 mm universal quality line scale and descriptive terms characterizing the odour attributes perceived. Real time computer data analysis was completed to facilitate the panel calibration discussion that followed. The individual pass or fail decisions and numerical line scale scores were entered into a customized Excel data analysis spreadsheet (CFIA). The individual inspector and majority panel, pass or fail decisions, mean line scores and descriptive attributes were determined and shared with the group. The majority pass or fail decision and mean line score were used as tools in the calibration process to show the individual inspectors in the panel how they compared with their counterparts following their initial assessments. A facilitated discussion followed, where samples were re-evaluated and discussed until consensus regarding the pass or fail decision, line-score and sensory attributes of each sample was reached. The cut-off between pass and fail sample referred to as the borderline was also established. Panel consensus in terms of the pass or fail decision, mean line scale and

Figure 3.1 - Sensory Ballot used by Expert Panel

Whitefish Sensory Evaluation - Odour

Dartmouth Laboratory - June 12, 2001

NAME: _____ SESSION: _____

SAMPLE CODE	DECISION		SENSORY SCALE	COMMENTS
	P	F		
1 394	_____	P _____	_____	F _____
2 450	_____	P _____	_____	F _____
3 462	_____	P _____	_____	F _____
4 700	_____	P _____	_____	F _____
5 845	_____	P _____	_____	F _____
6 801	_____	P _____	_____	F _____
7 571	_____	P _____	_____	F _____
8 431	_____	P _____	_____	F _____
9 060	_____	P _____	_____	F _____
10 077	_____	P _____	_____	F _____
11 523	_____	P _____	_____	F _____
12 081	_____	P _____	_____	F _____
13 585	_____	P _____	_____	F _____
14 399	_____	P _____	_____	F _____
15 985	_____	P _____	_____	F _____
16 320	_____	P _____	_____	F _____
17 935	_____	P _____	_____	F _____
18 780	_____	P _____	_____	F _____
19 875	_____	P _____	_____	F _____
20 697	_____	P _____	_____	F _____

line scale range and the related odour descriptive terms was obtained for each spoilage increment.

3.7 Expert Sensory Panel Assessment of Experimental Samples

Two sets of 20 randomized, three-digit, blind-coded whitefish samples were presented to the calibrated expert sensory panel. Five sample units/replicates of each of the eight treatments were randomly distributed throughout the two sets. Samples were presented on styrofoam trays identified with a three-digit, random number. The samples were thawed in the refrigerator overnight and allowed to equilibrate to room temperature as described in section 3.6. Each set of samples was presented in 20 individual sample booths. The inspectors moved randomly from each sample booth to the next. The inspectors evaluated the odour of each sample independently, using the ballot provided in Figure 3.1, to record their pass or fail decision, overall quality on a 100 mm quality line scale and descriptive terms characterizing the odour attributes perceived. The data was entered and summarized in an Excel spreadsheet producing the majority pass or fail decision and the mean, median and mode for the panel line score data. Samples without unanimous agreement by the panel were re-evaluated and attributes discussed to clarify the associated pass or fail assessment and line-score result and to reach consensus.

3.8 Setup & Operation of GCO Instrumentation

The Gas Chromatography – Olfactometry (GCO) static headspace method involved separation of headspace volatiles by GC with simultaneous detection of eluting compounds by a flame ionizing detector (FID) and a human sniff port. The GCO

instrumentation was a customized system consisting of a modified Varian 3400 gas chromatograph fitted with a flame ionization detector (GC/FID) and an additional custom-built sniff-port as a second detector (GCO) (Palto Alto, CA, USA). The system was controlled using the Star Chromatography Workstation Controller software, version 5.51 (Varian, Palto Alto, CA, USA). A Dynatherm Analytical Instruments, Model 890, Thermal Tube Desorber (TTD) was used as the column inlet, which provided and controlled the column carrier gas flow (N_2 at 10 ml/min). A heated transfer line connected the TTD to the Varian GC, consisted of a heated 1/8" SS sheath surrounding a 0.90 m length of internally uncoated 0.35 mm ID column tubing entering directly into the Varian GC column oven. The transfer line temperature was regulated by the TTD and held at 75°C. In the GC oven, the uncoated transfer line was connected to the 30 m x 0.35 mm Q-plot megabore column via a tapered glass butt-joint column connector. The column eluent stream was split using a "Y" butt-joint column connector. One leg of the Y connected to the flame ionization detector while the other leg connected to the custom-built sniff port. The column eluent in the sniff port (5 ml/min N_2) was diluted to 2 L/min with a make-up airflow with a constant temperature of 25°C and saturated humidity. The airflow was provided by a variable speed air pump monitored by an electronic flow meter, bubbled into a 2 L vessel containing distilled water with the temperature maintained on a hotplate. The human sensory responses were measured using a custom built slide-wire intensity recorder connected to a potentiometer. The signal from the slide wire potentiometer was conditioned in a custom built electronic signal box with a manual zero/range control to provide a 0 to 2 volt maximum signal. The signal from the signal box fed directly into the Star workstation ADC board, which recognized it as a second

detector. The FID was recognized as the first detector. The panelists manually slid the response bar in a right hand direction as an odour appeared and intensified and to the left as the odour intensity decreased or subsided completely. The signals from both the FID detector and the slide bar were displayed by the Star Chromatography Workstation (Palto Alto, CA, USA) in two windows simultaneously, while a third window displayed run time and vital statistics of the chromatographic run. The first window displayed the FID chromatogram and the second window displayed the “aromagram”, (volatile compounds with odour impact), as perceived by the panelist.

A chromatographic run was initiated by the sensory panelist, once the desorption tube was locked into the TTD desorption chamber, by switching a valve on the TTD. This valve re-directed the carrier flow through the desorption tube onto the chromatographic column, which initiated the ballistic heater surrounding the desorption tube (ambient temperature to 200°C in 60 seconds held for 5 min and then initiated the gas chromatograph. The GC was temperature programmed as follows: initial hold time 5 min at 35°C, ramped to 135°C over 10 min, then ramped to 220°C over 5 min, held at 220°C for 2 min for a total run time of 22 min. The recycle time was approximately 12 min to return to 35°C.

3.9 GCO Static Headspace Equipment, Sample & GC Cartridge Preparation

The static headspace method used a custom built, stainless steel headspace cylinder (McLachlan, 1997) to confine and concentrate a large headspace volume. The components of the 1 L capacity headspace cylinder include the cylinder base, cylinder wall, piston head, stainless hubs, Teflon beveled ring, nylon guide, stainless fitting and

viton o-rings (Figure 2.1). The cylinder components were assembled and 30 g of blended whitefish tissue sample was placed into the well of the cylinder base and screwed onto the cylinder wall. A glass thermal desorption cartridge (115mm x 6mm OD, 4 mm ID), (Supelco/Sigma-Aldrich Canada, Oakville ON) was packed with a 80 mm split-bed of Tenax and Carboxen (Supelco) in a 4:1 ratio respectively, (lightest molecules trapped on the Carboxen). Before adsorption of sample headspace, the cartridge was conditioned for 30 min on a Dynatherm Analytical Instrument, Model 10 Tube Conditioner (Dynatherm, Kelton, Pa.) under a flow of N_2 at 20 ml/min held at 200°C. Cleaned tubes were held in glass screw cap sealed tube holders, prior to use to prevent contamination by extraneous volatile compounds. The thermal desorption cartridge was secured to the piston at the top of the cylinder using a Swagelok connector with a vespel ferrule, forming an airtight seal. The whitefish tissue sample was allowed to equilibrate in the chamber for 20 min at ambient temperature (20°C). The headspace volume within the cylinder was compressed onto the GC cartridge using an automated screw press. The piston traveled 20 cm at a compression speed of 1 cm/minute and the flow rate onto the cartridge was 40 ml/minute, total headspace volume was 800ml. A water displacement device (Figure 3.2) was used to measure the precise volume of the headspace which passed through the GC cartridge for each whitefish tissue sample. The device consisted of a modified 2 L plastic (polymethylpentene) volumetric cylinder inverted into a 3 L glass beaker filled with water. A vacuum line was attached through a threaded, bored hole in the base of the volumetric cylinder with a toggle valve at the top of the apparatus. Opening the valve allowed the vacuum to fill the cylinder to the 100 ml mark. Flexible silicone tubing connected the Tenax cartridge to the volumetric cylinder. When the piston of the

headspace cylinder was compressed the displaced headspace volume passed through the GC cartridge and into the volumetric cylinder. The adsorbed headspace volume was determined directly from the displaced water volume in the volumetric cylinder. For subsequent measurements, the toggle valve was opened and the volumetric cylinder refilled. The GC cartridge with known adsorbed headspace volume was then desorbed in the GCO system. For time efficiency, three identical GC cartridges were used and were alternated between consecutive GCO sessions. Between samples, the stainless steel headspace cylinders were ultrasonically washed and heated in an oven to 110°C to deodourize them.

3.10 Calibration of GCO Panel using Chemical Standards

The GCO panel, comprised of four expert fish inspectors, was trained and calibrated for the GCO sniffing and slide bar response technique prior to evaluating the fish samples. A chemical cocktail was prepared using four pure chemical standards of known concentrations, retention times and descriptive terminology associated with the odour of each compound. The chemical cocktail was composed of 30 ppm of each standard as follows: propyl acetate with a fruity, pear-like odour; ethyl butyrate with a pineapple odour; isoamyl butyrate with a banana odour; and 2, 3-butadione with a caramel odour. Ten ml of the cocktail was measured into the well of the headspace cylinder apparatus, allowed to equilibrate at ambient room temperature for 20 min before being compressed onto the GC cartridge. The panelists were instructed to begin moving the sliding response bar to the right when an odour was perceived and further to the right if the odour intensified. If the odour intensity declined they were instructed to move it to

the left and back to zero if it disappeared. They were also instructed to describe the odour they perceived, as it intensified, as it changed in character or as it vanished. The experimenter recorded the odour attributes articulated by each panelist during each GCO sample run along with start and end times. The electronic slide wire provided an electrical signal to the Star Computer Workstation (Varian, Palo Alto, CA, USA). As a result, the computer recorded retention times, volatile intensity peak areas and generated aromagrams similar to GC/FID chromatograms. To increase detection frequency of eluting odour volatiles each panelist used a rapid breathing technique similar to that described by Hanaoka et al (2001).

3.11 Expert Panel Evaluation of Whitefish Samples by GCO

The whitefish GCO sessions were conducted over a one-week period. The GCO panel evaluated the volatiles eluting from the human sniff port for each of the eight whitefish spoilage increments presented in a randomized order. The spoilage increment samples were evaluated by each panelist once. Although each GCO chromatographic run was 22 min long, the panelist only evaluated the eluent stream for 19 min since all of the odour impact components were off the column by that time. The last three min at 220°C allowed late running compounds without odour impact to exit the column to ensure there was no ghosting on the next column run. The panel of four experts was divided into pairs who alternated with each other over consecutive GCO sessions. That is, for the first pair, as one person was performing GCO the other was resting and vice versa until all increments had been evaluated by both panelists. This procedure was then repeated for the second pair of GCO panelists. The method prevented sensory fatigue and allowed for

an efficient panelist exchange between GCO sessions. The panelists followed the same procedure they did for the calibration session.

3.12 Chemical Methods - Introduction

Since the composition of any fish species can vary due to numerous biological factors including size, age, sex, feeding environment and season of catch, it was necessary to determine the proximate composition of the eight whitefish spoilage increments. This was done in order to evaluate the biological variability of the sample set and the possible influence of this variability on the sensory attributes perceived for each treatment.

Levels of ash, moisture, protein, total fat and fatty acid content were determined by direct chemical analysis and carbohydrate was determined by subtraction. Traditional chemical indicators of spoilage, Freshness Index, ethanol concentration and total volatile base nitrogen (TVB-N) were also measured as a means of bench-marking the results of the expert panel and to examine their applicability to whitefish.

3.13 Ash & Moisture Content Determination

Ash and moisture content were determined using the method of the Canadian Food Inspection, Chemical Methods Manual, 2003, Chapter 2, Section 1 and Chapter 2, Section 2 respectively. The Thermolyne, Model F-A1730 blast oven (Barnstead International (Thermolyne), Dubuque, Iowa) with Barber-Colmen, Model 293C temperature gauge was used. Analyses were performed in triplicate.

3.14 Protein Content Determination

Protein content was determined by the Buchi-Kjeldahl method using the Tecator 1030 - Kjeltac Auto Analyzer (Tecator AB, Höganäs, Sweden) following the method of Canadian Food Inspection Agency, Dartmouth Laboratory Method No. SOM-DAR-CHE-99, (1999). Analysis was performed in triplicate.

3.15 Total Fat Content Determination

Tissue samples from each of the eight spoilage increments were dried to a constant weight in a VWR, Model 1410, Vacuum Oven (VWR International, West Chester, PA, USA) at 95°C, under a partial vacuum 457 mm of Hg. The samples were held under these conditions overnight, weighed and reheated until a constant weight was obtained to ensure they were completely dried prior to extraction. Total lipid content was determined on the dried samples using the Dionex ASE 200 Accelerated Solvent Extractor (Dionex Canada Inc., Oakville, ON) according to the Dionex method, Extraction of Lipids and Polychlorinated Biphenyls from Fish Tissue in a Single Run Using Accelerated Solvent Extraction, Application Note 337. Analyses were performed in triplicate.

3.16 Fatty Acid Analysis

The lipid fraction was extracted from the whitefish tissue samples according to the modified Folch described by Kates (1986). Methyl esters of the fatty acids were

prepared according to the AOCS Official Method Ce 2-66 using sulfuric acid as a catalyst for esterification. The fatty acid esters were analyzed using a Hewlett Packard gas chromatograph (GC) (Model 5890, Hewlett Packard, Palo Alto, CA, USA) equipped with split/splitless injector, flame ionizer detector (FID) and autosampler (Model 7673, Hewlett Packard, Palo Alto, CA). A silica GC capillary column SP-2560 (L = 100 m; i.d. = 0.25 mm; df = 0.25 μ m, Supelco, Oakville, ON) was used for separation. The linear velocity of the carrier gas, hydrogen was 0.5 m/s with split ratio set at 1:100. The column temperature was programmed from 70°C held for 2 min, increased at a rate of 15°C/min to 155°C and held for 25 min, then programmed at 3°C/min to 215°C. Final temperature was held at 215°C for 8 min. The injector and detector temperatures were set at 250°C. The fatty acid methyl esters were identified by comparison of retention times with a standard mixture of known composition (Mixture #461, NuChek Prep, Elysian, MN). Quantification of fatty acids was done using AOAC Method Ce 1b – 89. The fatty acid composition was expressed as a mass percentage (% w/w in g/g) of the total fatty acids. All analyses were performed in triplicate.

3.17 Freshness Index

The Freshness Index is developed from a high performance liquid chromatography (HPLC) method for determining the adenosine triphosphate (ATP) degradation products in biological samples (Burns et al, 1985). The Freshness Index (G-value) was determined for the eight spoilage increments following the method of Burns et al (1985). A Waters model 600E HPLC with a model 700 auto sampler and model 486 UV/visible detector was used (Waters, Milford, MA). The chromatographic column was

a RP-8 MPLC cartridge type (Brownlee Labs,) reversed phase analytical column (4.6 mm x 10 cm; 10 μ m particle size) directly coupled to a 3 cm MPLC guard column packed with the same material (Burns et al, 1985). The mobile phase was 0.01 M potassium phosphate buffer pH 4.5, isocratic run at 1.0 ml/min with a 10 μ L sample injection. The system was controlled with the Waters Millennium version 3.05 computer software. All analyses were performed in triplicate.

3.18 Ethanol Concentration Determination

The automated headspace sampling, gas chromatographic method of McLachlan et al (1999) was used to determine the ethanol content in the eight spoilage treatments. The Hewlett Packard (HP) Model GCD gas chromatograph with interfaced mass spectrometer detector (GC/MS) and a HP Model 7694 static headspace auto sampler was used (Hewlett Packard (Agilent Technologies), Palo Alto, CA, USA). A HP Inowax column (30 m x 2.5 mm), 0.25 μ m film thickness was used. The carrier gas helium was 0.5 ml/min and 1.1 psi at 50°C. The temperature program was: initial temperature 32 °C , held for 4 min; temperature increased at 65 °C/min to 140 °C, held for 1.3 min. Overall run time was 7 min. All analyses were performed in triplicate.

3.19 Total Volatile Base Nitrogen (TVB-N) Determination

Total Volatile Base Nitrogen was determined by a Kjeldahl method using the Tecator Model 1030 – Kjeltex Auto Analyzer (Tecator AB, Höganäs, Sweden), following the method of the Canadian Food Inspection Agency, Darmouth Laboratory, No. SOM-DAR-CHE-005-01 (2001). All analyses were performed in triplicate.

3.20 Statistical Analysis

The General Linear Models Procedure (PROC GLM) was used to analyze the GCO data collected from the expert panel (PROC GLM, SAS, 1989). The “General Linear Models Procedure” (PROC GLM) in SAS (1989) was used to describe the GCO results in terms of the following Analysis of Variance (ANOVA) model.

$$\text{Log}_{10}(\text{GCOPeak AreaResponse}) = a_j \times \text{Panelist} + b_i \times \text{KeyTerm} + c_k \times \text{Spoilage Increment} + d_{ij} \times \text{Spoilage Increment} \times \text{KeyTerm}$$

The terms “Panelist”, “Key Term” and “Spoilage Increment” are class variables; a_j , the panelist effect, b_i , Key Term effect and c_k , the location effect, and d_{ij} the combined Spoilage Increment and Key Term effects. The logarithm of the response was used so that the responses across Spoilage Increment $\times$ Key Term combinations would have a more homogenous variance. “Least Squares Means”, the estimated means if the design was balanced, that is, if there were no missing data, were estimated for each Key Term $\times$ Spoilage Increment combination and plotted as radar graphs (option LSMEANS in GLM, SAS 1989)

One-way ANOVA was performed on all proximate analysis, fatty acid and sensory evaluation line-score data using the Microsoft, Excel ANOVA data analysis tool (Microsoft® Excel 2000).

Linear regression analysis was also applied to the Freshness Index chemical indicator data, known as G-values (Burns et al, 1985) along with summary ANOVA statistics (Microsoft® Excel 2000). Student’s t-tests were also conducted on the Freshness Index G-values for the following spoilage increments pairs: D-9 and D-12, D-

12 and D-15 to test whether the D-12, G-value was significantly different from D-9 and D-15 G-values (Microsoft® Excel 2000).

Chapter 4

Results and Discussion

The discussion of results will begin by presenting the results of the calibration and experimental testing of the expert sensory panel, using traditional sensory evaluation procedures (Section 4.1 and 4.2). The fundamental background data related to proximate composition and fatty acid composition of each samples increment will follow (Sections 4.3 and 4.4). The results of the three chemical indicators of spoilage, Freshness Index, Ethanol and TVB-N concentration, conventionally used to benchmark sensory evaluations for various fish products in a regulatory, inspection setting will then be discussed (Sections 4.5, 4.6 and 4.7). The results of the main focus of the study, detection of volatiles with odour impact by GCO in tandem with GC/FID will be presented and discussed in Section 4.8.

4.1 Expert Sensory Panel Calibration Results

The controlled spoilage samples used to calibrate the expert sensory panel were presented in order from high pass to extreme fail (Comm-2, D-2, D-5, D-9, D-12, D-15, D-17 and D-19). The expert panel unanimously agreed on the pass or fail sample decision for each sample and the mean panel line-score values which represented the quality of each sample is summarized in Table 4.1. The minimum and maximum line-scores and standard deviations are also listed in Table 4.1 and were used as a tool by the discussion facilitator to identify spoilage increments that received a wide range of scores on the line-scale. For example, although the panel agreed that

TABLE 4.1: Fundamental statistical calculations for unstructured line-score¹ data collected from a panel of four fish inspectors for each spoilage increment

SPOILAGE INCREMENT	P/F²	MIN LINESCORE	MAX LINESCORE	MEAN³ LINESCORE	STD DEVIATION LINESCORE
COMM-2	P	13	22	16.8	3.9
D-2	P	6	22	14.0	6.6
D-5	P	24	29	26.5	2.4
D-9	P	28	33	29.8	2.4
D-12	BP/BF	45	59	50.5	6.5
D-15	F	60	78	66.8	12.0
D-17	F	68	85	78.8	7.5
D-19	F	78	99	92.8	10.0

1 Unstructured linescale: 0 mm = pass (fresh from catch); 100 mm = fail (extreme decomposition)

2 Where: P = Pass, BP = Borderline Pass, BF = Borderline Fail, F = Fail

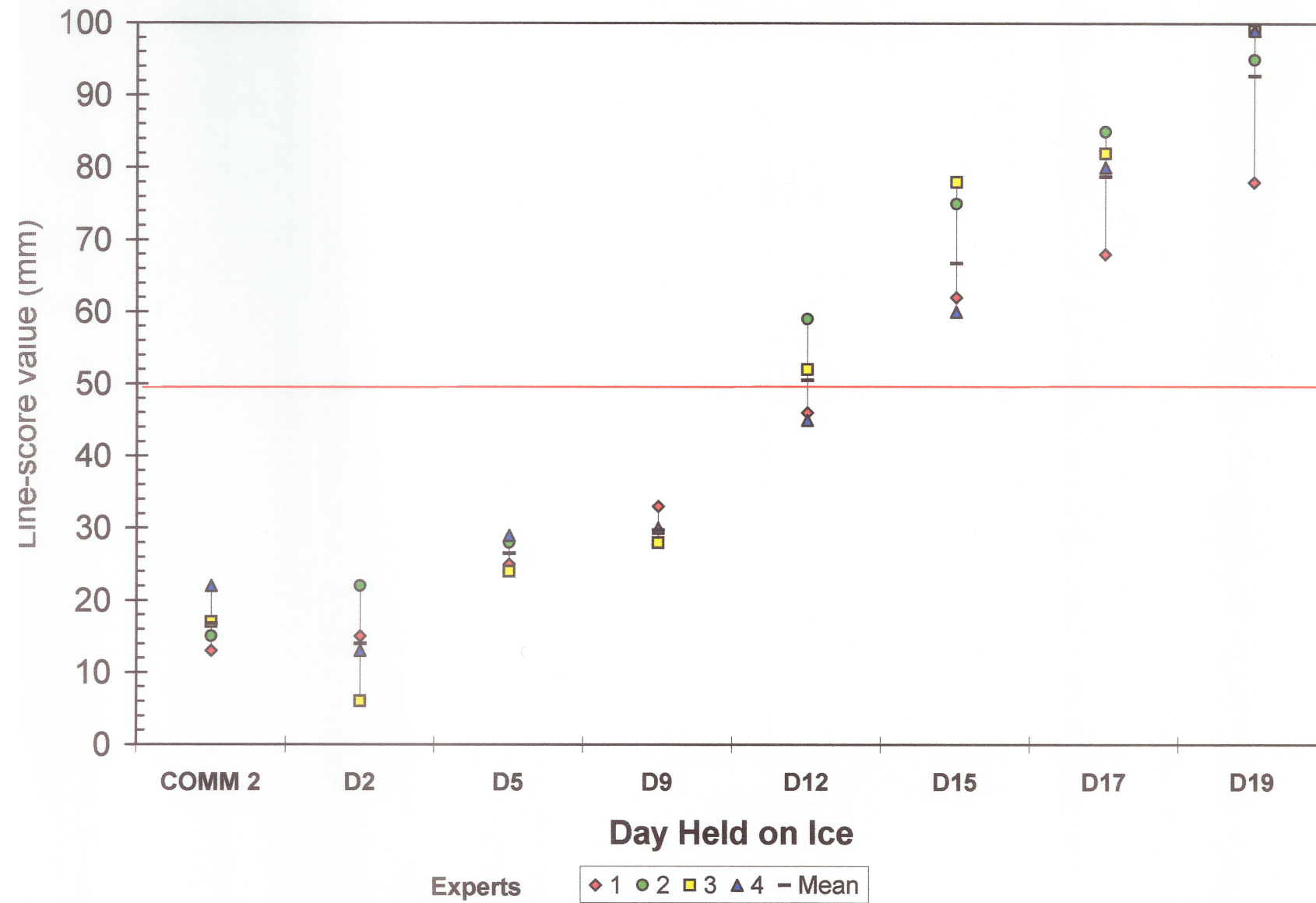
3 Mean of 4 expert panelist line-score determinations

spoilage increment D-19 was extremely decomposed compared to D-15 the line-scores used by the panel for the two samples overlapped as shown by the minimum and maximum values. Through discussion, the panel agreed that the mean line-scores better represented the quality level of each increment and adjusted their individual scores and agreed to use the scale for the experimental sets accordingly.

The spoilage increments (days held on ice) versus the individual panel line-score values were plotted against each other to demonstrate the continuum of spoilage as perceived by the calibrated panel (Figure 4.1). The figure shows the progression of spoilage over time from the high pass increments (Comm-2 and D-2) through to the extreme fail increments (D-17 and D-19) for individual panelists compared to the mean panel line-score. Increment D-12 was clearly defined as the pass/fail borderline since the panelists scores bridge the centre point of the line-scale (50mm).

The major descriptive terms for each spoilage increment identified by the panel are summarized in Table 4.2. Given the terms selected, increments Comm-2, D-2, D-5 and D-9 were of pass quality. Increments Comm-2 and D-2 were described with the same key terms characteristic of freshly caught whitefish which were, cucumber, grassy and melon. Increment D-2 was also described as slightly oxidized which could be attributed to a longer time period in frozen storage. Increments D-5 and D-9 samples lost the distinctive cucumber odour attribute and were otherwise described with identical descriptors. Overall, increment D-5 was characterized as grassy and melon while increment D-9 was determined to be neutral to slightly stale with slight odours of melon, and grassy. Increment D-12 was determined to have border-line quality, i.e. the point at

Figure 4.1: Expert Sensory Panelist & Mean Panel line-scores for Calibration - Set 1



**Table 4.2: Odour Descriptive Terms Determined by the Expert Sensory Panel
for each Spoilage Increment**

Term #	Term	C2	D2	D5	D9	D12	D15	D17	D19
1	cheesy							X	X
2	cucumber	X	X						
3	dairy sour								
4	faecal					X		X	X
5	fermented						X	X	X
6	fishy					X	X		
7	grassy	X	X	X	X	X			
8	melon	X	X	X	X	X			
9	musty					X			
10	neutral	X	X	X	X				
11	oily			X	X	X			
12	oxidized		X			X			
13	putrid					X		X	X
14	rotten meat					X	X	X	X
15	sickly sweet						X	X	X
16	sour					X	X	X	X
17	sour meat								X
18	sour milk								
19	stale	X	X	X	X	X	X	X	X
20	sweaty socks								
22	yeasty					X	X		X

which samples exhibit odours of decomposition. Approximately half of the D-12 samples passed and half failed. The D-12 increment received border-line pass assessments with the perception of stale and slight but non-persistent odours such as sour being perceived. The D-12 samples received border-line fail assessments when odours such as sour, yeasty and fermented were perceived at a slight but distinct and persistent level. Increments, D-15, D-17 and D-19 received definite fail quality assessments. Increment D-15 was characterized as distinctly sour, fermented and yeasty while D-17 and D-19 were intensely sour, cheesy, fermented, faecal and putrid.

4.2 Expert Sensory Panel Experimental Results

Once the panel was calibrated, two sets of 20 randomly presented, three-digit, blind-coded samples representing the spoilage increments were evaluated by the panel. Five samples of each increment were randomly distributed in the two experimental sets (Set 2 and Set 3) following the calibration (Set 1). Analysis of Variance (ANOVA) was performed on the line-score data for each spoilage increment (Table 4.3). No significant differences were found between panelists or within each panel member for all increments. Therefore the ANOVA results indicate that the panel was calibrated and consistent in using the scale for each spoilage increment across the five sample replicates. The individual panelist line-scores were plotted against the mean panel line-score (Figures 4.2 to 4.5) to evaluate the performance of each panelist compared to the overall group. Each figure was divided into four quadrants, i.e., data points in quadrant 2 represent a correct pass decision and data points in quadrant 3 represent a correct fail

**Table 4.3: Summary of ANOVA Output for Expert Sensory Panel
of Whitefish Spoilage Increments**

ANOVA - WH-COMM-2						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Panelists	33.69	3	11.23	0.38	0.7693	3.49
Within Panelists ¹	354.75	12	29.56			
Total	388.44	15				
ANOVA - WH-D-2						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Panelists	899.00	3	299.67	2.28	0.1110	3.10
Within Panelists ¹	2634.33	20	131.72			
Total	3533.33	23				
ANOVA - WH-D-5						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Panelists	484.79	3	161.60	2.28	0.1102	3.10
Within Panelists ¹	1416.17	20	70.81			
Total	1900.96	23				
ANOVA - WH-D-9						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Panelists	760.79	3	253.60	2.13	0.1281	3.10
Within Panelists ¹	2378.83	20	118.94			
Total	3139.63	23				
ANOVA - WH-D-12						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Panelists	720.83	3	240.28	0.87	0.4721	3.10
Within Panelists ¹	5511.67	20	275.58			
Total	6232.50	23				
ANOVA - WH-D-15						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Panelists	65.00	3	21.67	0.32	0.8095	3.10
Within Panelists ¹	1346.33	20	67.32			
Total	1411.33	23				
ANOVA - WH-D-17						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Panelists	567.46	3	189.15	1.31	0.2979	3.10
Within Panelists ¹	2881.50	20	144.08			
Total	3448.96	23				
ANOVA - WH-D-19						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Panelists	832.33	3	277.44	1.97	0.1511	3.10
Within Panelists ¹	2817.67	20	140.88			
Total	3650.00	23				

1 Represents Panel Error Variation

Figure 4.2: Mean expert panel line-score values (mm) vs panelist 1 (Sessions 1 to 3 - All Samples)

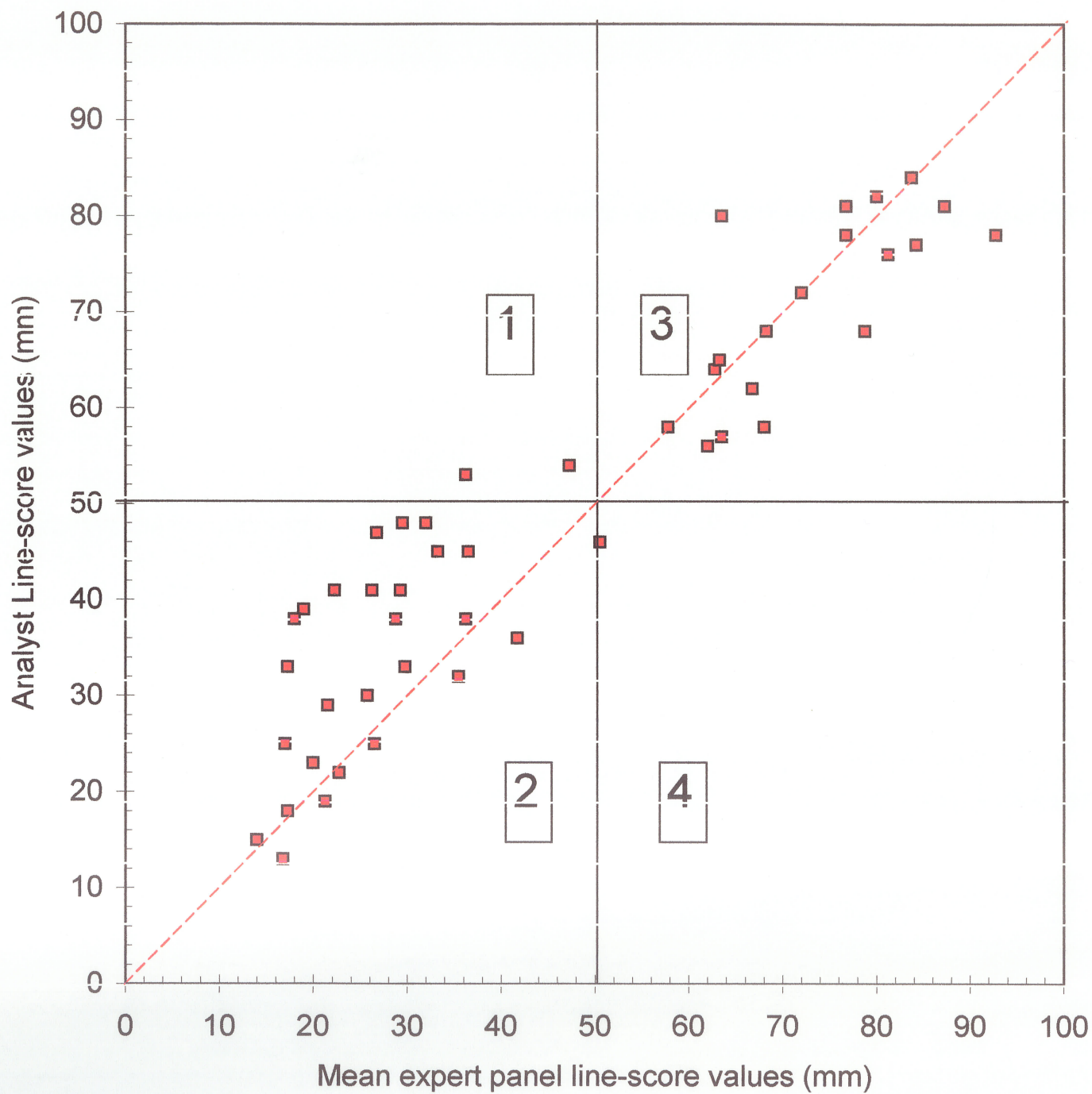


Figure 4.3: Mean expert panel line-score values (mm) vs panelist 2 (Sessions 1 to 3 - All Samples)

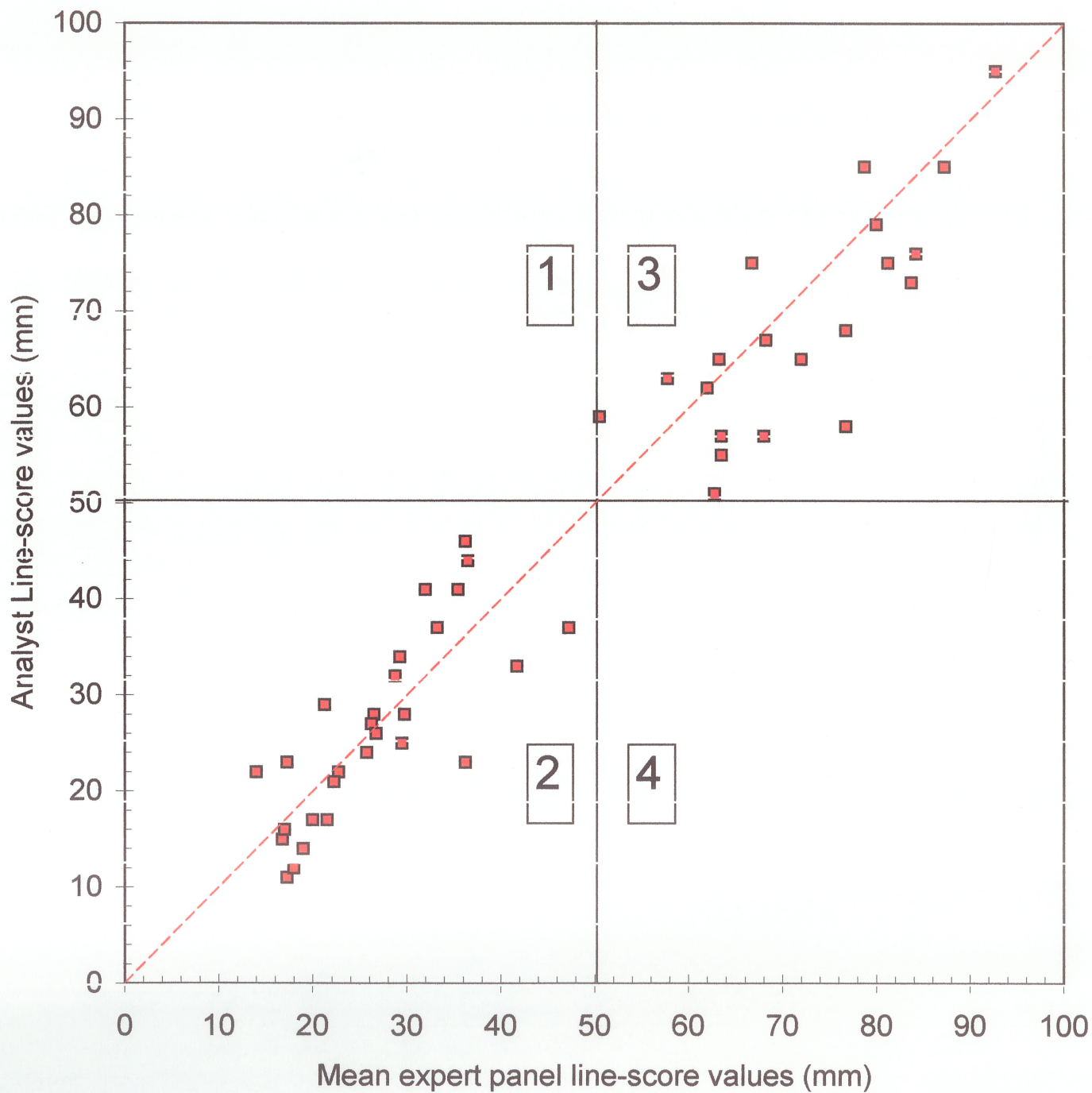


Figure 4.4: Mean expert panel line-score values (mm) vs panelist 3 (Sessions 1 to 3 - All Samples)

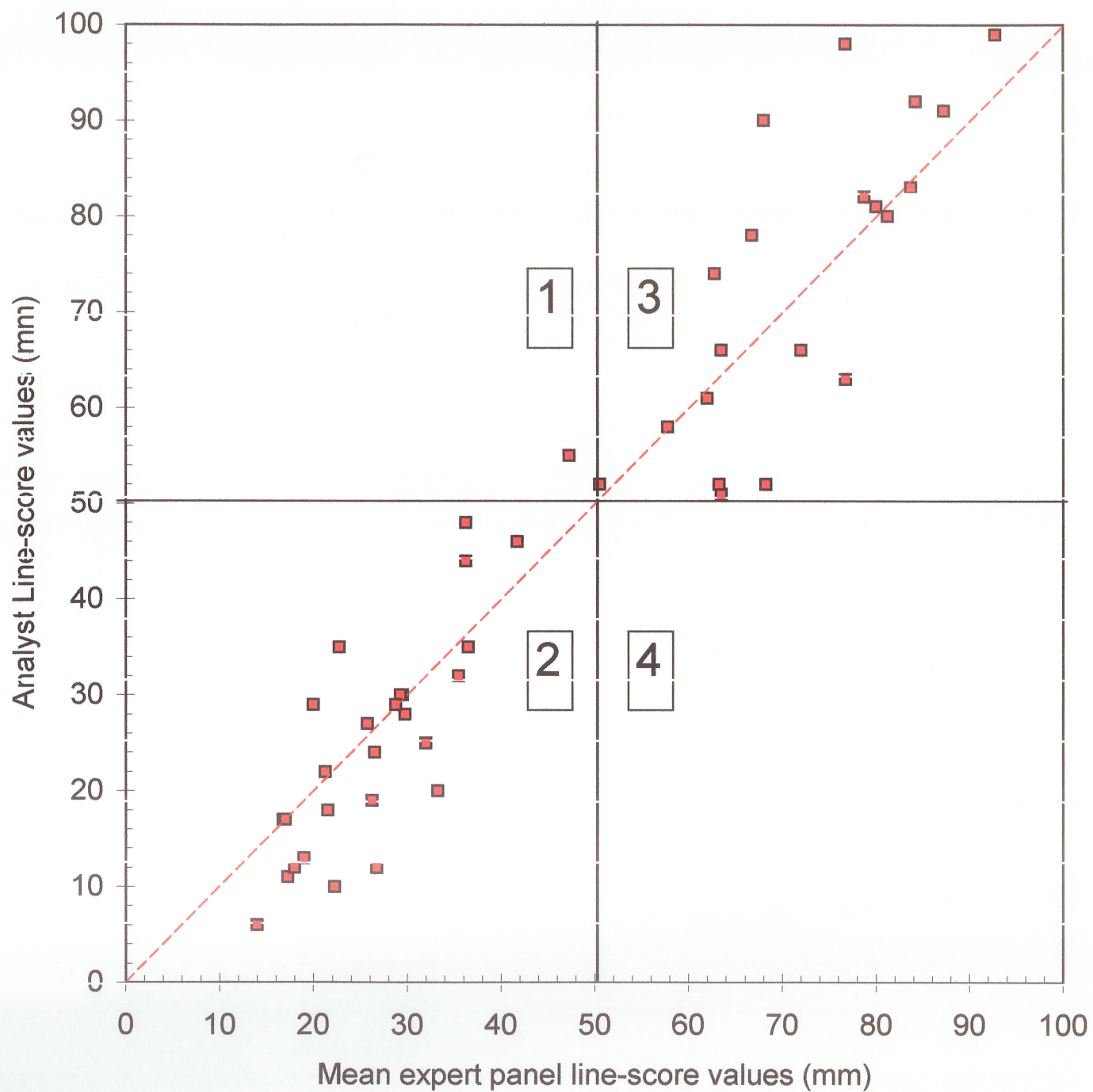
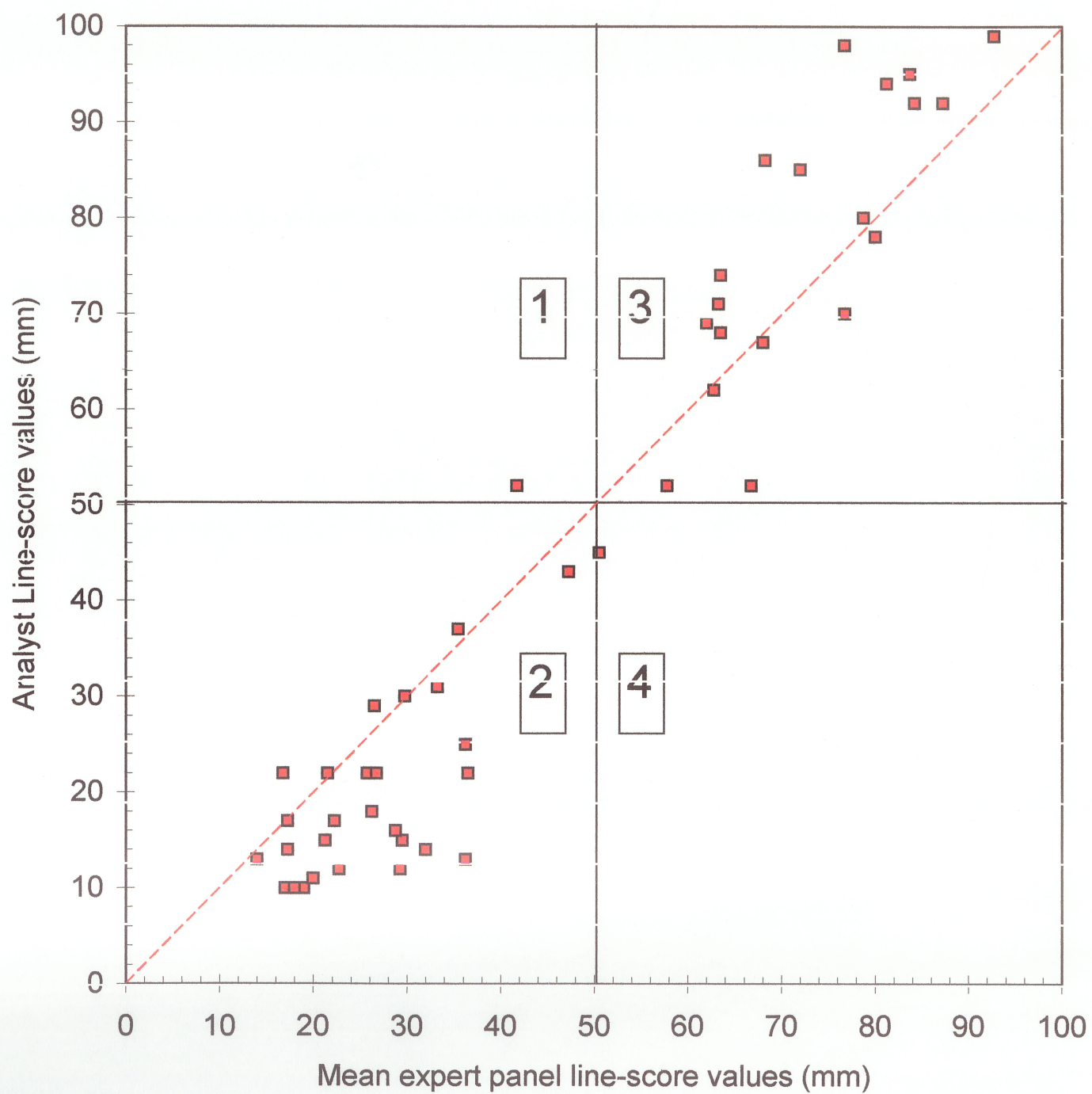


Figure 4.5: Mean expert panel line-score values (mm) vs panelist 4 (Sessions 1 to 3 - All Samples)



decision as compared to the mean expert panel line-score. Data points that appeared in quadrant 1 represent an incorrect fail decision, where the panelist failed the sample but the mean panel line-score was indicative of a pass decision. Data points that appeared in quadrant 4 represent an incorrect pass decision, where the panelist passed the sample but the mean panel line-score was indicative of a fail decision. The diagonal dashed line in each graph represents a best fit of panelist line-scores to the mean expert panel line-scores for a continuum of spoilage from 0 mm to 100 mm. Each figure shows that the line-score data points used by each panelist closely follow the diagonal line and that data points only appear in quadrants 1 (incorrect fail) and 4 (incorrect pass) around the borderline (50mm) region. Therefore, the figures clearly show that the line-scores used by each panelist closely followed the panel mean and that the panel performed consistently. Individual panelist line-scores and the mean panel line-score for each experimental test sample versus the days held on ice were also plotted for each experimental set (set 2 and 3) (Figures 4.6 and 4.7). These figures show that the panel's line-scores for the experimental samples (sets 2 and 3) followed the same trend identified for the calibration (set 1, Figure 4.1) even though the sample order was randomized. They also show the range of line-scores used by the panel for the experimental samples compared to the calibration are somewhat larger but that the mean panel score shows the incremental change in quality in the controlled spoilage series.

Colour coded pie charts were produced to show the key descriptive terms used to describe each increment and to show the similarities and differences between increments (Figure 4.8 – 4.15). The descriptive charts for Comm-2 and D-2 (Figures 4.8 and 4.9) show there were differences in the odour attributes between the two increments even

Figure 4.6: Expert Sensory Panelist and Mean Panel Line-scores for Experimental - Set 2

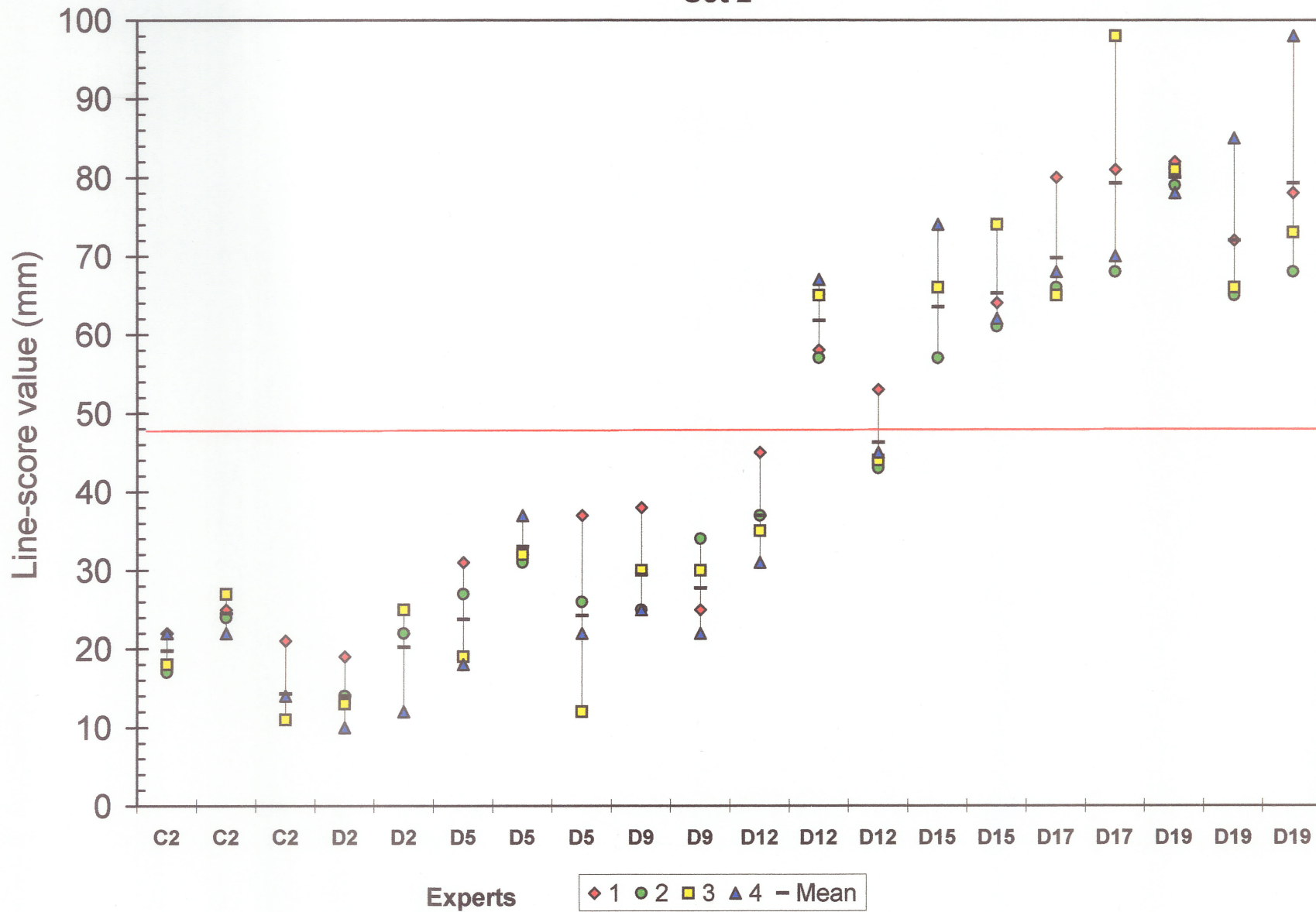


Figure 4.7: Expert Sensory Panelist and Mean Panel line-scores for Experimental - Set 3

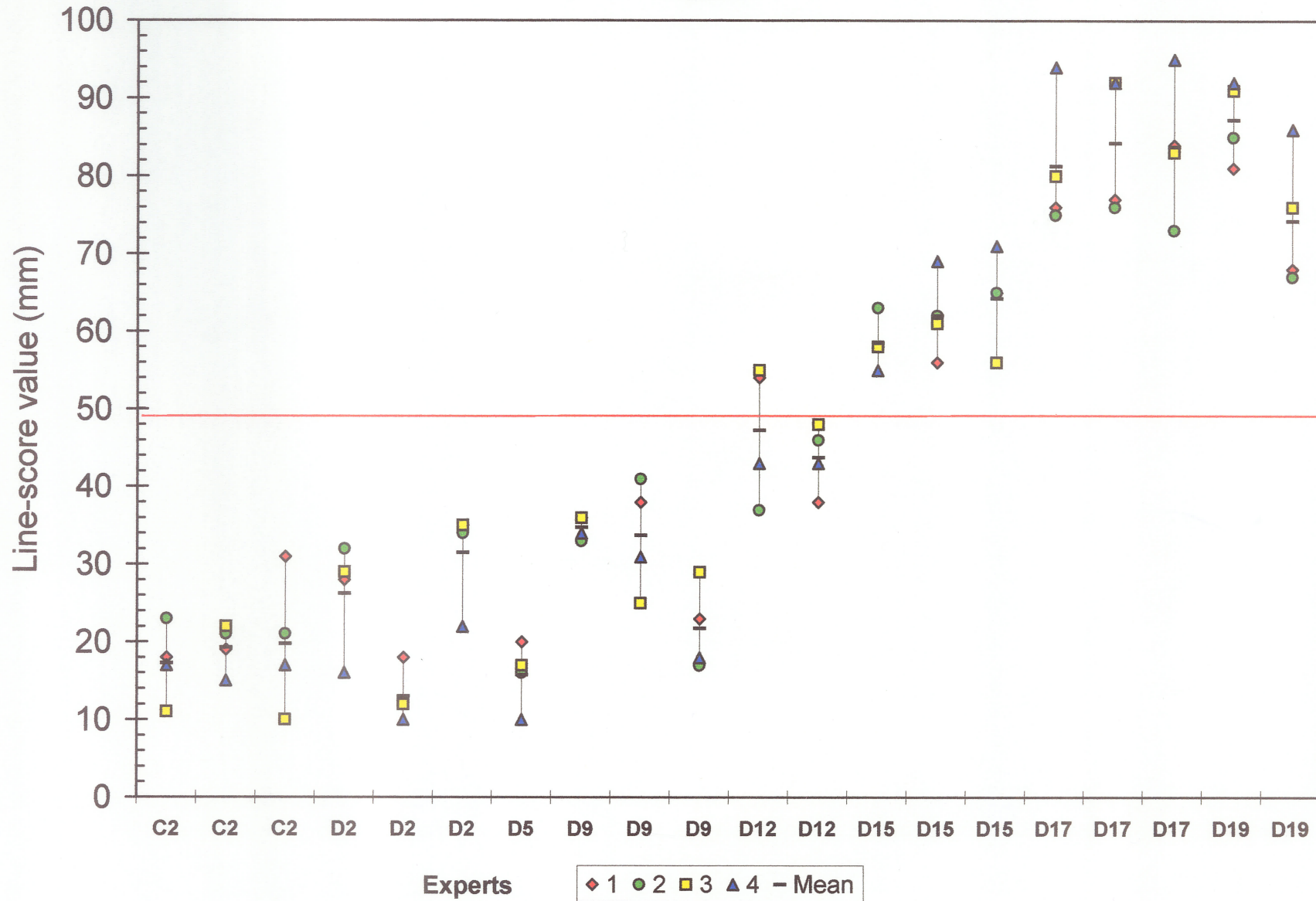


Figure 4.8: Frequency of Odour Descriptors used by Expert Sensory Panel to Describe Treatment Comm-2 Whitefish Sample

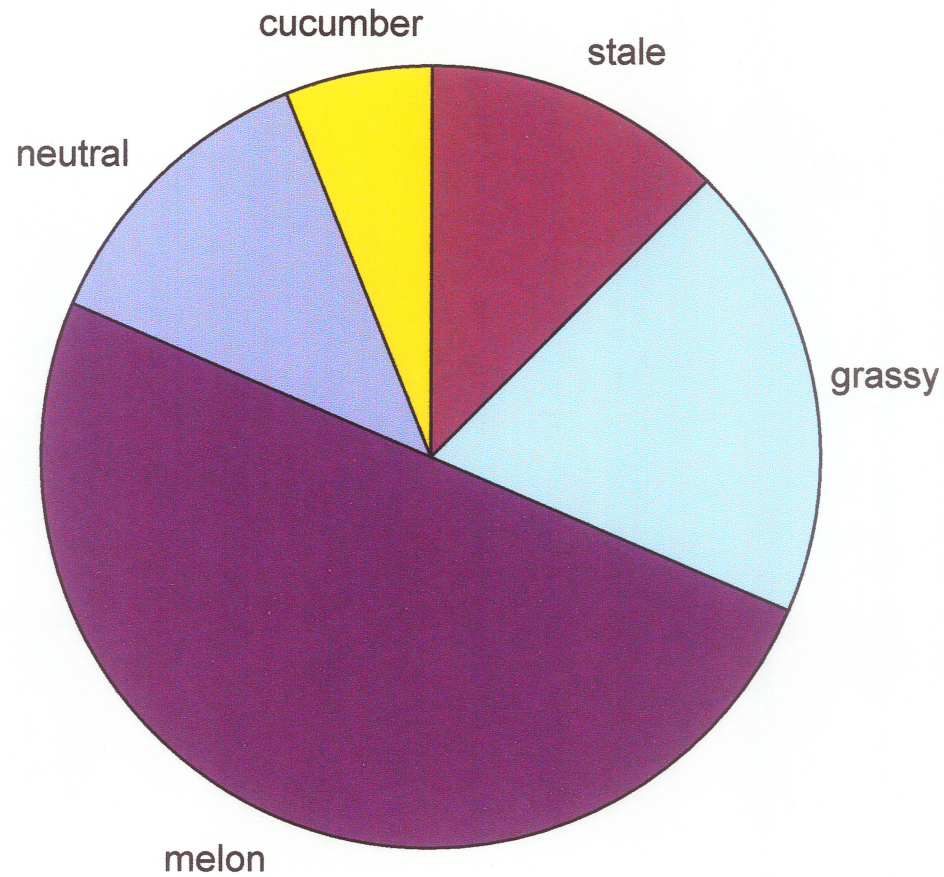


Figure 4.9: Frequency of Odour Descriptors used by Expert Sensory Panel to Describe Treatment D-2 Whitefish Sample

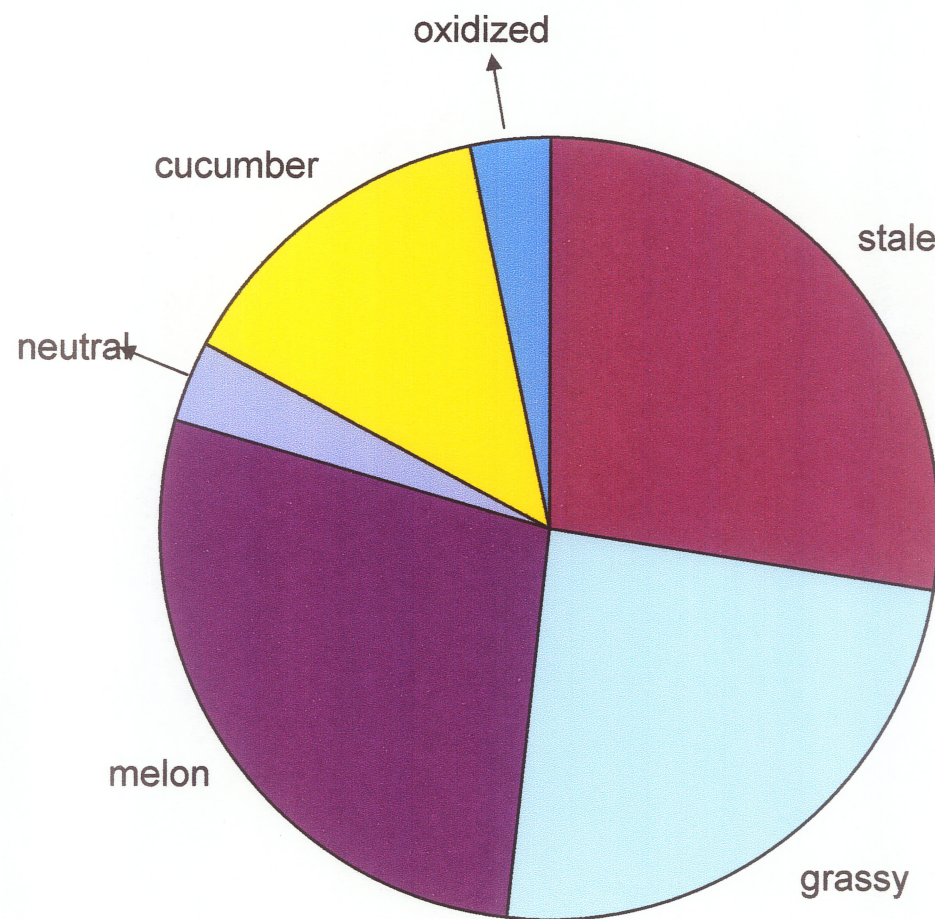


Figure 4.10: Frequency of Odour Descriptors used by Expert Sensory Panel to Describe Treatment D-5 Whitefish Sample

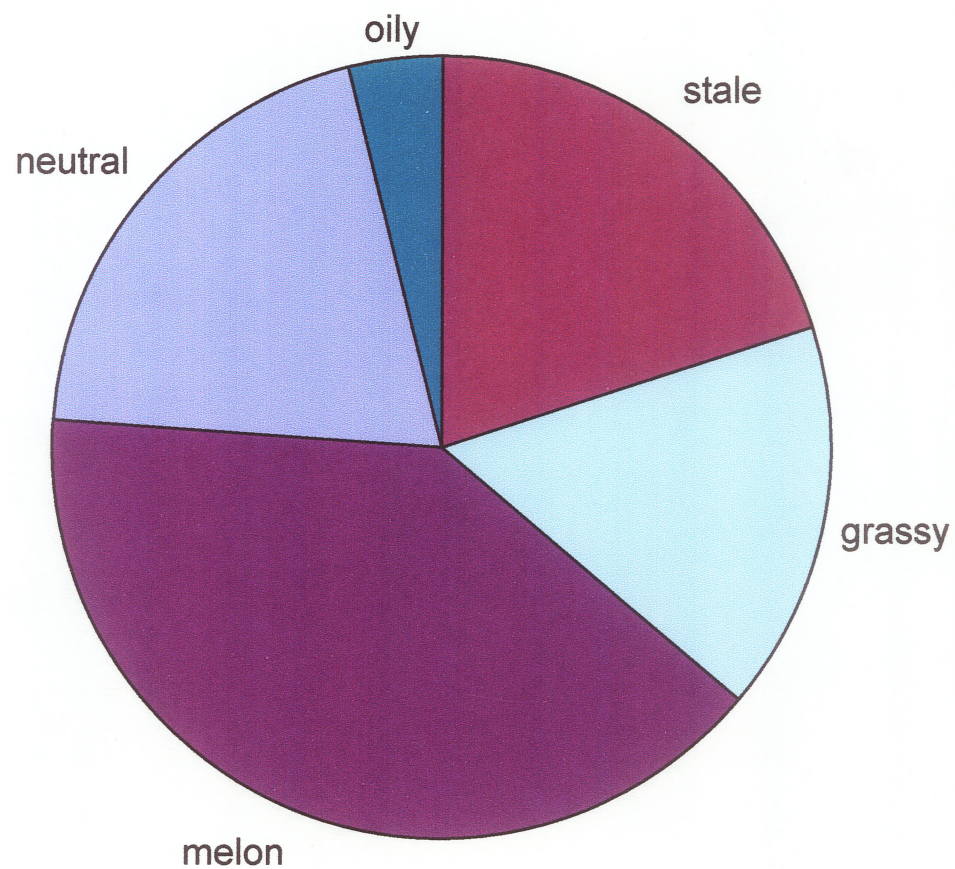


Figure 4.11: Frequency of Odour Descriptors used by Expert Sensory Panel to Describe Treatment D-9 Whitefish Sample

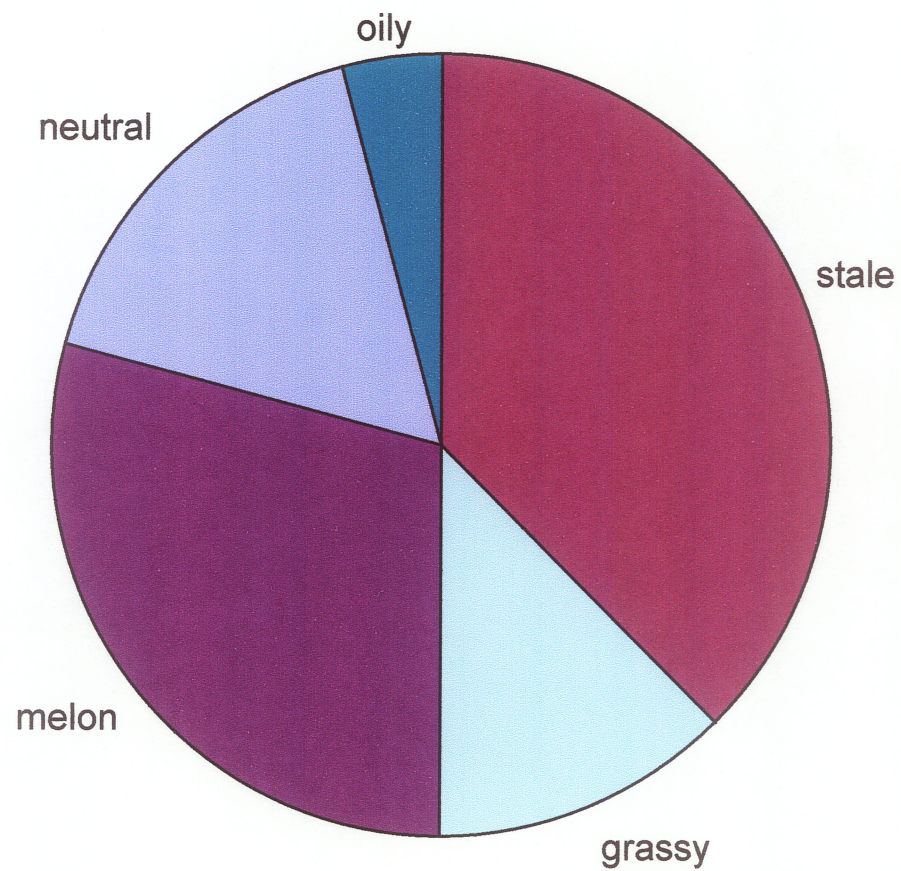


Figure 4.12: Frequency of Odour Descriptors used by Expert Sensory Panel to Describe Treatment D-12 Whitefish Sample

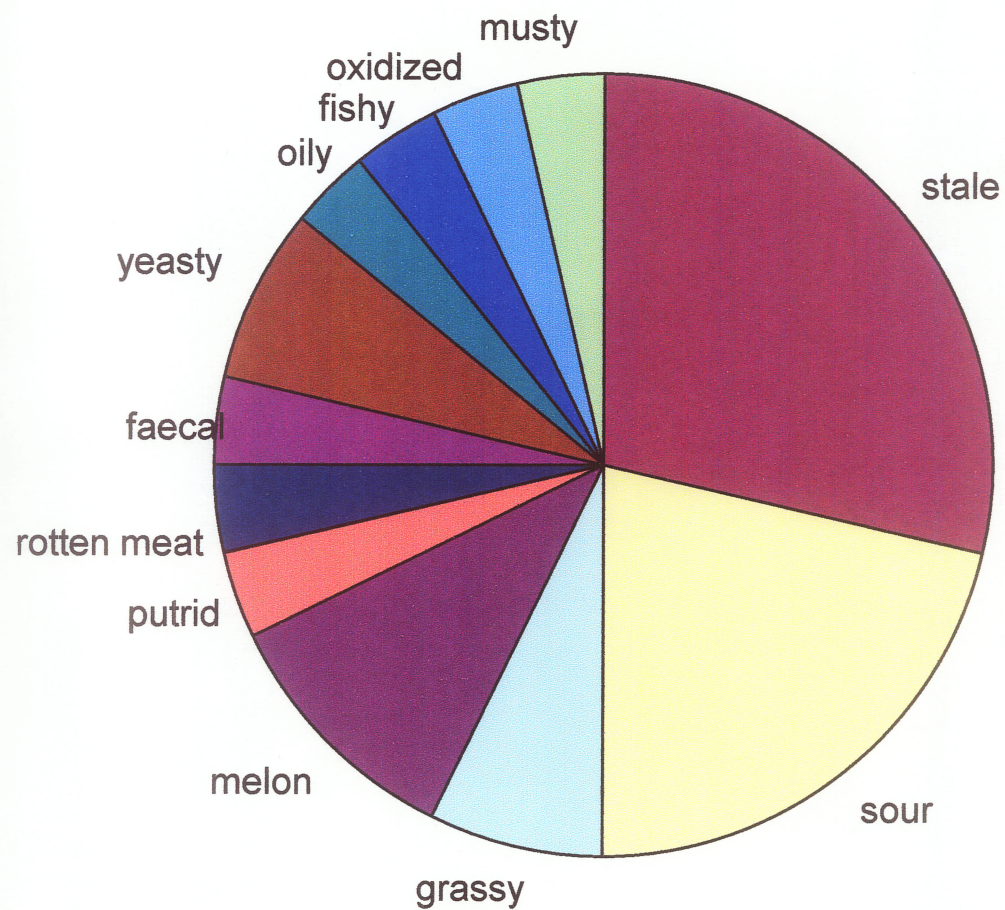


Figure 4.13: Frequency of Odour Descriptors used by Expert Sensory Panel to Describe Treatment D-15 Whitefish Sample

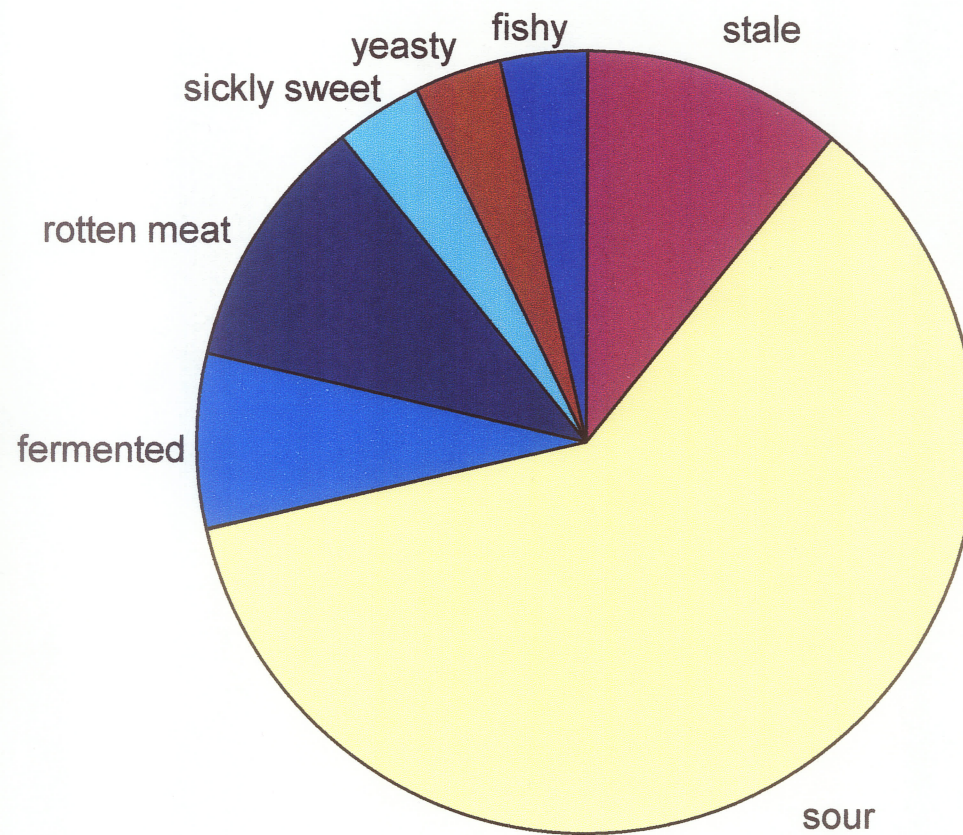


Figure 4.14: Frequency of Odour Descriptors used by Expert Sensory Panel to Describe Treatment D-17 Whitefish Sample

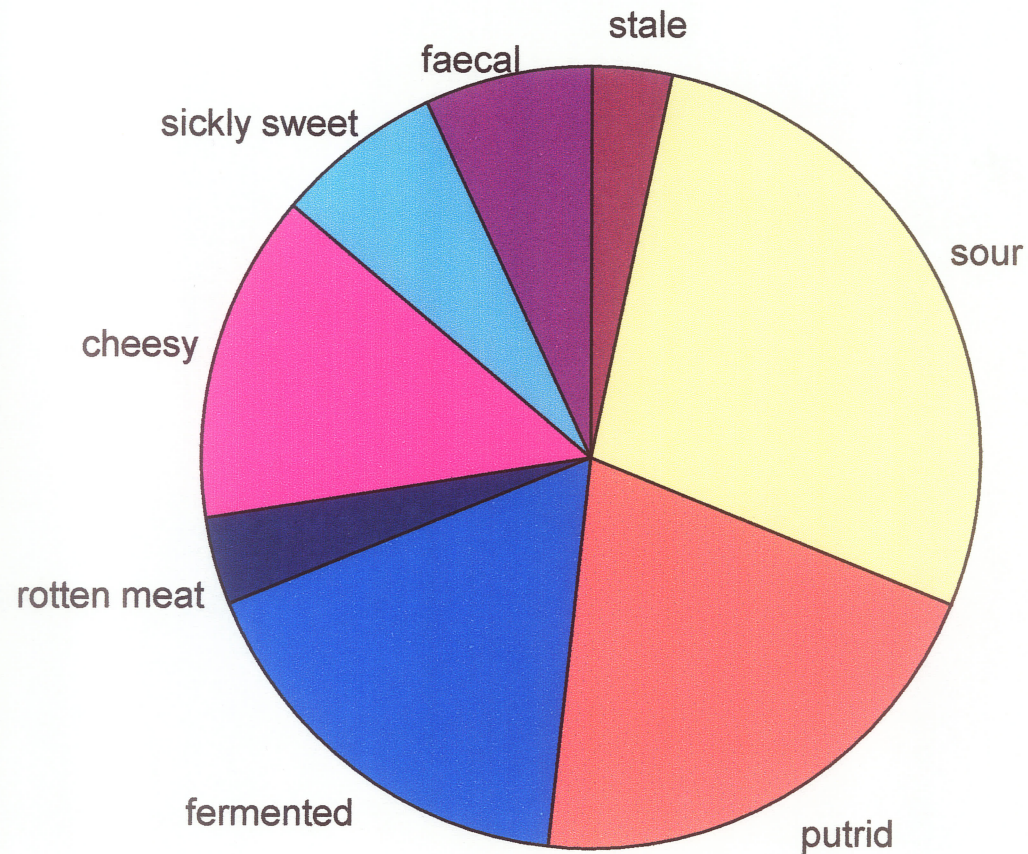
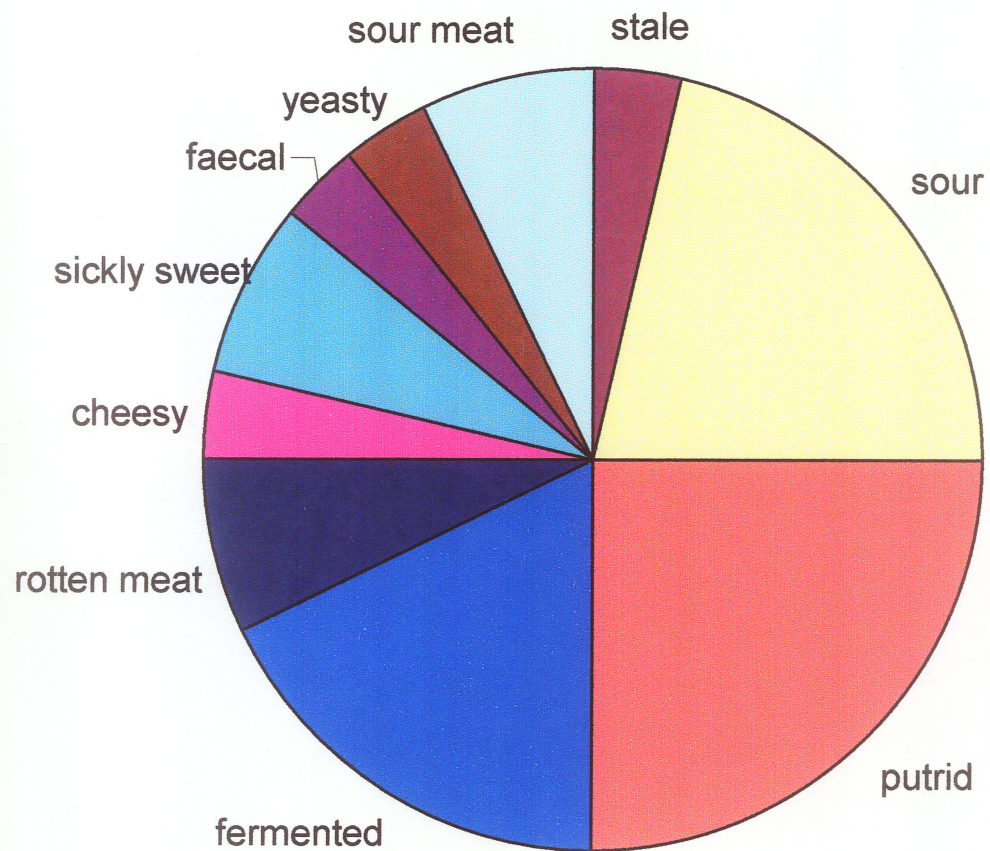


Figure 4.15: Frequency of Odour Descriptors used by Expert Sensory Panel to Describe Treatment D-19 Whitefish Sample



though they were both produced two days from catch. Comm-2 samples were described as predominantly melon and grassy, while D-2 was also described as cucumber and stale with greater frequency. The increased stale odours in D-2 could be attributed to the duration held in frozen storage (10 months) as opposed to Comm-2 which was held for one month in frozen storage. Increment D-5 (Figure 4.10) was also described as predominantly melon, grassy and stale but had lost the cucumber attributes identified in D-2 and to a lesser extent in Comm-2. Increment D-9 (Figure 4.11) had a notable increase in the detection of stale odours while still being described as melon and grassy. Increment D-12 (Figure 4.12) showed a significant change in odour attributes with the predominant attributes of stale and sour even though the attributes, melon and grassy associated with pass quality were still present. Numerous odours associated with decomposition were also identified for the first time in the controlled spoilage series in these samples. The presence of odours associated with pass and fail, confirms the finding that D-12 was of borderline quality. Increment D-15 (Figure 4.13) was described as predominantly sour with the absence of the melon and grassy. Increments D-17 and D-19 (Figures 4.14 and 4.15) were predominantly fermented, putrid and sour. The appearance of the term yeasty and a slight increase in the use of the term rotten meat, distinguish D-19 from D-17 which was also had a greater frequency of being described as cheesy.

4.3 Results of Proximate Analysis Data

The mean proximate analysis results are summarized in Table 4.4. All tests were conducted on three individual sample units per increment. No significant difference

Table 4.4: Mean¹ and Standard Deviation for Proximate Analysis of Whitefish Spoilage Increments

Spoilage Increment	Moisture Mean %	Std.Dv.	Protein Mean (g)	Std.Dv.	*Fat Mean (g)	Std.Dv.	Ash Mean (g)	Std.Dv.
WH-COMM2	75.00	2.48	18.29	0.52	11.03	0.75	1.07	0.08
WH-D2	76.63	1.81	18.16	0.36	11.07	0.91	1.09	0.03
WH-D5	78.75	1.64	18.07	0.21	11.44	1.98	0.96	0.01
WH-D9	78.65	1.23	16.86	0.68	10.07	1.00	0.96	0.02
WH-D12	77.09	1.71	17.90	1.30	10.25	1.07	0.94	0.02
WH-D15	78.70	1.02	18.13	1.00	12.35	1.34	0.93	0.02
WH-D17	78.91	1.09	17.00	0.96	11.49	1.11	0.91	0.03
WH-D19	78.97	0.70	17.44	0.48	11.59	1.58	0.94	0.03

¹ Mean of three replicates for moisture, protein, fat and ash

($p=0.05$) was found within or between increments for fat, protein or moisture (Tables 4.5). A significant difference was found within or between sample increments for ash (Table 4.5). This finding was not considered to be of practical importance due to the extremely small differences in the ash content among the spoilage increments relative to the other proximate data. The fat content has been shown to be the most notable proximate measurement based on historical information. Whitefish can vary in fat content from 2- 13%, with the average being approximately 8% (Hendzel, 1985). The fat content of the fish samples used in the current study had a range of 11.0 – 12.3%. This relatively higher fat content compared to the published average could be responsible for the detection of odour attributes by the expert panel of stale, oily and oxidized and GCO odour impact volatiles such as volatile #29 (stale/oxidized) (Section 4.8). These terms are associated with oxidation of fat which likely occurred during frozen storage. In addition, fatty acid determination was added to the study based on the relatively higher fat content of the spoilage increment samples compared to the published average of 8 % (Hendzel, 1985).

4.4 Results of Fatty Acid Analyses

The mean fatty acid content in each sample increment is summarized in Table 4.6. Saturated (SFA), monounsaturated (MUFA), omega-3 (n-3), omega-6 (n-6), other polyunsaturated with 2 or 3 double bonds per acid (PUFA) and polyunsaturated with four or more bonds double per acid (PUFA (≥ 4)) were measured for each increment. No significant difference ($p = 0.05$) in fatty acid content was found within or between increments (Table 4.7). The total SFA's contributed 21.2 -24.5 % and the total MUFA's contributed 37.8 –

**Table 4.5: Summary of ANOVA Output for Proximate Analysis
Data of Whitefish Spoilage Increments**

ANOVA: Moisture Content Data						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Spoilage Increments	16.24	7	2.32	2.47	0.6708	2.71
Within Spoilage Increments ¹	14.09	15	0.94			
Total	30.32	22				
ANOVA: Ash Content Data						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Spoilage Increments	0.09	7	0.01	10.48	< 0.0001	2.66
Within Spoilage Increments ¹	0.02	16	0.00			
Total	0.11	23				
ANOVA: Protein Content Data						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Spoilage Increments	6.48	7	0.93	1.56	0.2179	2.66
Within Spoilage Increments ¹	9.49	16	0.59			
Total	15.97	23				
ANOVA: Fat Content Data						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Spoilage Increments	11.51	7	1.64	1.01	0.4574	2.66
Within Spoilage Increments ¹	25.93	16	1.62			
Total	37.44	23				

1 Error Variation within Spoilage Increments

Table 4.6: Mean¹ Fatty Acid Content in Spoilage Increment Samples

Item	Sample	MUFA	Std.Dv.	n-3	Std.Dv.	n-6	Std.Dv.	Other PUFA	Std.Dv.	($\geq$) PUFA	Std.Dv.	Total PUFA	Std.Dv.	Total SFA	Std.Dv.
1	Comm 2	50.2	8.12	3.6	1.89	2.4	0.86	2.4	1.26	27.3	5.83	35.8	2.48	21.2	1.03
2	D2	37.8	12.52	3.7	0.64	3.1	0.72	2.7	0.30	30.8	10.18	40.3	10.57	24.5	2.48
3	D5	44.8	1.13	3.6	0.19	2.6	0.44	3.1	0.54	24.2	2.29	33.5	1.78	24.4	1.47
4	D9	41.0	6.33	4.1	0.12	3.4	0.72	3.5	0.74	26.2	7.12	37.1	7.11	24.5	0.60
5	D12	43.3	8.03	3.4	0.20	3.0	1.48	2.7	0.38	26.7	5.02	35.8	5.34	23.7	1.58
6	D15	45.5	2.39	4.3	0.98	2.7	0.49	3.3	0.44	24.0	0.39	34.3	1.47	22.7	1.58
7	D17	43.2	1.99	3.7	0.43	2.7	0.30	2.6	1.66	25.9	1.42	34.8	2.61	24.4	0.83
8	D19	46.9	3.20	3.4	0.45	2.4	0.39	3.9	0.73	23.2	3.62	32.9	3.55	21.8	3.35

1 = Mean of three analyses

MUFA = Monounsaturated Fatty Acid

n-3 = Omega-3 Fatty Acid

n-6 = Omega-6 Fatty Acid

PUFA = Poly Unsaturated Fatty Acid

PUFA ($\geq$) = Poly Unsaturated Fatty Acid with 4 or more bonds per acid

SFA = Saturated Fatty Acid

Table 4.7: Summary of ANOVA Output for Fatty Acid Composition of Whitefish Spoilage Increments

ANOVA - MUFA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	291.74	7	41.68	0.96	0.4927	2.66
Within Groups	696.64	16	43.54			
Total	988.38	23				
ANOVA - omega - 3						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.05	7	0.29	0.43	0.8668	2.66
Within Groups	10.83	16	0.68			
Total	12.88	23				
ANOVA - omega - 6						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.65	7	0.38	0.65	0.7064	2.66
Within Groups	9.27	16	0.58			
Total	11.93	23				
ANOVA - Other PUFA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5.36	7	0.77	1.00	0.4659	2.66
Within Groups	12.25	16	0.77			
Total	17.61	23				
ANOVA - PUFA (≥ 4)						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	118.70	7	16.96	0.58	0.7628	2.66
Within Groups	468.26	16	29.27			
Total	586.96	23				
ANOVA - Saturated Fatty Acids						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	36.49	7	5.21	1.57	0.2159	2.66
Within Groups	53.27	16	3.33			
Total	89.76	23				

50.2% of the fatty acids appearing in the sample increments. The remaining 25.3 – 41 % was composed of the n-3, n-6, other PUFA's and PUFA's (≥ 4). These results indicate that MUFA's and total PUFA's were the predominant fatty acids. SFA's and MUFA's are more stable and less susceptible to oxidation. PUFA's, however are highly susceptible to oxidation (Ackman, 1980) and would contribute the most to the volatile odour products of oxidation in all spoilage increments. The odour attribute "stale" identified by the expert sensory panel in all increments supports this conclusion. One would expect, however, to have fewer odour volatiles associated with oxidation in the Comm-2 samples because they were held in frozen storage for one month versus 10 months for the seven controlled spoilage increments. This conclusion is also supported by infrequent use of the term "stale" for Comm-2 compared to D-2. The oxidation of unsaturated fatty acids in fish or seafood involves the formation of free radicals and hydroperoxides (Khayat and Schwall, 1983). Many off-odour volatiles are formed from the breakdown of the hydroperoxides of unsaturated fatty acids (Pryzybylski and Eskin, 1995). These breakdown products form hydroxyl free radicals which are capable of interacting with almost any organic molecule it comes into contact with have been identified as one of the main initiators of lipid oxidation in fish and other biological tissues (Hultin, 1994). These free radicals reactions may contribute to a range of volatile odour impact compounds including, ketones, aldehydes, alcohols, hydrocarbons, acids and epoxides.

4.5 Results of Freshness Index

The Freshness Index is developed from a HPLC method used to determine the nucleotide degradation products of ATP that result following the death of a fish. The ratio of the degradation products called G-value is indicative of the progression of biochemical changes due to autolytic and bacterial action that have occurred in the fish post mortem (Burns et al, 1985). The nucleotide degradation reaction that generally occurs under ideal chilled handling conditions follows the sequence:



Where ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate, IMP = inosine monophosphate, INO = inosine, Hx = Hypoxanthine, Xa = xanthine and UA = Uric Acid (Burns et al, 1985). ATP and ADP are rapidly degraded following death; therefore the method measures the levels of AMP, IMP, INO and Hx. A ratio of the nucleotides called G-value was used to track the progression of spoilage in the increment samples. The G-value indicates the accumulation of Hx and also reflects the disappearance of AMP, IMP, and INO. The G-values were calculated from the following equation:

$$G = \frac{\text{Hx} + \text{INO}}{\text{INO} + \text{IMP} + \text{AMP}}$$

Table 4.8 summarizes the nucleotide content and the mean G-values of each increment. Figure 4.16 depicts the number of days on ice versus the mean calculated G-values and predicted linear regression G-values. The data shows that G-value steadily increased as days held on ice increased. This data is also supported by Figure 4.17 which shows the relationship of G-value to the mean panel line score. This figure shows that a borderline

**Table 4.8: Mean Nucleotide Content, G-value and Standard Deviation
for each Whitefish Spoilage Increment**

Spoilage Increment	IMP μmoles/g fish	HX μmoles/g fish	AMP μmoles/g fish	INO μmoles/g fish	G-value	G-value Standard Deviation
Comm-2	182.55	60.68	0.00	141.83	0.62	0.02
D-2	0.00	86.91	0.00	269.07	1.32	0.03
D-5	0.00	80.19	0.00	251.27	1.32	0.02
D-9	0.00	119.90	0.00	198.24	1.61	0.04
D-12	0.00	123.67	0.00	183.40	1.67	0.04
D-15	9.94	135.42	0.00	93.50	2.21	0.10
D-17	15.35	173.55	0.00	117.97	2.19	0.27
D-19	13.53	209.09	0.00	127.69	2.39	0.23

IMP = Inosine Monophosphate

HX = Hypoxanthine

AMP = Adenosine Monophosphate

INO = Inosine

Figure 4.16: Days on Ice vs Calculated G-Value Determined from a Ratio of AMP, IMP, INO and Hx Content in Whitefish Spoilage Increments

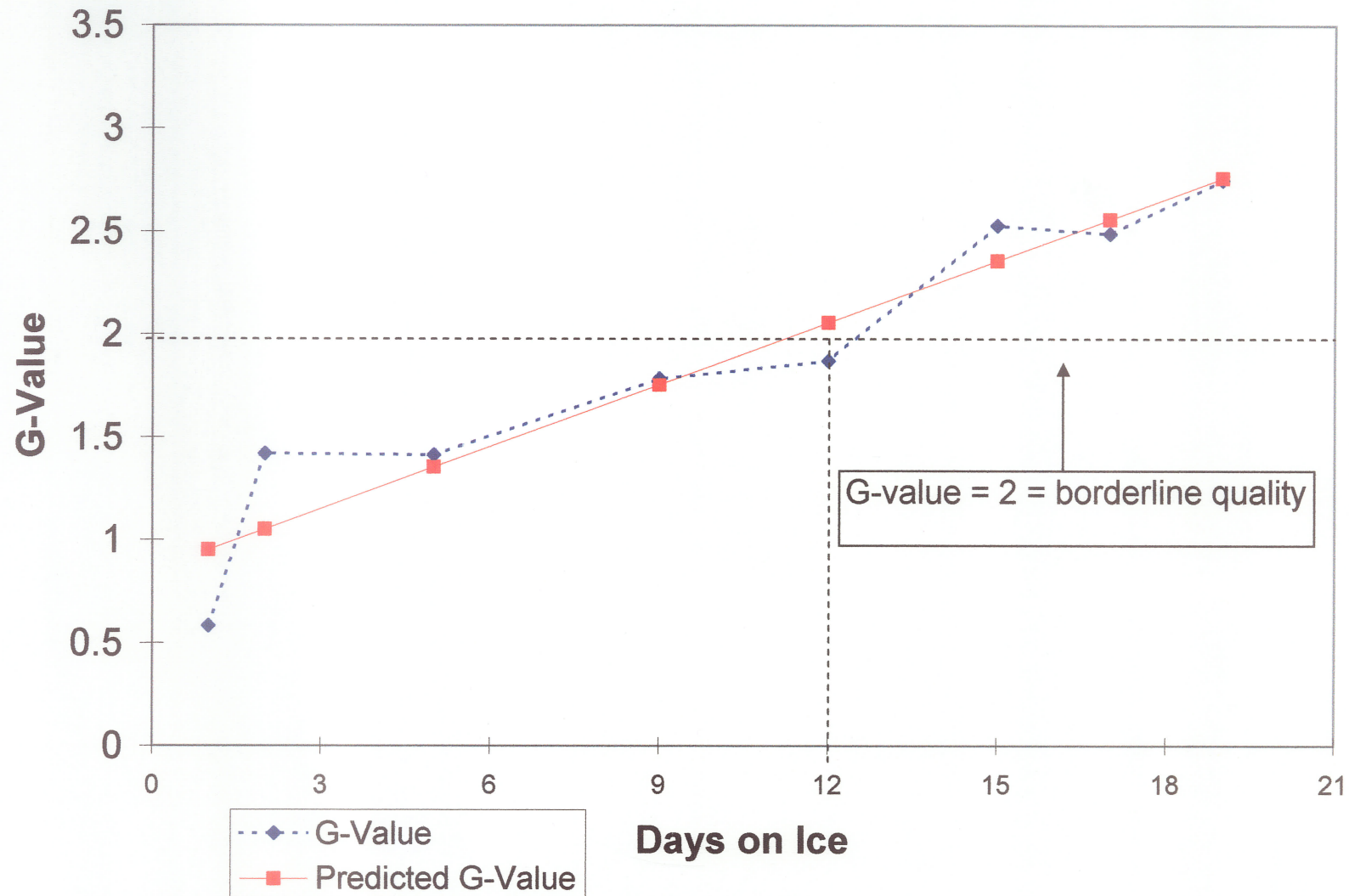
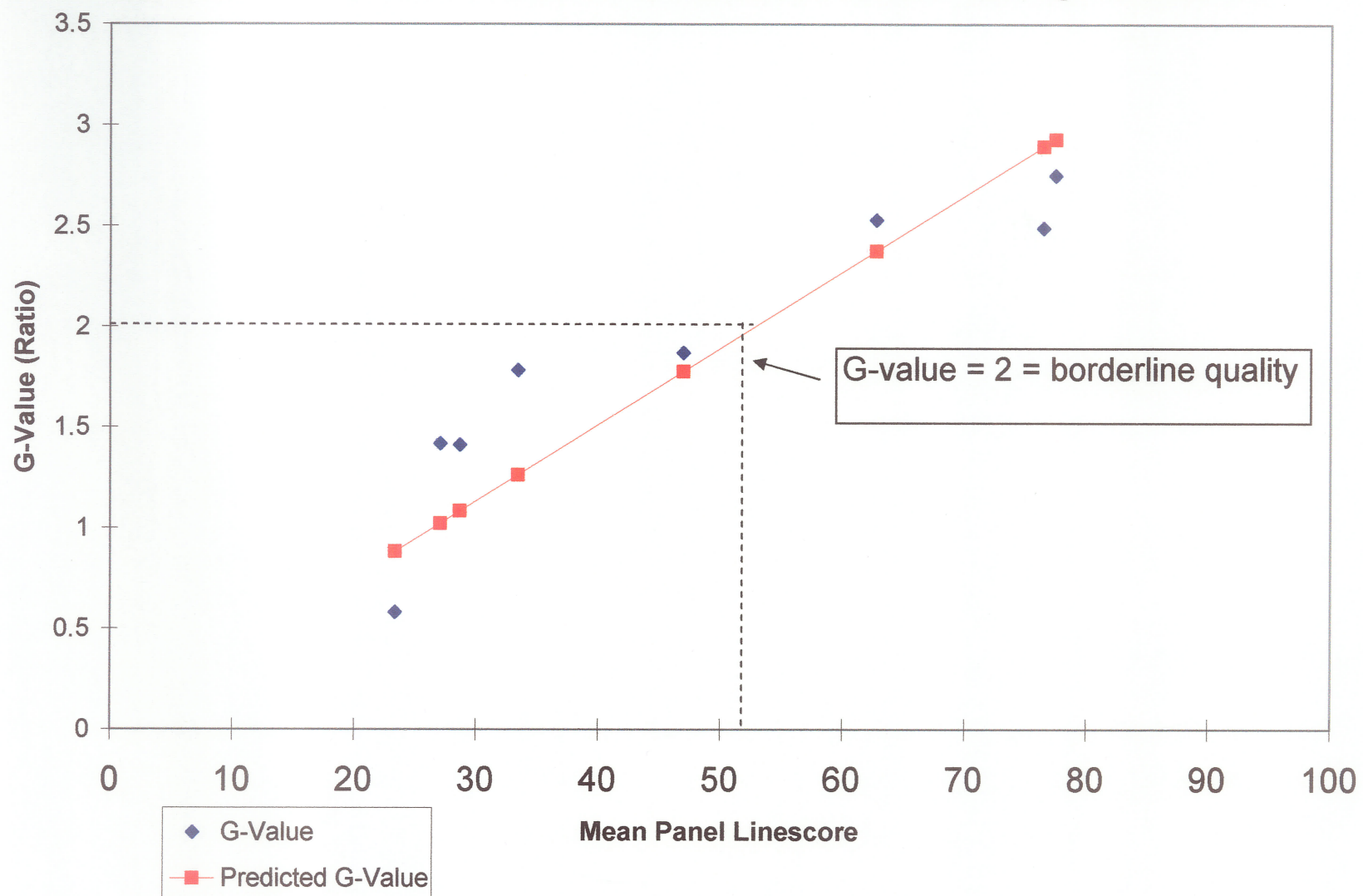


Figure 4.17: Mean Panel Line-score vs Calculated G-Value Determined from a Ratio of AMP, IMP, INO and HX Content in Whitefish Spoilage Increments



quality line-score approximately equal to 52 mm corresponds to a G-value equal to 2.0. If we take the G-value equal to 2.0 we can see that this relates to 12 d of storage on ice in Figure 4.16. Together, the two figures demonstrate that the G-value effectively tracked the progression of spoilage across the increments as well as benchmarking the borderline between pass and fail quality at G-value = 2.0. The ANOVA output (Table 4.9) indicates a significant linear relationship between G-value and days on ice at $p = 0.0557$. The Student's t-test was also conducted to compare the mean G-values between D-12 and spoilage increments adjacent to it (D-9, D-15). The G-value for increment D-12 was significantly different from D-15 ($t = 0.001$) and from D-9 ($t = 0.095$). Therefore the statistical results further support that the Freshness Index (G-value) is a good chemical indicator for differentiating and benchmarking whitefish spoilage over time. Nucleotide degradation products, hypoxanthine in particular, have been determined and used to track spoilage in whitefish and other fish products (Dugal, 1967, Jacober and Rand, 1982, Sikoski et al, 1986). Specifically, Dugal (1967) found a steady increase in Hx concentration from 0.25 $\mu\text{moles/g}$ to 1.52 $\mu\text{moles/g}$ in whitefish held on ice from 0 to 18 d and sampled on days 0, 2, 5, 7, 9 and 18 from the day of catch. He concluded that Hx formation may be a good indicator of fish freshness but that Hx concentration would vary in fish from different locations or season of catch. Thus, the Student's t-test was also used in the present study to compare mean hypoxanthine content between D-12 and spoilage increments adjacent to it (D-9, D-15). The hypoxanthine content in D-9 and D-15 was not significantly different from D-12 at $t = 0.74$ and $t = 0.45$ respectively. Therefore, the Freshness Index (G-value) was a better indicator for benchmarking the pass/fail borderline quality for whitefish than hypoxanthine alone.

**Table 4.9: Summary of ANOVA of Linear Regression:
Whitefish Spoilage Increment Days on Ice versus G-Value**

ANOVA Output of Linear Regression:					
	df	SS	MS	F	p-value
Regression	1	1.63	1.63	5.60	0.0557
Residual Error	7	2.04	0.29		
Total	8	3.67			

4.6 Results for Determination of Ethanol & Other Volatiles Determined by GC/MS

Ethanol has been shown to accumulate in the muscle tissue of marine fish, canned tuna and canned salmon as storage time increased (Kelleher and Zall, 1981, Lerke and Huck, 1977, McLachlan et al, 1999). It is believed that ethanol is produced via two chemical pathways in fish tissue over time: the breakdown of carbohydrates by microbial enzymes and more importantly by the deamination and decarboxylation of the amino acid alanine (Kelleher and Zall, 1981). Figure 4.18 shows the ethanol concentration found for each of the whitefish spoilage increments. Ethanol was not detected in any of the three sample units analyzed for increments Comm-2, D-2, D-5 or D-9. Ethanol appeared in two out of the three sample units analyzed for D-12 and were consistently detected in all samples analyzed for increments D-15 and D-17, with a spike in concentration in increment D-19. From this data it can be concluded that the presence of ethanol at a concentration as low as 1 µg/mL or 1 ppm is indicative of fail quality for whitefish while the absence of ethanol indicates pass quality whitefish. Therefore ethanol can be considered a good chemical benchmark for the pass/fail borderline because the ethanol findings correspond to the sensory quality assessment which found increment D-12 to be borderline pass/fail (Figure 4.1).

Interestingly, the GC/MS method used in this study to determine ethanol concentration also identified and determined the concentration of nine other chemical compounds shown in

Figure 4.18: Ethanol concentration in Whitefish Spoilage Increments

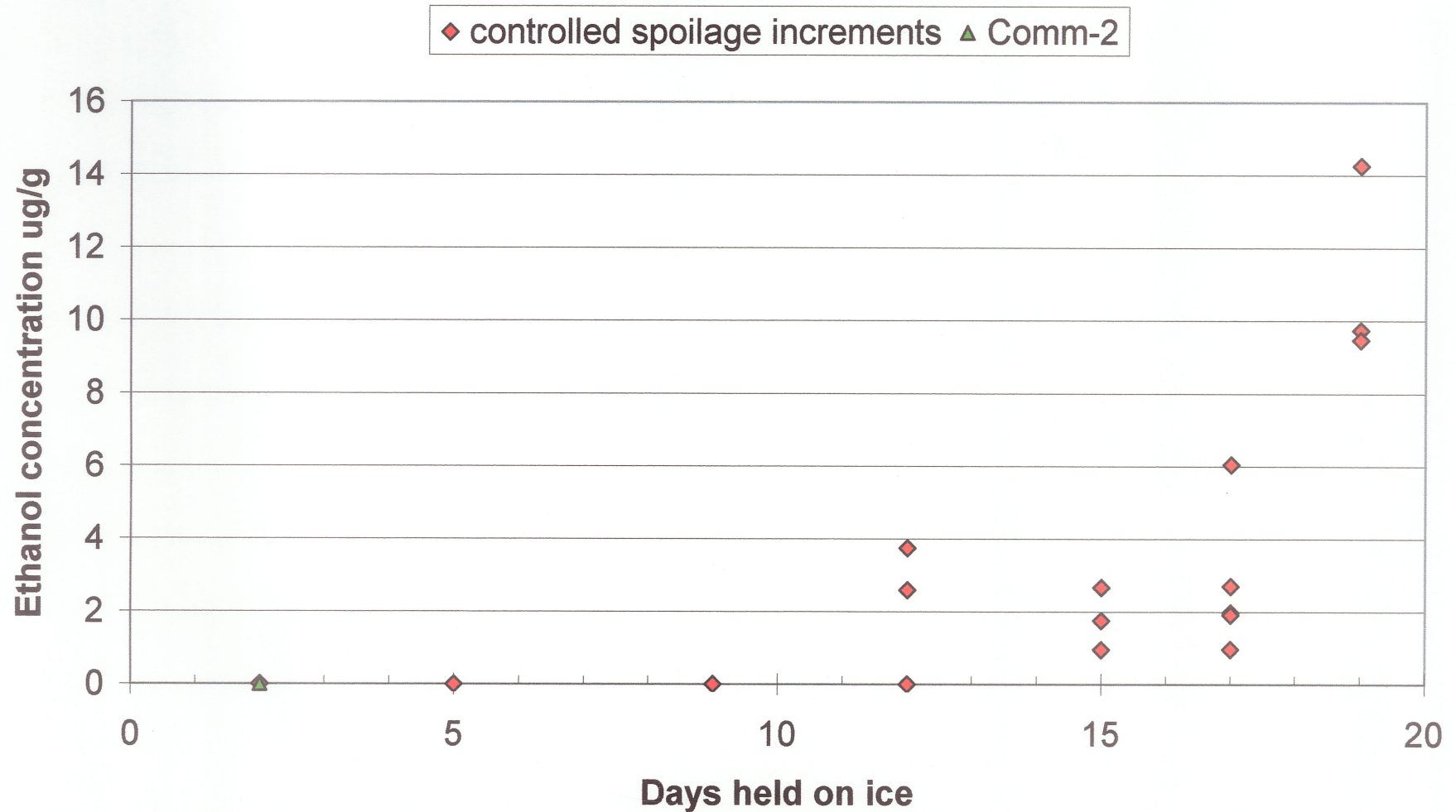


Table 4.10. Figure 4.19 shows the concentration of each chemical in each of the spoilage increments. From this graph it is evident that none of the other chemical components identified showed a trend that could be associated with the spoilage increments. All of the identified volatile peaks except ethanol are often associated with rancidity (McLachlan, 2003). It is interesting that propanal had a higher concentration than any of the other components and showed a decrease in concentration as duration in storage increased.

4.7 Results of Total Volatile Base Nitrogen (TVB-N) Determination

Total Volatile Base Nitrogen (TVB-N) was determined by Kjeldahl following the method of the Canadian Food Inspection Agency. Figure 4.20 shows the TVB-N concentration for each of the spoilage increments. At 15 d there was a notable increase in TVB-N levels which would be indicative of extensive protein and amino acid breakdown. TVB-N is especially useful for tracking spoilage in marine species which contain large amounts of trimethylamine oxide (TMAO). In marine species, the nitrogen measured is typically associated with the production of TMA following the reduction of TMAO. Freshwater fish species do not contain TMAO and therefore the nitrogen measured is a product of protein and amino acid breakdown (Lindsay, 1994). TVB-N is able to clearly distinguish the fail whitefish increments (D-15, D-17 and D-19) from the

Table 4.10: Summary of Chemical Compounds and their Peak Area Identified by GC/MS Method for Ethanol Determination

Peak ID No.	Chemical Compound	Whitefish Sample Treatment Concentration (Area Counts)							
		Comm-2	D-2	D-5	D-9	D-12	D-15	D-17	D-19
10	acetaldehyde	2220455	3620159	1913187	3277068	1833483	1393136	1025569	2533409
16	ethanol	0	0	0	0	1582594	1785242	2718994	10796575
17	propanal	13440828	21964759	9406894	8715450	10640090	9309410	9252117	6608153
21	butanal	1876416	3758181	1603241	1004258	1633785	1356718	1586544	823643
22	hexane	0	2014346	0	0	0	0	0	0
23	2-butanamine	0	0	0	0	0	0	0	985980
24	1-penten-3-ol	1161589	2788559	0	1679455	1002726	1675440	1007024	496107
25	2-ethyl furan	1825664	4370303	1929519	1516225	1735160	1589039	1246163	0
26	pentanal	1479634	2765053	2009865	953496	540736	0	0	0
29	hexanal	4063260	5346366	2794914	2011929	3029601	2421923	1488351	1613495
35	heptanal	1039428	1789560	1393697	0	1319321	0	0	0

Figure 4.19: Concentration vs. Days Held on Ice for all Chemical Compounds Identified by GC/MS Mehtod for Ethanol Determination

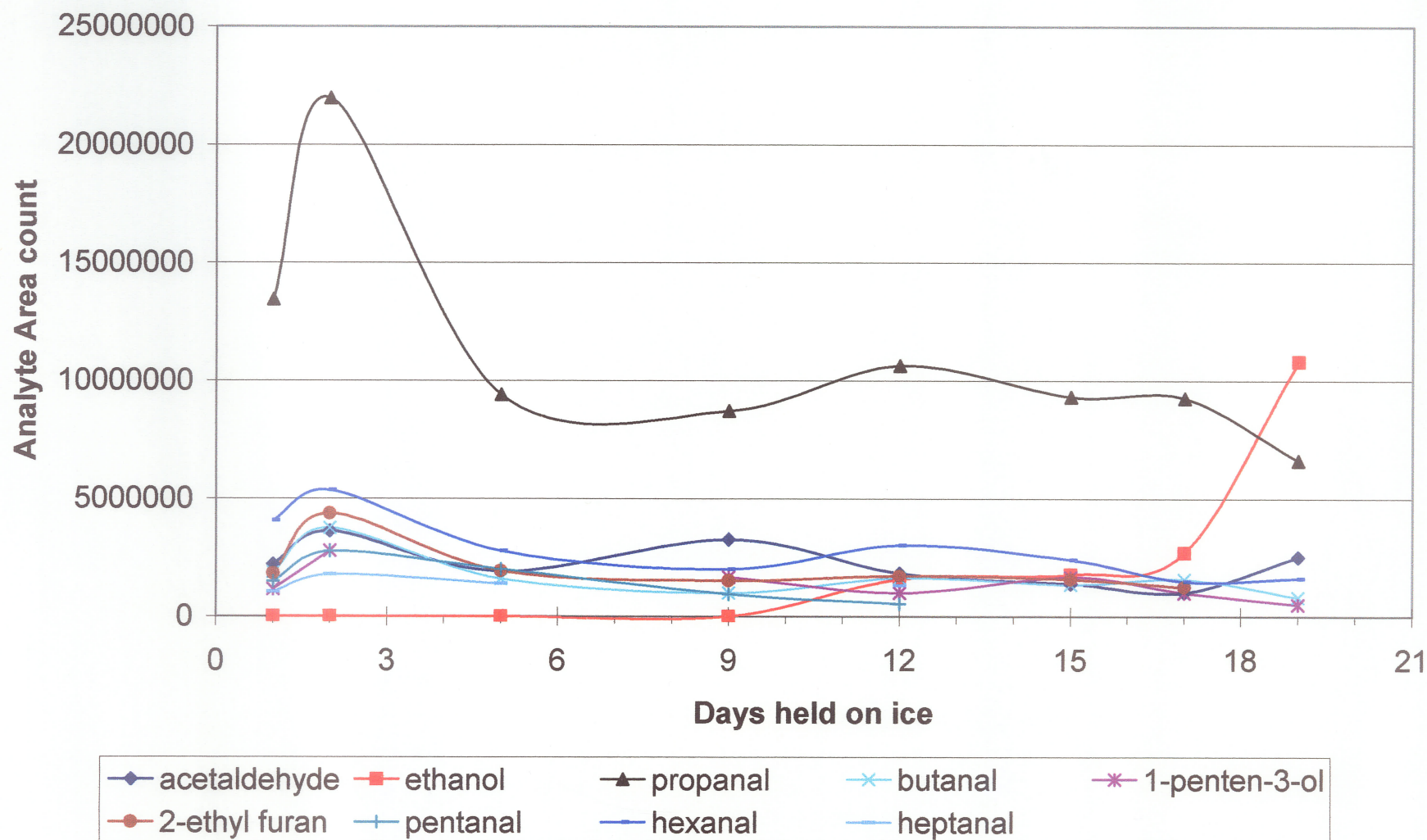
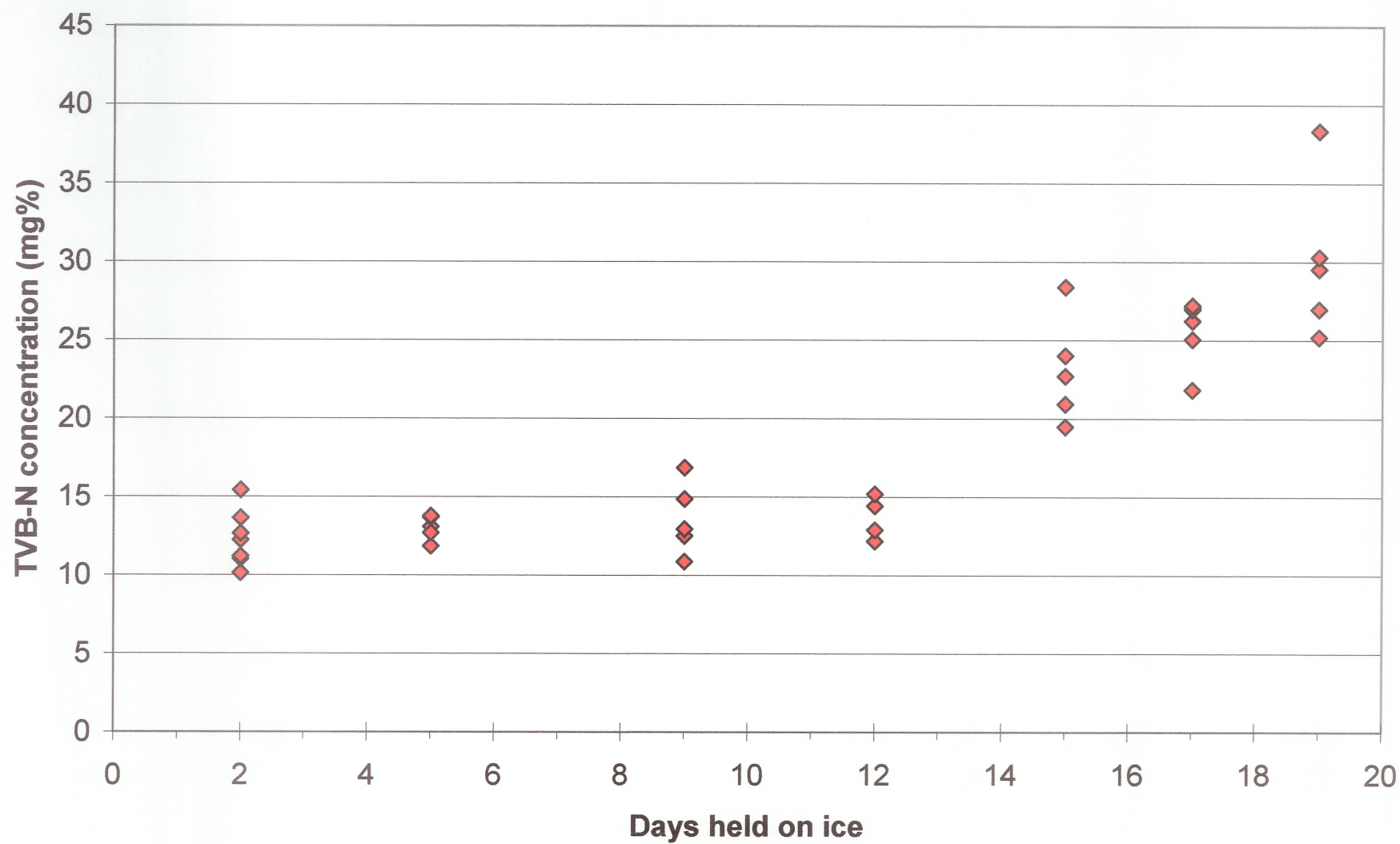


Figure 4.20: TVB-N Concentration for Whitefish Spoilage Increments



other increments at a concentration of 19 mg/100g. It is not however, able to distinguish the borderline quality samples (increment D-12) from the pass quality increments. Therefore it was not a useful quality indicator in benchmarking whitefish samples of borderline quality.

4.8 GCO Results

The panel of four experts collectively used a total of 402 terms to characterize the odours they perceived across the thirty two GCO sessions (4 panelists x 8 increments). The list of 402 terms was reduced to 133 terms by removing all qualifying terms such as slight, moderate and strong and by removing terms where only one panelist responded. The terms related to intensity, such as, slight, moderate or strong could be removed with confidence because the sliding response bar was used to measure odour intensity. Thirty seven key descriptive terms were selected from the 133 terms by grouping synonyms based on the main term used by at least two panelists for the volatile eluting at an average retention time. The grouping of like sensory terms generated by a trained sensory panel is an accepted practice utilized in sensory descriptive analysis methods (Poste et al, 1991, Meilgaard, 1991, Murray et al, 2001). For wine odour volatile research using the OSME method (McDaniel et al, 1989, Miranda-Lopez et al 1992a, 1992b) the researchers also used the criterion that at least two panelists in a four person panel had to identify an odour volatile and associate similar terms for it to be considered a key odour impact volatile. Table 4.11 lists the Key Terms selected by the experimenter, the mean retention time and the actual terms used by each panelist to characterize the volatiles with odour impact that eluted at that mean retention time.

**Table 4.11: List of 37 Selected Key Terms from Actual Terms used by the Panel
to Characterize the Volatiles with Odour Impact**

Term No.	Key Term	Mean Retention Time	Term - Panelist 1	Term - Panelist 2	Term - Panelist 3	Term - Panelist 4
1	sweet	1.39	sweet	sweet	sweet	NA
2	dimethyl sulfide	1.54	slight shellfish/ozone	slight fresh fish/sweet	dimethyl sulfide	canned corn/sl.haddock
3	solvent	1.90	solvent	NA	methane gas	NA
4	cheesy/fermented	2.93	cheesy/dirty socks	cheesy/fermented	cheesy/agar	cheesy/fermented
5	briny	3.82	briny	NA	briny	NA
6	caramel	3.83	strong sweet	butterscotch	caramel/butterscotch	caramel
7	scorch	4.43	scorch	burnt	hot vinyl	hot barbecue
8	sweet/green	4.81	green	sweet/fresh grass	sweet/plant leaves	sweet/floral
9	fresh/floral	5.49	fresh	floral	fresh/slight honey	fresh/floral
10	caramel/fruity	6.29	fruity	caramel/garden	butterscotch/green apple	caramel/green apple
11	green/fruity	6.70	planty/fruity	green/fruity	citrus/banana peel	garden/fruity
12	fresh grass	7.71	fresh grass	leafy/green	fresh/green	fresh grass
13	geranium	8.52	green/metallic	green/earthy	green/metallic	geranium
14	stale/acrid	8.97	stale/chalky	stale/acrid	NA	stale/paint sealer
15	musty/vegetable	9.25	vegetable/slight oxidized	NA	musty	musty/wet hay
16	sweet	9.84	NA	sweet	NA	sweet
17	fresh/green	9.94	fresh	plant leaves	fresh air/green	green
18	stale/musty	10.48	stale/oxidized oil	musty/basement	stale/oxidized oil	stale/musty
19	herbal/cilantro	11.14	sweet/green/herbal	planty/floral	green/herbs	herbal/cilantro
20	musty	11.48	musty	musty	musty	musty
21	fresh plants	12.00	green leaves	fresh earth	green plants	fresh/geranium
22	sulfur	12.26	sulfur	sulfur	sewer gas	slight faecal
23	geranium	12.34	geranium	metallic/green	geranium	geranium
24	sweet/floral	12.57	sweet/floral	floral	sweet/green	sweet/flowers
25	cucumber/melon	12.87	sweet/melon	cucumber	cucumber	melon
26	stale	13.16	stale	stale	stale/dusty	bark mulch
27	sour	13.35	sour milk	vinegar	acetic acid	sour/vinegar-like
28	green/planty	13.89	green/floral	green/leafy lettuce	green/outdoors	green/cilantro
29	stale/oxidized	14.48	oxidized	stale/oily	stale	stale/cardboard
30	floral/fresh	15.05	floral/pleasant	floral/outdoors	fresh/floral	floral/fresh dew
31	citrus	15.47	NA	NA	citrus/orange like	sour/lemon
32	cheesy	15.69	cheesy	cheesy	cheesy/agar	agar/sour
33	floral	15.93	floral	NA	NA	floral
34	mothball	16.08	dirty socks	indole/painty	mothball	chemical/butyric acid
35	green/fruity	16.56	green/pleasant	green/fruity	green/citrus	green/fruity
36	sulfur	16.60	NA	NA	sulfur	sulfur
37	pungent licorice-like	16.84	licorice/spicy	pungent/anise	stong/mint-like	licorice

Table 4.12 presents the summary analysis of variance (ANOVA) for the multivariate model used to describe the GCO sensory results. The “Panelist” and “Key Term” effects were significant at $p < 0.0005$ and the “Spoilage Increment” effect at $p = 0.0536$. The “Treatment x Key Term” interaction was significant at $p = 0.0956$. The combination of the “Key Term”, “Treatment”, and “Key Term x Treatment” effects were significant at $p = 0.0048$ (calculated from combinations of sums of squares and degrees of freedom, $(F_{142,401df}) = 1.46$).

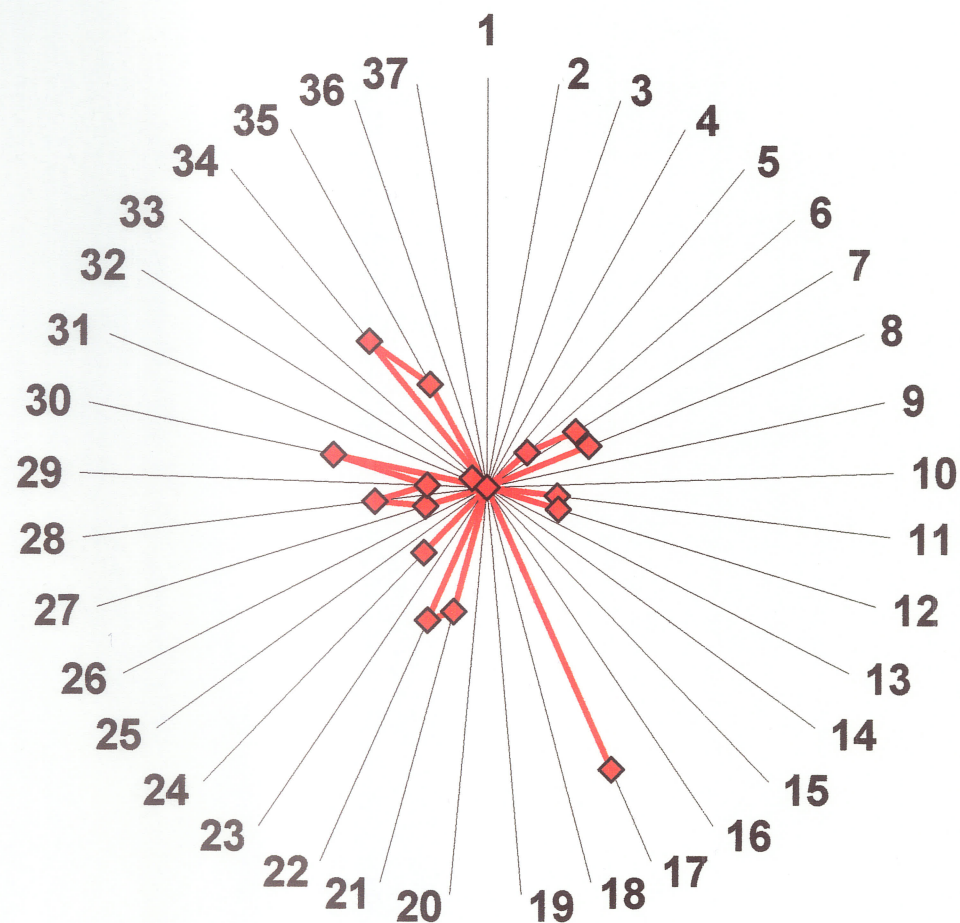
The multivariate model accounted for panelist variation in odour volatile intensity ratings and was used to normalize each panelist’s responses. In addition, it was used to estimate responses for panelists in the case when two out of four panelists identified an odour impact volatile. The estimate responses were the Least Square Means calculated by the model for each “Key Term × Spoilage Increment” combination and plotted as radar graphs (option LSMEANS in GLM, SAS 1989). Figures 4.21 to 4.28 show the “Least Squares Means” intensity ratings for the “Spoilage Increment x Key Term” response for each spoilage increment.

Using the radar graphs it is possible to describe each increment utilizing the Key Terms that have the greatest odour impact based on intensity and to relate them to the overall odour of the increment sample. In the radar graph for increment Comm-2 (Figure 4.21), the Key Term, fresh/green (#17) stands out as a high intensity odour volatile. This finding corresponds well to the results obtained from the expert sensory panel that assessed the odour of the whitefish samples directly.

**Table 4.12: Summary of ANOVA Output - Gas Chromatography/Olfactometry Data
using the General Linear Models Procedure (SAS, 1989)**

Anova Output - GCO					
Source of Variation	df	Type III SS	MS	F Value	P-value
Panelist	3	7.80	2.60	13.93	< 0.0001
Key Term	36	14.13	0.39	2.10	0.0005
Spoilage Increment	7	1.13	0.16	0.86	0.5363
(Spoilage Increment * Key Term)	99	22.84	0.23	1.24	0.0956

Figure 4.21: COMM-2 Volatiles with Odour Impact

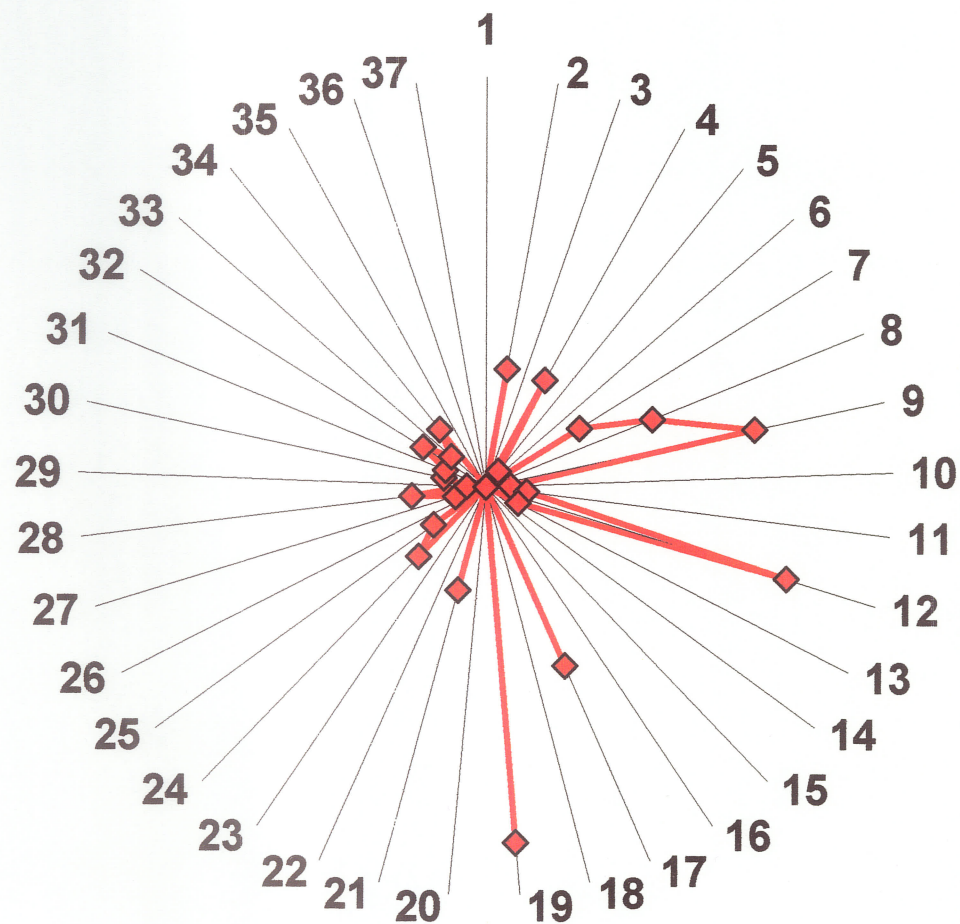


LEGEND

- 06 - caramel
- 07 - scorch
- 08 - sweet/green
- 11 - green/fragrant
- 12 - fresh grass
- 17 - fresh/green
- 21 - fresh plants
- 22 - sulfur
- 24 - sweet/floral
- 27 - sour
- 28 - green/planty
- 29 - stale/oxidized
- 30 - floral/fresh
- 32 - cheesy
- 34 - mothball
- 35 - green/fragrant

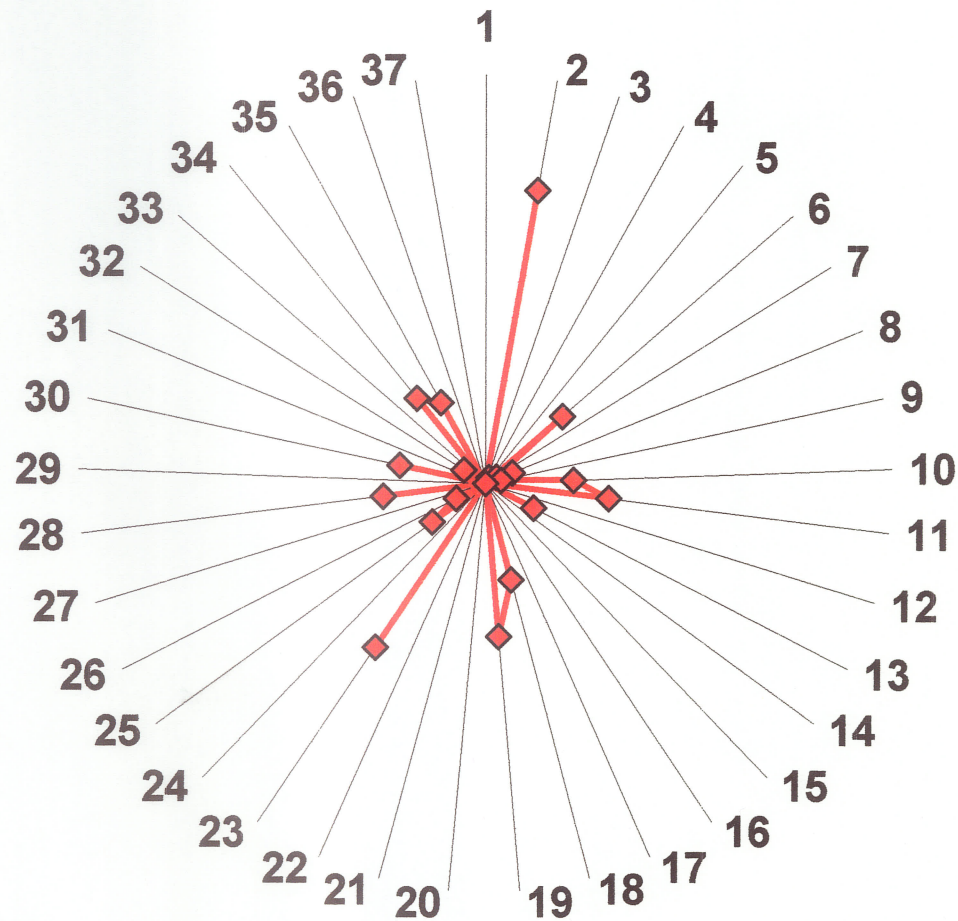
Figure 4.22: D-2 Volatile with Odour Impact

LEGEND



- 02 - dimethyl sulfide
- 04 - cheesy/fermented
- 05 - briny
- 07 - scorch
- 08 - sweet/green
- 09 - fresh/floral
- 10 - caramel/fruity
- 11 - green/fruity
- 12 - fresh grass
- 13 - geranium
- 17 - fresh/green
- 19 - herbal/cilantro
- 21 - fresh plants
- 24 - sweet/floral
- 25 - cucumber/melon
- 27 - sour
- 28 - green/planty
- 29 - stale/oxidized
- 30 - floral/fresh
- 31 - citrus
- 32 - cheesy
- 33 - floral
- 34 - mothball

Figure 4.23: D-5 Volatiles with Odour Impact



LEGEND

- 02 - dimethyl sulfide
- 04 - cheesy/fermented
- 06 - caramel
- 07 - scorch
- 08 - sweet/green
- 09 - fresh/floral
- 10 - caramel/fruity
- 11 - green/fruity
- 13 - geranium
- 18 - stale/musty
- 19 - herbal/cilantro
- 23 - geranium
- 25 - cucumber/melon
- 26 - stale
- 28 - green/planty
- 30 - floral/fresh
- 32 - cheesy
- 34 - mothball
- 35 - green/fruity

Figure 4.24: D-9 Volatiles with Odour Impact

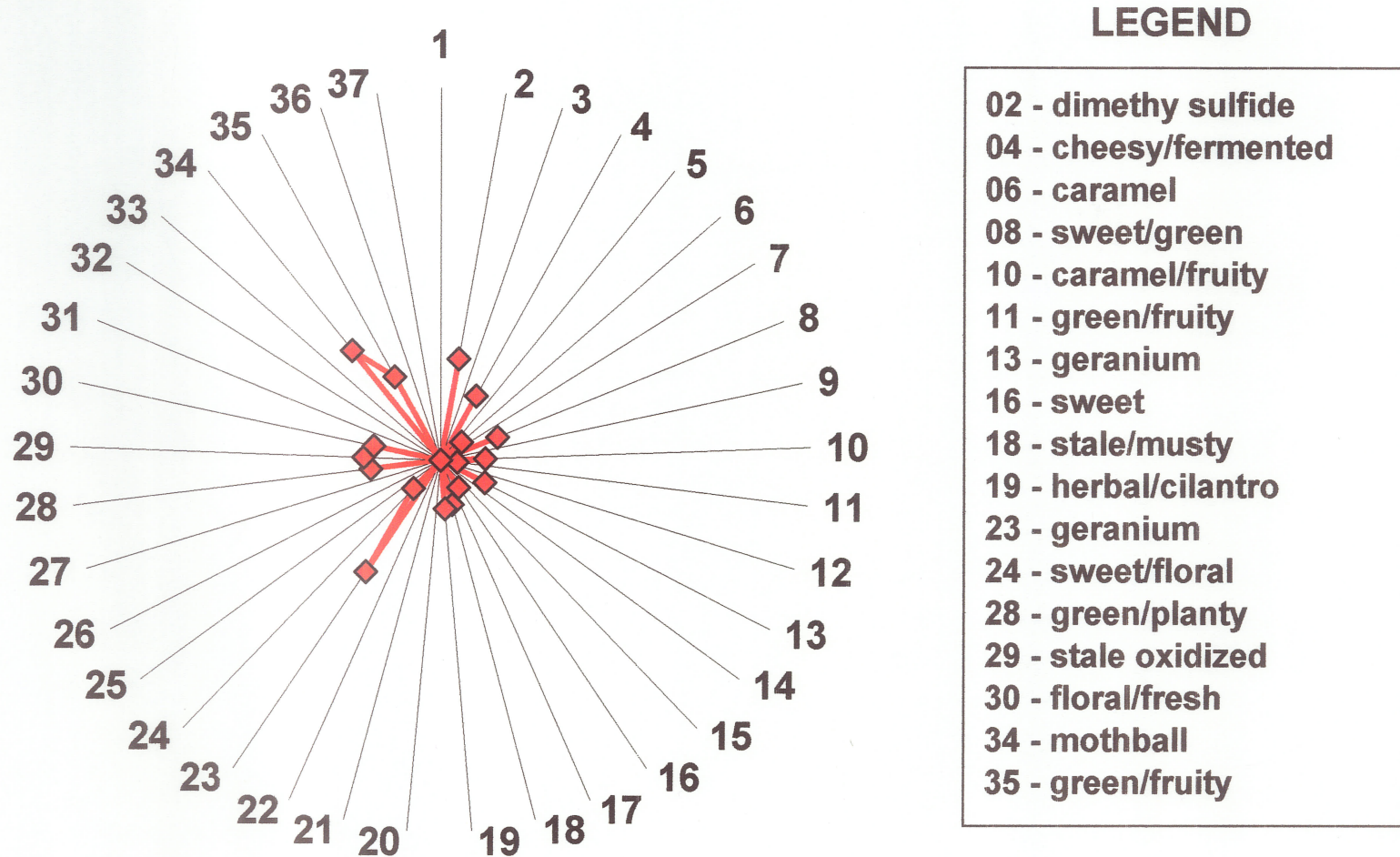
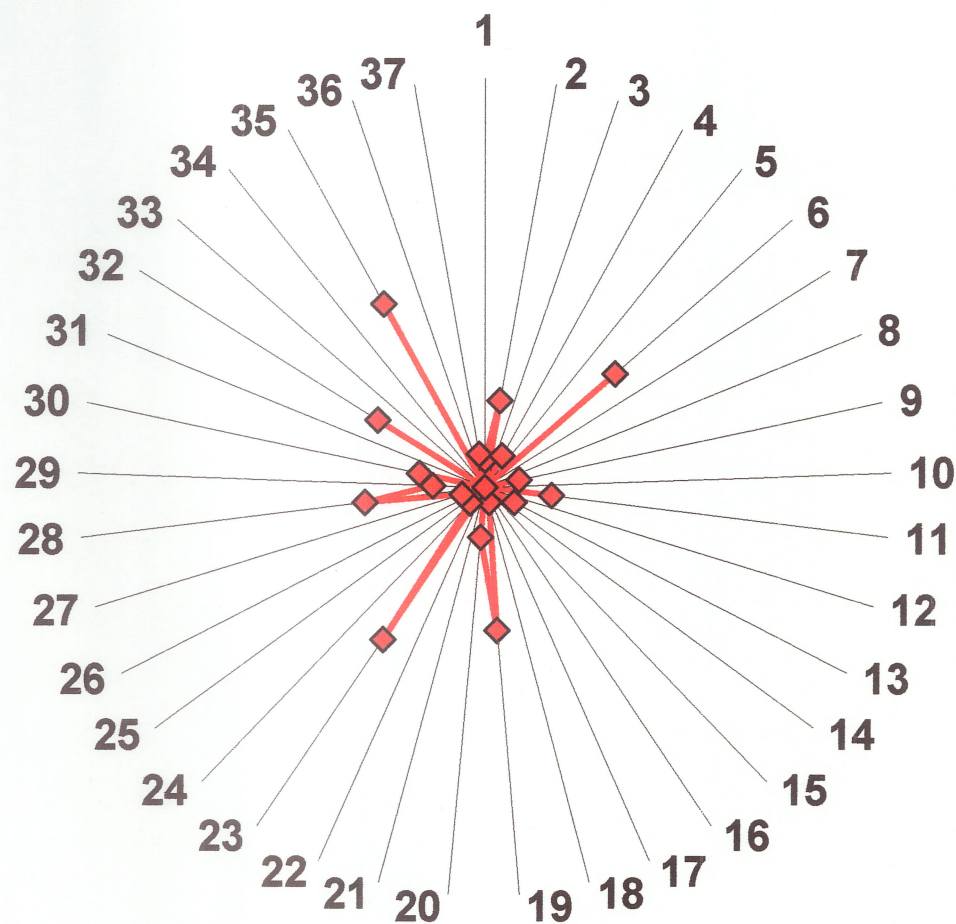


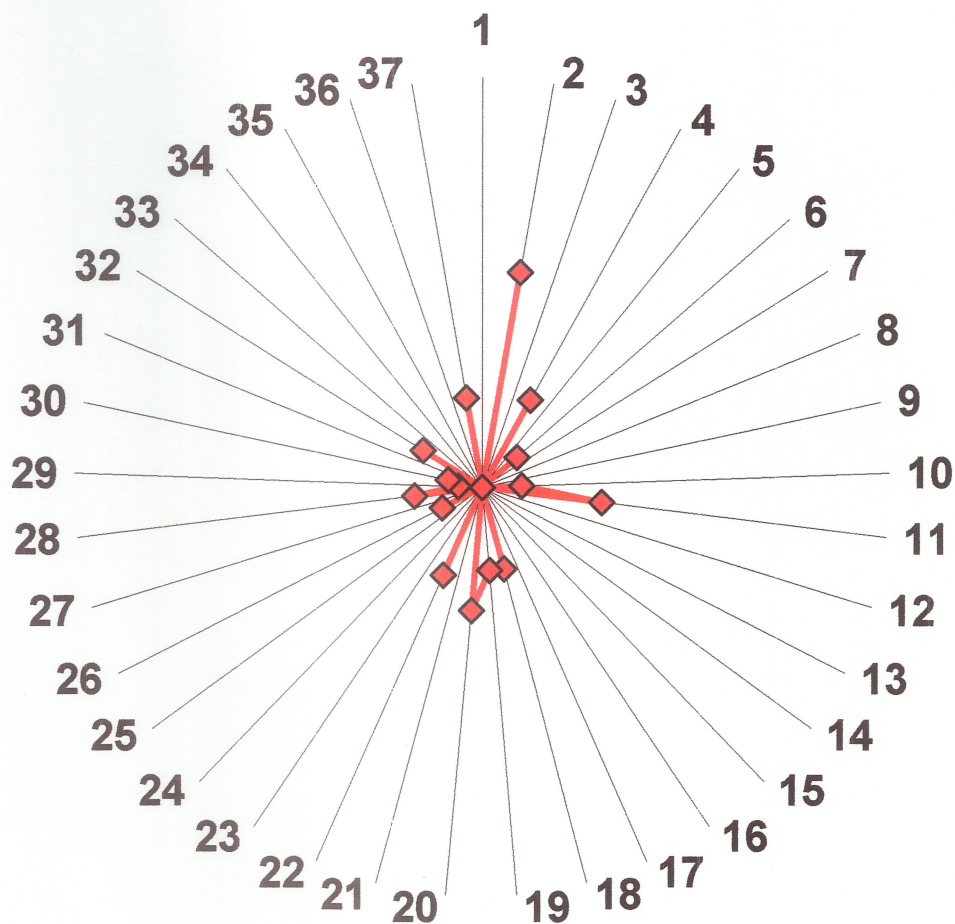
Figure 4.25: D-12 Volatiles with Odour Impact

LEGEND



- 01 - sweet
- 02 - dimethyl sulfide
- 04 - cheesy/fermented
- 06 - caramel
- 09 - fresh/floral
- 11 - green/fruity
- 13 - geranium
- 18 - stale/musty
- 19 - herbal/cilantro
- 20 - musty
- 23 - geranium
- 24 - sweet/floral
- 27 - sour
- 28 - green/planty
- 29 - stale/oxidized
- 30 - floral/fresh
- 32 - cheesy
- 35 - green/fruity
- 37 - pungent/licorice-like

Figure 4.26: D-15 Volatiles with Odour Impact



LEGEND

- 02 - dimethyl sulfide
- 04 - cheesy/fermented
- 06 - caramel
- 10 - caramel/fruity
- 11 - green/fruity
- 18 - stale/musty
- 19 - herbal/cilantro
- 20 - musty
- 22 - sulfur
- 26 - stale
- 28 - green/planty
- 29 - stale/oxidized
- 30 - floral fresh
- 32 - cheesy
- 37 - pungent/licorice-like

Figure 4.27: D-17 Volatiles with Odour Impact

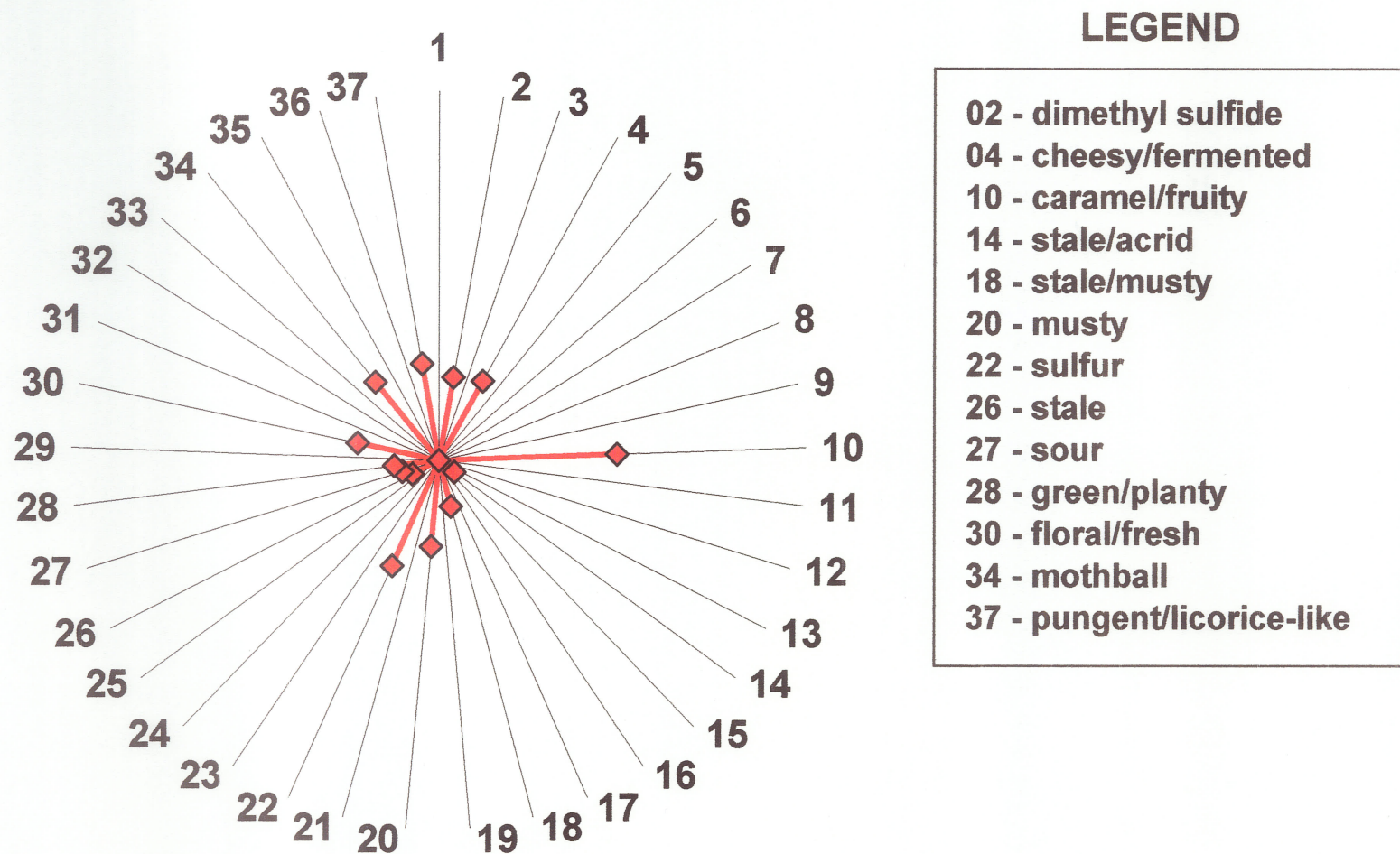
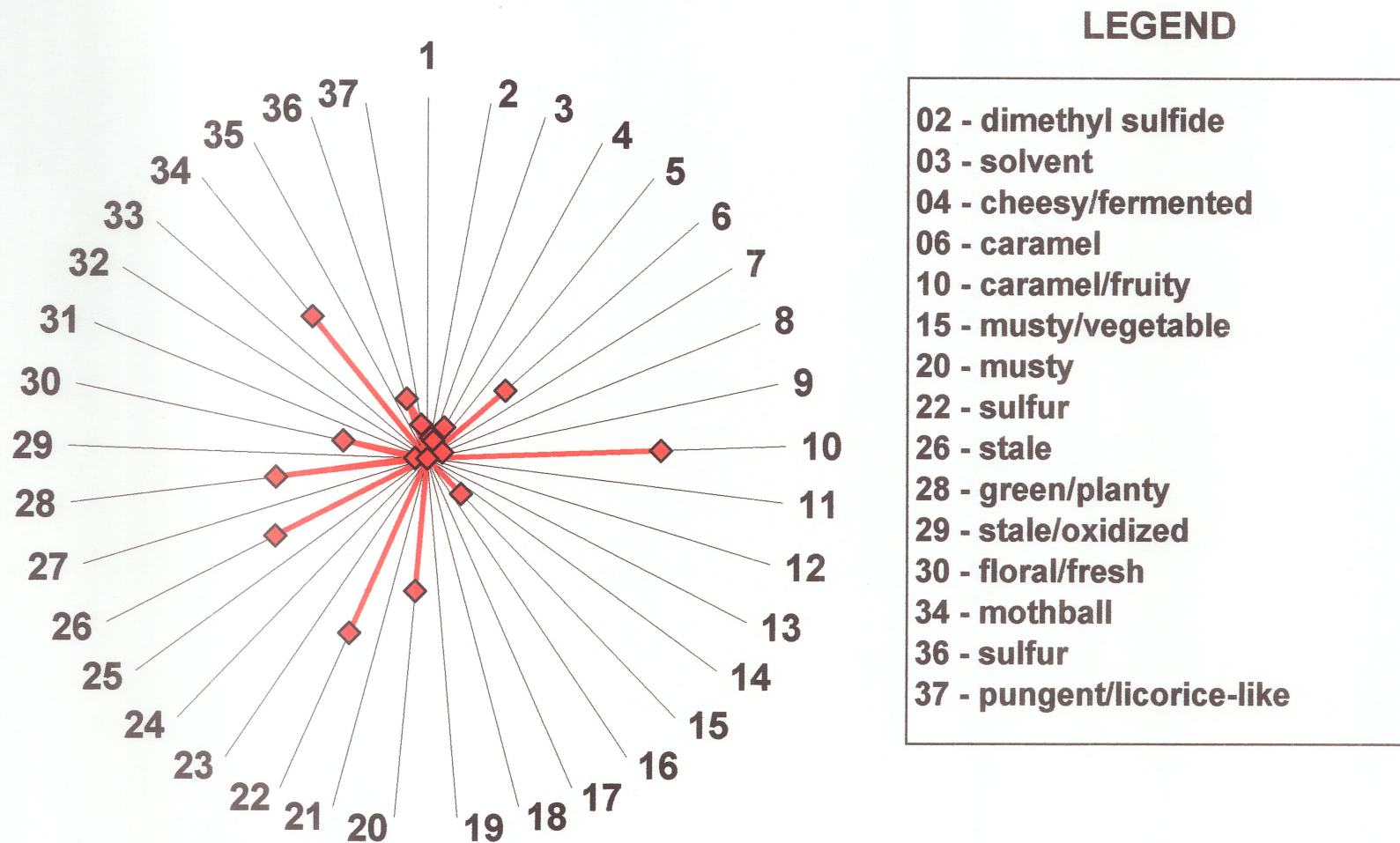


Figure 4.28: D-19 Volatiles with Odour Impact



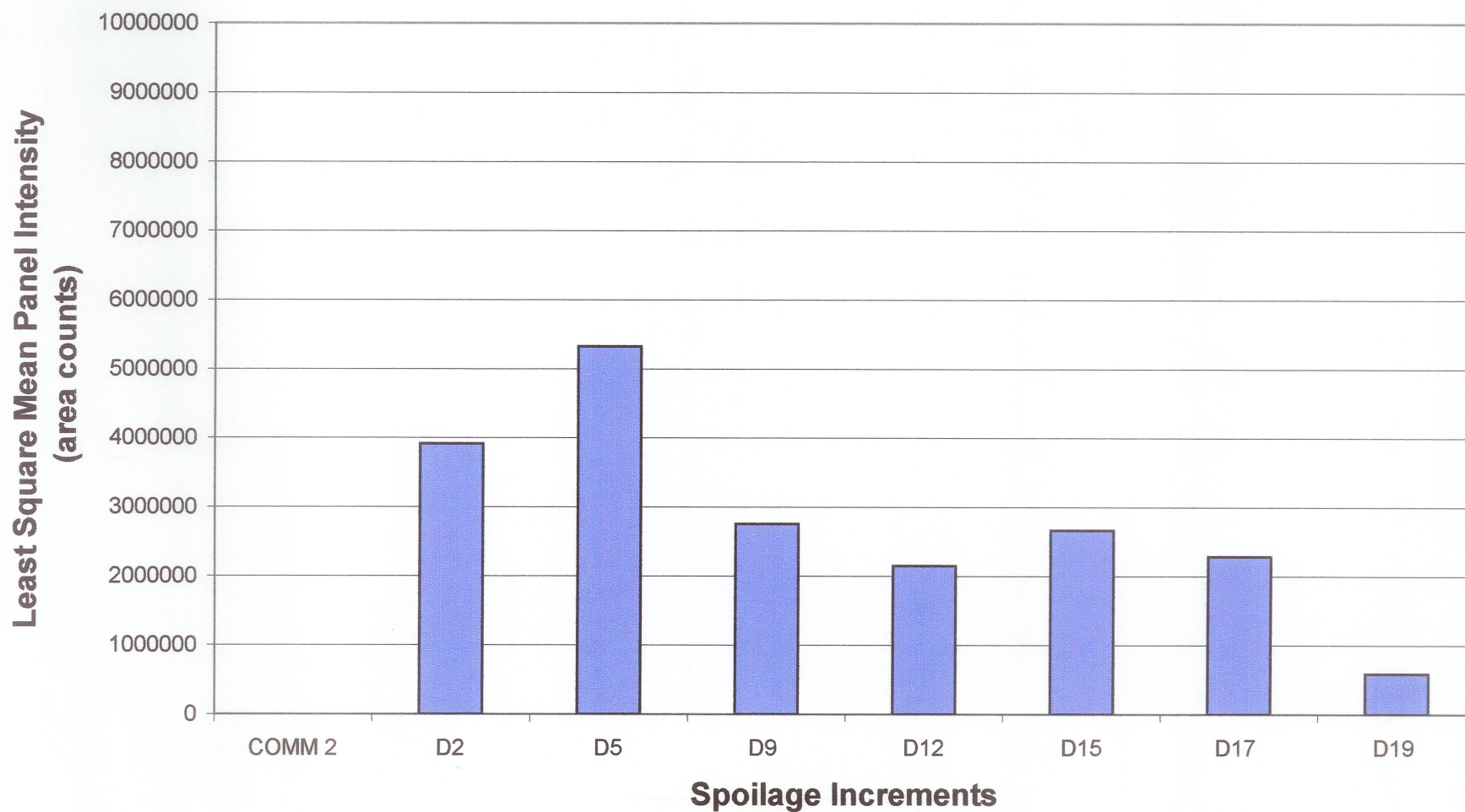
The expert panel described Comm-2 as grassy, melon and cucumber (Figure 4.8), with a mean quality line-score of 16.8 (Table 4.1). As shown in Figure 4.22, four odour impact volatiles stand out for the D-2 samples with the Key Terms, fresh floral (#9), fresh grass (#12), fresh/green (#17) and herbal/cilantro (#19). The presence of the Key Term, cucumber/melon (#25) is also noteworthy. These individual components also correspond to the odour terms identified by the expert panel which were, cucumber, grassy and melon (Figure 4.9) with a mean quality line-score of 14.0 (Table 4.1). For increment D-5 (Figure 4.23), the odour volatiles with the Key Terms fresh grass (#12) and fresh/green (#17) are notably absent from the radar graph. In addition, the intensity has decreased for fresh/floral (#9), herbal/cilantro (#19) and cucumber (#25). Instead, the key odour impact volatiles with the Key Terms of dimethyl sulfide (#2) and geranium (#23) are present. A change in the overall odour was also noted by the expert sensory panel with the D-5 increment being described as slightly grassy, melon and stale (Figure 4.10) and a mean quality line-score of 26.5 (Table 4.1). The D-9 radar graph (Figure 4.24) depicts a generally reduced intensity odour impact profile. The disappearance of the Key Term cucumber/melon (#25) is most notable. The decline in intensity of fresh odour attributes was also observed by the expert sensory panel for increment D-9 as indicated by the mean quality line-score of 29 (Table 4.1) and the sample being described as predominantly stale and slight grassy or melon in addition to slightly oily and oxidized (Figure 4.11). The D-12 radar graph (Figure 4.25) shows increased intensities for the Key Terms, caramel (#6) and green/fruity (#35). Also, four new Key Terms, although at low concentrations are present, musty (#20), sour (#27), cheesy (#32) and

pungent/licorice-like (#37). The presence of the new Key Terms along with terms present in the earlier spoilage increments clearly distinguishes the D-12 increment as a borderline quality sample. This data also corresponds to the expert sensory panel data which had an overall line-score mean of 50.5 (Table 4.1) with the odour descriptors representing pass and fail quality characteristics. The complete list of odour terms used by the expert sensory panel to describe D-12 is presented in Figure 4.12. Overall D-12 would be described as stale, oily and oxidized with slight grassy and melon notes when the increment passed and stale, slight fishy and slight sour, musty or faecal when the increment failed. The radar graph for D-15 (Figure 4.26) is differentiated from D-12 with the addition of the Key Term, sulfur (#22) and an increase in the intensity of musty (#20) and the loss of Key Terms, fresh/floral (#9), geranium (#23), sweet/floral (#24) and green/fruity (#35). This relates to the expert sensory panel data for increment D-15 which was described as predominantly sour and stale (Figure 4.13) with a panel quality line-score of 66.8 (Table 4.1). The radar graph for increment D-17 (Figure 4.27) shows a moderate increase in intensity for the Key Term, caramel/fruity (#10), the loss of herbal/cilantro (#19) and the presence of a new odour descriptor stale/acrid (#14). As with the D-15 increment, the majority of the individual odour descriptors identified for this sample are also associated with fail quality product. The expert sensory panel described increment D-17 as sour, fermented and putrid (Figure 4.14) with a mean quality line-score of 78.8 (Table 4.1). Finally, the D-19 radar graph (Figure 4.28) shows notably higher intensities for caramel/fruity (#10), musty (#20), sulfur (#22), stale (#26) and mothball (#34). In addition, a new Key Term, musty/vegetable (#15) was also present. These terms correspond to the expert sensory

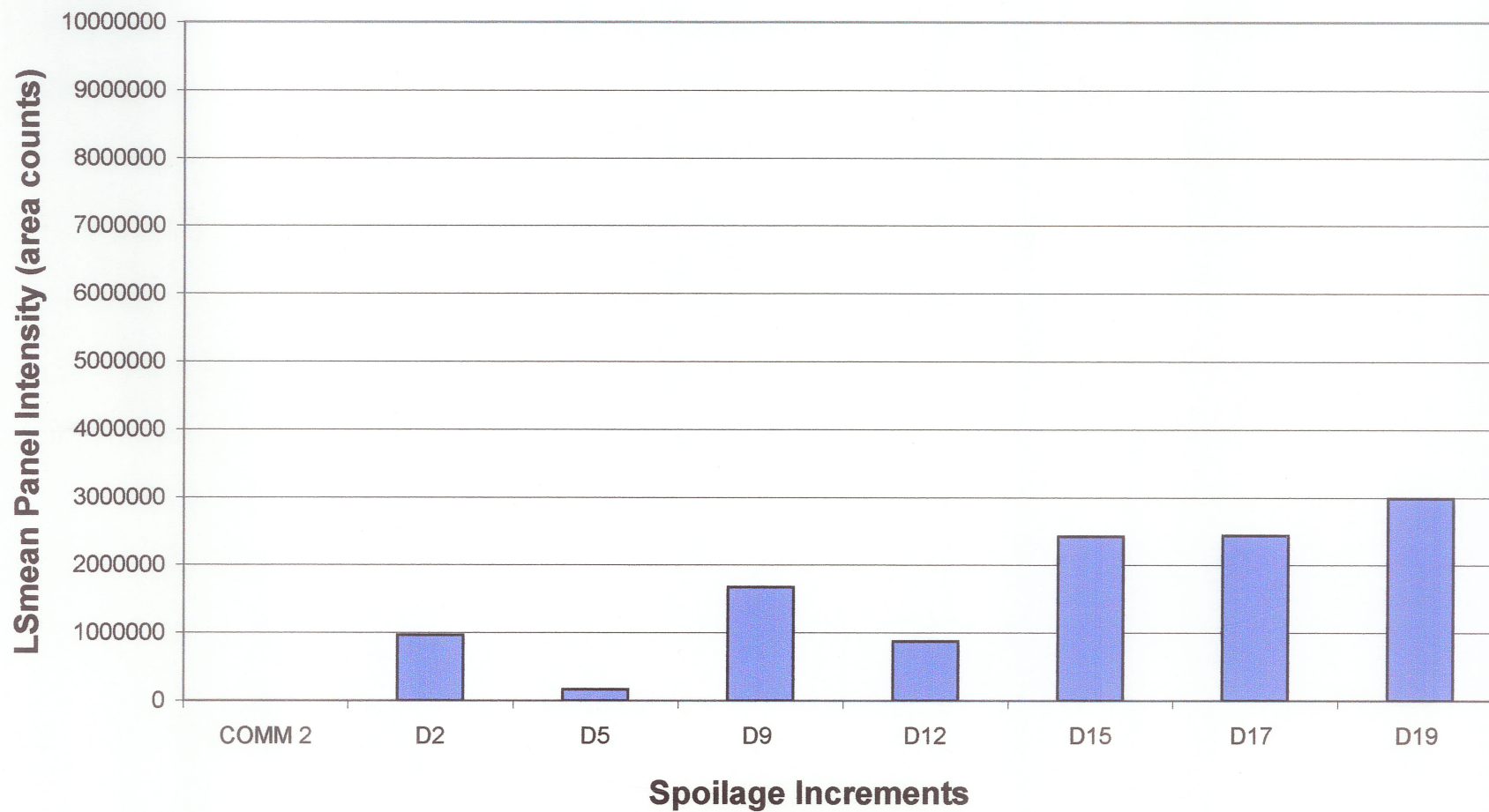
panel description of sour, fermented, putrid, and rotten meat (Figure 4.15) , with a mean quality line-score of 92.8 (Table 4.1).

It is also important to examine the Key Term identified across all spoilage increments. The odour volatiles with the associated Key Term that appeared in seven of the spoilage increments (excluding Comm-2) were dimethyl sulfide (#2), cheesy/fermented (#4), green/planty (#28), stale/oxidized (#29) and fresh/floral (#30). The intensity for most of these odour volatiles did not vary appreciably across spoilage increments, except for dimethyl sulfide (#2), where the greatest intensity was perceived in the pass increments and dropped gradually in the fail increments, with the lowest perceived level in increment D-19. This pattern is more clearly shown in Figure 4.29. A similar, yet reversed trend was evident for cheesy/fermented (#4), where it was given a higher intensity rating in the fail increments and a lower intensity rating in the pass increments (Figure 4.30). This finding could be accounted for by the fact that this odour volatile is only a minor component of the odour in fresh whitefish, but increases due to bacterial action or enzymatic activity during spoilage. Increment Comm-2 differed in that the odour volatiles dimethyl sulfide (#2) and cheesy/fermented (#4) were not perceived by any of the panelists. The most probable explanation for the absence of dimethyl sulfide (#2) would be that the Comm-2 fish were caught ten months later and were inherently different from the fish used in the controlled spoilage series due to biological variations such as sex, season of catch and differences in the aquatic environment. Dimethyl sulfide is a component typically found in fresh fish and seafood particularly in shrimp and scallops (Sims and McLachlan, 2000). The absence of the odour volatile cheesy/fermented (#4) could be attributed to a shorter time in frozen storage (one month)

Figure 4.29: Least Square Mean Intensity Ratings for Key Term #2 - dimethyl sulfide by Spoilage Increment



**Figure 4.30 Least Square Mean Mean Intensity Ratings for Best Term #4 -
cheesy/fermented by Spoilage Increment**



compared to ten months of storage for the controlled spoilage samples. The Key Term green/fruity (#11) appeared in all increments except D-17 and D19. This would indicate that either the associated chemical compound was no longer present or the physiological concentration was too low to be perceived in the later spoilage increments due to advanced decomposition and its associated odour volatiles. A discrete peak was not present on the GC/FID chromatogram at the corresponding mean retention time for any of the spoilage increments.

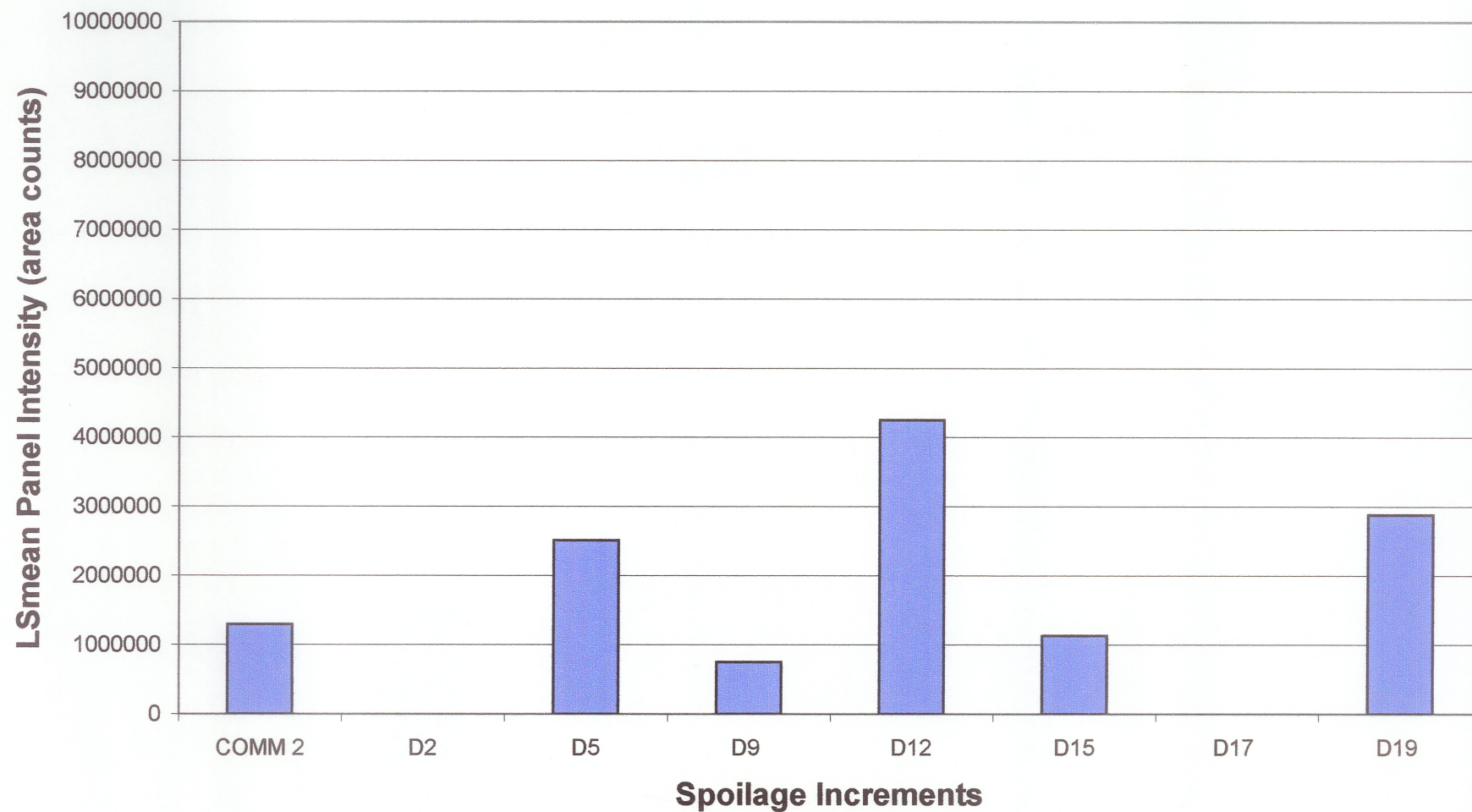
In general, the pass quality increments Comm-2, D-2, D-5, and D-9 were characterized by the odour volatile with Key Terms associated with fresh plants, flowers and fruit. The Key Terms were sweet/green (#8), fresh floral (#9), geranium (#13), fresh green (#17), fresh plant (#21), geranium (#23), sweet/floral (#24), cucumber/melon (#25), citrus (#31), floral (#33) and green/fruity (#35). These Key Terms were used for most of the pass increments but none of the fail increments except D-12 which was border-line pass/fail quality. The radar graph for D-12 (Figure 4.25) shows the presence of these Key Terms but at lower intensities than the pass quality increments.

Interestingly, the odour volatiles associated with Key Terms, fresh grass (#12) and fresh plant (#21) appeared in only Comm-2 and D-2 indicating that the associated chemical compound is probably only present in freshly caught fish, still in rigor and is depleted rapidly, even under ideal iced handling conditions. The odour volatiles associated with Key Term geranium (#23) appears in only D-5, D-9 and D-12. One might speculate that the chemical processes which are leading to a reduction in most of the fresh, characteristic odours are also resulting in the production of the odour component associated with geranium which itself disappears as the product further degrades.

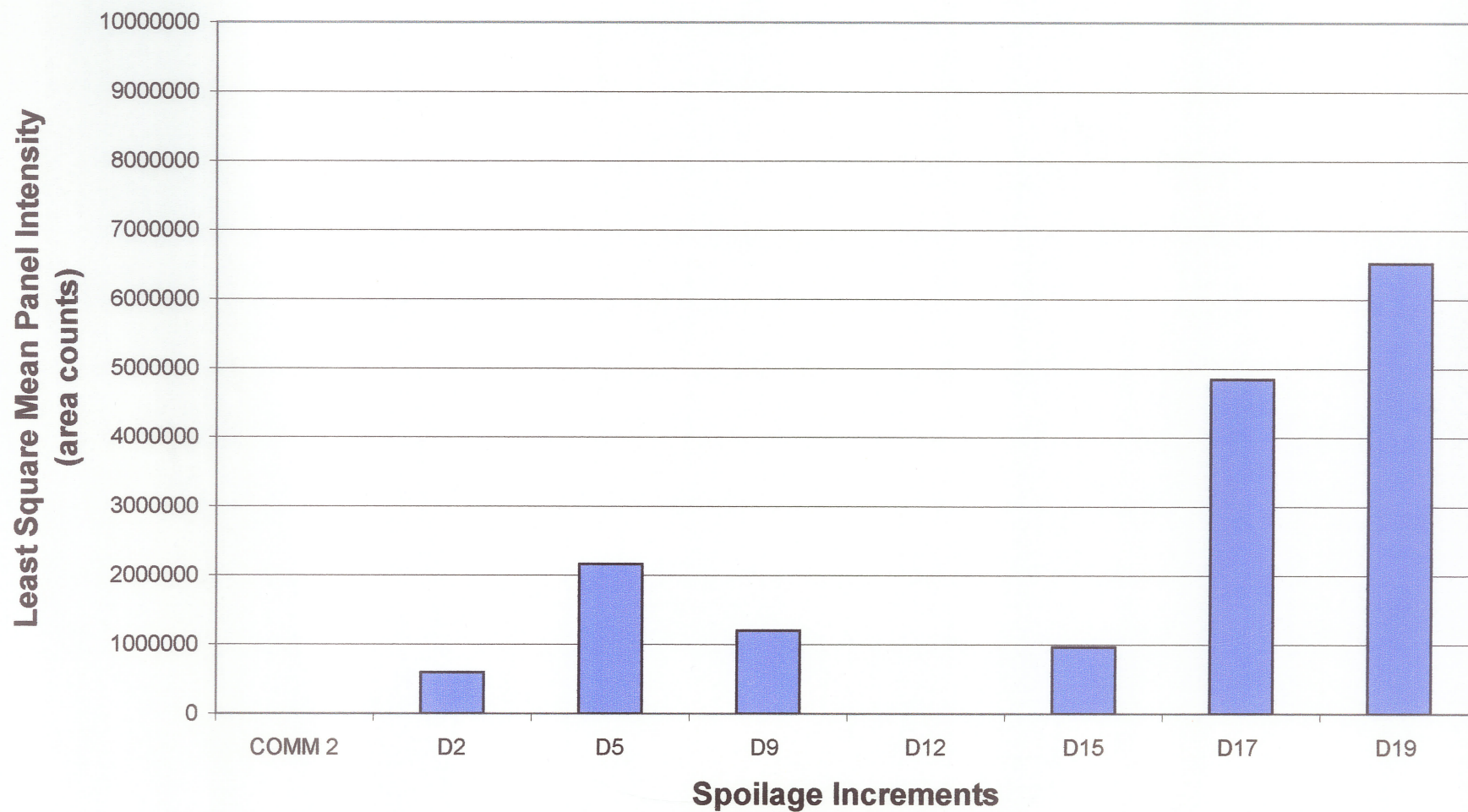
The pass/fail borderline increment D-12 and the fail increments D-15, D-17 and D-19 were characterized by odour volatiles that are usually associated with decomposition, autolytic breakdown and/or oxidation. The volatiles were associated with the Key Terms; solvent (#3), stale/acrid (#14), musty/vegetable (#15), musty (#20), sulfur (#22), stale (#26), sulfur (#36) and pungent (#37).

Several odour volatiles associated with Key Terms appeared sporadically across all increments. The odour volatile caramel (#6) was perceived in all increments except for D-2 and D-17 (Figure 4.31). Based on the corresponding GC/FID chromatograms for D-2 and D-17 it is likely that the volatile was present but appeared at concentrations too low to be perceived by the panel. The odour volatile caramel/fruity (#10) appeared in all increments except Comm-2 and D-12 (Figure 4.32). The intensity of caramel/fruity (#10) was low in the pass samples and much higher in D-17 and D-19 suggesting that the chemical compound associated with the descriptive term caramel/fruity (#10) appears to accumulate at higher concentrations for extremely decomposed whitefish samples. From the series of radar graph (Figure 4.21 – 4.28) it is evident that caramel/fruity (#10) plays a major role in the odour impact of D-17 and D-19 but to a much lesser extent in D-2, D-5, D-9 and D-15 samples. Interestingly, a discrete peak was not present on the GC/FID chromatogram at the associated mean retention time for any of the spoilage increments. For increment D-12 it is possible that the odour volatile, caramel/fruity (#10) was masked by another eluting volatile. This may also be true for Comm-2 or it may simply be inherently different due to varying biological conditions or may also have been masked by another volatile. The Key Term, stale/musty (#18) (Figure 4.33) first appeared in D-9

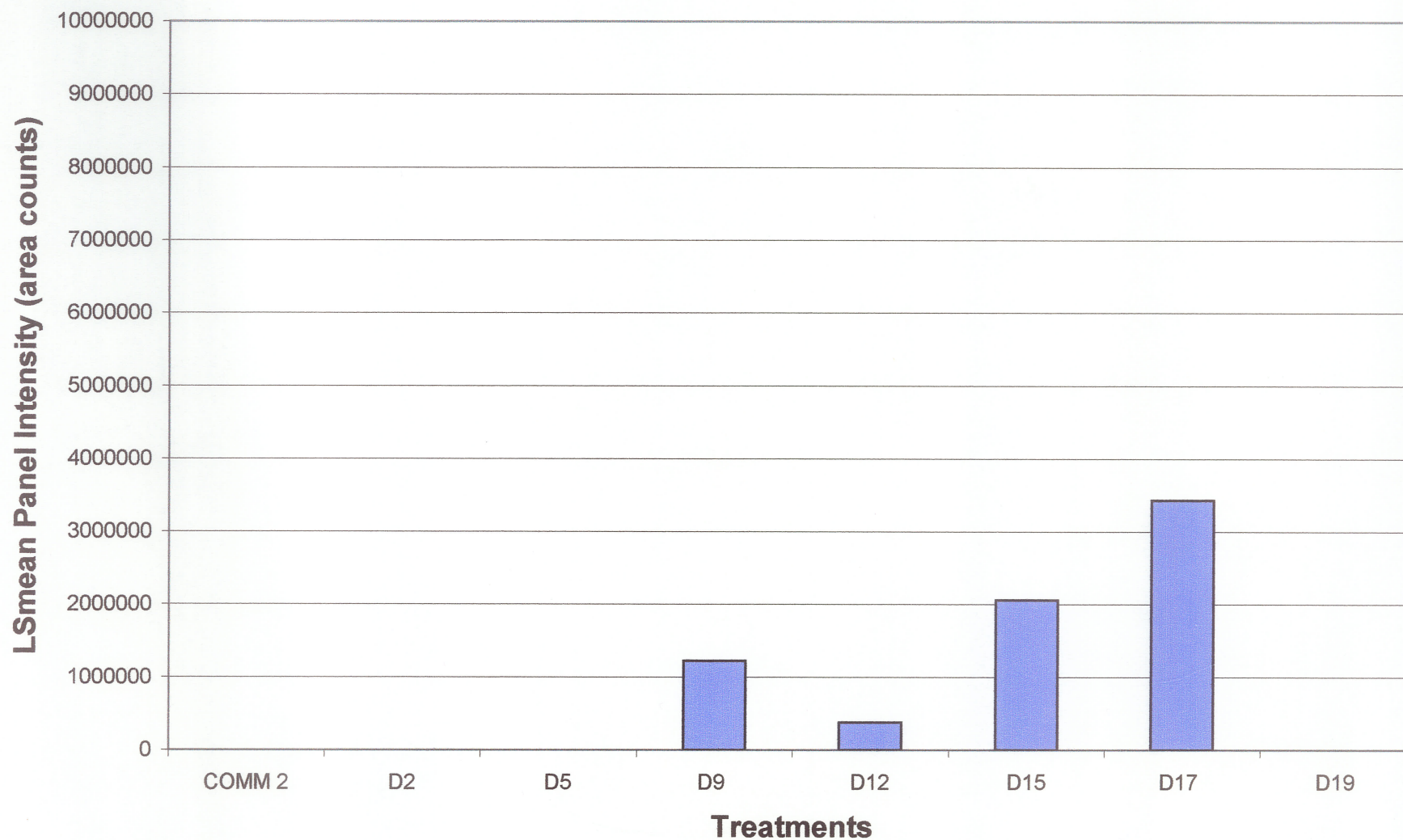
**Figure 4.31: Least Square Mean Intensity Ratings for Best Term #6 -
caramel by Spoilage Increment**



**Figure 4.32: Least Square Mean Intensity Ratings for Key Term #10 -
caramel/fruity by Spoilage Increment**

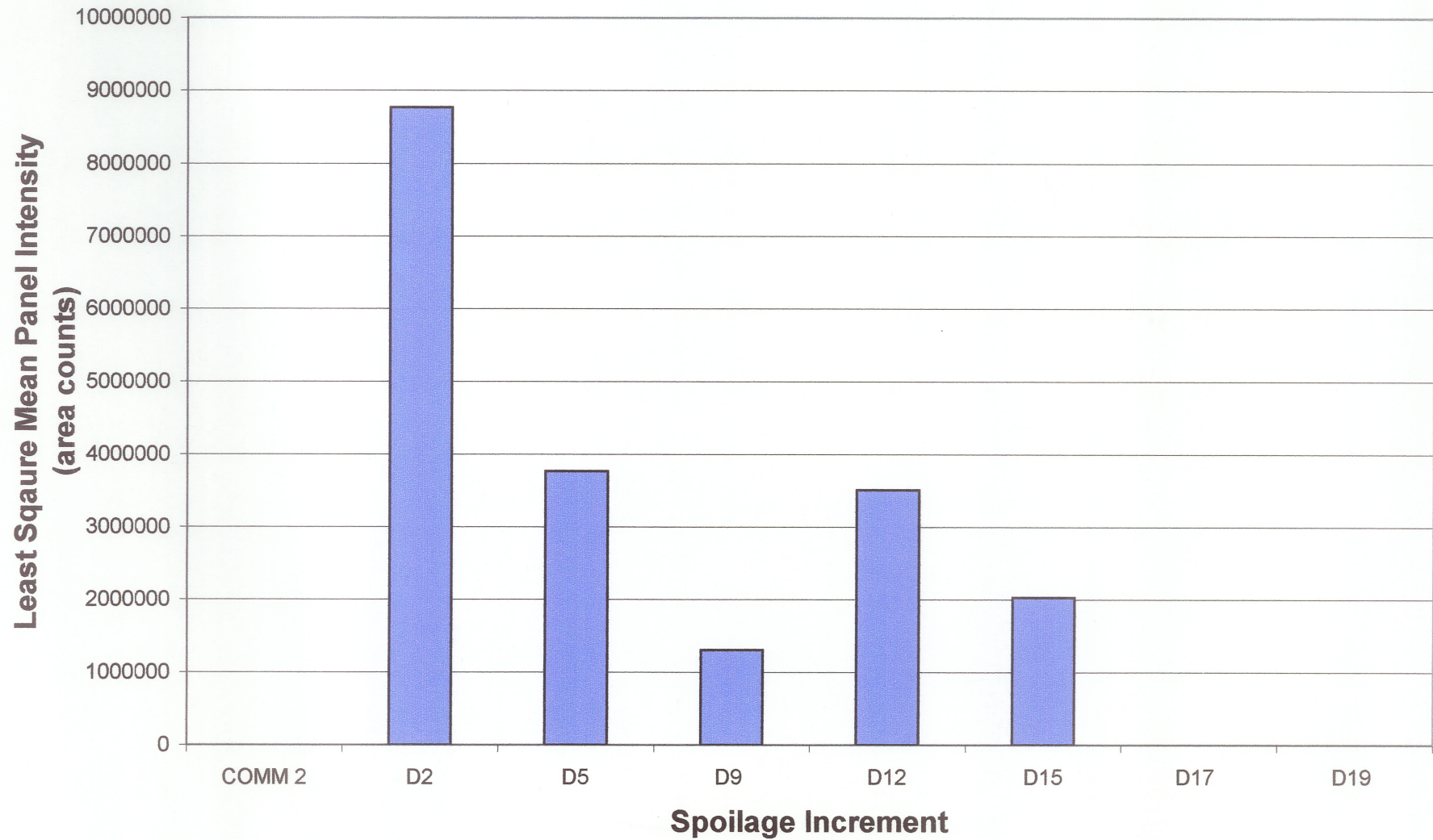


**Figure 4.33: Least Square Mean Intensity Ratings for Key Term #18 - stale/musty
by Spoilage Increment**



and continued to be present in D-12, D-15 and D-17 but was notably absent from D-19. The GC/FID chromatographs show a series of small conjoined peaks, just above baseline at the associated mean retention time for all spoilage increments. Therefore it is possible that the chemical compound associated with stale/musty (#18) is not present in D-19 due to depletion of the substrate used for its production; its concentration was below the perception level of the panel or it was converted into another chemical. The odour volatile associated with Key Term, herbal cilantro (#19) was perceived in increments D-2, D-5, D-9, D-12 and D-15. It appears that this component is associated mostly with fresher quality since the perceived intensity was more than double for increment D-2 compared to the other increments (Fig. 4.34) and was not perceived in two of the more highly decomposed spoilage increments. Notably, the odour volatile was not perceived in Comm-2, this may be explained by the fact that it was caught 10 months after the fish used in the controlled spoilage series. The odour volatile associated with Key Term, sour (#27) appeared in Comm-2, D-2, D-12 and D-17 (Figure 4.35). Similarly, the odour volatiles associated with Key Term, cheesy (#32) (Figure 4.36) appeared in Comm-2, D-2, D-5, D-12 and D-15 and mothball (#34) (Figure 3.37) appeared in all increments except D-12 and D-15. Like sour (#27) both terms appeared across the various spoilage increments without a notable trend in odour intensity. Finally, three Key Terms were only perceived by the panel in one increment. The odour volatiles associated with Key Terms sweet #1 was perceived in increment D-12, briny (#5) in increment D-2 and sweet (#16) in D-9 at relatively low concentrations (Figures 4.38 -4.40). Therefore the odour impact of each of the volatiles in the associated

**Figure 4.34: Least Square Mean Intensity Ratings for Key Term #19 -
herbal/cilantro by Spoilage Increment**



**Figure 4.35: Least Square Mean Intensity Ratings for Key Term #27 -
sour by Spoilage Increment**

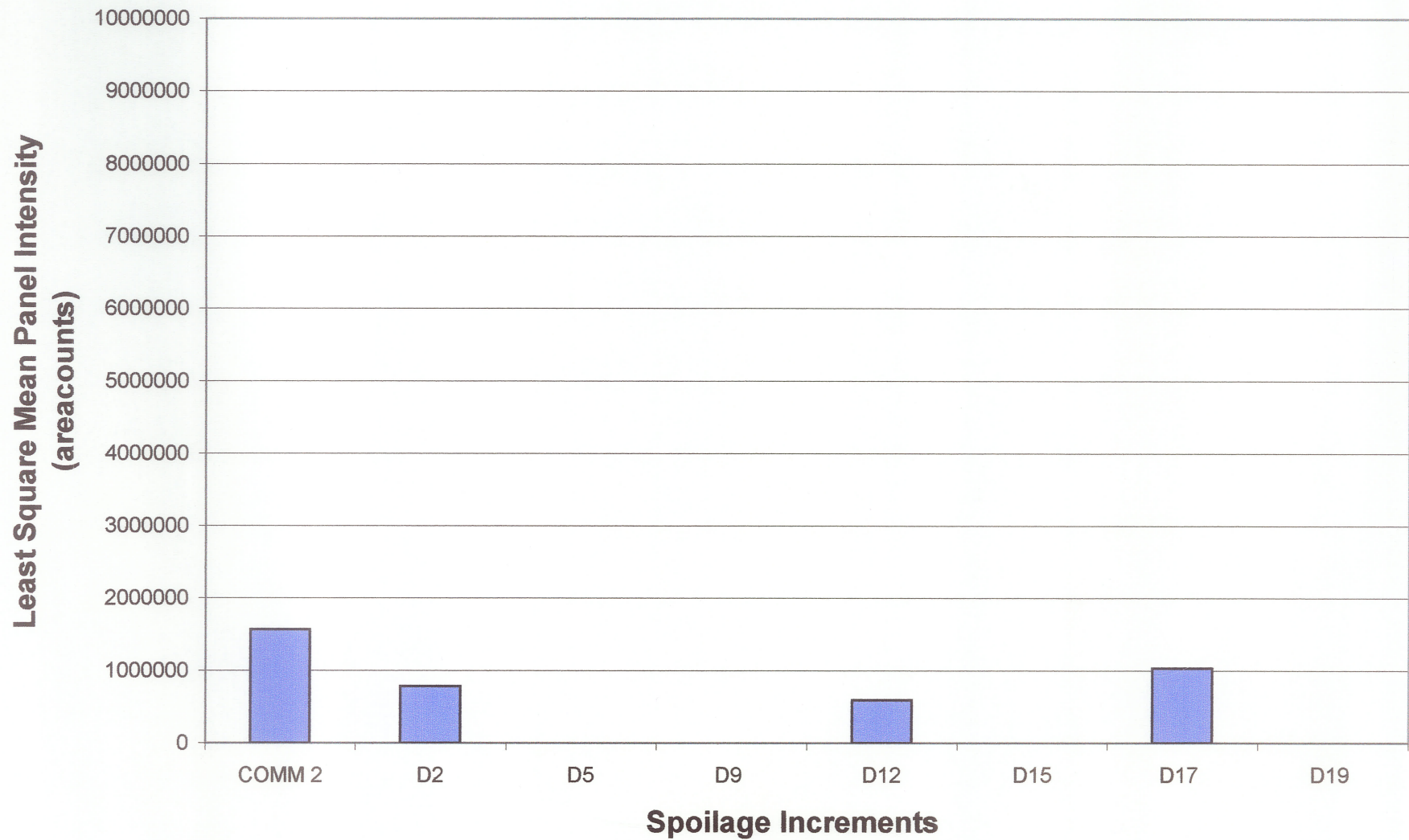


Figure 4.36: Least Square Mean Intensity Ratings for Key Term #32 - cheesy by Spoilage Increment

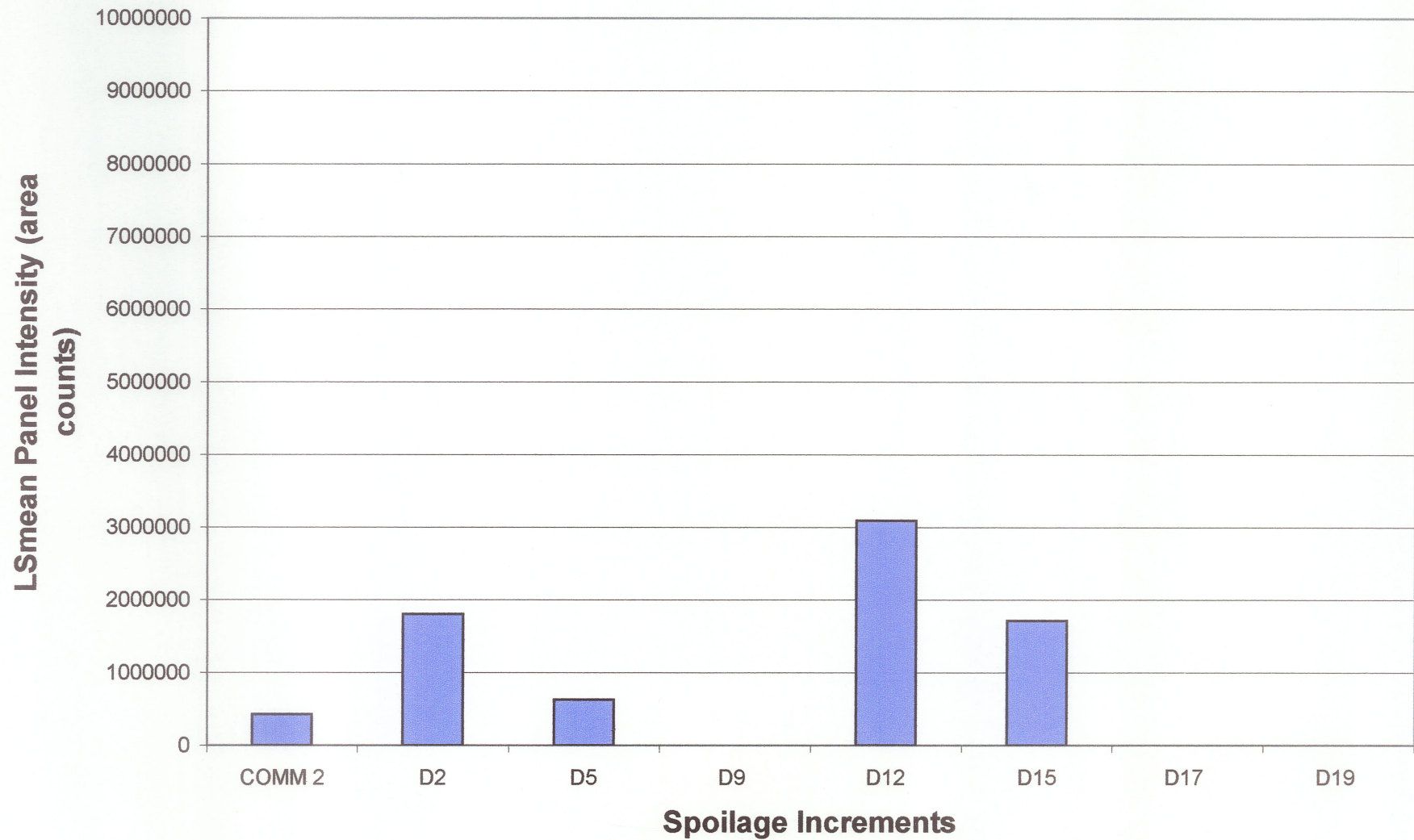
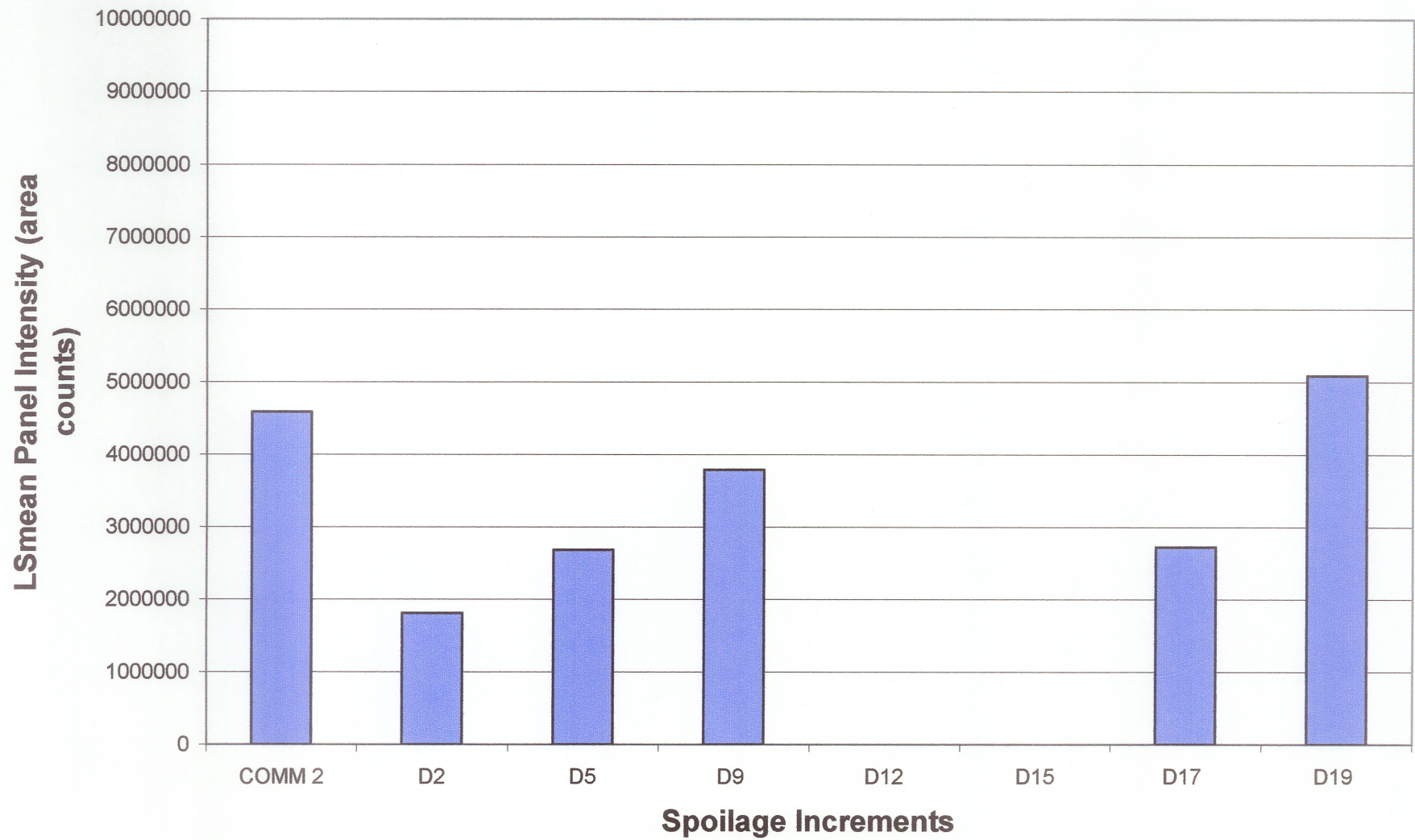


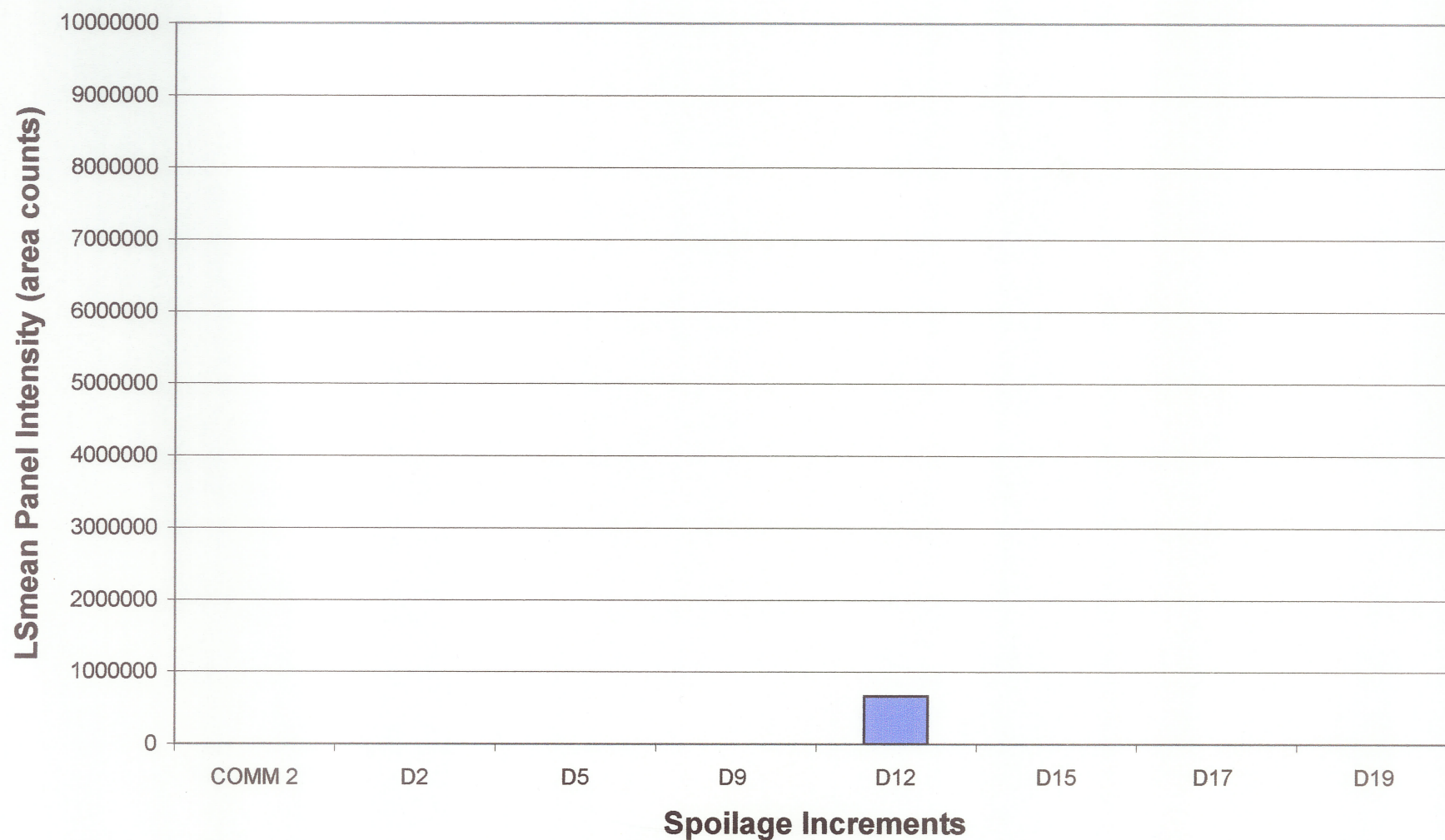
Figure 4.37: Least Square Mean Intensity Ratings for Best Term #34 - mothball by Spoilage Treatment



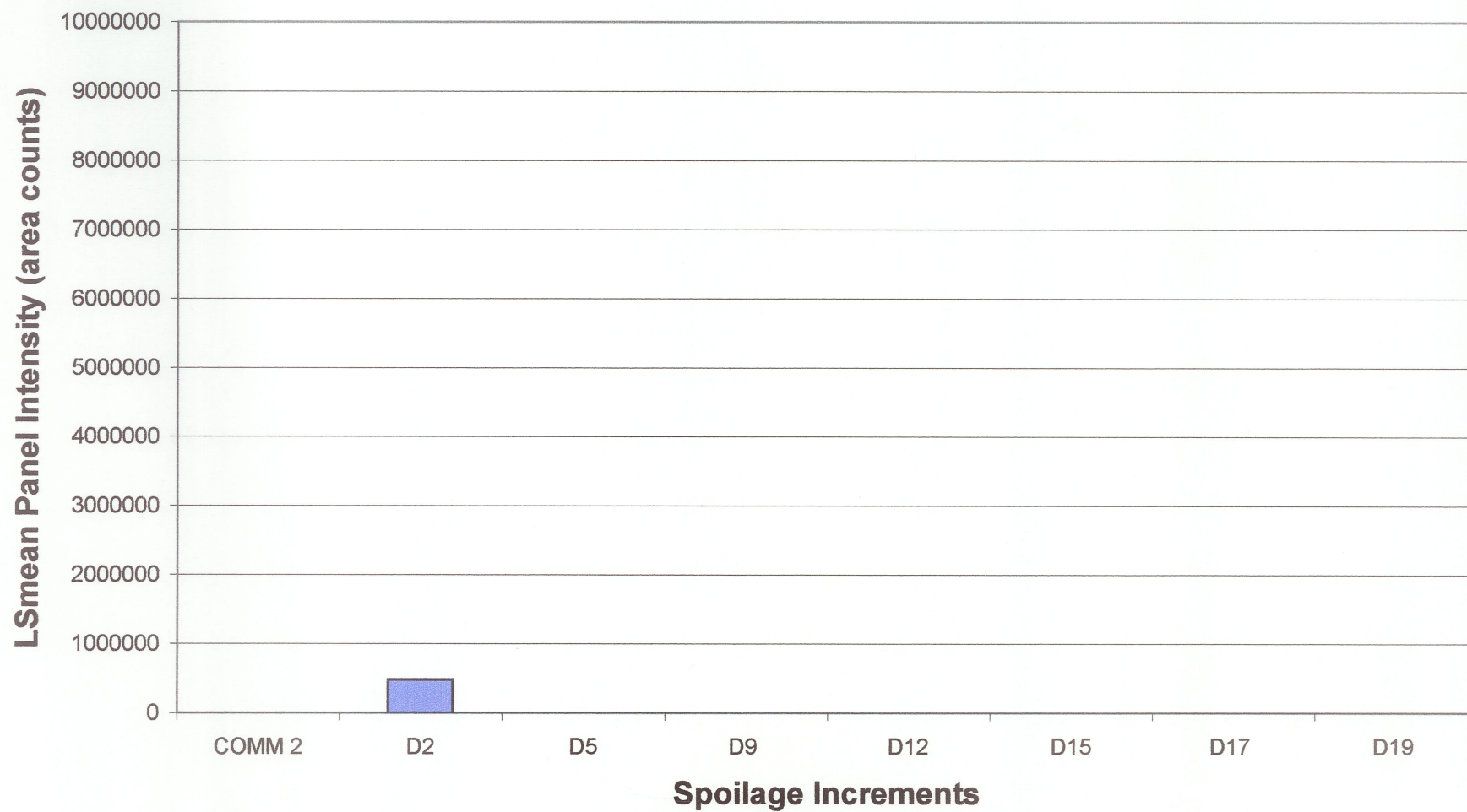
increments was probably very slight. In general the descriptive terms, sweet and briny were used to describe pass quality fish products.

It is important to re-emphasize that past studies have determined that GCO odour volatile intensity is not directly proportional to the physical concentration of a chemical compound detected by FID (Blank, 1997, Mistry et al, 1997). This was found to be the case in some instances in the present study. That is, a relatively small FID peak may have high odour intensity, such as the Key Term, fresh/green (#17) identified in the pass spoilage increments. While a relatively large FID peak may have low odour intensity such as Key Term, cheesy/fermented (#4), identified in all spoilage increments except Comm-2. Odour impact volatiles may also be small components of co-eluting volatiles that are not differentiated as discrete peaks such as Key Term, stale/musty (#18), identified in D-9, D-12, D-15 and D-17. In addition, the GCO panelist may be capable of perceiving an odour impact for chemical concentrations below the detection limit of an FID, such as Key Term, citrus (#31). Therefore FID detection alone can not be used to conclusively determine the chemical concentration of odour impact volatiles identified by GCO. Chromatography using GC/MS is necessary to not only determine the identity and concentration of an odour impact volatile but to detect potentially co-eluting chemical compounds that could be separated by changing the chromatography conditions.

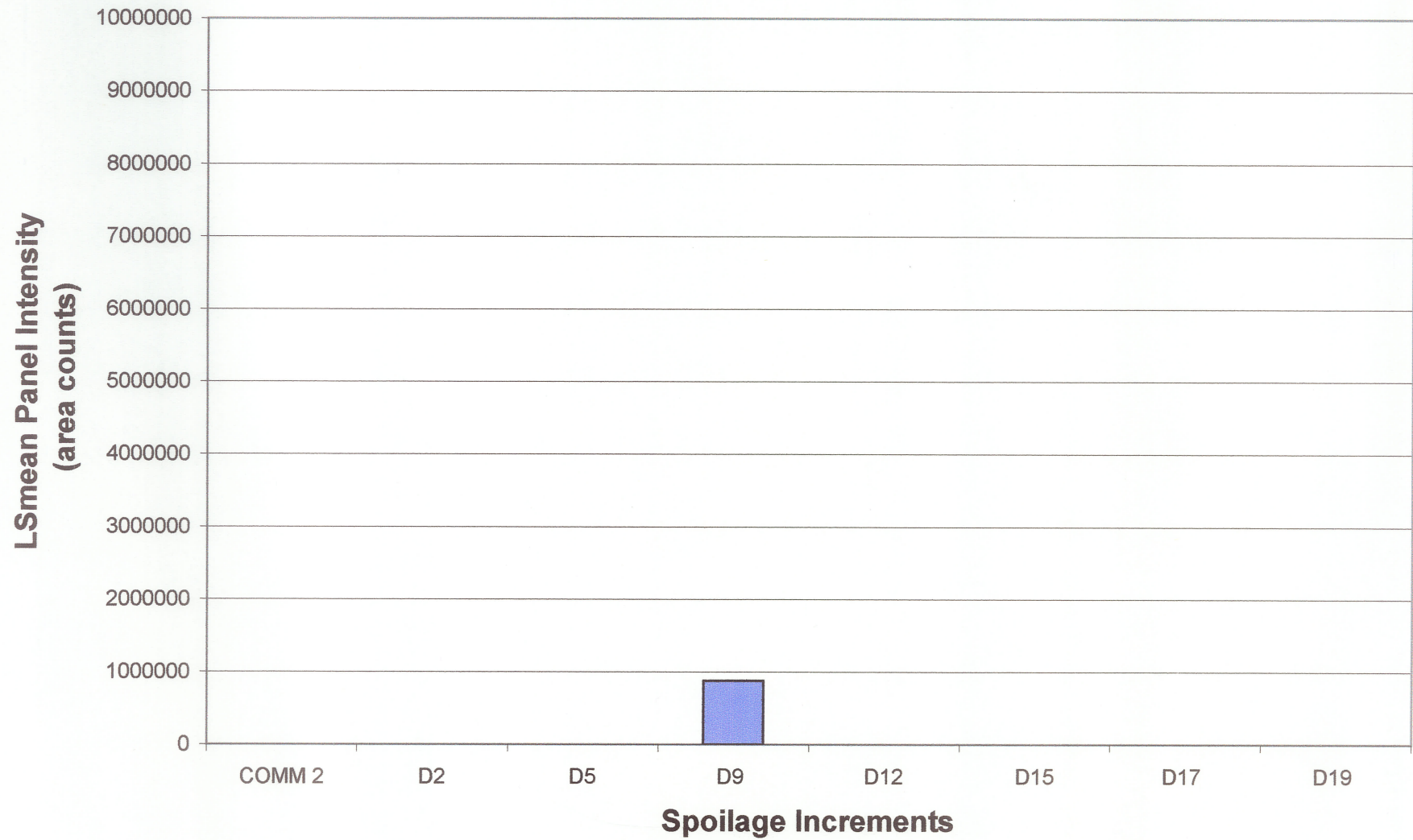
**Figure 4.38: Least Square Mean Intensity Ratings for Key Term #1 -
Sweet by Spoilage Increment**



**Figure 4.39: Least Square Mean Intensity Ratings for Best Term #5 -
briny by Spoilage Increment**



**Figure 4.40: Least Square Mean Intensity Ratings for Best Term #16 -
sweet by Spoilage Increment**



Chapter 5

Summary, Conclusions and Recommendations for Future Research

5.1 Summary

The objective of the first part of this study was to determine the quality of a series of eight spoilage increments of whitefish using an expert sensory panel and to benchmark the pass/fail borderline quality using three traditional chemical indicators of spoilage: Freshness Index, ethanol and TVB-N. The quality rating (pass or fail), associated mean panel line-score, and odour descriptions were determined for each spoilage increment. A progression in odour attributes from fresh to highly decomposed was found as the days held on ice increased for the spoilage series. The pass/fail borderline quality was found to occur at increment D-12 (twelve days held on ice). Freshness Index and ethanol were found to be good indicators of spoilage since both indices were able to benchmark the borderline quality of the spoilage series as determined by the expert sensory panel. The Freshness Index was also useful in showing the progression of autolytic changes over time. TVB-N correlated well with increments indicative of advanced spoilage but was not useful in benchmarking the borderline quality.

The objective of the second part of this study was to determine the proximate and fatty acid composition of each spoilage increment in order to evaluate the uniformity of the fish used in the controlled spoilage experiment. A secondary objective was to determine the possible contribution of these components to the sensory attributes perceived by the expert panel for each spoilage increment. No significant differences were found in moisture, protein and fat across all spoilage increments. The fat content was found to range from 11-12.3%, which was higher than the published average of 8%

for whitefish. No significant differences were found for the SFA, MUFA, and total PUFA contents among the whitefish spoilage increments. Overall, the whitefish spoilage increments contained a high level of total PUFA. Some of the off-odour volatiles associated with oxidation and rancidity could be attributed to the relatively high fat and total PUFA content.

The main objective of this thesis was to identify the key descriptive terms and to quantify the intensity of volatile chemical compounds with odour impact present in the whitefish spoilage increments using a static headspace, GCO method (McLachlan, 1997) and an expert sensory panel. In addition, it was the goal of this research to determine whether individual volatile compounds and their intensity changed significantly throughout the spoilage series and to identify what critical changes occur in odour volatile composition among the increments.

The GCO panel collectively used a total of 402 terms to characterize the odours they perceived across the thirty-two GCO sessions conducted. The list of 402 terms was reduced to 133 terms by removing qualifying terms related to intensity perception. A final list of thirty-seven volatiles with odour impact was selected by grouping synonyms of the main term used by at least two of four panelists. The mean retention time associated with each Key Term and the intensity of key volatiles appearing in each spoilage increment were determined from the aromagrams generated from the slide-bar responses of the expert GCO panel. The panel aromagram intensity ratings were normalized using a multivariate statistical model. The model was also used to estimate responses for panelists in cases when only two or three out of four panelists identified an odour impact volatile. The Least Square Means intensity ratings calculated from the

model were used plot radar graphs showing the key odour impact volatiles and the associated Key Terms for each spoilage increment.

Key odour volatiles that remained present across all spoilage increments were identified and intensity quantified. For example, Key Term dimethyl sulfide (# 2) was present in all spoilage increments except for Comm-2. The greatest intensity was perceived in the pass quality spoilage increments. The intensity gradually dropped in the fail quality spoilage increments. This result is not surprising since dimethyl sulfide is a component typically found in fresh fish and seafood. Similarly, the presence and intensity of Key Term stale/oxidized (#29) was found across all spoilage increments. Although the level remained relatively constant this odour volatile is likely one of the compounds associated with oxidation that occurs with storage.

Unique, key odour volatiles were determined for fresh quality (Comm-2 and D-2), pass quality (D-5 and D-9) borderline quality (D-12) and for fail quality (D-15, D-17 and D-19) whitefish. Comm-2 and D-2 both contained the Key Term, fresh/green (#17) which received relatively high intensity ratings. The radar graph for D-2 also indicated that Key Terms, fresh/floral (#9), fresh grass (#12) and herbal/cilantro (#19) contributed greatly to its overall odour. Increments D-5 and D-9 were distinguished by the appearance of Key Term, geranium (#23) which was not found in Comm-2 or D-2. Overall, D-5 and D-9 radar graphs were distinguished by the loss of a key volatile fresh/green (#17) and reduced intensity ratings for volatiles associated with the two freshest increments. Critical changes in the volatiles with odour impact were identified in the borderline quality, increment D-12. Odour volatiles identified in both the pass and fail samples were present in the D-12 samples. Like, D-5 and D-9, the radar graph

for D-12 showed decreased intensities for Key Terms such as fresh/floral (#9) and herbal/cilantro (#19) and the presence of geranium (#13). In addition four new Key Terms, musty (#20), sour (#27), cheesy (#32) and pungent/licorice-like (#37) were present, although at low intensities. The radar graph for increment D-15 was distinguished from D-12 with the presence of Key Term, sulfur (#22) and the increase in intensity of musty (#20). In addition, the Key Terms, fresh/floral (#9), geranium (#23), sweet/floral (#24) and green/fruity (#35) present in the fresher increments were lost. The radar graph for D-17 showed the presence of a new Key Term, stale/acrid (#14) and a moderate increase in the intensity of Key Term caramel/fruity (#10) which was present at low intensities in the fresher increments. Finally D-19 was distinguished by the presence of Key Term, musty/vegetable (#15) along with notably higher intensities for the Key Terms, caramel/fruity (#10), musty (#20), sulfur (#22), stale (#26) and mothball (#34).

Thus the GCO method was successful in identifying the key descriptive terms associated with volatiles contributing to the odour of each spoilage increment and quantifying their intensities. The method was also successful for examining and demonstrating the differences between spoilage increments and the critical changes in odour impact volatiles that developed for whitefish stored on ice over time. Unfortunately, the concentration of volatiles by GC/FID could not be determined conclusively for each key odour volatile using this GCO method.

5.2 Conclusions and Recommendations for Future Research

Mistry et al (1997) emphasize the importance of aroma-recombination studies that use the data provided from GCO, GC/FID and GC/MS to formulate a “chemical

cocktail” that simulate the complex odours or flavours of a food product. The data include, the identification of odour impact volatiles and their intensity determined by GCO, their concentrations determined by GC/FID and GC/MS and their identity and structure determined by GC/MS. Studies regarding aroma-recombination have been published for tomatoes (Buttery et al, 1987, 1990), dill herb (Blank and Grosch, 1991), butter flavors (Schieberle et al, 1993), Emmentaler cheese (Grosch and Preininger, 1994) and roasted coffee (Semmelroch et al, 1995). Sims and McLachlan (2000) were able to identify odour volatiles and their concentration in Atlantic scallops using a modified GCO method of McLachlan (1997) in tandem with GC/MS. Additional work is needed however, before aroma reference compounds can be developed.

Gas chromatography - olfactometry is a rapidly evolving science that has made strides in practical applications in food science through application of standardized sensory methods and optimization of GCO techniques tailored to the features unique to the food product. Odour-recombination studies are on the forefront as the next step in utilizing detailed odour volatile profiles and effectively using both chemistry and sensory science techniques. Fish and seafood products in particular are complex products that use sensory evaluation performed by sensory experts (inspectors) to distinguish pass from fail quality and the continuum of quality in between. Fish products have not been widely studied using GCO techniques and the demand for references that simulate the different quality levels remains high, especially by industry players who are implementing QMP and QMPI systems which include sensory assessments to meet CFIA, FSPP program requirements.

This study made valuable progress towards the development of an odour reference set for whitefish at various quality levels by identifying a number of key odour impact volatiles and their associated descriptive term which contribute to the overall odour of each spoilage increment. These volatile components are likely to form a foundation for the future development of odour references for whitefish. The quality assessment using an expert sensory panel provided important information with regard to the overall odour of each spoilage increment. The supporting background information including the proximate composition analysis and traditional chemical indicators of spoilage provided insight into the biochemical processes that contributed to the odour volatiles that developed in the whitefish spoilage series.

The multivariate statistical model applied to the GCO data was an invaluable tool for handling the large data set. The Least Square Mean intensity values plotted in each radar graph were used to identify the key odour impact volatiles in each increment and were based on significant relationships between the class variables; "Panelist", "Key Term", "Spoilage Increment" and combined "Spoilage Increment $\times$ Key Term" effect. This statistical approach has not been used by other researchers employing static headspace GCO methods.

The odour character and intensity of the key volatiles with odour impact contributing to the overall odour of whitefish from fresh to highly decomposed were identified in this study. GC/MS is required to determine the chemical identity and structure of the odour impact volatiles since it was not conducted in this study. The GC/MS data is necessary for the development of a stable, chemical reference odour set for whitefish.

Upon review of the data, and chromatographic conditions by the review committee, some concern was expressed over the use of “high speed” chromatography for the GCO work. The author acknowledges that the carrier flow is at the upper end for a megabore column (0.35 mm x 30 m). Two main factors were taken into consideration when establishing these conditions. There is a need to get the trapped components onto the column in a timely manner when using thermal desorption using the present external thermal desorption inlet (which is also supplying and controlling the carrier flow for the entire chromatographic run). This necessitated a higher flow rate. A second factor was the need to keep the chromatographic run short enough that the sensory analyst at the olfactometer did not suffer sensory and physical fatigue over the course of the chromatographic run. From the sensory analyst’s point of view 20 min of intense concentration involving GCO eluent “sniffing”, recording odour intensity and severely restricted mobility is their physical limit. Typically from previous work on scallops, the standard deviation for the retention time has been demonstrated to be anywhere from 0.02 to 0.14 when considering 20 chromatographic runs for a single analyst with most of the standard deviations falling in the 0.05 to 0.08 range, (McLachlan, 2004). This retention time standard deviation seemed adequate considering that the sensory analyst has to detect the peak, and record the change in intensity over time using a “slide wire” which generates a signal to the GC workstation. The slight variation in retention time between a series of 20 chromatographic runs from scallops with different odour profiles due to changes in spoilage is offset by the selective nature of olfactometry detection, and the odour signature / description provided by the sensory analyst detector.

This being said the author will take into consideration these concerns of the review committee in any future work and provide additional data on retention time reproducibility. In addition the author has discussed this issue with the laboratory head who established the original chromatographic conditions. The laboratory head respectfully acknowledges the concern and assured the author that steps would be taken to examine the chromatographic conditions in detail to address these concerns and will examine the effects of reduced flow rates on the chromatographic performance of the system, (personal communication, McLachlan 2004).

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